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Abstracts

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INCIDENCE AND PROGNOSTIC IMPLICATIONS OF IMMUNOPHENOTYPES IN THE ALL-BFM STUDY 83

W.D. Ludwig, F. Herrmann, H. Seibt-Jung, B. Komischke, E. Odenwald, J. Hofmann and H. Riehm

Immunophenotyping studies were performed in 615 children included in the ALL-BFM study 83. According to their reactivity with a panel of monoclonal antibodies recognizing B- and T-cell associated antigens (CD19/20/24; CD1/2/3/4/5/7/8) as well as other non-lineage-restricted antigens (CD10, HLA-DR), five subtypes could be identified: null-ALL (3.6%), c-ALL (78.2%), B-ALL (2.4%), pre-T ALL (2.9%) and T-ALL (12.4%). After a median trial duration of 21 months, event-free survival (EFS) was 61% for null-ALL, 72% for c-ALL, 27% for B-ALL, and 60% for pre-T and T-ALL. There was no significant difference between the immunologic subgroups in their response to remission induction therapy, whereas a significant difference in EFS could be observed in cALL vs. null-, pre-T/T- and B-ALL ($p \leq 0.05$). Further subclassification of the cALL group revealed a significantly shorter duration of EFS for children with CD20 + cALL ($p \leq 0.01$), suggesting that the prognosis in B cell lineage ALL is related to the degree of maturity of the malignant cells. Adjustment for clinical risk groups disclosed that the worse prognosis for CD20 + cALL is not due to more unfavorable risk factors within this subtype, whereas the negative prognostic influence of null- and pre-T/T-ALL is partly related to the association with other clinical features of known prognostic importance, such as age and white blood cell counts.

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SHIFTS IN EXPRESSION OF IMMUNOLOGIC PHENOTYPE OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) AT RELAPSE

H. Seibt-Jung, C.R. Bartram, J. Harbott, A. Raghavachar, G. Gassner, W.D. Ludwig.

Biologic features of childhood ALL-blast cells were analyzed in 102 children at diagnosis and relapse of their disease in order to better understand the pathogenesis of disease recurrence. Based on the reactivity with a panel of monoclonal antibodies (moAb) directed against B-cells (CD 19,20,24), T-cells (CD 1,2,5,7), and non-lineage-restricted antigens (CD 10, HLA-DR, TdT), 75 cases were classified as common-ALL, 16 cases as T-, and 4 cases as B-All. We found 5 cases of 0-ALL, and 2 cases were diagnosed as acute unclassifiable leukemia (AUL).Changes in the expression of surface antigens or TdT activity occurred in 27 (26%) patients. Loss of CD 1o (n=6) or CD 20 (n=9) at relapse was a frequent finding, indicating a shift to a more immature differentiation stage of B-progenitor-cell ALL. Conversely, 4 cases with common-ALL disclosed acquisition of CD 20 at relapse, and 1 patient showed evidence of a shift from common-ALL to mature B-ALL at relapse. Decreased TdT activity at relapse was found in 2 patients with T-ALL. One patient with AUL at diagnosis clearly revealed a myeloid phenotype at relapse, and only 1 patient with T-ALL showed a lineage switch to acute nonlymphoblastic leukemia.Immunoglobulin and T-cell-receptor gene rearrangement, analysed in 1o patients both at diagnosis and relapse, demonstrated clonal evolution in 4 cases. Results of sequential cytogenetic studies performed in 8 patients will be presented.

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LEUKEMIA KARYOTYPE IN 147 CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA OF ALL-BFM 83 STUDY GROUP

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Whereas chromosome constitution of leukemic cells is helpful for confirming diagnosis and classification in different types of leukemia, its prognostic significance is not yet clear. As therapy is the most important prognostic factor all our examined patients were treated uniformly according to the ALL-BFM 83 protocol.

Successful cytogenetic blast cell analysis could be performed at diagnosis in 147 children with acute lymphoblastic leukemia (ALL). Besides "normal diploid" cells (44.9%) four groups of aberrations could be distinguished: hypodiploidy (3%), pseudodiploidy (19%), hyperdiploidy (47-49 chr.) (10%) and hyperdiploidy (>49 chr.)(23.1%). Structural aberrations, e.g. t(8;14) in B-cell ALL or t(11;14) in T-cell ALL, as well as numerical changes, e.g. >50 chromosomes in c-ALL, were found to be specific.

As to prognosis, only children with c-ALL were evaluated. It could be shown, that patients who relapsed, exhibited more frequently structural aberrations as compared to patients in continuous complete remission.

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CORRELATION OF ONCOGEN EXPRESSION WITH THE MANIFESTATION OF DIFFERENT TYPES OF THE IMMUNOPHENOTYPE OF LEUKAEMIC CELLS

Helmke, M., Kemnitz, J., Buhr, Th., Freund, M., Dominis, M., Georgii, A.

The oncogen expression in the c-myc oncogen system in leukaemic cells occurring in acute leukaemias as well as in blast crises of chronic myeloproliferative disorders can - as our earlier studies have shown (KEMNITZ et al., 1986) - be differentiated in 3 types: 1. the positive synchronous expression (c-myc-onc$^+$: p-55-protein$^+$), 2. the negative synchronous expression (c-myc-onc$^-$: p-55-protein$^-$); and finally 3. the asynchronous expression (c-myc-onc$^+_-$: p-55-protein$^-$). The phenotype of leukaemic cells, respectively their immunophenotype, occurs predominantly in a homogeneous type of manifestation. The heterogeneous phenotype, characterized by positivity of cell markers also of other histogenetic lineages, however exists in 3 types: 1. the additive phenotype; 2. the hybrid phenotype; and 3. the combined phenotype. The study was based on the material (bone marrow and peripheral blood smears) of 68 patients with AL (62/68) and with blast crises of CMPD (6/68). The heterogeneous phenotype was analyzed in the following combinations: MY-7 : GP-IIb-IIIa; MY-7 : GLY-A; and MY-7 : Tdt. The expression of the c-myc oncogen system was studied with the method of gene hybridisation "in situ" with the DNA probe c-myc (9 KB, - 2.000 base pairs). The oncogen product, p-55-protein, analyzed with the I-PAP method. The results of our study with respect to the correlation of different types of the oncogen expression with the immunophenotype of the leukaemic cells have shown that the stability of the immunophenotype of the leukaemic cells - respectively the homogeneous immunophenotype - is correlated with a positive - predominantly synchronous - expression of the c-myc oncogen in the individual leukaemic cell.

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CSF-GENE EXPRESSION AND -SYNTHESIS BY BLAST CELLS FROM PATIENTS WITH ACUTE MYELOID LEUKEMIA

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Expression of the genes for GM-CSF, G-CSF and M-CSF was studied by Northern blot analysis in normal hematopoietic cells and blast cells from patients with acute myeloid leukemia (AML). Whereas normal hematopoietic cells failed to show constitutive mRNA synthesis for any CSF species, G-CSF transcripts were expressed in 45 % and GM-CSF transcripts in 30 % of the AML samples tested. M-CSF transcripts were not detectable. G-CSF and GM-CSF respectively were present in culture supernatants of 75 % of the leukemias, suggesting that constitutive CSF-gene expression may regularly result in the secretion of a functional CSF protein. These results further suggest that expression of this gene may contribute to the abnormal growth properties of AML.

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GENOTYPIC AND PHENOTYPIC ANALYSIS OF TdT POSITIVE AML's

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Terminal desoxynucleotidyltransferase (TdT), an intranuclear DNA polymerase is found in normal precursors of lymphoid cells, both of B and T type and has also been widely used as a marker of immature lymphoid malignancies. In a distinct proportion of acute myeloid leukemias, but not in normal myeloid precursor cells, TdT can also be demonstrated. In our experience, for instance, of 157 AML's phenotyped 19 could be shown by double staining experiments to contain also TdT. Some of these AML's also coexpressed the T cell marker CO7.

To further analyze these case we initiated an additional genotypic analysis and an in depth immunological evaluation of these leukemic blast cells.

For the genotypic analysis we are using the probes J_H, $T\beta$ and $T\gamma$ to analyze the immunglobulin and T-cell receptor gene configuration. For the phenotypic analysis we are using antibodies to surface and cytoplasmatic determinants of immature hemopoetic cells.

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ACUTE UNCLASSIFIED LEUKEMIA: PROGNOSTIC VALUE OF ELECTRON MICROSCOPY

E. Gunsilius, G. Heil, F. Klier, E. Kurrle, W. Heit, H. Heimpel,

10-20% of acute leukemias remain unclassified after extensive morphological, cytochemical and immunological investigation. Positive ultrastructural demonstration of endogeneous myeloperoxidase by mean of eletron microscopy (POEM) would classify these cases as early myeloid leukemias. We investigated 10 patients with newly diagnosed acute unclassified leukemia (AUL) for the demonstration of POEM. All patients received an ALL/AUL-related chemotherapy. In 5/10 cases POEM activity was found in the nuclear envelope, endoplasmic reticulum, Golgi complex and/or in some small cytoplasmic granules. In the other 5 cases, no POEM activity was found. These leukemias remained unclassified. The clinical outcome of the patients with POEM positive blasts seems to be poor (1/4 remissions, one patient is receiving induction therapy at present), compared to the POEM negative group (5/5 remissions), indicating that an ALL/AUL-related therapy might not be successful in these patients.
Our findings demonstrate that POEM is a sensitive tool to identify early myeloid leukemias within the AUL group. The presented results further indicate, that POEM might be of a prognostic value in AUL patients, a finding which has to be confirmed in a larger series of patients.

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HISTAMINE H2 RECEPTOR ACTIVITY IN LEUKEMIC BLASTS OF THE B-CELL LINEAGE

W.M.Maurer, A.Bolhar-Nordenkampf, D.Lutz

Histamine modulates various immune reactions. Its function is mediated by H2 receptors expressed on lymphocytes and monocytes. These are also found on granulocytes and platelets. Recently, we could show that histamine induced cAMP generation occurs in immature leukemic blasts of common ALL, O-ALL, B-ALL and occasionally AML, particulary with monocytic differentiation (M4,M5), (W.M.Maurer et al Scan.J.Haematol. 1986;37:438-442).

Incubation of c-ALL blasts with various H1 and H2 agonists as well as antagonists in the presence of histamine was performed in the concentration range of 10^{-8}-10^{-3} mol/l. The results indicate, that the receptor is of the H2 type. The order of potency of the histamine agonists is histamine > 4-methylhistamine > dimaprit > 2-methylhistamine > thiazolylethylamine. The H2 antagonist ranitidine was more effective than cimetidine; the H1 antagonists diphenhydramine and pyrilamine were able to inhibit cAMP synthesis only at a tenfold excess. Preinkubation of blasts with trypsin and bromelain followed by histamine stimulation results in a loss of cAMP synthesis, indicating that histamine acts via a proteoreceptor.

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IMMUNOLOGICAL MARKER ANALYSIS IN PATIENTS WITH ACUTE DE NOVO MYELOID LEUKEMIA (AML) : POOR PROGNOSIS OF CLB-ERY3$^+$, TDT$^+$ AND VIM-2$^-$ CASES.

I.Schwarzinger, P.Bettelheim, U.Jäger, U.Köller, W.Knapp, and K.Lechner

In a prospective trial 60 patients with de novo AML received "3+7" as induction, 1 consolidation and 6 maintainance courses (Blut Vol.53 No3, 1986). In order to investigate the prognostic value of immunological marker analysis the bone marrow blasts of these patients were analysed with a panel of 9 monoclonal antibodies (VID-1, VIM-D5, VIM-2, My7, My9, VIM-13, 63D3, CLB-ERY3, WT1) and an antiserum against the terminal deoxynucleotidyltransferase (TdT). Among the markers tested 3 were of prognostic significance:

marker		CR	CCR (mo/med)	Survival (mo/med)
CLB-ERY3	+	3/8	4	4
	-	31/40*	18*	17**
TdT	+	2/7	6	2
	-	39/53**	15	14*
VIM-2	+	37/54	16***	11
	-	4/6	5	11

*p< 0.01, **p< 0.05, ***p< 0.001; mo=months; med=median;
The prognostic significance of these 3 markers appears to be independent from other known prognostic factors.

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QUANTITATION OF THE mRNAs CODING FOR B- AND T-CELL SPECIFIC RECEPTOR PROTEINS IN LEUKEMIAS OF DIFFERENT MATURATION STAGES BY IN SITU HYBRIDIZATION WITH FLUOROCHROME-LABELED GENE PROBES III. ACTIVATION OF LYMPHOCYTE RECEPTOR GENES IN LEUKEMIAS CARRYING MARKERS ASSOCIATED WITH NON-LYMPHOID LINEAGE AFFILATION

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***E.Thiel, **B. Doerken

Activation of genes coding for receptor molecules expressed by lymphoid cells such as the immunoglobulin chains and T-cell receptor chains are assumed to be lineage specific.
It has, however, been shown that the responsible genes can be rearranged also in non-lymphoid leukemias.
We demonstrate that even transcription of these genes can occur in immature leukemias carrying myeloid markers. Quantitative determination of the mRNAs shows, that in some very immature cells the amount of the three mRNAs coding for the /u chain and the T and T chains may be higher than in mature lymphoid cells of the respective lineage, eventually due to aberrant transcription. Thus, even though we could show that the expression of T- and B-cell receptor molecules is quantitatively correlated to maturation, unusual transcription may occur. Therefore only multiparameter studies may give conclusive results.

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THE HETEROGENEOUS PHENOTYPE OF LEUKAEMIC CELLS IN ACUTE LEUKAEMIAS AND BLAST CRISES OF CMPD (IN THE SYSTEM OF MY 7: GP IIb-IIIa)

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Previous results have revealed that, in cases of heterogeneous phenotype of the leukaemic cells in the system of the heterogeneous phenotype MY 7^+: glycophorin A^+, there are 3 different types (KEMNITZ et al., Hum. Pathol., 1986):

1. an "additive phenotype", in which cells with different expression of the lineage markers exist simultaneously in an additive relation;
2. a "hybrid phenotype", in which one cell expresses markers of different lineages; and, finally,
3. a "combined phenotype", which incorporates both the additive and the hybrid phenotypes in one population of leukaemic cells.

Whether this occurs also in other combinations has been tested in the system of the myeloid marker MY 7 and the megakaryocytic lineage marker GP IIb-IIIa.

Material: Bone marrow and peripheral blood smears from 49 patients with acute leukaemias and blast crises (31 AML-FAB M1-6, 18 ALL-FAB L1-3, 4 blast crises of CML).

Method: Immunoalkaline phosphatase - single and double marking in "sandwich" modification. As result, we have found

- the additive heterophenotype in the system MY 7^+: GP IIb-IIIa in AML-M2 (1/5), M3 (1/3), M4 (2/12), M5 (1/3);
- the hybrid phenotype MY 7^+: GP IIb-IIIa in M1 (1/4), M2 (1/5), M4 (3/12) as well as in one blast crisis of a CML (1/4);
- the combined phenotype finally in M2 (1/5), M3 (1/4); M4 (1/12) as well as in one blast crisis of CML (1/4).

Therefore the existence of heterogeneous phenotypes could be considered as a result of "restricted promiscuity of lineage-associated gene expression" (according to GREAVES et al., Blood, 1986).

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MONOCLONAL ANTIBODY TC25 DEFINES AN ANTIGEN EXPRESSED ON SOME HEMATOPOIETIC PROGENITORS AND ALLOWS IMPROVED IMMUNOPHENOTYPING OF IMMATURE LEUKEMIAS

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Monoclonal antibody TC25 detects a 160kDa polypeptide antigen. This antigen was found on platlets, endothelial cells, a small percentage of normal human bone marrow cells and weakly on monocytes. Inhibition experiments with antibody TC25 and complement on CFU-GM cultures revealed a significant inhibition of colony growth. Testing more than 90 leukemias and lymphomas for their immunophenotype, TC25 reactivity was found to be restricted to certain types of leukemias. Only acute myelocytic leukemias (AML) of FAB M4 or M5 classification had a significant percentage of TC25 positive cells, while M1, M2, and AML of M3 type were distinctively negative. Interestingly,chronic myelocytic leukemia (CML) in myeloid blast crisis and refractory anemia with excess blsts did show reactivity. In the lymphoid lineage only a subgroup of T-cell acute lymphoblastic leukemia (ALL) was TC25 positive, while non-T ALL as well as more mature B-cell tumors were distinctively unreactive. Thus, TC25 detects a mesodermally defined antigen which appears specifically related to certain stages of hematopoietic differentiation, and, in turn, allows for more accurate immunophenotyping of immature leukemias.

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ESTABLISHMENT OF A L-BCGF SENSITIVE LEUKEMIC PRE B CELL LINE WITH A DELETION OF THE SHORT ARM OF CHROMOSOME 9

B. Wörmann, J.M. Anderson, D.C. Arthur, T.W. Le Bien

A cell line designated B-lineage 1 (BLIN-1) was established from the bone marrow aspirate of a patient with non T-ALL using low molecular weight B cell growth factor (L-BCGF). The cells express the B lineage associated surface antigens CD9, CD10, CD19, CD24 and are cytoplasmic IgM positive. A population of 3-5 % surface IgM positive cells is also consistently present. Southern blot analyses show rearrangement of the IgM and IgK genes. Northern blot analyses reveal transcripts for IgK and the membrane form of IgM. Karyotype analysis of the cell line shows a single nonrandom chromosomal abnormality with a deletion of the short arm of chromosome 9 at band p22, which is the locus of the alpha1 and beta1 interferon genes. This chromosomal aberration is identical to the one found in the initial leukemic bone marrow aspirate.
In the initial liquid cell cultures, L-BCGF enhanced the growth of BLIN-1 cells. After the first 3 weeks the cells could also be maintained in culture medium without L-BCGF. The doubling time of these cells, however, was about two times slower compared to cells grown in the presence of L-BCGF. After 5 months in culture this difference was no longer detectable, if the cells were maintained at a high cell density of 4-6 x 10^6 cells/ml. However, colony formation in methylcellulose has been strictly L-BCGF dependent. BLIN-1 provides an interesting model for examining the role of L-BCGF/L-BCGF receptor interaction, the self renewal of leukemic pre-B cells, and may aid in elucidating the molecular significance of $9p^-$ chromosomal abnormalities.

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INTERLEUKIN (IL) PRODUCTION BY AML BLASTS IN VITRO

J.Salamon[1], A.Köck[2], T.Luger[2], D.Lutz[1]

Blasts derived from fresh peripheral blood of AML patients (n=8; FAB:M2:3; M4:4, M5a:1) or frozen samples (n=3; FAB:M2,M4,M5a) were tested for their ability to produce interleukines. The mononuclear cell fraction was incubated for 48h either unstimulated or stimulated by 5µg/ml LPS or 50µg/ml silica. The cell free supernatants were kept frozen at -20° until used.
Serial dilutions were prepared for testing. IL-1, IL-2 or IL-3 activity was determined by measuring the proliferation of factor dependent cell lines D10, CTL-2 and 32DCL, respectively. IL-1 like activity of >100 U/ml (the arbitrarily designed standard produced by stimulated keratinocytes PAM212) was detected in supernatants of 7 unstimulated cell samples tested (M2:2; M4:4; M5a:1) and was absent in 4 (all frozen samples and 1 fresh M2). In all positive samples the production of IL-1 like-activity was enhanced by LPS or silica to more than 800 U/ml, whereas no induction was detected in the 4 negative blast populations. The nature of the IL-1 like activity tested could be further confirmed in two ways:

a) by blocking the activity of the supernatant using an α IL-1 Moab in the D-10 costimulator assay and
b) by demonstrating a positive intracellular and membrane binding of the same Moab.

In contrast, neither IL-2 nor IL-3 activity was detectable in any sample tested.

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DRUG TESTING IN ACUTE LEUKEMIA, IN VITRO ATTEMPTS TO BYPASS CHEMORESISTANCE.

F.C.Prischl and J.D.Schwarzmeier

Determining the in vitro incorporation of nucleic acid precursors into cellular RNA and DNA of leukemic blastcells is a reliable method to predict chemosensitivity or -resistance in vivo. Using criteria established in previous studies we evaluated 68 patients with acute leukemia. In 91% true positive and in 69% true negative correlations between in vitro and in vivo results were found. A striking observation was, that patients with an extremly high in vitro sensitivity seemed to relapse very early. Serial testing also revealed changes in the sensitivity profile to different agents. These data indicate, that highly proliferating cell populations might be eradicated by induction chemotherapy but chemoresistant cell clones are selected. To find out whether Ca^{++}channel blockers influence in vitro sensitivity to cytostatic agents we studied the effect of verapamil. In contrast to results reported on cell lines we found that in human blast cells verapamil itself showed a considerable inhibition of precursor incorporation in about 2/3 of the experiments. Coincubation of blast cells with adriamycin and verapamil lead to an enhancement of the inhibitory effect of adriamycin in 3 out of 9 cases only. We therefore conclude that patients showing drug resistance in vitro and in vivo probably do not benefit from including verapamil to treatment protocols.

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COMBINATION CHEMOTHERAPY FOR ADULT ACUTE MYELOID LEUKEMIA WITH MITOXANTRONE, CYTOSINE-ARABINOSIDE AND VP-16

H. Link*, M. Freund*, H. Diedrich*, H. Wilke*, M. Henke#, Th. Hecht#, H. Wandt+, R. Kuse§, A. Calavrezos§, and H.Poliwoda*

In phase I/II studies mitoxantrone proved to be effective in acute myeloid leukemia (AML). In combination with VP-16 up to 35 % of refractory AML-patients achieve a complete remission (CR). The issue of our study is to find out, if the addition of the very effective antileukemic Cytosine-Arabinoside increases the response rate at tolerable toxicity. We therefore combined Mitoxantrone (M) 10 mg/m^2 i.v. days 4-8, Cytosine-Arabinoside (A) 100 mg/m^2 continuous infusion days 1-8 and VP-16 (V) 100-120 mg/m^2 i.v. days 4-8 (MAV-protocol) for relapsed and refractory AML. 16 patients were treated median age was 50 (26-72) years, 11 patients were induced into CR. 4 patients died in CR from chronic infections or after consolidation therapy with MAV. The median duration of granulocytopenia $<$500/µl and of thrombocytopenia $<$ 25 000/µl was 23 (10-23) days and 22 (3-46) days respectively. We conclude that MAV-therapy is a powerful antileukemic combination, which deserves further clinical evaluation in untreated AML.

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HIGH DOSE ARA-C AND ETOPOSIDE IN THE TREATMENT OF REFRACTORY OR RELAPSING ACUTE LEUKEMIA

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We have treated 25 patients (12 m, 13 f) with AraC 3 gr /m^2 twice daily d 1-6 and VP16 100 mg/m^2 d 1-5. In 5 patients consolidation with a second course was given. 4 ALL, 21 AML (6 M1, 9 M2, 3 M4, 3 M5) are enrolled in the study. Mean age was 39.0 yrs (18 - 62). Seven of these patients had primary refractory leukemia, 6 had early ($\leq$ 6 mo) relapse, 5 were in refractory 1st relapse, 6 more had 2nd or following relapse. 1 patient had a 1st relapse after more than 6 mo but was heavily pretreated. 11 patients were pretreated with TAD, 12 with DAV, 1 with Amsa + VP16, 3 with Ara-triple V, 4 patients with ALL with BMFT - chemotherapy. Treatment results were 13/25 CR (11/21 AML, 2/4 ALL; 2/7 in primary refractory leukemia, 3/6 in early relapse, 3/5 in refractory 1st relapse, 4/6 in 2nd or following relapse, 1/1 with late relapse). There were 4 early deaths in induction, 1 death occurred in consolidation. Median calculated duration of complete remission was 4.6 mo, median survival 4.5 mo. Moderate hepatic toxicity was observed as well as mucositis, diarrhoea, erythema, infections, conjunctivitis but cerebellar toxicity only in 1 case. Mean duration of granulocytopenia below 500/cmm was 23.1 d (s = 6.56), and of thrombocytopenia below 20 000/cmm 21.6 d (s = 7.89) in patients responding to treatment.

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INACTIVATION OF LEUKEMIC CELLS BY ALKYL-LYSOPHOSPHOLIPID (ALP) AS MEASURED BY FOUR DIFFERENT METHODS

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ALP's are known to inactivate leukemic cells selectively by interfering with the phospholipid metabolism of the cell membrane. They may be useful for the treatment of marrow of leukemia patients prior to autologous transplantation. We studied the effect of ALP (BM 41.440) on lymphoblastoid cells of the REH line by comparing four methods: 1. exclusion of trypan blue indicating damage to the cell membrane; 2. the colorimetric MTT-test as a correlate of mitochondrial function; 3. clonogenic growth in limiting dilutions of suspension cultures and 4. growth of colony forming units in semisolid agar. REH cells were treated "in vitro" with ALP in concentrations up to 50 µg/ml for 30 min at37°C. The dye exclusion test proved to be the least sensitive. MTT was at background levels at a concentration at which 37% of cells excluded trypan blue. The proportion of clonogenic cells measured in limiting dilutions decreased to less than 10% of viable cells as compared to untreated cells. Similarly the agar culture showed only up to 10% of colonies of untreated controls. Moreover the growth of colonies decreased in favor of that of small clusters. We conclude from these experiments that leukemic cells are inactivated by ALP in several ways and their potential of growth is inhibited.

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INCREASE OF ANTIGEN-BINDING CELLS IN LYMPHATIC TISSUE OF RATS AFTER IN VIVO ADMINISTRATION OF DIPHENYLHYDANTOIN

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There are observations suggesting that the clinical situation of patients with AIDS may be improved by treatment with diphenylhydantoin (DPH), possibly due to the capacity of DPH to mask the T-cell virus receptor. Multicenter clinical studies on the effect of this drug in symptomatic HIV infection are in progress. While the influence of DPH on lymphocytes is well investigated, little is known about possible effects of DPH on the accessory cell system.
Wistar rats were injected with 50 mg of DPH (controls: 50 mg phenobarbital and 0.9 % NaCl solution, resp.) subcutaneously in both hindpads; 8 days later, 40 mg of alkaline phosphatase (AP) dissolved in 0.9 % NaCl solution were administered at the same site for antigenic exposure. Ninety min. and 24 h after injection of the antigen popliteal lymph nodes were removed and homogenized. The cells in the resulting suspension were counted and cytospin slides were prepared. In animals pretreated with DPH a significant increase of cells with membrane-bound antigen (4.84×10^5 after 90 min.; 8.6×10^5 after 24 h), as detected by a monoclonal anti-AP antibody, was observed as compared with the controls (0.38 and 0.02×10^5, resp., after 90 min.; 0.55 and 0.08×10^5, resp., after 24 h). Nonspecific reactions were excluded by staining cells from DPH-, but not AP-exposed animals and by immunocytochemistry with antibodies not specific for AP. HIV has been identified not only in lymphocytes but also in macrophages and on dendritic reticulum cells, indicating processing of the virus by accessory cells and/or infection of these cells with the virus. Recruitment of antigen-binding/presenting cells in the lymphatic tissue by DPH might reinforce cellular immunity, thus possibly adding to the favorable effect of this drug in HIV infection.

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QUANTITATIVE DETECTION OF HIV IN INDIVIDUAL CELLS BY MICROFLUORIMETRY AND FLOW CYTOMETRY AFTER IN SITU HYBRIDIZATION WITH A FLUOROCHROME-LABELED GENE PROBE

K. Pachmann, U. Pachmann, W. Mellert, V. Erfle, E. Thiel and B. Emmerich

The possibility of quantifying cellular mRNA by microfluorimetry of hybridized fluorochrome-labeled gene probes has prompted us to use this method also for quantitative detection of RNA virus transcripts in individual cells.
We were able to obtain a sufficiently high signal in individual cells to discriminate HIV-infected cells from noninfected cells. A modification of the in situ hybridization method even yielded positive hybridization results using flow cytometry.
Thus, this method will hopefully provide an additional tool for analysis of HIV infection and therapy monitoring.

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EPIDEMIC KAPOSI SARCOMA (EKS) AND MALIGNANT LYMPHOMAS (ML) IN PATIENTS WITH AIDS

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107 patients, 102 males and 5 females with a median age of 34 years were treated so far for AIDS in the Department of Internal Medicine. 42 of them developed an EKS and/or a ML as the initial manifestation of their disease. There were 9 cases of non-Hodgkin lymphoma (NHL) and 3 of Hodgkin's disease (HD). Patients with stage I or II did not receive systemic therapy for EKS. Time to progression in these patients was 3 to > 22 months. Three out of 15 pts. have stable disease without systemic treatment for 12+ to 22+ months. Pts. with advanced or progressive disease received various combinations of chemotherapy or Interferon. Only two of them died of EKS. Opportunistic infections were the most common cause of death.
In NHL histologic diagnosis was immunocytic (polymorphic or lymphoplasmocytic subtype), immunoblastic or lymphoblastic (Burkitt type) lymphoma. One patient was lost for follow up. NHL showed a rapid fatal progression in 8 patients with death occuring within 6 months after diagnosis. The primary manifestation of the disease was extranodal (bone, soft tissue) in 4 out of 9 pts..
The three pts. with HD had an advanced stage of the disease (IIB or IVB). Bone marrow infiltration was detected in both pts. with stage IVB disease. One patient did not respond to chemotherapy and died of progressing HD. Two pts. receiving combined chemotherapy are alive for 5 or 6 months after diagnosis.

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CYTOPENIA AND DEFECTIVE GROWTH OF HEMOPOIETIC PROGENITOR CELLS IN AIDS

A. Ganser and D. Hoelzer

Peripheral blood cytopenia due to increased vulnerability of hematopoiesis is tha main side effect of the virustatic drug azidothymidine as well as of the folic acid antagonist trimethoprim-sulphamethoxazole in the treatment of opportunistic infections in patients with AIDS.
These observations prompted us to investigate the hemopoietic system in these patients by analyzing the bone marrow morphology and the in vitro growth of the bone marrow derived as well as of the circulating hemopoietic progenitor cells CFU-GEMM, BFU-E, CFU-Mk, and CFU-GM. While more than 90% of patients with AIDS were lymphopenic, anemia was observed in 80%. Monocytopenia, neutropenia and thrombocytopenia were seen in about 35% each. Myelodysplastic changes including hypercellularity despite peripheral cytopenia, dyserythropoiesis, dysgranulopoiesis, and increased numbers of hypolobulated megakaryocytes were found in more than half of the 27 patients studied. In vitro culture studies revealed a decreased incidence of hemopoietic progenitors in the blood as well as in the bone marrow. While the incidence of the progenitors in the blood correlated to the number of CD4 positive lymphocytes and to the ratio of CD4:CD8 positive cells, the growth of the bone marrow progenitors could be increased but not normalized by the depletion of T-cells. Re-addition of T-cells again caused a decrease in colony formation the extent of which was inversely related to the CD4-CD8-ratio.
The failure to completely restore colony formation by T-cell depletion could indicate a loss of hemopoietic progenitor cells in patients with AIDS caused by direct infection with the human immunedeficiency virus. Our data demonstrate that the bone marrow is a target organ of this virus.

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SELECTIVE DECONTAMINATION FOR INFECTION PREVENTION - A REVIEW OF E.O.R.T.C. GNOTOBIOTIC PROJECT GROUP STUDIES SINCE 1969

G. Maschmeyer and F. Wendt

In five consecutive randomized multicenter studies since 1969, the Gnotobiotic Project Group of the E.O.R.T.C. investigated different clinical concepts for infection prevention in severely immunocompromised patients. Trial I compared rigorous antibiotic decontamination in strict reverse isolation with strict reverse isolation only and with open ward conditions. In trial II a standard mixture of non-absorbable antibiotics for gastrointestinal decontamination was tested against a selected combination of antibiotics based on susceptibility patterns of the initial fecal flora of each individual patient. The use of the systemic effect of cotrimoxazole was investigated in comparison to only non-absorbable drugs (neomycin/polymixin) in trial III. After a smaller pilot study using two 4-quinolones in different dose regimens (trial IV), the group actually studies ciprofloxacin against cotrimoxazole/polymixin, both in combination with non-absorbable antimycotics, for infection prevention in severely granulocytopenic leukemia patients.
Study results will be reviewed and actual concepts for antimicrobial prophylaxis in the immunocompromised host will be discussed.

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ACTION OF RECOMBINANT HUMAN GM-CSF ON GRANULOCYTE FUNCTIONS

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PMNs are the major host defence cells protecting the body against invasion of microorganisms. The defence involves their mobilization to the site of tissue injury, their local accumulation and the destruction of invading agents through enzymes and products of the oxidative metabolism. Recent publications have shown that GM-CSF besides its effect on progenitor cells is also able to interact directly with non-dividing end-cells, inducing an enhancement of their functional activity. We demonstrate the presence of high-affinity binding sites for GM-CSF on human PMNs. Maximum binding was achieved after incubation of 60 min at 37°C as studied in time-course experiments. We then explored oxidative metabolism, chemotaxis and elastase release of human PMN after stimulation with rH.GM-CSF.
Using chemiluminescence we observed that rH.GM-CSF alone stimulates the oxidative metabolism and that it also induces an increase of the response to PMA. This effect was not endotoxin-related. Chemotaxis and random migration were investigated using a modified Boyden chamber assay. Yeast activated serum was used as chemoattractant. It was evident that rH.GM-CSF inhibits chemotaxis as well as random migration. Elastase release was investigated after 90' incubation with rH.GM-CSF, either with or without f-MLP. The elastase activity was determinated by its amidolytic effect on a chromogenic substrate (Pyro Glu-Pro-Val-pNa). We could demonstrate that rH.GM-CSF induces an elastase release, an effect, which is enhanced by preincubation with f-MLP. These date suggest that GM-CSF produced at the site of infection inhibits migration and induces functional activities of PMN. It therefore may have some therapeutic potential in patients with impaired PMN functions.

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BIOTIN-LABELED DNA PROBES TO DETECT EPSTEIN-BARR VIRUS (EBV)-DNA

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The Epstein-Barr virus (EBV) is a member of the herpes virus group which cause latent and persistent infections. In immunocompromised individuals, especially in patients with AIDS, there is a remarkable high incidence of EBV-associated lymphomas. In order to demonstrate the presence of latent viral genomes, we prepared biotin-labelled DNA probes by the procedure of random priming using biotin-7-dATP. As an EBV - DNA probe we used Bam HI digested cosmid-DNA (CM 301-99) and in addition, a plasmid clone containing a part of the Bam HI R/K region of EBV-DNA. Southern blots were carried out with DNA prepared from cosmids and tissue culture cell lines. Specifically hybridizing DNA was detected by a streptavidin-alkaline phosphatase conjugate. This technique is highly sensitive: DNA amounts of 1-5pg can easily be detected. Therefore these probes are also useful at the single copy detection level. The stability of these biotinylated probes during storage at -20 ^{o}C, the possibility of repeated use and therefore immediate availability and the non-hazardous test conditions strongly favor the use of such probes in rapid clinical diagnosis.

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CMV - PROPHYLAXIS WITH A NEUTRALIZING HUMAN ANTI CMV MONOCLONAL ANTIBODY - PRELIMINARY RESULTS OF A PHASE I TRIAL IN BONE MARROW TRANSPLANT RECIPIENTS

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CMV-infection is the most frequent and most serious infectious complication in bone marrow transplant recipients: up to 40% of the patients suffer from interstitial CMV pneumonitis and the mortality of this condition has been consistently above 80%. No effective treatment or prophylaxis for CMV-infection is known, but it has been reported, that prophylaxis with CMV-hyperimmunogloulins (HIG) is capable of diminishing the severity of CMV-infection. Thus it seems likely, that highly specific antibodies might be effective for the prophylaxis and treatment of life threatening CMV-infection. Human monoclonal antibodies have so far not been tested in vivo. In an ongoing phase I trial we investigated the pharmacokinetics, tolerability and clinical efficacy of two human monoclonal antibodies with at least 100 times mor neutralizing activity against CMV, when compared to conventional HIG preparations. During the first trimenon following bone marrow transplantation six patients were treated prophylactically with 0.5 or 2 mg/kg every second week. Preliminary clinical, virological and pharmacokinetic results of this trial will be presented.

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LYMPHOCYTE SUBSETS AND ACTIVATED T CELLS IN APLASTIC ANEMIA

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Recently increased T helper/T suppressor ratios (Zoumbos et al. Br. J. Haematol.,1984, 58:95), as well as increased numbers of activated suppressor T cells (HLA DR+, Tac+) (Zoumbos et al., N. Engl. J. Med., 1985, 312:257) have been described in patients suffering from severe aplastic anemia (SAA).

In this study peripheral blood (PB), and furthermore bone marrow (BM) lymphocytes were investigated with respect to the T cell ratio, the T suppressor inducer subset and for the expression of HLA DR antigens by double staining experiments. In peripheral blood, no significant differences between SAA patients and controls with regard to the T cell ratio as well as the total number of HLA DR positive cells were detectable. Untreated patients however showed higher percentages of HLA DR+ suppressor cells than controls. Within bone marrow no significant differences between SAA patients and healthy controls could be found.

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IMMUNOHISTOLOGICAL ANALYSIS OF BONE MARROW LYMPHOCYTES IN APLASTIC ANEMIA

N. Frickhofen, H.P. Kiem, A. Raghavachar, H. Heimpel

Lymphocyte populations were analyzed in 45 bone marrow biopsies from 33 patients with aplastic anemia using frozen sections, a panel of monoclonal antibodies and alkaline phosphatase (APAAP) as the marker enzyme. Results were calculated as mean antigen-positive cells of 5 representative high power fields (HPF) and compared with lymphocyte patterns in biopsies from 12 normal donors. Absolute numbers of T cells ($CD7^+$, $CD3^+$), B cells ($CD19^+$, $CD22^+$) as well as CD4- and CD8-positive T cells were similar to controls in 14/20 biopsies from untreated patients. Lymphocyte numbers were decreased in 2 severely hypocellular biopsies and increased in another 2 biopsies, primarily due to changes within the CD8-positive T cell population. CD4-positive T cells were significantly increased in 4/20 cases resulting in an increased CD4/CD8 ratio (normal value in bone marrow: 0,1 - 0,5). Significant T cell activation could not be demonstrated with antibodies against the interleukin 2 receptor or 'Tal'. There were only few K and NK cells ($Leu7^+$, $CD16^+$) in normal as well as the majority of aplastic bone marrows; more than 3 cells per HPF could be shown in only 3 cases. During treatment with cyclosporin A the number of CD8-positive T cells significantly decreased - apparently independent of the treatment results.
In summary, imbalances of lymphocyte populations, detected with the above mentioned antibodies, are rare in aplastic anemia. The data argue against a pathogenetic role of NK cells in the majority of the patients. If marker studies have any predictive value regarding the results of immunosuppressive therapy is currently under investigation.

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Abstract withdrawn

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NATURAL HISTORY AND PROGNOSIS OF MYELODYSPLASTIC SYNDROMES

R. v. Hirschhausen and J.G. Saal

The myelodysplastic syndromes (MDS) are a nosologically diverse group of rare disorders of hematopoetic stem cells. In a retrospective study, the clinical and hematological data and natural history of MDS (FAB 1980 : RA 9; RAS 3; RAEB 5; RAEBT 2) were followed in 19 patients (male 15, female 4, median age 65 + 11 years). Whereas those with RA needed regular blood transfusions, no such supportive therapy was necessary in most patients with RAS, RAEB or RAEBT. In RA a high incidence (3/9) of monoclonal gammopathy was found. Transition to leukemia (AML 4; ALL 1) was seen more often in RAEB/RAEBT (3/7) than in RA/RAS (2/12). Transition from RA/RAS to RAEB/RAEBT, however, has not been observed. Median survival was long in RA (4.2 + 4.5 years) when compared with RAEB/RAEBT (1.5 + 0.6 years). Death was mostly due to infection (4/10) or bleeding complications (3/10). Although MDS can be regarded as preleukemia, natural histories differ profoundly between RA/RAS and RAEB/RAEBT. Thus, correct classification of MDS following the FAB MDS proposals is necessary for rational planning of therapy.

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CYTOGENETIC, IMMUNOLOGICAL AND CLINICAL CHARACTERISTICS OF MYELODYSPLASTIC SYNDROMES (MDS) AND ACUTE MYELOID LEUKEMIAS (AML) WITH ACQUIRED CHROMOSOME 5 ABNORMALITIES

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Acquired abnormalities of the long arm of chromosome 5 were observed in the bone marrow of 13 adults and 2 children (11 females and 4 males) with 6 myelodysplastic syndromes (MDS) und 9 acute myeloid leukemias (AML), respectively. In 4 MDS and 8 AML cases these abnormalities were due to a simple deletion, del(5q), in one MDS and one AML case due to translocations involving chromosome 6 and 10, respectively, and in another one due to a ring formation. Whereas in 3 MDS and 2 AML patients the del(5q) was the only abnormality, it was part of complex karyotype changes in all others.

Our findings can be summarized as follows:

1) In AML chromosome 5 anomalies may be found in any FAB-subtype. They are not disease specific. However, they are suspected to be associated with preceding cancerogen exposure.
2) MDS patients with a del(5q) as the only abnormality have a long disease duration, but may eventually develop acute leukemia. This process seems to be more common as previously estimated.
3) AML cases have a bad prognosis which seems independent of the genesis of the 5q abnormality and of the presence and/or type of additional karyotype changes. Interestingly, such leukemias are often biphenotypic with myeloid markers and TdT-positivity.

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GAMMA-INTERFERON (γ-IFN) FOR TREATMENT OF MYELODYSPLASTIC SYNDROMS (MDS)

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8 patients (6 males, 2 females, median age: 59 years) with MDS (RAEB: 3, RAEB-t: 4, CMML: 1) were treated with γ-IFN. The patients received 3 courses of γ-IFN in 4 week intervals. Each course consisted of daily subcutaneous injections of 0.1 mg/m^2 d 1-14. The effect of treatment is evaluated after 16 weeks. At the present time 6 patients are evaluable. No patient showed a reduction of blast cells in the bone marrow. 2 patients showed a slight improvement of peripheral blood cell counts: 1 patient, who required transfusions of red cells before treatment, normalized with hemoglobin levels, and 1 patient, who required transfusions of platelets before treatment, showed an increase of platelets to > 20.000/ul. 2 patients had stable disease, and in 2 patients disease progressed under treatment with γ-IFN. 3 of the latter patients were treated with "low dose ARA-C", in none of them improvement of peripheral blood cell counts was observed. All patients developed fever and transient myelosuppression under treatment with γ-IFN, serious side effects did not occur. Our preliminary results show that γ-IFN had no antiproliferative effects in patients with MDS, but may improve peripheral blood cell counts in some patients (by induction of cell differentiation?).

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SUCCESSFUL TREATMENT OF ATYPICAL LEUKEMIAS BY A FLEXIBLE LOW DOSE ARA-C REGIMEN ADAPTED TO THE FAB-CLASSIFICATION OF MYELODYSPLASTIC SYNDROMES (MDS)

R. Schlag, R. Zankovich, J. Walther and E. Thiel

46 consecutive patients (range 24-86 years) with MDS were treated with low doses of ARA-C (10 mg/m^2/12 h) up to 4 times. Therapy was initiated because of progressive cytopenias in all cases and performed either s.c. for outpatients or i.v. by continuous infusion. Duration of treatment was modified according to subgroups of MDS as proposed by the FAB-classification: 8-13 days for RA and RARS, 14-21 days for RAEB(T) and CMML respectively. Diagnosis was confirmed by means of bone marrow histology to establish further criteria for remission prediction.
Remission was proved by criteria similiar to those proposed by Tricot et al: 1. no further requirement for transfusion of erythrocytes, 2. increase in granulocytes $\geq$1000/µl, 3. increase in thrombocytes $\geq$50.000/µl and 4. decrease in bone marrow blast cells by at least 50 %. Quality of remission was grouped into CR (4 criteria fulfilled), GR (2-3), PR (1) and NC. Satisfying remissions (CR and GR) amounted to 66 % for RA (n=6), 60 % for RAEBT (n=15), 55 % for RAEB (n=11) and 46 % for CMML (n=13). One patient suffering from RARS obtained good remission (GR). Therapy was not repeated until recurrent cytopenia pointed to relapsing disease. Bone marrow morphology and chromosomal aberrations of bone marrow cells point for a cytostatic effect as the major cause for remission rather than induction of differentiation.

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TREATMENT OF MYELODYPLASTIC SYNDROME (MDS): LOW DOSE ARA-C OR MORE AGGRESSIVE CHEMOTHERAPY?

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43 patients (pts.) with advanced MDS were treated with chemotherapy because of severe cytopenia or transformation to AML. 34 pts. (1 RA, 7 RAEB, 5 RAEB-T, 10 CMML and 11 AML secondary to MDS) received low dose Ara-C (18 pts. 10 mg/m^2/12 h s.c. and 16 pts. 20 mg/m^2/d i.v.). 4 pts. achieved CR (2 RAEB-T and 2 AML secondary to RAEB-T). CR was maintained by 7 day courses of low dose Ara-C repeated monthly. Median duration of CR was 8 months (range 2+ to 12+ mo.).
A selected group of 9 pts. (4 RAEB-T and 5 AML secondary to MDS) was chosen for aggressive induction regimens (8 pts. for TAD-9 and 1 pt. for TAD-9/HAM). Selection criteria were good condition, young age (range 17 to 64 y.) and absence of contraindications for aggressive chemotherapy. In this group 6 pts. (4 RAEB-T and 2 AML secondary to MDS) achieved CR and 1 pt. (AML secondary to MDS) showed PR. Early deaths (2 pts.) were due to infectious complications. Median duration of bone marrow aplasia (leukocyte count below 1000/µl) after application of TAD-9 was 20 days (range 6 to 26 d.). CR was maintained by administration of cyclic chemotherapy. Median duration of CR was 11 mo. (range 2+ to 23+ mo.). BM smears obtained in CR showed a normal maturation of hematopoiesis, suggesting an eradication of the malignant cells. Our data indicate, that aggressive chemotherapy may be of benefit for a small number of selected MDS patients, showing a high remission rate without the risk of prolonged bone marrow aplasie.

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DOSE-DEPENDENT ANTIPROLIFERATIVE EFFECT OF DAUNORUBICIN ON LEUKEMIC BLASTS AND IN VITRO UPTAKE

M.E. Scheulen, U.B. Wandl and W. Reich

According to the mechanisms of drug resistance hitherto evaluated, the uptake of cytostatic agents by malignant cells is one of the determinants for responsiveness.
Mononucleated cells were isolated from peripheral blood of patients with acute leukemias and CML blast crisis by Ficoll gradient centrifugation and exposed to daunomycin in concentrations varying between 2 and 2.000 ng/10^6 cells/ml. Up to two hours aliquots were taken and intracellular daunomycin-concentrations were measured by high performance liquid chromatography (HPLC) after washing and extraction of the cells. In parallel experiments cells were plated in semi-solid medium stimulated with PHA-LCM and CFU-C were evaluated after 8 days in comparison to an untreated control.
In each of the 12 leukemic cell populations analysed, daunomycin-uptake was linear over the range of concentrations used. In contrast, it varied inter-individually between 16 and 58 %. The daunomycin-concentrations causing 50 % reduction of CFU-C were between 5,4 and 63 ng/ml. The calculated intracellular daunomycin-concentrations causing 50 % reduction of CFU-C varied between 1,7 and 13 ng/16^6 cells.
Up to now, a correlation between these data and the clinical outcome did not become apparent. Further analyses and a comparison with daunomycin pharmacokinetics and in vivo uptake are under investigation.

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ANTITUMOR ACTIVITY OF ANTHRACYCLINES IN HUMAN GASTRIC CANCER XENOGRAFTS DEPENDING ON TIME SCHEDULING

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50 intraoperative gained gastric cancer specimens were xenografted on NRMI-nu-nu mice from our own breeding section. 17 tumors are succesfully kept in serial passages from 3+ to 8+ passages(take rate 33%). Tumors were screened for chemotherapy sensitivity in 4th passage with the following agents: Adriamycin, 4-Epidoxorubicin, Theprubicin, Cisplatin, Carboplatin, Mitomycin, Etoposide, 5-Fluorouracil. In sensitive tumors a possible time scheduling dependend antitumor activity was investigated. So far one anthrazycline sensitive cell line(HM-4) has been treated with different time schedules of Adriamycin, Epidoxorubicin and Theprubicin. Three other cell lines(HM-5, HM-15, HM-22) are currently tested using the same schedules. Methods:To obtain comparable test conditions mice were distributed among control and treatment groups according to tumor size. Doses: ADM and THP 11mg/kg day 1; 5,5mg/kg day 1 and 8; 3,5mg/kg day 1,4,7 i.v.; EPI 12mg/kg day 1; 6mg/kg day 1 and 8; 4mg/kg day 1,4,7 i.v.. Results: ADM, EPI and THP induced significant tumor reduction. Single dose application of ADM, EPI and THP was inferior concerning tumor reduction compared to split dose schedules(day 1 and 8, day 1,4,7). Toxicity was high with ADM especially with single dose application. Side effects/antitumor-activity relation was better with split dose schedules.
Conclusions: Antitumor activity of ADM, EPI and THP was comparable. Using the same cumulative drug doses per schedule split dose schedules were more effective and less toxic than single dose applications. Toxicity of ADM was higher than of EPI and THP. Confirmation of these results in more cell lines has to be done.

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MELPHALAN DOSAGE IN MYELOMA PATIENTS WITH RENAL FAILURE

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There are no human pharmacokinetic data available concerning melphalan dosage in myeloma patients with renal insufficiency. Therefore, we compared plasma levels of the alkylating agent in 16 patients with no severe renal dysfunction (creatinine clearance $\geq$ 40 ml/min) to the data of 5 hemodialysis patients (serum creatinine about 10 mg/dl).

The following results were obtained (normed to the dose of 0.5 mg melphalan/kg i.v.; means $\pm$ SEM):

	$t_{1/2}$ (min)	AUC (min µg/ml)
Creatinine clearance $\geq$ 40 ml/min	66 $\pm$ 6	72.9 $\pm$ 5.1
Hemodialysis	89 $\pm$ 6	96.7 $\pm$ 15.2

$t_{1/2}$ = elimination half-life, AUC = area under the melphalan concentration-time curve.

In patients with renal failure, $t_{1/2}$ and AUC showed an increase of about one third.

These results suggest that severe renal dysfunction can slow the plasma and renal clearance of melphalan. We recommend a melphalan dose reduction of about 30 % in these risk patients.

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FURTHER STUDIES ON THE MECHANISM OF METHOTREXAT (MTX) AND 5-FLUOROURACIL (FU) SYNERGY

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In previous studies we have demonstrated increased cellular uptake of FU following pretreatment with MTX in an "in vivo" model. The objectives of this study were to (1) find out more about the mechanism of this interaction and (2) determine the dependency on the circadian timing of treatment. Sarcoma 180 bearing mice received MTX 150 mg/kg sc. or saline (control) followed 8 hrs later by ^{3}H-FU 65 mg/kg ip. and ^{14}C-guanosine 1 mg/kg ip. Two hours later tumors and the livers were removed. Significantly more ^{3}H-FU was detected in the acid soluble (AS) fraction, the fraction containing thymidylate synthase (TS) and the RNA fraction of tumor cells. No difference was seen in the liver. When treatment was started at midnight instead of 8 a.m. results for FU were similar for the tumor cells, but significantly less ^{3}H-FU was detected in the TS fraction of liver cells from mice pretreated with MTX. ^{14}C-guanosine was readily detected in the AS and in the RNA fraction. Significantly more radioactivity following MTX was only found in the group of animals where treatment had been started at midnight. In the liver only very little radioactivity from ^{14}C was measured. We conclude that (1) increased RNA incorporation of FU following MTX pretreatment is not likely to be a result of increased RNA synthesis, (2) circadian timing of antimetabolite application may lead to an improved therapeutic index. More studies are necessary to determine the effect of this approach on actual tumor growth.

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COMPARATIVE ANTITUMOR ACTIVITY OF CISPLATIN (DDP), JM8 AND JM9 AGAINST ESTABLISHED HUMAN TESTICULAR TUMOR CELL LINES IN VITRO AND IN VIVO

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DDP, the most active drug against testicular tumors (TT) has several serious toxic effects including nephro- and neurotoxicity. We evaluated the antitumor activity of JM8 and JM9, two new platinum analogs which have shown to have less toxic effects, against established human TT cell lines. Methods: In vitro antitumor activity of DDP, JM8 and JM9 was investigated by a H_3-Thymidin incorporation assay using 3 different human TT cell lines. Drug exposure lasted 1 h in concentrations between 1 µg/ml and 100 µg/ml. For the in vivo tests established human TT cell lines were heterotransplanted on nude mice and the 3 agents were administered i.p. at equitoxic doses (≙LD 10; DDP 3 mg/kg/d d 1-5; JM9 4,3 mg/kg/d d 1-5; JM8 12,5 mg/kg/d d 1-5). Antitumor activity was measured by comparing the growth curves. Results: In vitro: In all 3 cell lines tested DDP showed a significantly stronger antitumor activity than JM8 and JM9 when the same doses (µg/ml) were used. Dose response curves showed that more than 10 times higher concentrations of both analogs are necessary to produce the same amount of H_3-Thymidin incorporation inhibition. In vivo: In 3 of 4 cell lines DDP achieved a significant reduction of tumor volumes whereas both analogs only produced a slight retardation of tumor growth. Conclusions: From these data we conclude that both analogs have a significantly lower antitumor activity in vitro when compared to DDP at the same µg/ml concentration. At equitoxic doses both analogs seem to be inferior to DDP against heterotransplanted human TT cell lines. Thus it seems unlikely that JM8 or JM9 will be able to replace DDP in the treatment of TT.

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TUMORSPECIFIC CYTOTOXICITY OF PHYLLANTHOSIDE AND CHLOROQUINOXALINE SULPHONAMIDE ON HUMAN TUMOR XENOGRAFTS IN VITRO

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The in vitro cytotoxicity of Phyllanthoside (NSC 328426, PH) and Chloroquinoxaline Sulphonamide (NSC 339004, CHS) was studied on 2 human bone-marrows (HBM, CFU-C), P388 murine leukemia, and on human tumors propagated as xenografts in nude mice. The evaluation was performed by the clonogenic assay using a modification of the method described by Hamburger and Salmon. Effectivity was defined as a >70% reduction of colonies as compared to controls.

PH at the concentration of 0.01 µg/ml in continuous exposure was not active in both HBM and in P388, and was active in 9/25 tumors of human origin, namely 5/8 bronchogenic carcinomas, 1/2 melanomas, and 2/4 stomach cancers. CHS was not active at 1 µg/ml in both HBM and in P388, and was active in 7/23 xenografts, namely 4/8 bronchogenic carcinomas, 2/4 stomach cancers and 1/3 breast cancers.

In conclusion, PH and CHS showed tumorspecific cytotoxicity compared to HBM. Therefore, we propose to test PH, CHS and PAN in vivo in subcutaneously growing tumors using those tumors most sensitive in vitro.

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LACK OF CORRELATION BETWEEN CYTOTOXICITY OF AGONISTS AND ANTAGONISTS OF PLATELET ACTIVATING FACTOR (PAF-ACETHER) IN NEOPLASTIC CELLS AND MODULATION OF <^{3}H>-PAF-ACETHER BINDING TO PLATELETS FROM HUMANS IN VITRO.

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The 3 ether-lipids ET-18-OCH_3, SRI 63-154 and paf-acether, the TLP BM 41.440, the ester-linked 2-LPC and CV-3988, were tested for cytostatic/antiproliferative (<^{3}H>-thymidine uptake) and cytotoxic (trypan blue dye exclusion, human tumor clonogenic assay) activity in 11 neoplastic human cell lines (U 698-M, NaII-1, Su-DHL-4, RPMI 8226, K562-4, Li-A, HTB-47, HTB-38, CCL 218, N 59, N 63) and 1 ALL in vitro. 2-LPC and paf-acether showed either no or only minor activity and CV-3988 varying activity. There were no significant differences in the activity of ET-18-OCH_3, SRI 63-154 and BM 41.440, which showed IC_{50}- and LC_{50}- values </= 10 µg/ml after incubation periods </= 48 hours with or during continuous exposure to the cells. The latter 3 compounds were then tested for interaction with <^{3}H>-paf-acether binding to intact human platelets. ET-18-OCH_3 and SRI 63-154 reduced <^{3}H>-paf-acether binding in a time dependent manner. BM 41.440 did not show this interaction. Thus, since the in vitro cytotoxicity of these lipids did not correlate with their modulation of <^{3}H>paf-acether binding to human platelets, it was concluded that cytotoxicity of ether-lipids is not mediated by specific paf-acether binding sites similar to those present on human platelets. This finding is important for future design of antineoplastic lipids, since paf-acether-like activity might lead to limiting toxicity of these drugs.

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CYTOTOXICITY OF SN-2 ANALOGS OF PLATELET ACTIVATING FACTOR (PAF) IN CELLS FROM SOLID TUMORS AND HUMAN LEUKEMIAS IN VITRO.

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There is increasing interest in the antineoplastic activity of alkyl-lysophospholipid derivatives (ALP). The structural similarities of PAF with some ALP prompted some investigations analyzing the antineoplastic activity of three different PAF analogs with a chain length of 18 carbon atoms in the sn-1 position and an amide linkage in the sn-2 position of the molecule. The sn-2 formamide analog (A), the sn-2 acetamide analog (B), and a sn-2 trifluoroacetamide analog (C) of PAF were tested in vitro in comparison to the reference ALP ET-18-OCH_3 and the ester-linked 2-LPC for antiproliferative (<^{3}H>-thymidine uptake) and cytotoxic (trypan blue dye exclusion) activity in cells from eight freshly explanted human leukemias and eight solid tumors. All 3 analogs have shown dose- and time-dependent cytotoxic and antiproliferative activity, but have not revealed higher cytotoxicity than ET-18-OCH_3. In the human tumor clonogenic assay (HTCA) the most active PAF analog B was compared with ET-18-OCH_3 using HTB 47, HTB 38, CCL 218, HL-60, and K 562 cell lines. Both compounds showed clear suppression of the colony formation of human tumor and leukemic cells in a dose range between 1 and 20 µg/ml after 48 hours of incubation or during continuous exposure. However, colony formation was reproducibly suppressed to less than 30% of the controls by ET-18-OCH_3, which could not always be achieved by B. In conclusion, the high antineoplastic activity of the sn-2 acetamide analog of PAF in vitro commends this compound and other PAF derivatives for further investigation as experimental drugs in cancer therapy.
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HEPATIC ARTERY INFUSION WITH 5-FLUOROURACIL: DEPENDENCE OF SYSTEMIC 5-FLUOROURACIL LEVELS ON THE INFUSION RATE*

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Pts with isolated liver metastases of colorectal cancer were treated with hepatic artery infusion (HAI) with 5-fluorouracil (5-FU). 5-FU dosages ranging from 750 - 2000 mg were infused over 60 - 240 min, thus representing an infusion rate of 5 to 25 mg per min. Systemic 5-FU levels were determined by a simple and rapid HPLC method. During 30 hepatic artery infusions investigated so far, we could detect 5-FU in the systemic circulation. Depending on the infusion rate, steady state levels ranged from 2.4 µM to 150 µM. With infusion rates up to 10 mg/min, maximum systemic 5-FU levels were below 26 µM, and with rates over 15 mg/min they were always above 50 µM. The influence of the 5-FU dosage and HAI velocity is demonstrated in an individual patient: During 5-FU infusion of 1000 mg over 90 min, the serum levels peaked at 22 µM. An increase to 1500 and 2000 mg 5-FU over the same time period (16.6 and 22.2 mg/min, respectively) resulted in high systemic 5-FU concentrations of up to 76 and 144 µM, respectively. After a complete cycle over 5 days with the higher dosages and infusion rates, the patient experienced severe toxicity which made intensive supportive care necessary. In general, the increase of the 5-FU infusion rate from 5 to 25 mg/min resulted in an 10-fold increase of the mean systemic 5-FU levels from 7 to approximately 70 µM.
These results demonstrate an incomplete hepatic 5-FU extraction. However, the measurements of serum levels enable us to select appropriate 5-FU dosages and HAI rates to achieve locally high 5-FU concentrations in the liver with concomitant effective but nontoxic systemic 5-FU levels.

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REVERSAL OF RESISTANCE OF MALIGNANT CELLS IN CULTURE TO ACTIVATED CYCLOPHOSPHAMIDE BY LOW EXTRACELLULAR pH

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Recent studies have shown that a metabolic property of malignant cells - their aerobic glycolysis - can be exploited to increase the extracellular proton concentration selectively in malignant tumors in vivo. By parenteral administration of glucose the extracellular pH (pHe) can be reduced to 6,1 - 6,4 in primary and transplanted tumors of both animal and human origin. In a previous study we have shown that the cytotoxic action of bifunctional chloroethylating drugs can be enhanced by low pHe (Jähde et al., 1986). In this study we investigated whether an extracellular acidosis can also be used to sensitize cyclophosphamide (CP) resistant cells in culture to the action of this drug. As source of activated cyclophosphamide (aCP) mafosfamide was used. Resistance to aCP of BICR-M1Rk-d rat mammary carcinoma cells in culture was generated by repeated exposures to high concentrations of the drug.
When the parent cell line was incubated with aCP (mafosfamide concentration: 7,5 ug/ml) at pHe 7,4 survival of clonogenic cells was reduced to $1x10^{-4}$ of untreated controls. Under these conditions survival of resistant cells was only slightly impaired ($1x10^{-1}$). However, when CP resistant cells were exposed to aCP in acidic culture media (pHe 6,2) they exhibited approximately the same sensitivity to this alkylating agent as the parent cell line (survival: $3x10^{-4}$). The potential use of glucose as biochemical modulator for reversal of CP resistance in vivo is discussed.

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CELL BIOCHEMICAL CHANGES IN CYTOSTATIC DRUG TREATED HUMAN LEUKEMIC CELL LINES BY FLOW-CYTOMETRY: ESTERASE ACTIVITY, INTRACELLULAR PH, CELL VOLUMEN AND DNA-CONTENT

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Previous investigations have shown that flow cytometric methods are suitable for the determination of the effect of cytostatic drugs on human leukemic bone marrow cell samples (A.Neubauer et al., Blut 1987 in press). The purpose of this study was to investigate in more detail the cell biochemical changes in the drug resistant surviving cells of several human leukemia cell lines such as: T-cell lines CEM, JURKAT, MOLT4, B-cell lines FAJI, DAUDI, RPMI1788 and myeloblastic line HL60. Following incubation for 48 h with cytosine-arabinoside (ara-c), l-asparaginase, daunorubicin, prednisone, vincristine at 0.1, 1 and 10x the therapeutic plasma levels, the cells were simultaneously stained with ADB (1,4-diacetoxy-2,3-dicyano-benzene) to simultaneously determine the esterase and intracellular pH of the vital cells and with PI (propidium-iodide) to measure the DNA of dead cells. The intracellular pH decreased in the surviving cells of ara-c (5 µg/ml) treated cultures between 0.27 and 1.56 pH-units (DAUDI, MOLT4) and for daunorubicin (1 µg/ml) between 0.24 and 0.62 pH-units (JURKAT, HL60) while prednisone had no effect. The intracellular pH of l-asparaginase and vincristine treated cultures decreased in HL60, CEM and MOLT4 cell lines. Futhermore significant swelling of surviving cells could be observed in a range from 133 % to 212 % (daunorubicin; 1788, HL60), from 135 % to 197 % (ara-c; 1788, FAJI), from 123 % to 146 % (vincristine; 1788, MOLT4) of the untreated control-assays. The flow cytometric measurements have been useful to study the biochemical changes in drug resistant cells. This may be of future importance for the screening of leukemia patients under cytostatic therapy.

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IN VITRO TESTING OF IN VIVO ACTIVITY OF ANTILYMPHOCYTE SERA

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Treating aplastic anemia patients with different preparations of antilymphocyte and antithymocyte globulin (ALG, ATG) it turned out that a rabbit ATG resulted in significantly inferior remission rates compared to 2 horse antisera (Dtsch. med. Wschr. 112: 535-541, 1987). This observation prompted us to compare the properties of 2 clinically "inactive" with 3 "active" ALG and ATG preparations in vitro.

Antibody specificities were evaluated by a competitive ELISA using peripheral blood lymphocytes and mouse monoclonal antibodies (MoAb). The antisera contained comparable concentrations of antibodies, blocking the binding of MoAb to lymphocyte antigens. Highest concentrations were found for CD2 CD5 CD8; only rabbit ATG had significant anti transferrin receptor activity. Complement-dependent cytotoxicity was lowest with rabbit ATG but 50 % values were in a close dose range of 20-200 µg/ml for all antisera. Striking differences were noted when the anti-sera were used to induce lymphokine release from peripheral blood mononuclear cells: Only rabbit ATG and a specific lot of horse ALG, reported to be "inactive", were highly efficacious inducers of interferon- and cytotoxic factor-release, 6-10x more potent than PHA or OKT3. This lymphokine-inducing activity did not correlate with ^{3}H-thymidine uptake.

We would like to analyse other ALGs and ATGs with known therapeutic efficacy by the assays described since this could be one way to get insight into the mechanism of remission induction in aplastic anemia by these antisera.

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AN IMMUNOCYTOCHEMICAL AND ENZYMECYTOCHEMICAL DOUBLE STAINING PROCEDURE FOR HAEMATOPOETIC CELLS

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Immunocytological methods have opened new possibilities in typing and separating haematopoetic cells.Errors in these techniques may be caused by nonspecific binding of the monoclonal antibodies to Fc-receptors,by electrostatic and hydrophobic bonds,and by structures very similar to the antigen to which the antibody was raised.Furthermore immunological methods usually neglect morphology and biology of the cells.
In order to overcome these problems,we developed a double staining procedure for immunocytochemistry and enzymecytochemistry on a single cell level.We used alkaline phosphatase-anti alkaline phosphatase (APAAP) technique with Fast Blue BB or Newfuchsin as dying coupler to obtain optimal contrasts between the reaction products.As cytochemical tests we used ß-glucuronidase,acid phosphatase,dipeptidylaminopeptidase II and IV, alpha naphthyl butyrate esterase,naphthol-AS-D-chloroacetate esterase,naphthol-AS-D-acetate esterase and peroxidase.The difficulties in performing these combinations caused a series of modifications of both APAAP-technique and enzymecytochemical methods.By applying these techniques the cytology is well preserved and the methods are well reproducible.
As an example for their application we present our results concerning the antigen pattern of DAP IV + peripheral blood cells.

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SIMULTANEOUS DEMONSTRATION OF SURFACE MARKERS AND ENDOGENEOUS PEROXIDASE ACTIVITY: A NEW APPROACHE FOR ULTRASTRUCTURAL ANALYSES

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Ultrastructural studies of endogeneous peroxidase activity has been used in identifying early progenitor cells of granulopoesis (myeloperoxidase) and megacaryopoesis (platelet peroxidase). A third endogeneous peroxidase reaction, the platelet like peroxidase, serves as an ultrastructural feature of hairy cells and activated monocytes. We introduce a new method for electron microscopy that allows both the detection of immunological and cytochemical properties on a single cell level. This is of diagnostic value to identify subsets of cells coexpressing more than one differentiation marker. We were working with a median number of 100 000 cells seeded on ployester plastic foils coated with poly-l-lysine (70 Kd, Sigma) resulting in the attachment of the cells to the foil after 30 minutes. Labelling of surface antigens was accomplished by the immunogold technique. Subsequently the labelled cells were stained for endogeneous peroxidase activity using DAB-media. Fixation with 2.5 % glutaraldehyde and block staining with uranyl acetate was done according to conventional electron microscopy methods. Epon was used to embed the cell monolayer attached to the plastic foil. Thereafter, the foil was discarched and ultrathin sections were prepared. As and example the coexpression of the CD22 antigen and endogenous peroxidase reaction as an important diagnostic marker for hairy cells will be presented. In addition to the internalisation of the CD22 antigen by coated pits and vesicles was studied in certain cases of hairy cells. Preliminary findings suggest a similar antigen pattern in isolated B-cells from the peripheral blood.
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DEVELOPMENT OF HUMAN EXPERIMENTAL TUMOR MODELS FOR SOFT TISSUE SARCOMA

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The effect of chemotherapy in advanced human soft tissue sarcoma is not satisfactory. In order to develop tumor models for experimental chemotherapy, 40 human soft tissue sarcomas were transplanted subcutaneously into nude mice. Tumor take as defined by histologic evidence of viable tumor tissue was observed in 33 cases (83%). A rapid tumor growth (a x b ≥60 mm^2 after 3 months) was found in 26 (65%), and 23 tumors could be transplanted in serial passages. Using histopathological examinations all xenografts resembled closely the original tumors. 17 tumors with a very regular growth were selected as tumor models and characterized by different methods. The doubling time ranged from 3-16 days. 14/15 tumors showed regrowth after being frozen in liquid nitrogen. Experimental chemotherapy was done in vivo and in vitro. In subcutaneously growing tumors 3/5 tumors went into remission after treatment with standard drugs. In the clonogenic assay more than 30 colonies were observed in 6/10 tumors studied. In conclusion, experimental models for human soft tissue sarcomas with different sensitivities to drugs and different histological patterns were established following xenotransplantation of human sarcomas into nude mice.
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GROWTH OF HUMAN TUMOR CELLS IN PERFUSED POROUS CAPILLARIES

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One of the major limitations of the human tumor cloning system is its inability to test drug combinations and to provide pharmakokinetic flexibility. To overcome these limitations we have developed the perfused capillary cloning system, which is based on the utilization of special porous glas capillary tubes (Schott Glaswerke, Mainz). Human tumor cell lines were seeded into 0.3 % agar and were then filled into porous glas capillaries to allow the flow of medium with or without drugs. After 14 days of incubation at 37 C the number of colonies (> 50 cells) was determined and the cloning efficiency (CE) was calculated. Of 26 different types of capillaries best CE was found within tubes consisting of membranes with pore sizes between 8 nm and 16 nm. In tubes with smaller pore sizes the colony growth was less sufficient. Tubes with pores greater than 17 nm often did not provide adequate colony growth. The best CE was obtained in tubes modified with quaternary amonium base or amino groups at the surface. There was almost no growth in tubes with alcoholic OH-groups or strongly hydrophobic groups. With the optimal capillary tubes a mean CE of 39.1 % for MDA-231 and 31 % for Colo 201 was achieved compared to 53.3 % and 34 % for non porous capillaries. The flow of fresh medium did have an impact on colony growth with best CE at lower flow rates. In contrast to other perfused capillary systems (Knazak et al.) in our system tumor cells are growing as colony formations within the tubes and can be evaluated under the microscope, not requiring cell harvesting. This system could be of interest for in vitro investigation of new drug regimens in clinical oncology.

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COMPARISON BETWEEN CLONOGENIC ASSAY AND FLOW CYTOMETRIC VITALITY TEST FOR THE STUDY OF CELL RESPONSE TO CYTOSTATIC DRUGS

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Flow cytometric measurement of esterase activity using fluoresceine diacetate was performed in parallel with the clonogenic assay considered to be of particularly predictive value in cytostatic drug testing. The human leukemic cell lines HL-60, K562 and Reh were treated with adriamycin, ara-C, cis-platinum and 5-FU at various concentrations for either 1 or 24 hrs. Subsequently a liquid suspension culture lasting 3 days and a colony assay in soft agar lasting 3 weeks were initiated. The esterase activity as a measure of cell vitality was determined from cells in liquid culture at 12 hrs intervals. Cell survival and ID50 were extrapolated from the slopes in the time-response curves. With both assays largely identical results were obtained: HL-60 and K562 cells proved to be resistant to ara-C administered for 1 hr, whereas a 50% response was obtained in the case of Reh cells at 0.2 mg/ml. An effect of 5-FU was seen in all 3 cell lines only after 24 hrs at higher doses. Also the dose-dependent effects of adriamycin and cis-platinum were comparable with both tests. This study indicates that it might be possible to replace the long-lasting and only partially effective clonogenic assay by a flow cytometric test providing data within 3 days.

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TISSUE CONCENTRATIONS OF CEA AND CA 19-9 IN COLORECTAL (cr) CARCINOGENESIS EXEMPLIFIED BY THE ADENOMA-CARCINOMA-SEQUENCE (ADCAS) *

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Tissue concentrations were measured in specimens from 44 cr adenomas of various size, histology and cytology, 29 carcinomas and from 20 normal mucosa. Aliquots of tissue samples were homogenized, extracted with PCA, centrifuged and dialysed. CEA and CA 19-9 were determined by RIA (CEA-RIA 100, Pharmacia; ELSA-CA 19-9, CIS) and expressed as ng/mg resp. U/mg wet weight (mean±SEM).

	CEA	CA 19-9
normal mucosa	100.5 ± 9.8	36.6 ± 8.9
adenoma	161.6 ± 15.0	73.7 ± 18.8
tubular	172.6 ± 30.0	56.5 ± 17.0
villous	258.2 ± 73.8	47.5 ± 18.7
mild atypia	157.0 ± 18.7	82.7 ± 28.2
severe atypia	486.2 ± 164.0	30.8 ± 15.6
carcinoma	518.7 ± 166.7	101.2 ± 35.1

Our findings fit with the concept of the ADCAS revealing a significant ($p<0.05$) increase in tissue concentrations of CEA and CA 19-9 from normal mucosa through cr adenomas to carcinomas. In the adenoma group, CEA sig. increased with increasing villous component and the extent of cellular atypia present – the two decisive criteria in the ADCAS. In our opinion the ADCAS is a suitable model to study the largely unknown biological significance of tumor-associated antigens.

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IN SITU HYBRIDISATION FOR THE DETECTION OF AMPLIFIED EXPRESSED ONCOGENES:TECHNICAL APPROACH, SENSITIVITY OF METHOD AND COMPARISON WITH RESULTS WITH ANTI-ONCOPROTEIN ANTIBODIES

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Recently,the detection of amplified expressed oncogenes within tumours of the hematopoietic system has attracted interest of clinicians.Particularly,when the malignant clone is small or when the tumours' cellular composition is heterogeneous,procedures are of value which allow not only detection of oncogenic message but also preservation of cellular morphology.
14 cases of Non-Hodgkin lymphomas,virally-transformed NIH 3T3- and K 562 erythroleukemia cell lines were analysed for the presence of the abl mRNA.A semiquantitative system was used to determine preservation of morphological appearance,intensity of autoradiographic signal and background staining.Testing several technical approaches for in situ hybridisation,the following parameters turned out to be the most important variables:(i)type of fixative(paraformaldehyd superior to ethanol acetic acid) (ii)time of hybridisation (iii)posthybridisation treatment(RNAse concentrations between 0,1 µg/ml and 10 µg/ml without reduction of morphological quality but continuous reduction of background staining) (iv)time of autoradiographic exposure.Preparation of slides and composition of hybridisation mixtures were minor parameters.Using ^{35}S-labelled probes,distinct reduction of time necessary for autoradiographic exposure could be obtained but too high intensity of signal sometimes caused loss of morphological quality.^{3}H-labelled probes granted excellent preservation of morphological detail after longer periods of exposition.When results of in situ hybridisation were compared to results obtained by Northern blotting reasonable but less sensitivity was obtained by the former method.Whereas amplified expressed oncogenes can certainly be detected with this method the visualization of only few copies of mRNA will demand refined variations of in situ hybridisation.When a pannel of mono- and polyclonal antibodies was tested in parallel,clear cut evidence arose that at the present time the expression of cellular oncogenes should be studied on the protein- as well as at the RNA level.

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EVALUATION OF C-SIS mRNA EXPRESSION BY HUMAN MEGAKARYOCYTIC CELLS IN NORMALS AND PATIENTS WITH MYELOPROLIFERATIVE DISORDERS

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The B-chain of platelet-derived growth factor (PDGF) is encoded by the c-sis oncogene. PDGF synthesis has been shown in a number of cells, including megakaryocytes. In the context of an enlarged megakaryocytic progenitor cell compartment in myeloproliferative disorders and the known growth promoting activities of PDGF for stroma cells and hemopoietic progenitor cells, we tried to determine whether an elevated expression of c-sis in megakaryocytic cells can be observed in myeloproliferative disorders.
Since sufficient quantities of m-RNA cannot be obtained for solution hybridization in view of the rarity of megakaryocytic cells in human bone marrow and the low number of cells that can be picked from CFU-M colonies, we performed in-situ hybridization, which allowed detection of mRNA sequences at the single cell level. Pure preparations of megakaryocytic cells were either isolated from bone marrow by gradient centrifugation and fluorescence activated cell sorting (FACS) or derived from in vitro grown megakaryocytic colonies (CFU-M). Studies were performed with cells from normal volunteers (n=4), patients with CML (n=3) as well as from one patient with Essential Thrombocytosis and one patient with osteomyelofibrosis. Our studies demonstrated that neither immature and mature-megakaryocytes or cultured megakaryocytes from normals nor megakaryocytic cells derived from patients with myeloproliferative disorders displayed significantly increased hybridization signals compared to controls.
The available data suggest that at least some of the patients with myeloproliferative disorders do not express c-sis in megakaryocytic cells above basal levels, as assessed by in-situ hybridization. A possible biological role for c-sis and PDGF in myeloproliferative diseases remains to be determined.

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EXPRESSION OF abl- AND sis-ONCOGENES IN CHRONIC MYELOPROLIFERATIVE DISEASES BY GENOM HYBRIDIZATION IN SITU.

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CMPD's are characterized by histopathology and karyotyping mainly whereas phenotyping by membrane markers and genom identification by molecular methods are applied to a lesser extend only. Since abl-oncogene is strongly related to Philadelphia translocation, which does determinate the various categories of CMPD, the expression of this as well as of sis-oncogene was investigated within single cells from blood or bone marrow films among 27 patients by the method of in situ hybridization. Results of these findings were correlated with histopathology of bone marrow biopsies and karyotyping, both performed simultaneously by this laboratory. The Hannover-Classification of CMPD regarding the new categories of CML with megakaryocytic increase - CML.M - and Chronic Megakaryocytic Granulocytic Myelosis - CMGM has been applied (GEORGII et al., 1986).

Histological classification	Karyotyping Ph^+	Ph^-	not evaluable	Genom Hybridization abl^+/N	sis^+/N
CML.CT	5	0	7	12/12	0/12
CML.M	7	1	1	9/9	7/9
CMGM	0	1	2	0/3	0/3
PTH	0	1	0	0/1	0/1
P. VERA	0	1	1	0/2	0/2

Results show a strong correlation of positivity between Ph^+ and abl-oncogene-expression in all 21 cases of CML-CT and CML.M investigated so far. Moreover, the only case of CML.M with proven Ph-negativity as well as the 7 cases that could not be karyotyped for technical reasons have shown the abl-oncogene. The other 3 groups of Philadelphia-negative diseases, e.g. CMGM, Primary Thrombocythaemia and P. Vera showed no expression of these oncogenes.

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EFFECT OF RECOMBINANT INTERFERON ALPHA (rIFN-alpha-2) ON GENE DOSAGE AND EXPRESSION OF THE C-MYC ONCOGENE IN PHILADELPHIA CHROMOSOME POSITIVE (Ph^+) CML

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Recent studies with cells from patients with Ph^+ CML have shown a novel c-abl gene product arising from transcription of a chimeric gene which includes c-abl and bcr-gene sequences. Moreover, additional oncogenes may be involved in transformation and evolution of Ph CML. To assess the role of c-myc in Ph^+CML, c-myc gene and its expression was studied in 10 CML patients during the benign and accelerated phases of their disease. Since IFN-alpha has been effective in CML and has been reported to diminish the proportion of Ph^+ bone marrow cells, progress towards understanding the antiproliferative activity of IFN would be facilitated if it could be determined, what effect IFN has on the expression of specific cellular genes involved in the regulation of cellular proliferation and/or the process of neoplastic transformation. We therefore investigated the effect of IFN-alpha on the c-myc gene and its expression in blood and bone marrow mononuclear cells from CML patients undergoing IFN-alpha-treatment. Southern blot analysis revealed a normal c-myc gene in patients with benign phase CML. Occasionally, c-myc amplification was found during disease acceleration. In one patient with accelerated phase CML c-myc amplification disappeared following IFN-treatment, while the patient remained clinically stable. Before IFN-treatment, c-myc expression in bone marrow mononuclear cells from patients with benign-phase CML was found to be comparable healthy controls. Contrarily, disease acceleration was accompanied by a significant amplification of c-myc expression. We thus conclude that c-myc gene amplification and/or overexpression might play a crucial role in the evolution of CML-alpha from stable disease to blastic crisis.

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QUANTIFICATION OF $p21^{ras}$ EXPRESSION. $pp60^{v\text{-}src}$ EXPRESSION AND TYROSINE-PHOSPHORYLATION BY FLOW CYTOMETRY AND ELISA

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The expression and function of pp60v-src were measured in B31 and MS1, two avian sarcoma virus (ASV) transformed rat cell lines, and that of $p21^{ras}$ in the 14C cell line. In the 14C line the EJ^{ras} gene was placed under the transcriptional control of a steroid inducible mouse mammary tumor virus promotor /enhancer and introduced into rat1 fibroblasts. We used monoclonal antibodies which react with polypeptides encoded by each of the three human ras genes (Y13-259) with pp60v-src (EB7/GD11) and phosphotyrosine (1G2). Binding of the anti phospho-tyrosine antibody to P-Tyr residues was entirely inhibited by addition of excess phenylphosphate, whereas phosphoserine or phosphothreonine did not interfere with binding. ASV transformed cells were treated with Herbimycin A, a benzenoid ansamycin antibiotic, to decrease the expression and tyrosine kinase activity of pp60ßv-src in B31 cells. Herbimycin A caused decreases of 42 % in anti-pp60v-src and 58 % in anti-P-Tyr binding and induced a phenotypic reversion of the ASV transformed cell lines to an untransformed morphology characterized by actin rearrangement, and growth behavior. Flow cytometric evaluation of the protein tyrosine kinase expression ($pp60^{v\text{-}src}$) and activity (P-Tyr residues) was fast and easy, and correlated well with the ELISA, phosphoamino-acid analysis by two dimensional electrophoresis and measures of cell phenotype. EJ^{ras} expression in the 14C line was induced by $1x10^{-7}$ M dexamethason over 48 hours. $p21^{ras}$ measurement in induced and uninduced 14C cells by FCM corresponded well with mRNA analysis and changes in morphology. Qualitative and quantitative analysis of oncogene expression in many human tumors helps to get additional information about the nature of tumors and might have consequences for prognostic and therapeutic considerations. - II. Med. Univ.-Klinik Wien, A-1090 Wien, Garnisongasse 13

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POLYMORPHISM OF THE HA-RAS ONCOGENE LOCUS IN HUMAN CHRONIC LYMPHATIC AND CHRONIC MYELOGENOUS LEUKEMIA

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Chromosomal aberrations with translocations of genes and allelic polymorphisms seem to play an important role in the development of human leukemia. Especially the members of the ras-oncogene family are suggested to be involved in the pathogenesis of human hemoblastosis. We have studied the restriction length polymorphism of the Ha-ras oncogene in human chronic lymphatic (CLL) and chronic myelogenous leukemia (CML). White blood cell DNA from fifteen patients with CLL, sixteen patients with CML and twenty-six healthy blood donors were investigated. After cleavage with the restriction enzyme BamH I, common and a few rare Ha-ras alleles were found in the controls by Southern-Blot analysis, indicated by restriction fragments different in size. In four of the CLL patients Ha-ras alleles were detected that could not be found in the control group. In the CML group the restriction fragment pattern was totally different. In twelve of the CML patients one Ha-ras allele was found that occurred only twice in the controls and twice in the CLL patients. An amplification of the Ha-ras locus was not observed in any of the blood samples tested. Our data indicate an involvement of rare Ha-ras alleles in human chronic leukemia and the linkage of a certain allele to CML.

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CHROMOSOMAL STUDIES IN 24 PATIENTS WITH SECONDARY ACUTE MYELOID LEUKEMIAS (AML) AND MYELODYSPLASTIC SYNDROMES (MDS)

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Chromosomal banding studies of bone marrow and/or peripheral blood cells of 24 patients with acute myeloid leukemias or myelodysplastic syndromes following prior treatment by chemo- and/or radiotherapy for malignant (n=22) or benign (n=2) disease were performed. Chromosomal anomalies were found in 21 patients (88 %). The aberrations most often encountered were: -5/5q- (52 %), hypodiploid chromosomal patterns or complex aberrations (43 %), anomalies of # 3 or 17 (29 %) and -7/7q- (19 %). 5 out of the 21 patients with chromosomal anomalies had an abnormal Karyotype not at all suggestive of secondary leukemia (46,XY,11q-;47,XY,+8;47,XX,+11; 47,XY,+8,t(9;11)). The fact that some patients with so-called secondary acute myeloid leukemias or myelodysplastic syndromes have normal Karyotypes or chromosomal aberrations not at all typical for secondary hematopoietic neoplasias let us to speculate that some of these patients have a de novo, i.e. therapy-unrelated blood disorder. If this view can be corroborated by the well known clinical and hematological features of de novo versus secondary leukemias, then this subset of patients should be treated by conventional chemotherapy.

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ANOMALIES OF THE SHORT ARM OF CHROMOSOME 9 IN HEMATOLOGIC DISORDERS

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Clinical, hematological and cytogenetic data of 32 patients with loss of part of the short arm of chromosome 9, (9p-), are reviewed. There were 20 ALL, 7 NHL, 3 AML, 1 RAEB and 1 CML-BC. The cytogenetic findings were heterogenous: 13 del(9)(p21), 5 del(9)(p12), 4 i(9q), 3 unbalanced translocations involving 9p12 and 7 involving 9p21. Ten patients showed in addition known specific translocations for determined subgroups of ALL, NHL and CML.

In T-NHL, 3 children with 9p-, T-immunoblastic lymphoma originating from common thymocyte and presenting with mediastinal mass and pleural effusion may constitute a definite subgroup with good prognosis. All other patients had a poor outcome and no specific association between the karyotypic change, immunophenotype and clinical outcome could be ascertained. The previously suggested specific association of 9p- with T-ALL and "lymphomatous features" could not be confirmed. When the hypothesis, that the gene coding for methylthioadenosinephosphorylase situated in 9p21-pter is lost in these cytogenetic anomalies, could be verified, it would be possible to kill selectively the affected cells with purine-antimetabolites like metothrexate.

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HUMAN RECOMBINANT GRANULOCYTE COLONY STIMULATING FACTOR - ACTIVITY IN HUMAN LONG TERM BONE MARROW CULTURES

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Human recombinant granulocyte colony-stimulating factor (rhG-CSF) is a stimulator of granulopoiesis in vitro and in vivo. G-CSF supports survival and colony growth in vitro from granulocytic and monocytic hemopoietic progenitor cells (CFU-GM) and promotes the generation of CFU-GM from a population of immature progenitors (pre-CFU) in suspension culture of normal human bone marrow. We investigated the influence of G-CSF on stem or progenitor cells of myelopoiesis in long term cultures of normal human bone marrow (LTBMC). In the presence of rhG-CSF, excessive proliferation occured in LTBMC, necessitating frequent depletion of cells from LTBMC. rhG-CSF did not interfere with the formation of a typical stromal cell layer within 7 to 10 days of culture, nor with the migration of hemopoietic progenitor cells into the stromal cell layer. Nonadherent (supernatant) phase and stromal cell layer of LTBMC were analyzed for their content of viable cells, of CFU-GM and immature pre-CFU progenitor cells. Results indicated, that the kinetics of LTBMC cell growth was affected by rhG-CSF, particularly in the early phase of LTBMC, and that hemopoiesis in LTBMC was shifted towards granulopoiesis. However, the duration of the productive time period of LTBMC was not altered by rhG-CSF as compared to control LTBMC in the absence of rhG-CSF. These resultats suggest a role for G-CSF preferentially in the commitment and proliferation of progenitor cells in the granulopoietic compartment of hemopoiesis.

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INFLUENCE OF IRRADIATION ON DIFFERENT CELL POPULATIONS OF HUMAN LONG-TERM BONE MARROW CULTURES*

P. Brühl 1, H.-G. Mergenthaler 1,2 and P. Dörmer 1

One of the main features of human or murine long-term bone marrow cultures (ltbmc), is the development of a heterogeneous adherent cell population, that apparently simulates the native hemopoietic microenvironment. The nature of the interaction of both, hemopoieteic stem cells and their supporting stroma is still unknown. However, they can be distinguished by their differential radiosensitivity.

Ltbmc were set up in microtiter-plates and fed weekly by removal of half the non-adherent cell population and addition of an equal volume of fresh medium. After 2-3 weeks when confluent stromal layers had been established, the cultures were irradiated with different doses. Each week the number of GM-CFC in the non-adherent cell population was determined. Cultures which have been irradiated with a dose as low as 0.5 Gy reached a plateau phase which was far below the control group. If ltbmc were set up with irradiated bone marrow cell suspensions, the same survival pattern was obtained. However, after irradiation with 4.0 Gy a confluent stromal cell layer could be established in only 21 % of the culture wells. Preformed stromal cell layers irradiated with doses up to 20.0 Gy were still able to support in vitro hemopoiesis of "recharged" allogeneic bone marrow cells. No alteration of the hemopoietic inductive microenvironment was observed. Further studies are concerned with the radiosensitivity of hemopoietic stem cells separated from contaminating stromal cells by means of monoclonal anti-HLA class II antibodies and nylon-wool adherence.

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IMMUNOLOGICAL PHENOTYPING IN SITU OF MYELOID COLONIES IN AGAR CULTURES.
H.Schmetzer, H.H.Gerhartz

A technique to immunologically stain hematopoietic colonies in agar cultures would make possible the analysis of clonal aggregates in situ without destroying their architecture and avoid the necessity of picking or washing the cells from semisolid media. We developed such a method which gives satisfying morphological and immunological results within reasonable time. Fixation of agar plugs in 0.05% glutaraldehyde and their subsequent drying on polysiloxane coated slides turned out to be essential to avoid unspecific staining of the agar. Following this the cultures were incubated for 1.5 hours with the primary antibodies and finally developed in an indirect enzyme-immunoassay using an alkaline phosphatase-conjugated secondary antibody. A comparison of the reactivity of myeloid colonies stained in agar or in methylcellulose (mc) showed that some reactivity was lost in agar - especially at the relatively thick edges of the agar plugs. Therefore, agar cultures were set up directly on the slides with the result of thinner agar layers without any loss in plating efficiency. In this system 70-90% of clones were positive for VIM-D5, and much less reactivity was found with antibodies to earlier differentiation antigens (My 10, J 5) with corresponding proportions of positive cells obtained from mc cultures. Thus, the advantages of staining complete agar cultures were not compromized by a decreased reactivity of the assay.

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INHIBITION OF IN VITRO HEMATOPOIESIS BY HEPATITIS A VIRUS
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Inoculation of human bone marrow with hepatitis A virus (HAV) resulted in a dose- and duration of incubation-dependent suppression of hematopoietic progenitor (CFU-GM, BFU-E, CFU-Mix) growth in vitro. Monocytic progenitors appeared to be least affected. While HAV inactivation by heat or ß-propiolactone and neutralisation by specific antibodies completely abrogated hematopoietic inhibition, depletion of adherent bone marrow cells as well as enrichment of My 10^{+} progenitors did not alter the pattern of suppression, which also seemed to be independent of the presence of HuIFN- ,- ,- , and TNF. These findings support the concept that direct infection of progenitor cells by HAV may be responsible for hematologic changes commonly seen during early phases of infectious hepatitis and possibly for some cases of bone marrow failure.

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BENZENE INHALATION AND THE ERYTHROPOIETIC CELL SYSTEM IN MICE

H.J. Seidel, G. Beyvers, E. Barthel

BDF_1 mice have been exposed to 300 and 900 ppm of benzene 6 hours daily, 5 days/week, up to 16 weeks. The erythroid cell system was studied using in vitro cultures for BFU-E and CFU-E, the early and the late erythroid stem cell, bone marrow differentials and peripheral blood counts. In addition, Fe^{59} was used to measure the kinetics of the system and the regenerative capacity was tested after bleeding. It turned out that the CFU-E were the most sensitive cells, reduced after 2 weeks with 300 ppm. Morphologic changes in the erythroblasts E_1 - E_5 were not seen at this benzene concentration. The Fe^{59} study indicated an enhanced turnover of the erythroblastic cells in the benzene-exposed, slightly anemic mice.

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REGULATION OF HEMOPOIETIC CELL REGENERATION AND MIGRATION AFTER THIAMPHENICOL TREATMENT ANALYSED BY MATHEMATICAL MODELING

B.Bungart*,M.Loeffler*,H.Goris+,S.Schmitz*,W.Nijhof+

We intended to validate a recently postulated theory of intramedullar hemopoietic regulation (1) whose basic postulates are:(a) self renewal and cyclic activity of stem cells are independently controlled;(b) these contols come from intermediate granuloid (G) and erythroid (E) cell stages;(c) G and E have different impacts on stem cell self renewal (G more than E) and cyclic activity (E more than G);(d) all E and G cells in the marrow and the spleen participate in a systemic control. Thiamphenicol (TAP) selectively influences these G and E cell stages and therefore allows to study feedback regulation. Furthermore TAP affects the marrow to spleen ratio of progenitor cells hence giving insight into systemic control of the total(marrow + spleen) hemopoiesis. Computer simulations of our data (previous paper) show: 1) TAP suppresses cell divisions in CFU-E, erythroblasts and myeloblasts; 2) the decline of CFU-S and the simultaneous increase of BFU-E and CFU-GM during TAP treatment agree well with postulates (a)-(c) indicating a feedback mediated recruitment of stem cells to differentiation; 3) Only massive cell migration from the marrow to the spleen can explain the overshooting splenic CFU-S, BFU-E, CFU-E and CFU-GM counts; 4) Irrespective of the changed contributions of marrow and spleen hemopoiesis (with their differing microenvironments) the overall hemopoietic cell production appears well controlled just compensating the mild anemia and neutropenia. This finding supports the concept of systemic control superimposed on the local microenvironmental control. A regulation of cell migration seems to exist which links systemic demands with microenvironmental properties. (Supported by the DFG Lo 342/1-1, FRG); (1) Wichmann, Loeffler (Eds): Mathematical modeling of cell proliferation, CRC-Press 1985 ; *:Med Klinik I,Jos-Stelzmann-Str 9, D 5 Koeln 41, FRG; +:Lab Physiol Chem, Bloemsingel 10, 9712 KZ Groningen,Netherlands

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EFFECT OF LIPOPROTEINS AND INSULIN ON HUMAN ERYTHROID PROGENITOR CELLS

G. Konwalinka, D. Geißler, Ch. Breier, Ch. Wiedermann, J. Patsch, H. Braunsteiner

A serum-free agar system has been developed for the clonal growth of early and late erythropoietic prgoenitor cells (BFU-e and CFU-e). It consists of Mc Coy's medium, deionized and delipidated bovine serum albumin, transferrin, lipoproteinfraction I (LpFrI) ($d < 1.21$ g/ml) or low density lipoproteins (LDL) ($d < 1.020$-1.063), and an adequate dose of step III erythropoietin (Epo). In search for the growth supporting lipoprotein species in the LpFrI the main density lipoprotein fractions from human plasma, namely very low density lipoproteins (VLDL) ($d < 1.006$ g/ml), LDL, high density lipoproteins$_2$ (HDL_2) ($d < 1.063$-1.125), HDL_3 (d 1.125-1.21 g/ml) were prepared by ultracentrifugation and analyzed for their capacity was found to be concentrated mainly in thsoe fractions enriched for LDL, HDL_2 and HDL_3 in the presence of crude Epo.
However, on ly a weak erythroid colony formation was found in the presence of LpFrI or LDL, if recombinant Epo has been used instead of step III Epo. An additional factor was required in the presence of recombinant Epo which was found to be insulin. The concentrations of insulin required for optimal growth were much higher than can be considered pyhsiologically relevant.
In conclusion, our results show that the only method practical for identifying factors essential for cell growth, is the use of a cell culture system in which all components of the medium are well defined.

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CHARACTERIZATION OF CLONOGENIC LEUKEMIC CELLS IN PATIENTS WITH MYELODYSPLASIA

C. Aul, A. Heyll

Recently established culture techniques have allowed the clonal growth of blast cell progenitors (CFU-L) in patients with AML. It has been suggested that the in vitro characteristics of CFU-L reflect the biological behavior of individual AML clones. In contrast to de novo AML, patients with myelodysplasia (MDS) are usually characterized by a slowly progressing course. In this study we examined whether the indolent course of MDS is related to specific abnormalities of CFU-L growth.
Using the colony assay be Minden et al. 27 untreated patients (RA n=5, RAEB 5, RAEB/T 12, DMML 5) were investigated at the time of diagnosis. In 17 cases blast cell colonies containing up to 100 immature cells could be grown from T-depleted blood cells. Plating efficiency varied between 15 and $628/10^5$ E^- MNC and was not found to correlate with the peripheral blast cell concentration. Following short time exposure of cells to Ara-C (1 µM), a substantial reduction of colony formation (43 ± 16 %) was observed in all patients. In contrast to previous studies in patients with AML, self-renewal of CFU-L was uniformly found to be low, ranging from 0 to $99/10^5$ cells. No significant differences could be demonstrated between the FAB groups.
From these data we conclude that the proliferative state of CFU-L is not different in patients with MDS and AML. Clonogenic cells in MDS are characterized by low capacity for self-renewal which may limit the expansion of the malignant cell clone and explain the slow evolution to overt AML.

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DIFFERENT RESPONSE TO RECOMBINANT INTERFERON ALFA-2 IN THREE HISTOLOGIC SUBTYPES OF HAIRY CELL LEUKAEMIA

W.Hill*, R. Schlag**, E. Thiel **,***, R. Burkhardt*,+

28 patients with hairy cell leukaemia got rec. Interferon alfa-2 b during a prospective study started in October 1984. The criteria used for inclusion and exclusion in the study, its implementation and the response were those given in the protocol of H.M. Golomb 1986. 26 patients were evaluable. The initial dose was $2x10^6$ IU/m² subcutaneously three times weekly in 15 patients and $1x10^6$ IU every day for one month followed by $1x10^6$ IU three times weekly in 11 patients. The degree of infiltration was evaluated according to the hairy cell index (HCI) every 3 to 6 months. Three histologic subtypes could be differentiated: ovoid (n = 17), convoluted (n = 3), and indented (n = 6). 22/26 patients (85%) had a response: 4 complete (CR), 8 partial (PR) and 10 minor (MR). All 17 patients with the ovoid subtype (100%) had a response: 4 CR, 6 PR, 7 MR; 6 of these with low and 11 with higher dose. Only 5/9 patients (56%) with the convoluted and indented subtypes had a response: 2 PR, and 3 MR. 10/11 patients responded with low (91%) and 12/15 with higher dose (80%). The spleen decreased objectively in 14/19 patients with splenomegaly. Patients with low infiltration (HCI 0.50) had a higher rate of response (100%) than those with higher infiltration (HCI 0.50) (79%).

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INVESTIGATIONS ON THE MECHANISM OF ACTION OF INTERFERON -α_2 (IFNα_2) ON HAIRY CELLS (HC)

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IFNα has become a major therapeutic agent in HCL. Its mechanism of action has not been defined however.
Investigating HC from more than 20 patients we determined the influence of IFNα_2 on the incorporation of radiolabeled nucleic acid precursors, on the number and activity of NK cells before and during treatment and on the immunophenotype of HC during long term in vitro incubations. The results demonstrate that IFNα_2 leads to an enhanced incorporation of ^{3}H-uridine into HC in vitro, thus indicating a possible role for the induction of RNA synthesis in vivo. NK cells seem to play no major role in the regression of HC, since we did not find consistent correlation between the activation of these cells and the response to therapy. Finally, IFNα_2 does not alter antigenic determinents on HC in vitro, which makes a direct maturating or differentiating effect of the compound on HC in vivo rather unlikely.

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In Vitro Induction of Tumor Necrosis Factors in Hairy Cell Leukemia: Enhanced Release before Therapy and Normalization during Effective Therapy with Interferon Alpha or after Splenectomy.

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Hairy cell leukemia is a malignancy of a late stage of B-cell differentiation and usually presents with pancytopenia. In order to identify a factor which might be responsible for the pancytopenia, especially granulocytopenia and bone marrow fibrosis we analysed as a potential factor the cytotoxin production in peripheral blood mononuclear cells (PBMNC) of HCL patients.
PBMNC from patients with hairy cell leukemia (HCL) were isolated and induced by mitogen (PHA), phorbolester (TPA) and staphylococced enterotoxin B (SEB) to secrete the two distinct cytotoxic polypeptids TNF-α and TNF-β. The production of TNF-β was ten fold enhanced in PBMNC of HCL-patients before any therapy as compared to normal controls. The induction of cytotoxins with the combination PHA/TPA was also enhanced. Cytotoxin production in PBMNC during a successful therapy with rIFN-alpha2C was reduced to a normal level. Surprisingly, the enhanced levels of cytotoxins were also reduced after successful splenectomy.
It is possible that an enhanced TNF production in vivo might be important for the granulocytopenia and the typical bone marrow alterations and that an effective therapy reduce the enhanced TNF-production in this malignancy.

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HTLV-II IS NOT IMPLICATED IN THE PATHOGENESIS OF THE B-CELL TYPE OF HAIRY CELL LEUKEMIA

T. Lion, N. Razvi, B. Brownstein and J. Schwarzmeier

Two different isolates of HTLV-II from variant T-cell forms of hairy cell leukemia have been reported. These findings represent the only instances of the virus being detected in a human malignancy.
We have performed molecular genetic studies in 16 patients with hairy cell leukemia and were not able to detect HTLV-II sequences in the hairy cell-genome at our limits of sensitivity (as low as 2,6 pg viral DNA of 8 kb length /10 µg of human DNA, equivalent to 1 viral genome per 5 cells).

Since our results are consistent with the serological findings in this disease published recently by others the data suggest that HTLV-II is probably not involved in the pathogenesis of the B-cell type of hairy cell leukemia.

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GAMMA-INTERFERON IN THE TREATMENT OF LOW-GRADE LYMPHOMAS: A CRITICAL REVIEW OF A PHASE-I/II-STUDY

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In a phase I/II-study the efficacy and toxicity of recombinant gamma-interferon (γ-IFN) was investigated in 25 patients, 14 with chronic lymphocytic leukemia (CLL), 8 with immunocytic lymphoma (ICL) and 3 with multiple myeloma (MM). 21 of the patients were previously treated with one or more combinations of cytotoxic agents.
Initial doses of γ-IFN between 10 and 150 $\mu g/m^2$ were administered daily (Mo. to Fr.) subcutaneously or i.v.. In non-responders, the γ-IFN dosage was escalated or combined with alpha$_2$-IFN. The combination of both IFN was used because of the synergistic effects in inhibiting tumor growth in vitro.
20 out of 25 patients are evaluable for response. In two patients a partial remission and in 3 patients a minor response could be achieved. 9 patients had a stable disease and 6 showed a progression of the disease. However, a minimal initial response of leucocyte counts or enlarged lymph nodes was observed in almost all patients with stable or progressive disease, too. One out of 3 patients receiving the combination of alpha- and γ-IFN had a response.
In conclusion, the results demonstrate only a poor response rate in low-grade NHL. Further studies using different dosages and schedules or combination with other biological response modifiers are required.

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B-CLL IN EARLY PHASE RESPONDS TO RECOMBINANT ALPHA-2b INTERFERON THERAPY AS DETERMINED IN A PILOT STUDY

E. Thiel, R. Schlag, W. Hill, G. Kettner, D. Flieger and H.W.L. Ziegler

Recombinant alpha interferon (rIFNa) has been reported in several studies to be active in low grade lymphomas, but some reports of small numbers of patients with CLL suggested that CLL is poorly responsive to rIFNa. These patients, however, had rather advanced disease and were extensively pretreated. We, therefore, asked whether IFN treatment would be more efficient in the early phase of the disease. Since life expectancy has been demonstrated to be shortened in CLL, we decided to treat 7 patients (5 M, 2 F) less than 60 years old in early clinical stages (6 stage 0, 1 stage 1) after informed consent with Intron A (Essex Pharma) (5 x 10^6 I.U. s.c. three times a week). Due to minimal side effects self-treatment in outpatient form was possible in every case. In all cases flow cytometry demonstrated an objective response evidenced by the decrease of circulating lymphoma cells very early (1-3 weeks) after starting treatment. Complete remission, however, was reached only in the one patient with lowest WBC (15.000-20.000) before treatment, whereas the other cases exhibited partial remissions with normalization or $>$ 50 % reduction of the WBC, but still having monoclonal lymphoma cells in circulation. The extent of response was shown to depend on the WBC and the onset of disease at start of therapy. These results underline that rIFNa may be effective in early phase of disease with low tumor burden in spite of treatment failures in progressed disease.

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MOLECULAR GENETIC ANALYSIS OF HIGH GRADE NON HODGKIN'S LYMPHOMA AND HODGKIN'S LYMPHOMA

M. Kneba., I. Bolz, M. Bergholz, G.A. Nagel, M. Krönke and G. Krieger

We have analyzed 22 large cell high grade Non Hodgkin's lymphomas (NHL) and 8 Hodgkin's lymphomas (HL) by using the Southern blot technique with immunoglobulin gene (Ig) and T-cell antigen receptor (TCR) cDNA probes. The results were compared with the histologic and immunphenotypic analysis. In 18/22 cases of NHL the DNA analysis confirmed the immunohistological diagnosis as demonstrated by distinct clonally rearranged Ig- or TCR-genes. In 1 case of Ki-1-NHL of presumed B-cell type a clear TCR-gene rearrangement in the absence of any Ig-gene rearrangement was observed as clear evidence of the T-cell origin of this tumor. One case of AILD showed an Ig-gene rearrangement suggesting the evolution into a B-cell NHL. In two cases which were histologically diagnosed as of probably B-cell type no Ig- or TCR-gene rearrangements could be shown. In the 7 cases of HL no clear monoclonal Ig- or TCR-gene rearrangements could be found.
The results and clinical follow up data will be presented and the clinical significance of the molecular genetic analysis will be discussed.

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MUTATIONS OF c-myc AND c-mos GENES LEAD TO ABERRANT TRANSCRIPTION OF c-myc AND TO ACTIVATION OF c-mos IN FRESH TUMOR CELLS OF A PATIENT WITH IMMUNOCYTOMA.

F.Csaikl, L.Müllauer, M.Schwabe, U.Csaikl and J.Schwarzmeier

The involvement of oncogenes residing on chromosome 8 has been strongly implicated in the pathogenesis of human B-cell neoplasms. We report on the genomic alterations of the c-myc and c-mos genes in a newly diagnosed case of immunocytoma. Using a set of five restriction enzymes we found various mutations of the two oncogenes. In the case of c-myc one of the enzymes revealed a germline configuration only, whereas the other four gave aberrant patterns. Cell fractions enriched for T- or B-cells showed different patterns with two of the enzymes but, identical mutations with the other two. The analysis of c-mos was performed with four restriction endonucleases, one of them revealed a genomic alteration in B-cells only.
Northern blot analysis for c-myc revealed in both T- and B-cells, instead of a normal 2,7 kb, a 3,1 kb transcript. An additional 4,4 kb transcript was found in the B-cell clone. In the case of c-mos a 5,0 kb transcript could be demonstrated only in B-cells. This is the first time that a c-mos transcript was found in a hematological malignancy. As mutations of c-myc could be detected in B- as well as in T-cells the involvement of stem cells in the malignant transformation of this case of B-cell lymphoma has to be discussed.

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EXPRESSION OF A NOVEL ACTIVATION ANTIGEN DEFINED BY MONOCLONAL ANTIBODY 7F7 ON NON-HODGKIN LYMPHOMAS

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We have recently discovered a novel activation antigen defined by monoclonal antibody 7F7 (T.F. Schulz et al., submitted). This single chain membrane glycoprotein of 85 kd is found on peripheral and germinal centre B-cells, on follicular dendritic as well as on some vascular endothelium. Although not found on resting T-cells it is strongly expressed on CD4+ as well as CD8+ T-cells activated by either PHA, PWM or anti-CD3 antibodies and its expression on B-cells in markedly enhanced by stimulation with PWM or antibodies to the C3d/EBV-receptor, CR2 (CD21). This molecule is expressed as early as four hours after PHA stimulation. In this study we determined the expression of this activation marker on more than 90 Non-Hodgkin lymphomas (NHL) in order to assess its usefulness as a marker for proliferation of malignant lymphoid cells. NHL of early B-cell stage (ALL, lymphoblastic lymphoma) were consistently negative for antibody 7F7 in spite of their usually high proliferative activity. On the other hand germinal centre-derived NHL (centrocytic-centroblastic/centrocytic-, and centroblastic NHL) showed a very srong expression of this antigen. In other NHL of intermediate B-cell stage (CLL, immunocytoma) 7F7 antigen was absent or only weakly expressed. Among late B cell NHL the highly malignant immunoblastic lymphoma stained positive for this marker whereas hairy cell leukaemias and multiple myelomas which both contain only a small population of rapidly growing cells, were stained only in a minority of cases. We conclude that expression of 7F7 antigen does not always indicate proliferative activity. Based on the in vitro findings we would therefore postulate that expression of the 7F7 molecule indicates a state of activation which is not necessearily accompanied by cell division.

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LOCALIZED EXTRANODAL NON HODGKIN LYMPHOMAS - EXPERIENCE OF THE 3rd MEDICAL DEPARTMENT OF THE HANUSCH-HOSPITAL VIENNA

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More than 1100 NHL have been treated at our department during the last 10 years. If CLL and IZ are excluded 43 patients (6%), 18 male, 25 female, median age 62 years (range 26 - 80 years) presented with stage IE (n=38) or localized involvement of Waldeyer's region (n=5) most often. Immunoblastic lymphoma (n=13, 18% of all IB cases), followed by CB (n=12, 14% of all CB) presented with primary extranodal disease. In low grade NHL with the exception of IC initial extranodal involvement was comparabel rare (5% in CB/CC). The most frequent localisation were the gastrointestinal tract, expeciall the stomach (n=10). 7 of 12 CB had stomach involvement. 10 patients had localized skin disease (CB/CC:4, CB:2, IB:2, LB:1, CC:1). 9 of our patients presented with isolated brain tumors (6 of 13 IB). Rare localisations were testes, lung and breast. Pathologic staging was done in 10 patients, all of them presented this gastrointestinal disease. B-symptoms were present in 60%. Treatment was evaluable in 41 patients. Complete remission rate was 75%. Treatment failures were seen most often in brain tumors, treated with irradiation + CHOP. Durable remissions were seen in 31 patients. The influence of treatment on long term prognosis will be discussed with special regard to the question of primary irradiation or chemotherapy.

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TREATMENT AND PROGNOSIS OF PRIMARILY EXTRANODAL STAGE I AND II HIGH-MALIGNANT NON-HODGKIN'S LYMPHOMAS

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87 out of 159 unselected high-malignant NHL of the years 1976-1986 had primarily an extranodal manifestation. Stomach (n=30), mouth/pharynx/nose-region (n=18), and skin/muscle/soft tissue/bone (n=14) were most frequently involved. The median age was 60 (18-84) years. Immunoblastic (n=24), centroblastic (n=37), and unclassifiable (n=26) lymphomas showed no survival difference and were evaluated together. The 10 years survival probability was 72% for stage IEA (n=44), 61% for IIEA (n=28), and 39% for IIEB (n=12). There was no survival difference compared to cases with nodal involvement only. Originally, stages I and II were treated by radiotherapy (RT). Numerous relapses led to start treatment with polychemotherapy (PChT) followed by RT. The relapse-free survival of only 29% in stage IA after RT (n=12) increased to 85% after PChT (n=11) or sequential combination of PChT and RT (n=43). In stages IIA the combined treatment (n=26) yielded 72% and was superior to RT (n=8; 47%) or PChT (n=4; 50%) as well. Ten patients with stages IEA, IIEA, and IIEB relapsed in the same body half, another 10 transdiaphramatically or generalized. 82% of the extranodal lymphomas were initially treated with PChT, 75% of them with CHOP.
By the sequential combination of PChT and RT the uncertainty of incomplete staging, which often has to be accepted in the elderly, and the risk of undertreatment become less important. The remission maintaining effect of combined modality treatment in contrast to RT alone may also explain the good long-term results in these patients with high median age.

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COMBINATION CHEMOTHERAPY (CABOPP/VIM) FOR CLINICAL STAGE I AND II OF HIGH-GRADE MALIGNANT NON-HODGKIN'S LYMPHOMAS

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Management of early stages of aggressive Non-Hodgkin's Lymphomas (NHL) is still controversial. Initial therapy varies considerably and may include radiotherapy or chemotherapy alone or combined modality programs. This report deals with the results of a chemotherapy program which we used for the initial treatment of clinically localized NHL of unfavourable histology (Kiel classification). Therapy was started with a combination of Cyclophosphamide (500 mg/m^2, day 1), Adriamycin (50 mg/m^2, day 1), Bleomycin (1 mg q 8 h x 15), Vincristin (1 mg/m^2/day, days 1,8), Prednison (50 mg/m^2/day, days 1-10) and Procarbacine (100 mg/m^2/day, days 1-10) (CABOPP). Treatment was repeated every 3 weeks. In patients (pts) with complete remission (CR) after a maximum of 4 cycles of CABOPP, this regimen was continued for a total of 6 cycles. In pts with progressive disease or with only a partial remission after 4 cycles of CABOPP, chemotherapy was changed to a combination of VP-16 (90 mg/m^2/day, days 1,3,5), Ifosfamide (1200 mg/m^2/day + Mesna, days 1-5) and Methotrexate (30 mg/m^2/day, days 1,5) (VIM). 15 pts (6 with stage I and 9 with stage II of the disease) entered the study. All 15 pts achieved CR, 12 pts (80%) with CABOPP alone and 3 pts (20%) after additional chemotherapy with VIM combination. In 6 pts with bulky disease, additional radiotherapy was given to the involved area following chemotherapy. With a median follow up of 19 months (range 4-36), all 15 pts are still alive without any evidence of the disease. On the basis of these results, CABOPP/VIM program appears to be an effective therapeutic tool for the treatment of early stages of aggressive NHL. With regard to the less favourable results of radiotherapy alone in patients with clinical stage II disease, initial chemotherapy with CABOPP/VIM seems to be a valuable alternative.
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TREATMENT OF ADVANCED BURKITT-TYPE LYMPHOMA WITH VAC CHEMOTHERAPY, TOTAL BODY IRRADIATION AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT)

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Six patients with advanced Burkitt-type lymphoma were treated for induction of remission with vincristine (VCR), adriamycin (ADR) and cyclophosphamide. Autologous bone marrow was harvested during clinical and hematological remission and purged with a pan-B monoclonal antibody (Y29/55). Leukemia-lymphoma ablative therapy consisted of 4 x 1400 mg/m^2 cyclophosphamide, VCR and ADR, followed by a 6-10 gy TBI. The cryopreserved marrow containing 1-6 x 10^6 CFU-C/kg body weight was retransplanted for rescuing.

Two patients with stage III disease were transplanted in first remission, a third patient presenting with stage IV had achieved only partial remission. Three other patients who had suffered multiple relapses occurring after treatment according to the BFM B-NHL protocols were treated with ABMT as well. One of these patients died from severe veno-occlusive disease 2 weeks after autografting; a second patient, transplanted while still having abdominal masses, is in CCR 7 weeks after cessation of therapy. All other patients are in CCR for 27 to 53 months.

ABMT offers a very effective therapeutic approach for the treatment of patients with advanced Burkitt-type lymphoma.

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CYTOXAN (CTX) + TOTAL BODY IRRADIATION (TBI) WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN ADULT LYMPHOBLASTIC LYMPHOMA (LblL) IN FIRST COMPLETE REMISSION (CR). A REPORT OF THE NON-HD-LYMPHOMA COOPERATIVE STUDY GROUP, ITALY.

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Prognosis of LblL was recently improved by sequential chemotherapy (CT).The aim of this study is to improve long term survival of LblL patients (pts.)in CR by intensification with high-dose CT and TBI followed by ABMT. A modified LSA_2-L_2 was used as induction regimen (VCR 1.4 mg/mq d.1,8,15,22,29; ADM 30 mg/mq d.8, 15,22; CTX 750 mg/mq d.15,22,29; L-Ase 10.000 U/mq d.8-14; PDN 40 mg/mq d.1-29; intrathecal MTX 12 mg. d.3,10,17,24; DNR 50 mg/mq d.43,46,50; ARA-C 200 mg/mq continuous infusion d.43-49; MTX 400 mg/mq d.64 with Leucovorin rescue). If CR was attained, 1000-1400 cc of BM were criopreserved at recovery. Six pts. whose BM at diagnosis were infiltrated were purged with ASTA-Z (70-100 µg/ml). At the median time of 2 months from CR pts. underwent CTX (60 mg/kg) d.1,2 followed by TBI (10 Gy single dose) d.4 and BM reinfusion d.5. Up to April '87, 22 pts. entered the protocol and 19 are presently evaluable: 11 males and 8 females, median age 19 ys. (range 15-49), in stage II and III one pt. each, in stage IV seventeen pts.. 15 pts.(79%) showed mediastinal and 13 (68%) BM involvement. 17 out 19 pts. achieved CR (89%), 1 PR while 1 NR died. Of 17 CR, 4 refused ABMT, 2 are scheduled to and 11 underwent ABMT. Presently 9 out 11 post-ABMT pts. are in CR (82%), off therapy, 5 to 23 months (med.15 mo.). All pts. tolerated well the procedure and the haemopoietic recovery was prompt.

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IN-VIVO-IMAGING OF HODGKIN'S LYMPHOMA (HD) WITH RADIOLABELED MONOCLONAL ANTIBODIES (Moab) AGAINST HODGKIN AND REED-STERNBERG (H&RS) CELLS.

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Moab H-RS-1 developed in our laboratory against the HD derived cell line L428 detects a glycoprotein of 120 kd restricted to H&RS cells and a rare subpopulation of normal cells. After protein A purification from crude ascites, the Moab was labeled with ^{131}I and injected into 4 patients (pts.) with HD. Each pt. received 0.5 mg H-RS-1 with 48 to 67 MBq ^{131}I. Scintigrams were performed daily up to 6 days and best results were obtained after 2-4 days. Cervical and mediastinal lymph nodes were detected when larger than 2 cm. In one pt. hyperuptake was observed in a clinically normal spleen with histologically proven involvement by HD. The specificity of the uptake was tested by injection of a control anti-alpha-fetoprotein Moab labeled with ^{123}I (111 MBq). A moderate but significant specificity index of 1.3 was observed which correlated with differences in imaging patterns.

These preliminary results demonstrate that in-vivo-imaging in patients with HD is feasible. The recruitment of patients continues and results of modifications of the imaging technique in order to increase sensitivity and specificity will be reported.

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GENOTYPES AND IMMUNOPHENOTYPES OF HODGKIN'S DISEASE DERIVED CELL LINES

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The analysis of Hodgkin (H) and Reed-Sternberg (RS) cells is hampered by the scarcity of the neoplastic cells and contamination with by-stander cells. Cell lines established from patients with Hodgkin's disease (HD) are monoclonal cell populations and due to unlimited cell growth provide abundant material. We examined the immunophenotypic and genotypic features of the HD derived cell lines HDLM-2, KM-H2 and L-428. Cell lines were cultured under standard conditions. Expression of surface markers was examined by immunofluorescence staining in suspension; cells were analyzed under microscope and by flow cytometry. DNA extraction, Southern blot analysis and hybridization with T-cell receptor (TCR) and immunoglobulin heavy chain (J_H) probes was done according to established procedures. The cells share the following markers: CD15+ (Leu-M1), CD30+ (Ki-1), HeFi-1+ (raised against L-428), HLA class I and II antigens, proliferation markers. Whereas L-428 is devoid of any further markers, HDLM-2 is CD2+ (E-receptor) and KM-H2 is CD9+ and CD21+ (both B-cell associated). The gene status of the cell lines is as follows: HDLM TCR β/γ rearranged, J_H germline; KM-H2 TCR β/γ germline, J_H rearranged; L-428 TCR β germline, TCR γ rearranged, J_H rearranged. The combined information on geno- and immunophenotypes suggests that the cell lines are of lymphoid origin (HDLM-2: T-cell lineage; KM-H2: B; L-428: B). Extrapolation of the results to in-vivo H-RS cells indicates that H-RS cells are either of T- or B-cell origin.

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TREATMENT OF HODGKIN'S LMPHOMA RESISTANT TO COPP/ABVD WITH CCNU, ETOPOSID, VINDESIN AND DEXAMEHTASONE (CEVD).

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32 patients with advanced Hodgkin's lymphoma resistant to COPP and ABVD were treated with a salvage chemotherapy regimen consisting of CCNU, etoposid, vindesine, and dexamethasone (CEVD). 27 patients were treated because of primary resistance to COPP/ABVD and 5 patients were treated in early relapse (3-7 months) after COPP/ABVD induced complete remission. 14/32 patients (44%) achieved complete remission, and 4 patients partial remission, with an overall response rate of 56%. Two partial responders achieved complete remission by additional radiotherapy. 4/5 patients in early relapse after COPP/ABVD achieved complete remission. Consolidation radiotherapy was given only in one complete responder. Median duration of complete remission is longer than 10 months and median survival is longer than 26 months. The treatment was very well tolerated. Main side effects were leukopenia, thrombocytopenia, mild nausea/vomiting and cushingoid side effects. CEVD is a very active and well tolerated salvage chemotherapy regimen in patients with Hodgkin's disease resistant to or relapsing after COPP and ABVD.

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ABVC-SALVAGE CHEMOTHERAPY FOR RELAPSING OR RESISTANT HODGKIN'S DISEASE

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From 1979 to 1986 we treated 35 patients (pts.) with primary or secondary resistant Hodgkin's disease (HD), female 7 pts., male 28 pts., age 16 - 57 years, median 31 years.Classification according to histological subtypes are: lymphocyte predominant (LP)=2, nodular sclerosis (NS)=8, mixed cellularity (MC)=21, lymphocyte depleted (LD)=4. Treatment consisted of Adriamycin 60 mg/m^2, day 1; Bleomycin 15 U, day 1 + 15; Vinblastine 6 mg/m^2, day 1, CCNU 100 mg/m^2(max. single dose 160 mg),day 1. Each treatment cycle was repeated on day 28 or after hematological recovery. The following results were achieved: complete remissions (CR) 17/35 pts. (48,6%), partial remissions (PR) 9/35 pts. (25,7%), no change (NC) 5/35 pts. (14,3%),progressive disease (PD) 4/35 pts. (11,4%). Response rates (CR + PR) by histology are: LP= 2/2; NS= 7/8; MC= 16/21; LD= 1/4. Pretreatment characteristics of responders(26 pts.) were: COPP= 14 pts., combined modality= 5 pts., primary radiotherapy= 5 pts., COPP (12 pts.) and BVC (2pts) at time of relapse, and of non-responders including NC: COPP= 7 pts., BVC= 1 pt, and combined modality= 1 pt.. The overall response rate (CR+PR) was 74,3% of pts Duration of response was 3+ to 74 months (median 21 months). After an observation period of more than 5 years, five pts. are in continuing CR. Toxicity consisted mainly of nausea and vomiting in all pts. Hematological toxicity in the form of leucocytopenia and thrombocytopenia was moderate in general, however, considerable for heavily pretreated pts. Therefore, a prolongation of therapy free intervalls became necessary in 39/174 treatment cycles. No treatment related deaths or episodes of bleeding occurred.
In conclusion, ABVC therapy in relapsing or primary resistant HD is effective and results in a considerable number of complete and long term remissions.

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TRANSIENT DNA DEMETHYLATION DURING DIFFERENTIATION OF K562 CELLS

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Since DNA methylation has been implicated in the process of specific gene selection, we investigated changes in the overall methylation of K562 leukemic cells after induction of differentiation with low doses of 1-β-D-arabinofuranosylcytosine (ara-C). K562 cells were chosen because of their capability to differentiate in presence of a variety of chemical agents. The nucleoside analog ara-C used in the present studies has been shown to exert differentiation capacity on leukemic cells.

For the present experiments K562 cells in log phase of growth were cultivated for various time periods in presence of 100 ng/ml ara-C. Differentiation, first observed after 2 days, was assessed by morphological and immunological markers. At various time points, DNA was isolated and the overall methylation was estimamated by nearest neighbor analysis in presence of labeled α-[^{32}P]dGTP. Our results clearly demonstrate that as early as 12 h after application of ara-C, the DNA of K562 cells shows a dramatic decrease in the overall methylation. Uninduced K562 cells have an overall methylation of 30% which decreases 12 h after differentiation induction to 12% and is reestablished to the original level after 24 h. These results imply that during cell differentiation, methyl cytosines in DNA are initially partially erased and, thereafter, reestablished, presumably in a new pattern.

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GM-CSF INDUCTION OF "MONOKINE"-GENE EXPRESSION AND PROTEINSYNTHESIS BY NEUTROPHILS.

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Neutrophils share a number of common features with monocytes including their common cellular origin, their phagocytic nature and several similar receptors and surface antigens. Therefore, our interest has been focussed on whether neutrophils also share with monocytes the capacity of producing soluble factors (monokines) such as interleukin 1 (IL 1), tumor necrosis factor-alpha (TNF-alpha) or granulocyte-colony stimulating factor (G-CSF). We show by Northern blot analysis that: 1) Neutrophils can be induced to synthesize transcripts specific for IL 1, TNF-alpha and G-CSF, moleculary indistinguishable from mRNA for these monokines obtained from activated monocytes. 2) Neutrophils can be induced by one signal only to secrete IL 1 (but not TNF-alpha and G-CSF) in their culture supernatant biologically and antigenically indistinguishalbe from monocyte-IL 1. 3) T cell lymphokine GM-CSF is a physiological inducer for neutrophil-IL 1.
Taken together, these findings describe a new role for GM-CSF by extending its modulating function on neutrophils to the synthesis of biological relevant proteins.

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PRODUCTION OF HEMATOPOIETIC GROWTH FACTORS BY HUMAN T AND B LYMPHOCYTES

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Lectin stimulated blood mononuclear cells comprising monocytes, B and T lymphocytes are known to secrete both granulocyte/macrophage colony stimulating factor (GMCSF) and interleukin 3 (IL 3). In order to determine the cellular origin of these factors more precisely, we examined blood mononuclear cell preparations depleted of monocytes, a series of T cell clones and B cell lines. RNA was extracted at different time points after lymphocytes were established in culture with or without phytohemagglutinin/phorbol myristate acetate (PHA/PMA). The identity of secreted CSFs was determined by a murine colony forming assay and by a human chronic myeloid leukemia (CML) blast proliferation assay carried out in the presence or absence of antibodies to IL 3 and GMCSF.

The results demonstrate that PHA/PMA-stimulated blood lymphocytes synthesize both IL 3 and GMCSF mRNA. Similar results were obtained with one T cell clone. Bioactivity results in the CML assay were also consistent with the production of IL 3 and GMCSF by these cells. B lymphocyte lines were negative for IL 3 and positive for GMCSF mRNA only after stimulation. In addition stimulated B lymphocyte conditioned medium stimulated the formation of murine monocyte colonies and therefore contained macrophage colony stimulating activity (MCSF). These results strongly suggest that human T lymphocytes are the sole hematopoietic cell source of IL 3 and are an additional source of GMCSF. B lymphocytes can also synthesize GMCSF mRNA and as well secrete MCSF.

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THE EFFECT OF RECOMBINANT INTERLEUKIN-2 (IL-2) ON HEMOPOIETIC PROGENITORS IN IN A LONG TERM BONE MARROW CULTURE (LTBMC).

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Recombinant hemopoietic growth factors might facilitate the engraftment in autologous bone marrow transplantation (ABMT). In a LTBMC (Dexter system) we assessed the effect of human interleukin-2 (IL-2). The main biological function of IL-2 is the activation of IL-2 sensitive T cells inducing helper-, suppressor- or cytotoxic functions. In addition natural killer cells can be stimulated through the action of IL-2 and certain subpopulations of the B cells are responsive to IL-2 as well.

3 experiments with normal bone marrow have been performed. Cultures containing rec. IL-2 (10 U/ml) were compared to controls. Half of the medium, containing the non-adherent cells, was replaced weekly. The non-adherent cells in the supernatant were counted and assessed in a semisolid culture assay (CFU-C, BFU-E, CFU-GEMM). The average of 3 experiments is shown in the following table:

		Day 0	7	14	28	42
Tot. No. of cells/ml	without IL-2	$2x10^6$	$1.73x10^6$	$4.46x10^6$	$0.58x10^5$	$1.0x10^5$
	with IL-2		$3.3x10^6$	$5.8x10^6$	1.9×10^5	$1.45x10^5$
Tot. No. of CFU-GM/ml	without IL-2	337^6	139	126.7	4.6	n.d.
	with IL-2		475	125.0	7.4	n.d.

Conclusions: During the culture period there is a 2- to 3.2-fold increase in the total number of non-adherent cells/ml in the cultures containing IL-2. For CFU-GM/ml there was only a significant difference between the cultures containing IL-2 and the controls at day 7 with a 3.7-fold increase. In summary, the use of IL-2 in patients undergoing ABMT might be a promising approach to increase the endogenous production of various hemopoietic growth factors responsible for the engraftment and reconstitution of the peripheral blood cells.

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CYTOKINE INDUCED PROLIFERATION OF NORMAL AND LEUKEMIC B CELL PRECURSORS

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The regulation of B cell lymphopoiesis is not fully understood. As part of a study on the proliferation and differentiation of immature B cells we have investigated the influence of various cytokines on normal and leukemic human B cell precursors.
Bone marrow blasts from patients with B cell precursor ALL (BCP-ALL, non T-ALL) were incubated with a wide range of concentrations of low molecular weight B cell growth (L-BCGF), interleukin 1 (IL-1), interleukin 2 (IL-2) and gamma interferon (gamma IF). L-BCGF induced an increase in ^{3}H-TdR incorporation in 75 % of BCP-ALL, whereas the 3 other cytokines had no proliferative effect. L-BCGF also induced increases in absolute cell numbers and colony formation in a methylcellulose assay. IL-2 had no proliferative effect, despite the presence of high affinity receptors and the alpha (p75) and beta chain (p55/Tac) of the IL-2 receptor (shown by cross - linking).
In order to study normal B cell precursors we isolated CALLA positive, sIgM negative cells from normal bone marrow, fetal bone marrow and fetal liver by fluorescence activated cell sorting (FACS). L-BCGF consistently induced a 4 - 8 fold increase in ^{3}H-TdR incorporation in 8 different experiments, whereas IL-1, IL-2 and gamma-IF had no effect.
Our results suggest a major role for L-BCGF in the proliferation of B cell precursors. They also show that the majority of BCP-ALL qualitatively respond to external proliferative stimuli similar to their normal, putative counterparts.

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INVESTIGATIONS ON THE MECHANISMS OF RESISTANCE TO TUMOR NECROSIS FACTOR.

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A subclone of the human cervical cancer cell line ME 180 was selected which showed 50% cell kill at a concentration of human recombinant TNF of 1.36×10^{-6} mg/ml, thus being equally sensitive to the cytotoxic effects of TNF as the mouse fibroblast L cells. ME 180 cells were made resistant to a concentration of 4.5×10^{-2} U/ml by gradually increasing the TNF concentration in the culture medium over 7 months and 40 passages. Whereas only slight gains in resistance to TNF were observed during the first 30 passages, there was a rapid increase in resistance thereafter. The resistant clone continues to be TNF resistant in absence of TNF after more than 8 months and 40 passages. No difference could be shown in the number and affinity of TNF receptors between the sensitive and resistant clone. SDS-PAGE of lysates of the resistant and sensitive clone showed identical bands. There was identical sensitivity to cytotxic drugs such as actinomycin D and mitomycin. However, sensitivity of the TNF sensitive clone to the cytotxic effects of gamma-interferon was significantly higher than that of the TNF resistant clone. Currently, we test the expression of mRNA for TNF, determine the influx and efflux of TNF and the growth pattern of resistant and sensitive clones in nude mice.

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RECOMBINANT TUMOR NECROSIS FACTOR INHIBITS IN VITRO GROWTH OF NORMAL HUMAN MEGAKARYOCYTIC PROGENITOR CELLS

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It has been shown that human tumor necrosis factor is cytotoxic or cytostatic to a variety of malignant human cells in vitro.Recently the inhibitory effect of recombinant human tumor necrosis factor (rhTNF-α) on the in vitro growth of normal multilineage,erythroid and granulocyte-macrophage progenitor cells has been demonstrated.However,little is known about its effect on normal human in vitro megakaryopoiesis.In this study the effect of rhTNF-α on the in vitro growth of normal human bone-marrow derived megakaryocytic progenitors (CFU-Mk) was tested in a clonal culture system containing 30 % human plasma,5 % PHA-leucocyte conditioned medium,0.9 % methylcellulose,$5x10^{-5}$M 2-mercaptoethanol and 1 U/ml rh-erythropoietin.Addition of rhTNF-α (1 U - 300 U/ml) to unseparated bone-marrow cells resulted in a dose dependent inhibition of CFU-Mk,at a concentration of 300 U/ml rhTNF-α there was complet inhibition of the colony growth of CFU-Mk in all experiments.The inhibitory effect of rhTNF-α could be selectivly blocked by preincubation with antiserum specific for TNF-α or addition of the antiserum in early incubation period.Removal of adherent cells and T-lymphocytes from the bone-marrow target cells caused no alteration of the suppressive effect of rhTNF-α on CFU-Mk.It is concluded that rhTNF-α markedly inhibits the growth of CFU-Mk and the inhibitory effect does not appear to be mediated by autologous monocytes and T-lymphocytes in the bone-marrow samples.

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HUMAN BLOOD MONOCYTES AND MONOCYTE DERIVED MACROPHAGES SYNTHESIZE AND SECRETE A HEPATOCYTE-STIMULATING-FACTOR DIFFERENT FROM INTERLEUKIN-1 AND FROM TNF WHICH INDUCED HEPATIC ACUTE-PHASE-PROTEIN SYNTHESIS

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In response to inflammatory events, hepatic synthesis of a number of proteins designated as acute-phase-proteins is strongly induced. Based on experminents in mice, where interleukin-1 was found to be a strong stimulator of SAA - and factor B-synthesis it was suggested that Il-1 is the inducer monokine for hepatic acute-phase-synthesis. However, several other acute phase proteins, mainly in the rat system, where not or only weakly stimulated by Il-1.
We found that cultivated human blood monocytes secrete a nondialyzable factor which strongly induced synthesis of the rat acute-phase-protein alpha-2-macroglobulin (a2M) in rat hepatocyte primary cultures. In contrast to Il-1 which is synthesized by monocytes, but not by monocyte-derived macrophages (after in-vitro maturation), HSF is synthesized by both monocytes and macrophages. HSF activity could be separated by Il-1 by gel chromatography and eluted with a molecular weight of about 25 kD. Although cultivated blood monocytes secreted low amounts of HSF without endotoxin stimulation, HSF secretion war markedly stimulated by incubation of monocytes with LPS.
Experiments with rec. TNF as well as with anti-TNF revealed no identity of HSF with TNF. Also interferon alpha, beta and gamma had no effect on the synthesis of a2M in rat hepatocytes excluding an identity of INF with HSF.
We conclude, that hepatic acute-phase-protein synthesis is induced by a hepatocyte-stimulation-factor HSF, which ist secreted from monocytes and monocyte-derived macrophages. Secretion of HSF ist stimulated in the presence of endotoxin. HSF is not identical with Il-1, TNF or IFN.

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EFFECT OF RECOMBINANT INTERFERON ALPHA 2C (rIFN-a2) ON IN VIVO FC-DEPENDENT PHAGOCYTIC ACTIVITY OF THE RETICULOENDOTHELIAL SYSTEM (RES)

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Based on our previous observations of an IFN-related significant decrease of platelet half-life in patients with excessive thrombocytosis, we have investigated the influence of rIFN-a2 on the in vivo Fc-dependent phagocytic activity of the RES. We did this by measuring the rate of clearance of autologuous ^{51}Cr-labeled red blood cells (rbc) sensitized with anti-D alloantibody before and 3 months after initiation of IFN treatment. 6 pts. with excessive thrombocytosis due to a myeloproliferative syndrom (MPS) were studied.
All patients responded favourably to IFN treatment; the median platelet count decreased from $1,112 \times 10^9/l$ to $493 \times 10^9/l$. Statistical comparison of each individual's rbc clearance curves revealed a distinct and significant decrease of RES function during IFN therapy ($p < 0.0001$; log rank test). This somewhat surprising finding of impaired Fc-mediated phagocytosis during IFN treatment might represent a dose-related phenomenon; alternatively, since IFN is known to intensify the expression of Fc-receptors on platelet and leukocyte surfaces, it may possibly enhance unspecific binding of IgG to platelets and increase the number of IgG binding sites on phagocytes of the RES. Both mechanisms might lead to increased platelet uptake and subsequent overloading of the RES causing impaired Fc-receptor-mediated clearance of additional immune particles, such as IgG coated rbc. In conclusion, the shortened platelet half-life in pts. with MPS under IFN treatment is unlikely to be caused by increased phagocytic activity of the RES: it rather seems to stem from other mechanisms such as increased intravascular platelet consumption.

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ACCESSORY CELL MEDIATED GROWTH INHIBITION OF GRANULOCYTE-MACROPHAGE PROGENITORS (CFU-GM) BY HUIFN-α IN VITRO

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The effects of two preparations of HuIFN-α, partially purified IFN-α Ly and recombinant IFN-α 2a, on granulocyte-macrophage progenitor cells (CFU-GM) were tested. As target cells we used low density bone marrow cells depleted simultaneously of monocytes and T lymphocytes with specific monoclonal antibodies and rosetting with anti immunoglobulin antibody-coated erythrocytes. We observed no inhibition of CFU-GM colony growth by both preparations of HuIFN- (concentration range 5 - 500 U/ml). However, selective depletion of target bone marrow cells from either monocytes or T lymphocytes resulted in a dose dependent growth inhibition of CFU-GM by HuIFN-α. Further separation of T lymphocyte containing target cells in T4 or T8 positive subset preparations revealed a differential inhibitory effect on CFU-GM colony formation. We noticed a 86 ± 4 % (± S.D.) survival of CFU-GM for T4 and a 54 ± 2 % (± S.D.) survival of CFU-GM for T8 containing fractions after enclosure of 50 U/ml rHuIFN-α 2a in culture. Target cell preparations containing monocytes showed a 65 ± 10 % (± S.D.) survival of CFU-GM (rHuIFN-α 2a, 50 U/ml). Inclusion of indomethacin (10^{-6} M) did not change the inhibition pattern making it unlikely that CFU-GM growth inhibition is mediated by Prostaglandin E. We, therefore, conclude that (a) accessory cells are necessary for HuIFN-α mediated CFU-GM growth inhibition, (b) monocytes or T lymphocytes are responsible as accessory cells, (c) T8 subsets are the main subsets responsible for T lymphocyte-mediated inhibition of CFU-GM by HuIFN-α.

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THE FUNCTIONAL HETEROGENEITY OF INTERFERON-ALPHA RECEPTORS ON HUMAN B-CELLS

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We questioned whether a detailed analysis of binding data obtained with radioiodinated IFN-alpha for several malignant B-cells might reveal further clues to understand IFN-sensitivity. Both IFN-sensitive (HCL, JOK-1, Daudi) and IFN-resistant (CLL, NHL, Namalwa) cells displayed high and low affinity binding sites for IFN-alpha with apparent Kp's ranging from 1.2×10^{-10} to 5×10^{-9} M. The number of binding sites (400 - 12000) varied considerably but, the ratio of high : low affinity receptors was in the equal order of 0.33 on all cells. In all instances we obtained a Hill co - efficient of less than 1, which is indicative of site-site interactions. In addition, we analyzed the binding data by an "average affinity profile", which describes the affinity of the receptor as a function of the percentage of receptors occupied by IFN-alpha. This graphical analysis depicted that both high and low affinity receptors on IFN-resistant cells fit into a model of negative cooperativity. However, only the high affinity receptors on IFN-sensitive cells were found to be in a positively cooperative state. Alternatively, a certain high affinity receptor state on IFN-sensitive cells supports the consecutive binding of additional IFN molecules within a narrow molar range (positive cooperativity), which is contrasted by a sharp decline in receptor affinity on IFN-resistant cells (negative cooperativity). We propose a model which describes the IFN-alpha receptor as a functional complex of multiple interacting binding sites, and oppose the current view of the existence of two classes (high and low affinity) of receptors. Since only the high affinity receptors are alleged to trigger an IFN-mediated signal, our results implicate that only those cells whose receptors are capable to transform to a particular high affinity state are likely to respond similarily to IFN-alpha. Our model provides a working hypothesis to explain and to delineate IFN-sensitivity on the receptor level.

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ACTIVATION OF HUMAN TEFLON-CULTURED MACROPHAGES FOR TUMORCYTOTOXICITY IS DEPENDENT ON THE STAGE OF MATURATION

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Macrophages (MO) are derived from circulating blood monocytes (mo). Most studies on human mo/MO biology and function have been done using mo. These are still immature precursor cells and conclusions drawn may be questionable as mo have to undergo terminal differentiation before they reach relevant tissue sites of inflammation. We have analyzed the ability of mo-derived, teflon-cultured MO to respond to activation to increased tumorcytotoxic function using recombinant human interferon(rHuIFN)-gamma, rHuIFN-alpha2, rHUGM-CSF, rHuIL-2, rHuIl-1alpha, anti-DC16 antibodies and lipopolysaccharides (LPS) as mediator molecules. It could be shown that the response of MO to the most potent activator molecule IFN-g greatly depends on the terminal differentiation from the mo stage to the mature MO. Whereas mo could be activated only moderately, MO increased their cytotoxicity by a factor of up to 400. IGN-g activation positively correlated with the effector cell numer, the time of incubation and the dosage used, and was lost within 48 hours. LPS was active only in the microgram range. IFN-alpha2 activated MO as well but a two log higher concentration than IFN-g was required. GM-CSF was only slightly effective whereas incubation with G-CSF, IL-1 and IL-2 or triggering of the maturation specific, low affinity receptor for IgG (CD16) did not result in MO activation. In conclusion, responsivenes to activating lymphokines appears to be a function of cellular maturation acquired during terminal MO differentiation. Teflon-cultured mo-derived MO should be ideal effector cells to facilitate the study of activation of human MO. Moreover, such cells rather than blood mo may be suitable effector cells for adoptive immunotherapy in cancer patients.

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BIOLOGICAL RESPONSE MONITORING DURING IN VIVO TREATMENT WITH CYTOKINES: THE USE OF BETA-2 MICROGLOBULIN AND NEOPTERIN MARKERS

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Elimination of malignant cells is believed to involve various antigen-specific and unspecific immune-defense mechanisms. Of these, the function of cytotoxic T Lymphocytes and of activated macrophages has been attracting particular attention. We tested simple biochemical parameters such as beta-2 microglobulin and neopterin, thought to relate to both of these functions for their capacity to monitor patients treated with escalating doses of such cytokines. Both markers were assessed daily in serum and urine samples of eight patients, suffering from advanced renal cell carcinomas, and treated with various dose levels of rIFN-gamma. Dose levels selected were 10, 100 and 500 µg i.m. once weekly. Also investigated were patients treated in an ongoing study with rIFN-alpha, who received 1, 10 or 100 µg dose levels according to the same schedule. Results obtained in the IFN trial indicate that significant and dose related responses can be seen with both markers. As little as 10 µg already induced detectable responses in individual patients. Optimal responses during repeated once-weekly applications were seen with 100 µg in the majority of patients, while a down regulation was frequently seen at the highest dose level.

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CLINICAL SIGNIFICANCE OF INTERFERON ANTIBODIES

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In a clinical trial 25 patients with chronic myelogenic leukemia were treated with 5 Mio. I. E. IFN-2b administered subcutaneously three times weekly. Of 23 evaluable patients 9 developed a haematologic and 5 a partial haematologic response. Before and during therapy these patients were monitored for anti-IFN-α antibodies. Three different assays were employed: 1. an ELISA utilizing peroxidase labelled IFN-α 2b, 2. an immunoradiometric assay (IRMA) and 3. a neutralization bioassay employing Wish cells and VSV. During the course of the treatment five patients developed measurable IFN-α -antibody titers. Rapid changes of the anti-IFN-titer were measurable in these patients during IFN treatment. Therefore, sera from patients were collected under strict conditions in order to minimize the changes of the IFN antibody titer induced by the injected IFN-α. After emergence of the specific antibodies the five pts did not responded to or relapsed under IFN therapy. The leukocyte and the anti-IFN-negative patients differed significantly. These data demonstrate that anti-IFN-α antibodies can abrogate therapeutic effects of IFN.

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THE ROLE OF GROWTH FACTORS IN HUMAN TESTICULAR CANCER - IN VITRO-INVESTIGATIONS

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The effect of the proliferation rate of 3 established human undifferentiated embryonal carcinoma cell lines (H12.7, 1428A, 2102EP) by the following substances was investigated: Epidermal growth factor (EGF), insulin like growth factor I (IGF I), multiplication stimulating activity (MSA; IGF II), platelet extract (PDGF), estradiole (E2), insuline, transferrin, LDL & HDL lipoproteins and dexamethasone. The growth characteristics of the cell lines growing in a serum containing and serumfree medium were determined by ^{3}H thymidin incorporation assay. Results: 1) Reducing the supplemented serum concentration in the medium resulted in a reduction of the growth proliferation. 2) Insulin, transferrin and LDL & HDL lipoprotein were essential for proliferation in serumfree medium. 3) The ^{3}H thymidin incorporation of the cells growing in serumfree conditions was compared to the cells growing in serumfree medium supplemented with the different growth factors. It could be shown that dexamethasone has only a marginal inhibiting effect in high concentration (10 µm) in line 1428A and 2102EP; EGF and IGFII had a significant growth stimulating effect but only for line H 12.7 which has a high potential for differentiation whereas the completely undifferentiated cell lines 1428A and 2102EP were not responding to these growth factors. Anti-EGF monoclonal antibody reduced the proliferation rate in line H12.7, not in the other lines. Estradiole or PDGF had no significant growth stimulating or inhibiting effect. Conclusion: EGF and IGFII are growth factors only for the differentiating line H 12.7 and not for the other cell lines although they have EGF-receptors, indicating a dysregulation or possibly a maximal autocrine stimulating pathway by oncogene activation. The role of growth factors in human testicular carcinoma has further to be defined in different stages of differentiation.

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LYMPHOCYTE SUBSETS AND LAK-CELL INDUCTION IN PATIENTS WITH GASTRIC CANCER BY IN VITRO AND IN VIVO APPLICATION OF RECOMBINANT HUMAN INTERLEUKIN-2(rh-IL-2)

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Adoptive immunotherapy (AI) is a promising approach in the treatment of solid tumors. So far, there is no data on the effect of AI in gastric cancer. Prior to initiating a clinical study we assessed the spontaneous and in vitro IL-2 induced NK and LAK-activity in 15 patients with advanced cancer of the stomach. Spontaneous NK-activity of patients with gastric cancer was comparable to that of healthy individuals. Patients with very advanced stages seem to have a reduced NK-activity. In vitro IL-2 induced LAK-activity in patients with gastric carcinoma was similar to that of the controls. The maximum of LAK-activity was reached between day 3 to 6 of incubation. The optimal concentration of IL-2 (Bioferon, Laupheim, FRG) was 500 U/ml. Autologous serum did not inhibit the induction of LAK-activity.

In 2 patients continuous intravenous infusion (CIVI) of rh-IL-2 produced a marked lymphocytopenia followed by leucocytosis and lymphocytosis (up to 30 000/µl) with the maximum at 24 to 48 hours after discontinuation of IL-2 infusion. Surface marker studies by FACStar revealed a DR-expression on almost all T-lymphocytes. 30-40 % of all lymphocytes were CD3+DR+. There was evidence of an increase of CD3+CD8+Leu7+ - cells. The functional properties of these lymphocyte subsets are currently under investigation. The peripheral lymphocytes of the treated patients exerted a significant ex vivo LAK-activity.

In conclusion, the presented experimental data and the preliminary in vivo findings suggest that adoptive immunotherapy may be effective in patients with gastric carcinoma.

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ACTIVATION OF HUMAN GRANULOCYTES AND B LYMPHOCYTES BY THE ANTI CD 24 MONOCLONAL ANTIBODY VIBE3

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Recently several activation signal transducing membrane proteins have been defined on human T and B lymphocytes. We present here a structure, expressed both on human granulocytes and on human B cells which has in both cell types a signal transducing capacity. We detected this structure when searching for monoclonal antibodies with the capacity to activate human granulocytes. As a parameter of granulocyte activation we assessed the triggering of the respiratory burst, an essential component of the antibacterial function of granulocytes, by measuring the fluorescence of dichlorodifluorescindiacetate (DCF) with a flow cytometer. In this way the intracellular production of hydrogen peroxide is detected. Interestingly we found the monoclonal antibody VIBE3, established in our laboratory and specific for the CD24 antigen, which leads to an activation of the respiratory burst upon crosslinkage with a goat anti mouse $F(ab)_2$ immunoglobulin preparation.
Since this antigen is also expressed on B lymphocytes we were further interested if B cells can also be activated via this surface antigen. As an early parameter of B lymphocyte activation we assessed intracellular calcium concentrations by measuring the fluorescence of the calcium sensitive probe fura 2 in a spectrofluorometer. It was found that B lymphocytes, i.e. malignant cells from a CLL patient and tonsil B lymphocytes show an increase in free cytoplasmic calcium levels upon crosslinking of the CD24 antigen. Crosslinkage of CD24 with VIBE3 antibody also led to an impressive increase in DNA synthesis of PMA stimulated B cells. Thus we provide evidence that the CD24 antigen is a signal transducing structure both in human granulocytes and B lymphocytes.

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COOPERATIVE EFFECTS BETWEEN BONE MARROW (BM) STROMAL CELLS AND T CELLS: UPREGULATION OF HLA-CLASS II ANTIGENS ON MONOCYTOID STROMAL CELLS BY NATURAL INTERLEUKIN 3 (IL 3) IN LONG TERM BONE MARROW CULTURES

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Stromal cells from BM constitute a major source of hematopoetic growth factors in vivo. Moreover, they may as well serve as target cells for factors secreted by activated T cells. Monocytoid stromal cell layers established from human long term BM cultures were found to homogeneously express HLA-DR, -DQ, and -DP antigens if cultured in the presence of T cell conditioned medium (TCM) containing IL 2 and IL 3. In contrast, cultures were only weakly positive for HLA-DR and -DP, and negative for HLA-DQ if cultured in the absence of TCM. Simultaneously, TCM conditioned the continuous proliferation of myeloid progenitors in proximity to the stromal layer, whereas cultures in its absence died off within the first 2-3 weeks. Upregulation of HLA-class II on monocytoid cells (stroma cells) was distinct from that mediated by IFN-y since neither IFN-y was added to the cultures nor was it secreted during the first 50 days of culture. Moreover, strong HLA-class II expression occurred simultaneously with the proliferation of myeloid progenitors from stem cell precursors in these cultures. Within the context of bone marrow transplantation (BMT), the activation of HLA-class II restricted inducer T cells in early phases after allogeneic BMT coincides with significant increases of peripheral leukocyte counts in most instances and thus indicates hematopoetic reconstitution. These inducer T cells are thought to play an important role by "feeding" the hematopoetic system with IL 3 and thus further upregulate regeneration of a stem cell pool as a prerequisite for hematopoetic recovery.

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A NOVEL CELL SURFACE GLYCOPROTEIN IS ACTIVATION REGULATED IN HUMAN T CELLS

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Monoclonal antibody (mAb) H25 has been shown to react with human large granular lymphocytes/natural killer (NK) cells (Bai et al., Eur. J. Immunol., 13:521, 1983) and human bone marrow myeloid and erythroid precursor cells (Wisniewski et al., Blood, 69:419, 1987). We found expression of the H25 cell surface glycoprotein to be species specific and highly restricted to the hematopoietic system. T lymphocytes (CD3+) freshly isolated from peripheral blood did not react with mAb H25. However, expression of the antigen could be induced in both fresh and continuously cultured T cells upon activation via different pathways.

Fresh purified CD3+ T lymphocytes expressed the H25 antigen, following stimulation of the T3/Ti and the T11 complex, respectively. Induction of the H25 antigen was preceeded by the expression of transferrin receptor, Tac, and HLA-DR surface antigens, regardless of the activation pathway. When CD3+ cells were stimulated via the T3/Ti complex in the presence of interleukin-2, a proportion of these cells acquired natural killer activity and phenotype. This subpopulation was specifically identified by the H25 mAb.

Thus, we could demonstrate that mAb H25 recognizes a cell surface glycoprotein which is found on biphenotypic (CD3+, N901/NKH1+) lymphocytes displaying mature T cell and NK cell properties; this cell population appears to be clinically relevant in that it may contribute to graft vs. host disease and/or graft vs. leukemia effect in bone marrow transplant patients.

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SURFACE CHARACTERISTICS OF HUMAN PERIPHERAL BLOOD BASOPHILS

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A combined toluidine/immunofluorescence staining procedure was used to investigate the surface determinants of human basophils with a panel of well established monoclonal antibodies (mAbs, n=60). The close relationship of basophils to other mature myeloid cells was indicated by the reactivity of a number of myeloid associated antibodies (mAbs against the LFA-1 family (CD11a, CD18), mAbs against lactosylceramide (CD_w17), anti gp 150 mAbs MCS2 and MY7 (CD13), anti gp 67 mAb MY9 (CD33) anti Fcγ-receptor mAb CIKM5 (CD_w32), anti CR1 mAb E11 (CD35), and the anti glycolipid mAb VIM-2). Basophil surprisingly express at least three activation linked structures not detectable on mature neutrophils, i.e. the p45 structure (CD38), the p24 structure (CD9) detected by CD9 mAb BA-2, and the receptor for interleukin 2. CD16 mAbs directed against the 50-70kd Fcγ-receptor of neutrophils did not stain basophils at all. This was also the case with CD12 mAb 63D3, recognizing the monocyte/granulocyte associated p200 antigen and with CD14 mAbs VIM-13 and Mo2, detecting the monocyte specific structure p55. In FACS analyses purified basophils obtained from CGL-patients revealed identical results. In summary, basophils display a unique immunological phenotype and possibly represent an activated cell population.

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SURFACE PHENOTYPE ANALYSIS OF HUMAN MONOCYTE-DERIVED MACROPHAGES: EXPRESSION OF OSTEOCLAST-ASSOCIATED ANTIGENS AND OTHERS

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Cultivation of blood monocytes (mo) on teflon foils is a suitable in vitro model to study various aspects of the terminal maturation mo undergo in vivo upon migration from the capillary bed into body cavities and tissues. Blood mo differentiation produces competent effector macrophages (MO) in specific and non-specific immunity and brings about the great diversity within in the cell family in terms of morphology, biochemistry and function like Kupffer cells, microglia cells, osteoclasts, etc. The mo origin of the latter has been questioned when monoclonal antibodies (mAB) developed against osteoclast antigens could be shown not to react with blood mo and tissue MO (1). Using immunostaining of single cells as well as a sensitive cell-ELISA we have screened more than 185 mAb on their selective reactivity with mature MO but not blood mo. Besides the known expression of MAX antigens, the transferrin receptor and BA-2, some myleoid antigens (SC9, HE10, 14B6, 12B1), the low affinity receptor for IgG (CD16), and 2 osteoclast antigens (13C2, 23C6) could be detected on the surface of mo-derived MO. The antigen 13C2 appeared on more than 90 % of the MO on culture day 7 at high density. The reactivity of the 23C6 mAB developed at a later culture stage, was weaker than with the 13C2 and never exceeded 25 % of the mature MO. The results allow the detailed definition of a characteristic surface phenotype of end-stage matured MO. The $13C2^{+}$ $23C6^{+}$ phenotype of mo-derived MO may reinforce the hypothesis of the mo origin of osteoclasts. Teflon MO should provide tools with which to study the regulated expression and potential function of these osteoclast associated antigens.

(1) Horton et al., Cancer Res. 45: 5663, 1985.

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SPECIFIC AND SENSITIVE DETECTION OF TUMOR CELLS IN SEROUS EFFUSIONS WITH THE USE OF MONOCLONAL ANTIBODIES

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AIM: To improve the detection of tumor cells in serous effusions using monoclonal antibodies (MAb) to CEA and EMA.
METHOD: MAbs CEA-84 and E 29, cytoposin preparations, immunocytochemical staining (indirect alkaline phosphatase).
PATIENTS: 39 benign, 103 malignant (78 carcinomas (Ca), 7 mesotheliomas, 18 other tumors); 39 ascitic, 104 pleural effusions.
CONVENTIONAL CYTOLOGIC DIAGNOSES: In benign diseases: all "negative for malignancy". In malignant diseases: 36 "positive for malignancy": 5 "suspicious", 62 "negative".
IMMUNOCYTOCHEMISTRY: In benign cases, CEA-84 stained no cells at all, anti-EMA gave a very weak granular staning of mesothelia in 2 cases. EMA-staining was positive in 47 Ca cases, 2 mesothelioma, and 1 teratoma, CEA-84 reacted with 28 Ca and 1 teratoma. CEA staining occurred only in gastrointestinal breast and lung Ca. EMA-positive cases were found in all types of Ca.
CONCLUSIONS: EMA staining considerably improves the detection of Ca cells in serous effusions. Additional staining for CEA ist able to provide information about the site of the primary tumor.

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Abstract withdrawn

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IMMUNOTOXINS DIRECTED AGAINST HUMAN OVARIAN CARCINOMA CELL LINES

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Several immunotoxins (ITs) active against human ovarian carcinoma cell lines have been developed (Cancer Res 45, 751, 1985; J Clin Invest 76, 1261, 1985). The activity of the ITs was affected by the number of target antigens on the cell surface, the cellular uptake of the monoclonal antibodies and the toxin sensitivity of the target cells. Because ITs, particularly those made with ricin A chain, often have low activity, several drugs were evaluated for their ability to enhance the *in vitro* activity of ITs reactive with ovarian carcinoma cells. ITs made with ricin A chain (kindly provided by Cetus Corporation, Emeryville, CA, USA) and an IT composed of an anti-transferrin receptor monoclonal antibody and *Pseudomonas* exotoxin were used. The activities of these ITs could be enhanced by verapamil, dansylcadaverine and trifluoperazine. Comparing ID50 values of inhibition of protein synthesis, the amount of enhancement ranged from 2- to > 25-fold, was dependent on the drug concentration and was greater at short incubation periods. Enhancement was also seen in a colony formation assay. The enhancing agents did not decrease the specificity windows of the ITs. ITs with and without modulating drugs could become a new class of anticancer agents.

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INDUCTION OF AN ANTI-ANTIIDIOTYP-ANTIBODY CROSSREACTING WITH COLON CARCINOMA DETERMINANT (CO17-1A) IN HUMANS: PHASE I-STUDY

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Treatment with mouse monoclonal antibody (AB) preparations to colorectal carcinoma (CRC) determinants turned out to be rather ineffective with concering inhibition of tumor growth. Therefore the induction of cytotoxic AB in humans by vaccination with an antimouse-idiotype AB seems to be an alternative approach to raise specific humoral as well as T-cellular imune ractions. In this study 42 patients (pts) with disseminated CRC have been vaccinated with polyclonal aluminumhydroxiprecipitated goat AB (AB2) specific for Co17-1A mouse monoclonal AB (AB1). Doses and schedule: from 0.5 with increasing doses to 16mg s.c. day 1,8,15,43 (cumulative dosages 2-64) with (15 pts) and without (27 pts) additional booster 4 weeks thereafter. 23 pts received concomittant chemotherapy 5-FU/high dose folinic acid. Development of serum AB to goat anti-ID (AB3) and to mouse monoclonal AB (AB1) as well as to goat determinant were observed by ELISA with a median follow up of 5 (1-18) mos. 26/42 pts developed significant titers of AB3 after a medium time of 18 (2-28) weeks. An additional effect on AB3 titers could be achieved by the application of serial booster injection in intervalls of 4 weeks. Fever and chills for 6 hrs were observed as side effects, erythema/oedema at the site of injection occurred only after the injection of more than 4mg single dose. This report demonstrates the possibility of inducing antigen-specific imune reations by internal image vaccination in humans for the first time. Antiidiotype vaccination in CRC merits evaluation in further clinical phase II trials.

[1]Abt. Hämatologie/Onkologie, [2] Abt. Immunologie, [3] Abt. Abdominalchirurgie, all Medizinische Hochschule Hannover, [4] Wistar Institut of Anatomy and Biology, Philadelphia, U.S.A.; Supported by Sandoz Forschungsinstitut

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CONJUGATES OF PLATINUM WITH MONOCLONAL ANTIBODIES

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The effectiveness of the drug cis-diamminedichloroplatinum (II) (cis-DDP) has been proved in various human tumor situations. To enrich cis-DDP in neoplastic tissue the conjugation to monoclonal antibodies (MAB) directed against tumor associated antigens seems reasonable. The binding of cis-DDP to MAB may be achieved by different methods: 1. cis-DDP may be complexed by a chelating substance e.g. diethylenetriaminepentaacetic acid (DTPA) bound to MAB via the cyclic anhydride procedure, 2. cis-DDP may bind to thiol groups introduced into MAB by modification with the heterobifunctional reagent N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP). Applying both procedures cis-DDP was conjugated to MAB directed against the T-cell antigen CD7 and against MHC class II antigen. The conjugates formed were shown to mediate antiproliferative capacity as determined in a cell proliferation assay using the cell-lines CEM,MOLT3,IM9 and JM. Cell-lines preincubated with specific MAB-cis-DDP conjugate showed inhibited cell proliferation as compared to the untargeted cell-lines. Cell-lines preincubated with a MAB-cis-DDP conjugate directed against an epitope not expressed on cell surface were not affected. The complex MAB-cis-DDP produced by the DTPA procedure proved to be relatively stable when stored for 8 weeks at 4° C. The results obtained indicate that MAB-cis-DDP conjugates mediate specific antiproliferative capacity.

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COMBINED ADJUVANT TREATMENT WITH CMP-CHEMOTHERAPY AND TAMOXIFEN IN POSTMENOPAUSAL PATIENTS WITH STAGE II BREAST CANCER: A CONTROLLED PROSPECTIVE RANDOMIZED STUDY

P. Aiginger, E. Kubista, H. Salzer, P. Sevelda, M. Langer, A. Staffen, J. Spona and C.C. Zielinski

Although adjuvant chemotherapy has been shown to be advantageous in certain patients with stage II breast cancer, no ideal treatment modality has been yet found for postmenopausal patients suffering from this disease. We have performed a controlled prospective study in 84 postmenopausal patients with stage II breast cancer, in which patients were randomized to receive either adjuvant chemotherapy (CMF, six courses) or tamoxifen (20 mg/day for two years) or both. After a median observation time of 48 months, 36.9 % of the patients had experienced a recurrence of the disease. 52.9 % of the patients with CMF therapy versus 36 % of patients with tamoxifen treatment compared with 16 % with combined chemo- and hormonetherapy had developed metastases (p 0.0089). We conclude from this part of the investigation that combined adjuvant CMF- and tamoxifen-treatment might be advantageous in postmenopausal patients with stage II breast cancer. We have found that patients with ER 30 fmol/mg had a significantly shorter relapsefree interval and a significantly higher incidence of metastases (36.4 %), as compared to patients with ER 30 fmol/mg (13.2 %; p 0.0011). The incidence of metastases was similar in patients with ER concentrations of 10 fmol/mg (33.3 %) and those with ER 10-30 fmol/mg (33.3 %), but significantly lower when ER concentrations were either 30-100 fmol/mg(10 %) or 100 fmol/mg (17.9 %; p 0.01, respectively). These results could illustrate that the clinically relevant cut-off range between ER-positivity and -negativity may lie at 30 fmol/mg in postmenopausal patients, and thus be higher than assumed previously.

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TREATMENT OF METASTATIC BREAST CANCER: FLEXIBLE CASE ADAPTED DECISIONS OR FIXED STRATEGY?

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A complex strategy which considers 8 subgroups of metastatic breast cancer (MBC) patients and recomends 8 different primary therapies for these subgroups and up to 8 consecutive lines of therapies has been used by the Regional Study Group of the Tumor Center Ulm. From Jan. 83 to Dec. 85 347 pts were prospectively recruited, 335 were evaluable. 38% of pts were stratified to a high risk group defined by at least 1 out of 7 criteria. A total of 804 consecutive lines of therapies were evaluated. Deviations from the proposed strategy were recorded as protocol violation and the survival of high and low risk pts without and with violations of the protocol was evaluated. The data demonstrates that median survival of all pts is 25 mos which corresponds to data of other groups. The survival of pts without protocol violations (n=165) is not different from those with protocol violations (n=170) (p=0.035). When subgroups of pts are considered, it can be shown that survival of low risk pts without protocol violations (n=117) is longer than of low risk pts with protocol violations (n=91) (p=0.0015). In high risk pts survival was longer in the group with (n=79) compared to the group without (n=48) protocol violations (p=0.0070). This data suggests: Both, a fixed strategy and flexible case adapted decisions may considerably influence the survival of pts with MBC. Second, in order to find out which sequence of therapies should be given to subgroups of MBC pts it is necessary to accept a fixed treatment strategy and to analyse the results.

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A THREE DAY SCHEDULE OF HIGH DOSE LEUCOVORIN(L) PLUS 5-FLUOROURACIL(F) IN UNTREATED PATIENTS WITH PROGRESSIVE AND ADVANCED COLORECTAL CANCER-PHASE I/II STUDY

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High doses of L enhances cytotoxicity of F in vitro. Clinical data with L plus F in colorectal cancer sustain this finding.
A phase I/II study was carried out to determine the recommended dose for phase II studies. In addition first data on antineoplastic activity were ascertained. L 300mg/m² i.v. was given on day 1,2,3 followed by F 500mg/m² i.v. day 1,2,3 one our after L. F was escalated step by step in 100mg/m² doses until untolerable toxicity(myelotoxicity WHO 3, mucositis/stomatitis WHO 2) occured in 2 out of 5 pts.. Cycles were repeated day 22-28. 5 pts. recieved 500mg/m² F. Dose limiting toxicity was seen on dose level 700mg/m² F. Thus the recommended dose in this setting was 600mg/m² F. So far 21 pts of this ongoing study have entered this dose level. They all had measurable and progressive disease. 17 pts. (44 cycles) are evaluable for toxicity (≤1 cycle) and 16 pts. for preliminary response (≤2 cycles). 4 pts are too early to evaluate. Patient characteristics: male 11; female 6; mean age 58 yrs.(34-75); mean Karnofsky PS 85%(60-100); toxicity WHO grade: leukopenia 1°(4), 2°(5), 3°(1), 4°(2 ; both pts. were irradiated prior to chemotherapy in the pelvis, one died of septicemia); thrombocytopenia 1°(2), 2°(1); mucositis/stomatitis 1°(4), 2°(3), 3°(1); diarrhea 1°(1), 2°(2), 3°(1), nausea/vomiting 1°(6), 2°(2). Preliminary response in 16 pts.: CR 1, PR 5, MR 4, NC 4, PD 1, toxic death 1. Conclusions: This schedule seems to be as active as the often used 5-day regimen with acceptable toxicity. The reduction of the treatment time to three days is an advantage in terms of palliation and outpatient treatment.
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BW 494/32 - A NEW MONOCLONAL ANTIBODY FOR IMMUNOTHERAPY OF EXOCRINE PANCREATIC CARCINOMAS (PACA)

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Because of unsatisfactory results of palliative chemotherapy or radiation we try to involve Mabs into the treatment strategies for paca, as BW 494/32. BW 494/32 represents a new Mab which might allow "cold" treatment of paca because of a) positive antigen expression by G1 or G2 paca, b) positive ADCC in Cr-51-release-assay (Bosslet et al. 1986), c) positive ISC with I-131-BW 494/32 $f(ab)_2$ fragments in patients (Montz et al. 1986) as well as in xenografts in nude mice (Klapdor et al. 1986). We now would like to report on first studies a) in nude mice bearing the human paca ISCH84 and ASCH84, treated with 400-600 µg/d for 11-21 days (i.p.) and b) in patients suffering from advanced paca: 8 ♂, 2 ♀, 44-73 years of age, with positive ISC or IH, 6 after proceeding chemo- or radiation therapy. 5 patients were treated with increasing doses up to a total of 150-210 mg, 5 with total doses of 490 mg (30 mg/d after 100 mg at day 1), under control of Mab and HAMA serum concentrations. The animal studies showed a significant dose dependent inhibition of tumor growth of ASCH-84 when treatment was started at time of transplantation, however no effect in nude mice bearing established tumors of tumors of ISCH84. The patients showed no side effects except of 1 allergic reaction. 2/10 showed a significant decrease of serum CEA, 1/10 regression of liver metastasis and necrosis of the primary tumor. Basing on these data we are just starting a multicenter trial with BW 494/32 as adjuvant therapy after tumor resection.

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MULTIMODALITY THERAPY OF ADVANCED AND UNRESECTABLE ESOPHAGEAL CARCINOMA

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Eleven patients aged 48-76 with a locally advanced or distantly metastasized esophageal carcinoma were treated with cisplatinum 120 mg/m² and vindesine 3 mg/m² an day 1. This was followed on day 3 by bleomycin (10 U/m² loading dose and 10 U/m² iv 24 hr infusion x 4 days). Vindesine was administered on days 8, 15 and 22.
Median two cycles were given prior to radiotherapy . Five patients received one to three additional cycles of chemotherapy after local radiotherapy (median 50 Gy).
One patient underwent surgery after chemo- and radiotherapy, but at surgery there was no tumor.
Eight patients had squamous cell carcinoma, 1 pt had small cell carcinoma, 1 pt had adenocarcinoma, 1 pt had undifferentiated carcinoma.
The median survival for the eleven pts is 20 months (range 5 to 57+ mos).
Four pts are clinically free of tumor 29+ to 57+ months.
Three pts died with local recurrence after 5, 7 and 8 mos.
Two pts developed local and distant recurrence after 11 and 20 mos and died.
Two pts died not tumor related after 18 and 24 mos.
In conclusion the three drug regimen is practicable in patients with advanced and unresectable esophageal cancer. The survival data are encouraging. Because drug toxicity was considerable with severe myelosuppression vindesine administration had to be prolonged in halve of the pts. Now we started a new trial with chemotherapy containing cisplatinum and 5-FU and simultaneous radiotherapy.

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TREATMENT OF DISSEMINATED MID-GUT CARCINOID TUMOURS WITH RECOMBINANT ALFA-INTERFERON

C. Doberauer, N. Niederle and C.G. Schmidt

Recently, encouraging results in treatment of carcinoid tumours with human leukocyte-derived interferon-alfa and fibroblast derived interferon-beta were reported. The aim of this pilot study was to assess the clinical effects of human recombinant interferon-alfa (rIFN-α2b) in patients with advanced mid-gut carcinoid tumour and carcinoid syndrome.
Seven patients with progressive ileal or coecal carcinoid tumours and liver metastases were treated with rIFN-α2b at a dosage of $2\text{-}4 \times 10^6$ U daily or every other day, subcutaneously. Six patients had symptoms of carcinoid syndrome.
Stable disease lasting 5 to 37+ months (median, 16 months) was noted in 6 patients and 1 patient had progressive disease. A temporary decrease in urinary excretion of 5-hydroxyindoleacetic acid by more than 50 % was observed in 5 patients. Four patients were completely or partially relieved of flushing, diarrhea, obstruction or abdominal pain. The side effects were negligible with the exception of mild hyperthermia, headache and confusion, but only during the first week of therapy.
Treatment with rIFN-α2b offers good palliation to patients with disseminated mid-gut carcinoid tumours and carcinoid syndrome.

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TREATMENT OF COLORECTAL CANCER WITH THE MONOCLONAL ANTIBODY 17-1A: A PHASE II STUDY.

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Recent claims of remissions obtained by treatment of colorectal cancer with the 17-1A IgG2a monoclonal antibody (moab) remain to be substantiated. In this phase II trial 14 consecutive patients with progressive, measurable, metastatic or inoperable histologically proven colorectal cancer were treated with a single infusion of 200 mg 17-1A, given in 500 ml 0.9% NaCl in 90 minutes.

After a median follow-up of 694 days, 12/14 patients have died. Median survival of all patients is 140 days (range 10-728 days). No partial or complete remissions according to WHO criteria were observed. However, one patient showed a drop of elevated liver function tests and CEA-titre. Anti-CEA immunoscintigraphy demonstrated the disappearance of liver metastases, while CT-scan and sonography documented the continued presence of lesions of unchanged size. A second patient progressed during the first month after 17-1A therapy, but then developed extensive calcifications and prolonged stabilisation of his liver metastases. Both patients are alive and well for 728+ and 563+ days after 17-1A administration. With the exception of a corticoid-responsive periorbital edema during infusion, no significant side effects were observed. Currently, sequential patient sera are analyzed for a humoral human anti-mouse immune response. In conclusion, no objective remissions were obtained, however, the observed changes may indicate some biological acitivity of the 17-1A moab and deserve further investigations.

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PREOPERATIVE(NEOADJUVANT) CHEMOTHERAPY IN LOCAL ADVANCED GASTRIC CANCER WITH ETOPOSIDE/ADRIAMYCIN/CISPLATIN(EAP) - PROMISING RESULTS

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25 pts. with irresectable(stated by laparotomy) local advanced gastric cancer or local recurrent gastric cancer were treated with EAP which has shown to be high effective in this tumor. Second look operation with curative intention was done in case of objective response after EAP. Chemotherapy: Adriamycin 20mg/m² i.v. day 1,7; Cisplatin 40mg/m² i.v. day 2,8; Etoposide 120mg/m² i.v. day 4,5,6; Repeat day 22 - 28. Eligibilitycriteria: measurable ± evaluable disease; age ≤65 yrs.; WHO PS ≤2; no prior chemo-/radiotherapy; normal renal-, liver-, cardiac- and bone marrow function. Pts. characteristics: male 20; female 5; mean age 52 yrs.(20-65); WHO PS 1 (35%), WHO PS 2 (65%); local advanced 22; local recurrent 3. Results(chemotherapy): CR 7(28%); PR 11(44%); CR+PR 18(73%); NC+PD 6(24%); 1 toxic death. Toxicity WHO grade: leukopenia 1°(2), 2°(14), 3°(8), 4°(1); thrombocytopenia 1°(14), 2°(4), 3°(3); nausea/vomiting 1°(8), 2°(11), 3°(4); neurotoxicity 1°(1); no nephrotoxicity.
13 pts underwent second look operation; 2 refused re-operation; 3 pts.(1 CR, 2 PRs) are assigned for operation. Results: pCR 6; NED 3; R1-resection 2 (NED after two more cycles of EAP); irresectable 2: No increased morbidity by second look operation after EAP.
Median remission duration and median survival not yet reached. There were two relapses in 11 pts. with NED after chemotherapy and surgery(1 CNS- , 1 local + CNS relapse). Conclusions: 28% of CR after EAP and 44% NED after chemother. plus surgery is promising and might offer new possibilities for the treatment of this prognostic unfavourable tumor. Preoperative chemotherapy did not increase morbidity or mortality after second look operation.
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T-CELL DEPLETION AND "IN VITRO" TREATMENT OF MARROW WITH ABSORBED ATG FOR PROPHYLAXIS OF GRAFT-VERSUS-HOST DISEASE (GVHD) IN DOGS.

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In allogeneic marrow transplantation GVHD is induced by T-cells contained in the marrow graft. In the dog as a preclinical model T-cell depletion by antibody rosettes was compared to "in vitro" treatment of marrow with absorbed rabbit antithymocyte globulin (ATG) for prophylaxis of GVHD. Donors were DLA-homozygous and recipients were DLA-heterozygous and -haploidentical littermates. Recipients were conditioned with 9 Gy total body irradiation. Seven dogs received untreated marrow and died within 4 weeks of transplantation from severe GVHD. "In vitro" treatment of the marrow with absorbed ATG at a concentration of 1:200 prevented GVHD in 9 of 11 dogs and induced stable graft-host tolerance. A monoclonal pan-T antibody was used for rosetting of T-cells with sheep red blood cells covered with staphylococcal protein A. More than 99,9% of T-cell-rosettes could be removed by separation on a density gradient. CFU-GM were retained in the interphase and two autografted dogs showed prompt and complete recovery. Two of 4 allografted dogs rejected the marrow graft and two died from GVHD.

Our results indicate that depletion of T-cells from the marrow graft using a monoclonal antibody is inferior to "in vitro" treatment of the marrow with ATG for prevention of GVHD across major histocompatibility barriers. The reason for the superiority of ATG may be either more effective opsonisation of T-cells and removal "in vivo" or inactivation of T-cells by other mechanisms. Supported by the Wilhelm Sander Foundation.

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THE CRITICAL T CELL DOSE CAUSING GRAFT-v-HOST DISEASE IN T CELL DEPLETED HAPLOIDENTICAL BONE MARROW TRANSPLANTS

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Recently limiting dilution analysis (LDA) has shown that leukemic patients receiving a HLA-identical T cell depleted graft with less than 1×10^5 T cell per kilogram do not develop graft-v-host disease (GvHD) (N.A. Kernan, Blood 68: 770,1986). In order to determine whether this "critical" T cell dose is the same in the haploidentical situation, we investigated the residual T cells in our transplants using the method of Kernan. Depletion of T cells from bone marrow was achieved by differential agglutination with soybean agglutinin (SBA), followed by depletion of cells forming rosettes with sheep red blood cells (E^-). LDA of untreated marrow revealed frequencies of cloned T lymphocytes ranging from 1/15 to 1/534, thus indicating that the cloning efficiency must be higher than 50 % in our culture system. In T cell depleted marrows, LDA detected 1/485-1/30570 clonable T cells. The absolute number of T lymphocytes were in the range from 1×10^4 to 1×10^6. Expressed as T cell per kilogram recipient weight, the T cell dose was found below 1×10^5 in all patients. No GvHD was observed in these patients with one exception. This patient had been transfused with 6×10^4 T cells per kilogram. We conclude from this preliminary data that the critical T cell dose causing GvHD is in haploidentical transplants between 10^4 - 10^5 T cells per kilogram. Therefore it seems that the critical T cell dose causing GvHD might not be much different from that reported in the case of HLA-identical grafts.

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CORRELATION BETWEEN SPECIFICITY AND FUNCTION OF GRAFT-VERSUS-HOST-REACTIVE (GVHR) AND AUTOREACTIVE T CELL LINES TO THE OUTCOME OF ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT)

C. Mauz, M. Haen, G. Ehninger, P. Wernet, and E.M. Schneider

Activated T cell lines derived from BMT patients were successfully established by culturing peripheral blood mononuclear cells (PBMC) in partially purified Interleukin 2 at one to 2 days before clinical manifestation of acute GvH disease. These T cell lines were CD4+ and proliferated in primed lymphocyte tests (PLT). It was a consistent finding that a subpopulation responded to host cells alone whereas a different one proliferated to autologous stimulators, and both recognized antigen in restriction with HLA-class II antigens. In 4 T cell lines the anti-host reactive subpopulations were blocked by monoclonal antibodies (moAb) to HLA-DQ and the autoreactive component was inhibited by anti-HLA-DR moAb in some cases, and by broadly reactive anti-HLA-DR, -DP, -"DY" moAb in all cases. Functionally, PLT lines from patients with a lateron uncomplicated hematopoetic recovery exhibited helper function for responsiveness by graft cells and concomittantly suppressed responses by host cells. Three patients were unique in that their activated T cell lines lacked the anti-host reactive component as well as the restricted suppression of host cells. Despite their primary normal hematological reconstitution these patients relapsed within 140 to 510 days after BMT. Prospective and retrospective studies aim at the possible interrelations between specificity of pre-GvH T cell lines, and the manifestation of clinically mild or severe acute and chronic GvHD. Results may be of potential value to unravel distinct versus related T cell responses to ensure stable remission by Graft versus Leukemia (GvL) reactivity concommittantly with host-specific tolerance in vivo.

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T-CELL DEPLETED ALLOGENEIC BONE MARROW TRANSPLANTATION. RESIDUAL T-LYMPHOCYTES AS MEASURED BY LIMITING-DILUTION ANALYSIS CORRELATE WITH BMT-ASSOCIATED COMPLICATIONS

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We studied in selected leukaemic patients the connexion between T-cell depletion of allogeneic bone marrow grafts and bone marrow transplantation (BMT) associated complications such as graft versus host disease (GvHD) and host versus graft rejection (HvGR). Bone marrow of donors (HLA identical, MLC negative siblings) was incubated with the monoclonal antibody (MoAb) Campath 1 and autologous complement. Samples of bone marrow mononuclear cells (BMMNC) prior to and following T-cell depletion were polyclonally activated by phytohemagglutinine under limiting dilution conditions and individual wells were tested for Il2 secretion and cytotoxicity. GvHD and HvGR were diagnosed clinically by peripheral blood cell counts, bone marrow morphology, skin biopsies and genetic markers. Undepleted BMMNC's show high incidences of Il2 producing as well as cytotoxic T-lymphocytes (frequencies ranging from 1/3-1/30). Risk of GvHD is clearly indicated by frequencies of cytotoxic T-lymphocyte precursors (CTLp) after depletion: ineffective depletion of CTLp's with frequencies ranging from 1/8-1/30, as incidently observed, was found to be in strong correlation with GvHD. In contrast HvGR went along with reduced CTLp-frequencies (1/1000-1/1500). Remarkably frequencies of Il2-secreting T-lymphocytes (HTLp) after depletion were found to be in any case higher than CTLp-frequencies but yet in the same correlation with GvHD/HvGR.
Thus, functional frequencies of residual T-cells in T-cell depleted bone marrow grafts give a clear correlation with the appearence of GvHD and HvGR.

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SELECTIVE ELIMINATION OF MALIGNANT MYELOID BLAST CELLS WITH MONOCLONAL ANTIBODIES AND HUMAN COMPLEMENT

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Autologous bonemarrow transplantation may under certain conditions, be an alternative to conventional chemotherapy or allogeneic bonemarrow transplantation, provided that clonogenic leukemic cells can be removed. For this reason we recently have established a purging protocol for acute lymphoblastic leukemia (ALL) (1). At present we are working on a purging protocol for acute myeloid leukemia (AML) blasts, as AML is the most common acute leukemia, especially in adults. We are using for this purpose our monoclonal antibody VIM2 which reacts with clonogenic leukemia cells (AML-CFU) (2,3), but not with normal day 14 CFU-GM cells . We are now able to demonstrate that this antibody can even used with human serum as complement source and efficiently lyses clonogenic leukemia cells in a model system.

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CHEMOTHERAPY AS ALTERNATIVE TO TOTAL BODY IRRADIATION IN PATIENTS TREATED WITH BONE MARROW TRANSPLANTATION

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While conventional pediatric antineoplastic therapy favours chemotherapy and surgery in an attempt to reduce, delay or delete radiotherapy, the necessity of TBI (total body irradiation) as part of pretransplant conditioning regimens in the field of BMT (bone marrow transplantation) has rarely been questioned. However, the development of alternative regimens to TBI seems to be of importance, since late irradiation effects have been reported after TBI and the occurence of secondary malignancies is of particular concern in patients, who would have benefited from BMT otherwise.
17 patients with various forms of high-risk or chemotherapy-resistant tumours or leukemias received different pretransplant conditioning regimens, but all without the use of TBI, followed by allogeneic or autologous BMT. 3 of the 17 patients had various forms of leukemia and all received high-dose busulfan-(16mg/kg) containing protocols in preparation to BMT. 9/17 patients survive. Median survival time of surviving patients is 607+ days (range 36-1124+ days), and of non surviving patients 42 days (range 9-485 days). In view of the potential side effects of TBI, further alternatives to TBI should be investigated particulary since TBI might still be used for the patient, who failed a non TBI-containing pretransplant protocol.

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ALLOGENEIC BONE MARROW TRANSPLANTATION IN THE ABSENCE OF AN HLA-IDENTICAL SIBLING: PRELIMINARY TÜBINGEN EXPERIENCE

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Twelve patients (age range 7-34 years, median 19 years) were transplanted from donors other than HLA identical and/or MLC negative siblings. Ten patients had partially matched related donors, and 2 were transplanted from unrelated donors. The diagnoses were: AML 2nd CR (N=1), ALL/AUL 2nd and 3rd CR (N=4), SAA (N=1), and CML accelerated phase or blast crisis (N=6). Ciclosporin plus methotrexate was usually given as GvHD prophylaxis. Engraftment was documented in 10 patients. 7 out of 9 evaluable patients developed aGvHD (grade II, N=2; grade III and IV, N=5). Four patients died from infections and pneumonitis, two patients relapsed. Six patients are alive with a median follow up of 4 months (range 1-12 months). The group of patients transplanted is small and the follow up is short. Nonetheless, the frequency of aGvHD in such patients was higher than in HLA/MLC-matched patients and, moreover, appears to be higher than reported by others for similiar series.

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MICROANGIOPATHY AS A FREQUENT COMPLICATION OF ALLOGENEIC BONE MARROW TRANSPLANTATION - INDICATORS AND RISK FACTORS

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Microangiopathy causing severe hemolytic uremic syndrome has been reported as a rare complication of ciclosporin (CsA) prophylaxis in allogeneic HLA identical bone marrow transplantation. We observed clinical and biochemical changes indicative of generalized microangiopathy in 41 of 54 (76%) allogeneic marrow graft recipients treated with CsA as compared to none in 11 patients receiving methotrexate (MTX) prophylaxis and in 2 recipients of syngeneic transplants. Severe microangiopathy resembling thrombotic thrombocytopenic purpura developed in 7 of 54 patients and was fatal in 5. Microangiopathic changes were preceded by a decrease of activated partial thromboplastin time and fibrinogen to subnormal levels indicating activation of coagulation. Simultaneously factor VIII related antigen rose to 323$\pm$35% in patients with moderate and to 605$\pm$105% in patients with severe microangiopathy reflecting generalized endothelial damage. Severity of microangiopathy was highly correlated with the grade of acute graft-versus-host disease (GvHD) ($p<0.0001$) and weakly with cumulative CsA concentrations ($p<0.05$). It was not influenced by age, diagnosis, stage of the disease or by a short course of MTX in addition to CsA. In summary our data suggest an altered pathophysiology of acute GvHD in CsA treated allogeneic marrow graft recipients causing microangiopathy in combination with direct CsA toxicity.

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BONE MARROW TRANSPLANTATION IN ACUTE LEUKEMIA

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Bone marrow transplantation (BMT) was performed on 76 patients, 4 to 49 years old, suffering from acute lymphoblastic (ALL) and acute non lymphoblastic leukemia (ANLL). Before BMT the patients were in principle treated with 60 mg cyclophosphamide per kg body weight on 2 consecutive days followed by a total body irradiation. Life table analysis shows that 35 % (25% for ANLL and 45% for ALL) of the patients are alive at least 5 years after BMT and 28% are disease free. Of these 76 patients only 41 could be shown to be in first remission of ANLL or ALL or second remission of ALL. 40.5% of these patients are without relapse and alive up to 7 years after BMT compared to 17% of the other patients who were not in complete remission of their disease or in second or later remission of ANLL or in third or later remission of ALL. During the first 100 days 14 out of 20 patients died because of infections and only 6 because of a relapse. Later on main cause of death is relapse of the leukemia. 70% of the patients who relapsed were not in complete remission or in the second (ANLL), third (ALL) or following remission when BMT was performed. BMT provides a good therapy for acute leukemia in patients with ANLL in first remission and patients with ALL in second remission.

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ALLOGENEIC MARROW TRANSPLANTATION (BMT) FOR CHRONIC MYELOID LEUKEMIA (CML) AFTER CYTOREDUCTIVE THERAPY WITH RECOMBINANT ALPHA-2B INTERFERON (IFN ALPHA-2B)

D.W.Beelen,N.Niederle,K.Quabeck,O.Kloke,H.Sayer,U.Graeven, U.W.Schaefer and C.G.Schmidt

Based on the assumption that previous busulfan (BU) therapy might increase the risk of pulmonary complications after total body irradiation and BMT, we used IFN alpha-2b as cytoreductive therapy prior to BMT in 11 patients (pts) (3 females,8 males;median age 33 yrs.,range 22-49 yrs.) with chronic phase CML.Prior to introduction of IFN alpha-2b therapy, 9 of these pts had been treated with BU (n=8) or hydroxyurea (n=1) over a period of 48 to 1698 days (median 466 days). The duration of IFN alpha-2b therapy ranged between 131 and 607 days (median 269 days). A hematologic remission (as defined by normalisation of the peripheral-blood cell counts and disappearance of all clinical signs of the disease) was achieved in 10 of 11 pts. BMT was performed after a median interval of 23 days (range 16-260 days) from cessation of IFN alpha-2b therapy. Nine pts received marrow from an HLA-identical sibling donor and 2 pts from an HLA-compatible donor,respectively. Four of 11 pts (36%) developed fatal interstitial pneumonia (IP) (CMV-associated IP n=2,idiopathic IP n=2). All 4 pts had been previously treated with BU over a period of 543 to 1698 days (n=3) or with hydroxyurea (n=1),respectively. Six of 9 pts, who had an HLA-identical donor are alive between 6 and 21 months, resulting in a cumulative disease-free survival estimate of 67% at 21 months post BMT. These favorable results show that IFN alpha-2b is effective in inducing hematologic remissions even in pretreated pts. Since major pulmonary complications after BMT occured exclusively in pts,who had been extensively pretreated with BU or hydroxyurea, IFN alpha-2b therapy might be advantageous for pts designated to BMT.

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INTENSIFIED CONSOLIDATION CHEMOTHERAPY WITH HIGH-DOSE BUSULFAN (BU) AND CYCLOPHOSPHAMIDE (CY) FOLLOWED BY AUTOLOGOUS MARROW TRANSPLANTATION (ABMT) FOR ACUTE MYELOID LEUKEMIA (AML) IN FIRST REMISSION (CR 1)

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Ten patients (pts) (4 females, 6 males; median age 35 yrs., range 16-45 yrs.) with AML in CR 1 (median interval from diagnosis to CR 56 days, range 20-157 days) underwent ABMT after a preparative regimen consisting of BU (4 mg/kg/day orally on 4 consecutive days) followed by CY (60 mg/kg/day i.v. over 2 days). The duration of CR before ABMT ranged between 145 and 423 days (median 211 days) and marrow was harvested at a median of 187 days from entering CR (range 114-410 days). Prior to cryopreservation, no attempt was made to eliminate residual clonogenic leukemic cells from the marrow graft. The median time to attain a WBC count over 1000/µl was 22 days (range 13-31 days). Sustained recovery of a platelet count over 20.000/µl was reached at a median of 44 days (range 21-67 days). The duration from ABMT to discharge ranged between 29 and 67 days (median 50 days). As of May 25,1987 seven of 10 pts are alive and in continuous CR with a median follow-up of 8 months, resulting in an actuarial disease-free survival of 65% at 15 months post ABMT. Causes of treatment failure were leukemic relapse (n=2) at 2 and 3 months post ABMT,respectively, and fatal septicemia during aplasia (n=1). One of the relapsing pts was successfully treated by reinduction therapy and is alive in CR 2 at 1.2 yrs. post ABMT. This analysis indicates that ABMT after BU in conjunction with CY as preparative regimen is associated with hemopoietic toxicity, which is similar or even more pronounced to that observed with intensive induction chemotherapy regimens. Clinical trials will be necessary to demonstrate the antileukemic efficacy of this approach as compared to conventional postremission therapy.

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AUTOLOGOUS TRANSPLANTATION OF BLOOD-DERIVED HEMOPOIETIC STEM CELLS: A NEW CONCEPT OF STEM CELL RESCUE AFTER MYELOABLATIVE TREATMENT

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To use circulating blood as primary source of stem cells to repopulate bone marrow is a concept which reflects the physiological pattern in which fetal hemopoiesis develops. In man, the hemopoietic repopulating ability of normal circulating stem cells on a complete and permanent basis was first demonstrated by our group in a patient with malignant non-Hodgkin's lymphoma. Two years after autologous blood stem cell transplantation (ABSCT) this patient is fully reconstituted and in perfect health. Meanwhile we have performed a total of 16 ABSCT's in the following diseases: AML(CR1)6, AML(CR2+)3, ALL2, malignant lymphoma 3, and sarcoma 2. It was the purpose of this phase 1 study to evaluate the kinetics of hemopoietic reconstitution after myeloablative treatment and ABSCT. Preliminary conclusions are: 1 hemopoietic reconstitution occurs faster than after bone marrow transplantation if circulating stem cells are collected under selected conditions (AML and ALL in CR1, not heavily pretreated), 2 the pattern of hemopoietic reconstitution seems to correlate with the patients's pretreatment, 3 stem cell yield by successive leukaphereses can be greatly improved by using the stem cell overshooting after transient chemotherapy-induced myelosuppression. There are certain practical advantages of using blood derived stem cells instead of marrow derived stem cells. Besides this, the ratio between normal hemopoietic stem cells and clonogenic tumor cells in the peripheral blood of patients with malignant lymphopoietic disorders in remission may be in favour of the former, a hypothesis yet to be proven. In so far the peripheral blood seems to be an important, probably overlooked stem cell source in the concept of stemm cell transplantation. - Med.Klinik und Poliklinik, Strahlenklinik, and Institut für Immunologie und Serologie, University of Heidelberg, 6900 Heidelberg

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IN VITRO EFFECT OF REC. GM-CSF ON HEMOPOIETIC PRECURSORS FROM PATIENTS WITH PROLONGED PANCYTOPENIA AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION.

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Fifty patients with acute leukemia in remission have been transplanted with an autograft purged with mafosfamide. 32 of the 50 patients (64%) are alive and in complete remission following ABMT. 5 patients (10%) did not achieve a stable reconstitution of the peripheral blood cells. 10 are cytopenic (granulocytes $<$ 1.5 /nl, platelets $<$150/nl). The median follow-up for these patients after ABMT is 162 days (range 110 to 320 days). We investigated the effect of human rec. GM-CSF (Behring-Werke, FRG) on hemopoietic precursor cells from those patients with a delayed reconstitution. In a semisolid culture assay (Messner and Fauser) we evaluated mixed hemopoietic colonies (CFU-GEMMT), erythroid bursts (BFU-E) and granulocyte-macrophage colonies (CFU-GM). GM-CSF (from 1 to 100 ng/ml) was assessed with autologous plasma versus heterologous plasma from normal donors. The average number of CFU-GM/ml in the autologous system was 44 vs. 46 in the heterologous. In normal bone marrow donors (n=5) the average number of CFU-GM is 261, and 110 in those patients with a complete reconstitution after ABMT. Using GM-CSF (100 ng/ml) an increase in the cloning efficiency for CFU-GM of 51% in the autologous and 89% in the heterologous system was seen. For those patients with normal peripheral blood counts the increase in the autologous system was only 17% and in the heterologous a decrease of 18% occured.
In summary, patients with a delayed reconstitution of the peripheral blood have a low number of determined hemopoietic precursor cells and lack a sufficient production of hemopoietic growth factors. Therefore, the in vivo application of recombinant growth factors such as GM-CSF might prove useful to increase mature granulocytes and macrophages. Indirectly erythrocytes and platelets could be influenced via an increased production of growth factors by macrophages/monocytes.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR POOR PROGNOSIS GERM CELL TUMORS

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Malignant germ cell tumors are considered to be among the cancers presently curable by systemic chemotherapy. However, a minority of these tumors - identifiable by: a) extragonal primary origin and incomplete resection, b) failure of initial adequately administered platinum-based chemotherapy, c) extremely high tumor markers or a slow rate of decline of markers, and d) extremely bulky visceral metastases, - are presently not curable. We transplanted 4 patients defined by these prognostic criteria. These patients presented with massive intraabdominal, intrahepatic, and/or pulmonary metastatic choriocarcinoma. One patient presented with a HCG of 750.000 and demonstrated a slow decline in tumor markers. Two patients had residual tumor masses post resection of a mediastinal germ cell tumor, one of them with yolk sac elements. All patients were conditioned with VP-16 1500 mg/m^2, cisplatinum 120 mg/m^2, and cytoxan 120 mg/kg, followed by autologous bone marrow transplantation. Hematologic reconstitution occurred within 17-24 days. All 4 patients are alive, well, and free of disease with a follow up time between 4 to 25 months. Our preliminary data suggest that high dose of combined chemotherapy followed by autologous bone marrow transplantation might provide long term control or even a cure for patients with germ cell tumors with poor prognosis.

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AUTOLOGOUS AND ALLOGENEIC BONE MARROW TRANSPLANTATION IN ADVANCED HODGKINSs DISEASE

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Seven adult patients with relapsed Hodgkin's disease have been treated with high-dose chemotherapy followed by bone marrow transplantation (BMT). Six patients were rescued by autologous cryopreserved and one patient by allogeneic bone marrow. The median age was 23 years (range 22-34 years). All of them have been treated with the CVB-protocol consisting of CYCLOPHOSPHAMIDE 1500 mg/m^2 days -6, -5, -4, -3, BCNU 500 mg/m^2 day -6, VP-16 250-300 mg/my days -6, -5, -4. Three patients were treated with additional involved field irradiation. Additional total nodal irradiation was used for the patient who received an allogeneic graft. The toxicity consisted of damage to mucosal and skin barriers, fever and infections during granulocytopenia and of heart failure (3 patients). Hematopoiesis recovered in all patients. Six patients achieved a complete remission. One patient only went into partial remission, he eventually died from bronchopneumonia. As of May 1987 five patients remain in remission. The response rate in this heavily pretreated group of patients is very encouraging. This therapeutic approach should therefore be evaluated in a larger number of patients with poor prognosis.

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COMPARISON OF ALLOGENEIC AND AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR THE TREATMENT OF ADVANCED REFRACTORY LYMPHOMA

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Preliminary data from allogeneic and autologous bone marrow transplantation (BMT) indicate that approximately 30% of patients with advanced lymphoid malignancies may be cured. However, a large number of patients will not have a histocompatible donor and graft-versus-host disease (GVHD) and its sequelae add considerably to morbidity and mortality. We are therefore comparing allogeneic BMT in patients with a histocompatible donor versus autologous BMT in patients without a suitable marrow donor. In this ongoing study, both groups receive the same cytoreductive immunoablative regimen. Twenty patients (age range: 22-41 years; median: 27 years) with advanced lymphoid malignancies received allografts and a complete remission was achieved in 15 patients; seven of these 15 patients are alive and in complete remission for 1-26 months (median: 9 months) after BMT. Five patients died of interstitial pneumonia and one with chronic GVHD/bacterial pneumonia. Five patients who had achieved only a partial response died with progressive disease. Two patients were too early for evaluation when they died within three weeks after bone marrow transplantation with fungal infections. Twelve patients (age range: 24-50 years; median: 31 years) with advanced lymphoid malignancies without bone marrow involvement received the same preparative regimen followed by autologous marrow reinfusion. Eight patients are alive and in complete remission for 1-7 months after autologous marrow infusion. Three patients have relapsed. One patient died with multi-organ failure due to pre-existing polyneuropathy. More patients are needed to allow a comparative analysis. Updated results will be presented.

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MANAGEMENT OF PATIENTS WITH CARCINOMA OF UNKNOWN PRIMARY: THE RECOGNITION OF PROGNOSTICALLY DIFFERENT SUBGROUPS AND THEIR INPUT ON TREATMENT REPORTS

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156 pts with carcinoma of unknown primary (CUP-Syndrom) were reviewed retrospectively from 1977-1987. The median age was 57 years, male 63 %, female 37 %. The histology showed 76 (49 %) adenocarcinoma (ACUP), 25 (16 %) undifferentiated carcinoma (UCUP), 15 pts (9 %) squamous cell carcinoma (SQCUP), 25 pts (16 %) various histologies including extragonadal germ cell tumor, 16 pts (10 %) unclassified. The survival rate was markedly different with respect to the extend of disease and the presenting site of the metastases. We subdivided the pts into 4 groups: group I: primary localized, non-lymphonodal manifestation, 40 pts (26 %); group II: primary lymphonodal manifestation, 26 pts (17 %); group III: primarily disseminated disease, 68 pts (43 %), group IV: primarily fatal prognosis, 22 pts (14 %). Group I and II received radiotherapy and/or surgery, group III chemotherapy and group IV only palliative care inclusive palliative radiotherapy. The preliminary evaluation of median survival in 87/156 pts is 14,7 mots and stratified by group: 21 mots (group I), 17,5 mots (group II), 9 mots (group III) and 3,7 mots (group IV). From these data we conclude that in pts with CUP-syndrom the localization and extend of the metastases are the main prognostic factors. For reports on treatment results in CUP-Syndrom pts it is mandatory to stratify the pts into these prognostic different subgroups. Furthermore, therapeutic implications arise from this knowledge and will be explained in detail.

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CISPLATIN BASED SEQUENTIAL CHEMOTHERAPY IN FAR ADVANCED NONSEMINOMATOUS TESTICULAR CANCER (NSTC)

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13 patients (pts) with far advanced disseminated NSTC were treated with the following regimen: 2 courses with protocol A consisting of vinblastine 6 mg/m^2 iv d 1 and 2, bleomycin 30 mg/24 hours cont.inf. d 1-3 preceded by 30 mg iv push d 1, and cisplatin 120 mg/m^2 iv d 4, followed by 2 courses with protocol B consisting of ifosfamide 2 g/m^2 iv d 1-3, VP 16 110 mg/m^2 iv d 1-5, and cisplatin 120 mg/m^2 iv d 5. Courses were repeated at 3 week intervals.
11 pts are evaluable for efficacy. 9 achieved a continuous com - plete remission with a median duration of 11+ months (range, 4-21+), 6 by chemotherapy alone, 3 by additional surgery. Both incomplete responders died 7 and 10 months, respectively, after start with therapy.
1 pt died due to disease after the 1st course, 1 due to an in - fection during granulocytopenia grade 4 after the 2nd course. 7 pts had granulocytopenia grade 4, and 10 infections requiring antibiotic treatment occurred. 4 pts showed thrombocytopenia grade 3. Other objective toxicities were mild.
These preliminary data suggest, that this regimen has substantial activity in far advanced NSTC, but myelotoxicity is severe and lifethreatening.

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CISPLATIN, ETOPOSIDE, AND VINDESINE FOR ADENOID CYSTIC CARCINOMA OF THE HEAD AND NECK

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Adenoid cystic carcinoma of the head and neck is characterized by slow progressive growth often occurring more than 10 years after initial diagnosis. control of local disease is best achieved with combined surgery and radiation therapy. Distand metastases are frequent but the role of chemotherapy in the management of this rare tumor has not been defined.

Nine patients with adenoid cystic carcinoma were treated with intravenous cisplatin 100 mg/m² day 1, etoposide 100 mg/m² days 3, 4, 5 and vindesine 3 mg/m² day 1 (PEV) every 4 weeks. Change to alternative chemotherapy consisting of ifosfamide 1500 mg/m² days 1 - 5 instead of cisplatin was necessary in 4 patients because of nausea and vomiting (3 patients) and ototoxicity (1 patient). Five out of 9 patients primary received surgical treatment. No prior radiotherapy had been given.

A complete response (28+month) was observed in one patient, 4 patients achieved partial remission (PR) and in 4 patients disease stabilized (NC). No progress was seen (PROG).

Median progression-free survival was 24 months (range, 4-36). No patient died. The authors conclude that PEV is effective in adenoid cystic carcinoma of the head and neck. Further investigations of less toxic regimes are necessary.

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BULKY DISEASE TESTICULAR CANCER: AN EFFECTIVE REGIMEN OF DOUBLE-DOSE CISPLATIN/HIGH DOSE VP16/BLEOMYCIN

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Despite the high chemosensitivity of testicular cancer still 30-50% of the pts. with advanced bulky disease die under treatment with conventional or standard chemotherapy regimen. Because a demonstrated dose response curve for Cisplatinum in testicular cancer pts., the following regimen was designed for pts. with bulky disease testicular cancer (lung n >5, each > 2cm; or n> 20, <2 cm; mediastinal tumor > 5 cm, retroperitoneal mass $\geq$ 10 cm, liver, bone or CNS-involvement): Cisplatinum 35 mg/m² d 1-5, VP16 120 mg/m² d 1-5, Bleomycin 15 mg/m² i.v. d 1, 8,15, q d 22-29, 3-4 cycles. 116 pts. entered the protocol (age 28 (16-66) years , KI 80% (50-80%), 6 pts. pretreated, 96 germ cell tumors incl. 18% seminoma, 20 extragonadal tumors). 8 pts. are not evaluable for response (2 early tumor death, 2 early toxic death (septicemia), 3 left protocol, 1 l.f.u.). Results: CR 46%, NED 26%, CR+NED 72%, PR 22%, NC/P 6%; relapse rate 10% (2 pts. had only pseudoprogression). After a median follow up of 32 (16-55) mos. the overall survival for the entire population is 70%. Toxicity: Nausea/vomiting °3(90), °4(4%); lung °3(10%); renal °3(2%), °4(%); peripheral neuropathy was a major problem: (2%)°3, (20%) °2; 1 lethal cardiotoxicity. Conclusion: This regimen is very effective in this high risk group with 70% living and 65% living without disease after a minimum follow up of >2 years. The toxicity is high, particularly neurotoxicity. However, the possible advantage of double-dose Platinum over standard-dose Platinum has still to be evaluated in a prospective randomised study.

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SECONDARY SURGICAL MANAGEMENT OF REMAINING TUMOR MANIFESTATIONS AFTER CHEMOTHERAPY IN NONSEMINOMATOUS GERM-CELL TUMOR (NSGCT).

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The fact that thoracic metastases and retroperitoneal lymphnodes didn't completely disappear after chemotherapy in a large percentage of Nonseminomatous Germ-Cell Tumor (NSGCT) made a following secondary lymphadenectomy and/or thoracotomy necessary.From 1979 until now 61 patients with residual abdominal and/or pulmonary disease had a secondary surgery.20 patients had a thoracotomy,29 a retroperitoneal lymphadenectomy and 12 patients had both of them. These 12 and 6 other patients had primary Bulky tumor manifestations (lymphnodes larger than 10 cm,organ metastases larger than 5 cm).
Secondary surgery revealed three types of histology:MTU was found in 6 patients,MTD in 26 patients and necrotic fibrous tissue was found in 29 patients.
32 patients had secondary treatment with thoracotomy.The histology in surgery was MTD in 13 patients and necrotic fibrous tissue in 19 patients.Of the 41 patients,who had a retroperitoneal lymphadenectomy,in 6 patients MTU in 18 patients MTD and in 17 patients necrotic fibrous tissue was seen.
Of all 61 patients,54 patients remained tumor free after secondary surgery from 8 to 108 months (median 46 months).Though the relapse free survival rate was 88,5 %,7 patients reached only partial remission and relapsed after 2 to 10 months.6 of them died,1 patient was lost follow up.4 of these 7 patients had MTU and the other 3 patients MTD.
The fact,that no patient with necrotic fibrous tissue relapsed,documents poor prognosis for patients with active tumor in secondary surgery.

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HORMONE THERAPY OF PROGRESSIVE AND METASTATIC RENAL CELL CARCINOMA (RCC) WITH HIGH DOSE-TAMOXIFEN (HD-TAM)

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Renal cell carcinoma occasionally shows response to the anti-estrogen tamoxifen. This may be due to estrogen- and progesteron receptors found on RCC tumor cells. Additionally HD-Tam seems to have a direct anti-tumor effect. So it may be possible, to increase the response rate with hormonal therapy of RCC by using HD-Tam. Since Dec. 1985 25 pts. with progessive, metastatic RCC have been treated with Tamoxifen 100 mg/m² p.o. daily. 21 pts. are evaluable for response and toxicity (4 are too early to evaluate). Patient characteristics: male 18, female 3; mean age 57 years (39-72); mean Karnofsky PS 80%; metastatic sites: lung 9, liver 1, two sites 5, three sites 5, four sites 1 pt. Results: CR 1, PR 3, CR+PR 4(19%), NC (in progressive disease) 10 (48%), PD 7 (33%). 1 PR + 3 NC occurred in 6 pts. progressive under Interferon/Vinblastin/Tamoxifen (30 mg daily). Median response duration (months) for CR + PR + NC: 7+. Median survival: 9+ mos, for responders 11+, for nonresponders 5,5. Toxicity (WHO grade): Hepatotox. 1^0 (3/21), 2^0 (1/21); nausea/vomiting 1^0 (1/21), 2^0 (1/21); weakness 1^0 (2/21); no myelotox.; thrombosis 4/21 (1 deep vein thr. of the leg, 2 intraabdominal vein.thr. due to tumor compression, 1 letal lung embolisme after surgical stabilisation of a broken leg). Conclusion: HD-Tam in RCC seems to increase response rates with moderate side effects compared to conventional hormone- and/or chemotherapy.

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HIGH-DOSE MEDROXYPROGESTERONE ACETATE IN PATIENTS WITH ADVANCED RENAL ADENOCARCINOMA

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High-dose medroxyprogesterone acetate (MPA) is effective in patients with advanced carcinoma of the breast or of the endometrium. However, it's efficacy in metastatic renal cell carcinoma is controversial.
Twenty patients with disseminated and progressive histologically proven renal adenocarcinoma were treated with high-dose MPA using the following regimen: loading dose 1000 mg i.m. daily for 10 days; maintenance 200 mg 3 times a day orally. The patients had not been pretreated with hormones and/or cytostatics.
Objective remissions were not achieved. Eleven patients showed stable disease lasting 3-12 months (median 7 months). These patients were primarily characterized by a longer disease-free interval (median 9 months) than patients with progressive disease (median 2 months). Median survival was 22 months for patients with stable disease and 7 months for patients with progressive disease. Toxicity was low. Pulmonary embolism, possibly related to high-dose MPA, occurred in 1 patient.
It may be suggested that the stable disease observed reflected a selection of patients with a less malignant spontaneous course of disease rather than a response to MPA. Therefore, treatment with high-dose MPA cannot be recommended for patients with advanced renal cell carcinoma.

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PHASE II STUDY WITH THP-DOXORUBICIN IN SMALL CELL BRONCHOGENIC CARCINOMA

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THP-Doxorubicin is a new anthracycline with efficacy in experimental systems comparable to adriamycin. In clinical phase I / phase II studies the dose-limiting toxicity was leukopenia. At the MTD (70 mg/m^2, every 3 weeks) no other meaningful toxicities were observed. We performed a phase II study in patients with small cell cancer of the lung (70 mg/m^2 every 3 weeks). Ten patients were untreated because age and performance status did not permit conventional aggressive chemotherapy. Three patients had relapsed after initial response to the ACO combination. Median age was 74 years (range 45-86), median Karnofsky scale was 60% (range 50-80). 11 patients had extensive disease, 2 limited disease. 10 patients are evaluable at the present time. One achieved a complete remission and 5 a partial remission. 5 responders were untreated, one had relapsed after prior successful treatment. Besides leukopenia no other meaningful toxicities were observed. Leukopenia WHO grade III, II, I and 0 was found in 2, 5, 0, and 3 patients. The subjective tolerance of the treatment was excellent. We conclude that THP-Doxorubicin is a very active drug in untreated patients with small cell cancer of the lung. The good subjective tolerance makes this drug very suitable for use in combination therapy regimens.

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CLINICAL EXPERIENCE WITH A COMBINED MODALITY THERAPY FOR SOFT TISSUE SARCOMAS (STS)

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During the last 10 years 133 pts with STS were admitted at our hospital. Sixty-four pts had primarily an inoperable or an metastatic disease. Forty-five pts presented distant metastases without evidence of local failure. Lung metastases were most common (60 %) followed by liver metastases (26 %).
Confronted with a high incidence of metastatic disease we developed a multi modality strategy for primary therapy of STS. Dependent on size, site, grading and surgical radicality pts with high risc factors (initially large tumor, high grade histology, microscopic tumor after surgery) underwent adjuvant chemotherapy (CHT) with Ifosfamide 5 g/m^2 d_1 and Adriamycin 50 mg/m^2 d_1 every three weeks for a total of 6 cycles. CHT was started after wound healing of an adequate operation. In addition radiotherapy was given simultaneously with 5000 r (200 r/d) to a large volume completed with a boost of 1.500 r to the tumor bed whenever possible. Sixteen pts underwent this regimen.
Two early pts received CYVADIC instead of Ifo + ADM. Three pts refused CHT after two resp. three cycles. Two pts had leucocytopenia < 1.0. No thrombocytopenia < 20.000 was observed. Main toxic problems were nausea, vomiting and alopecia. Concerning radiotherapy three pts had severe erythema with epitheliolysis requiring interruption of irradiation for two weeks. After a median follow-up of 24 (7-108) mos 13/16 (81 %) pts were free of disease. 3/16 (19 %) developed distant metastases, two of them died so far.
In our experience a combined modality therapy is feasible and might improve the prognosis for pts with high risk STS.

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IMMUNOGLOBULIN GENE REARRANGEMENT IN CHRONIC PHASE AND LYMPHOID BLAST CRISIS OF CHRONIC MYELOGENOUS LEUKEMIA

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Chronic myelogenous leukemia (CML), a malignant disorder of the hematological stem cell, can develop lymphoid blast crisis. This does not seem to represent lineage infidelity but the wide differentiation potency of the transformed stem cell. In order to detect early steps in lymphatic differentiation in CML we studied rearrangements of the immunoglobulin genes in peripheral leukemic cells of eighteen cases of CML in chronic phase by Southern-Blot analysis. Simultaneously gene rearrangements in two cases of overt lymphoid blast crisis were determined. Heavy chain (biallelic in both cases), but no light chain gene rearrangements were found in both cases with lymphoid blast crisis. In five of the eighteen patients with CML in chronic phase, a rearrangement of the heavy chain gene was detected. The intensity of the hybridization signals in these cases with rearranged heavy chain gene was high even though no lymphoid blasts were detected in peripheral blood or bone marrow. All these patients had no signs of blast crisis at the time of study and three of them are still in chronic phase fifteen months to thirty-six months after study. One patient died of sudden central bleeding, another is lost of follow up.
In conclusion, the discovery of rearrangement of heavy chain gene in 28% of the patients with CML in chronic phase suggested that this was an inherent characteristic of the malignant cell clone and did not represent the expansion of a second lymphoid cell clone. Whether this phenomenon is associated with development of lymphoid blast crisis awaits further study and confirmation.

(gene probes were kindly provided by P. Leder, Boston / USA)

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TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA (CML) WITH LOW DOSES OF RECOMBINANT INTERFERON ∝2 B

H. Diedrich, M. Freund, P. v. Wussow, R. Eisert, B. Metzner, H. Link, H.J. Wilke, H.-J. Schmoll, and H. Poliwoda

25 patients (13 m, 12 f, median age 46.8 yrs; 18.4 - 75.9 yrs.) with Philadelphia - chromosome positive CML have been treated with interferon ∝2 B. 13 patients had pretreatment with busulfan, 1 with busulfan and hydroxyurea, 1 with IFN alpha and gamma combined, 10 were not pretreated. 1 patient received 3 x 10 mio U IFN ∝2 B/week, the others 3 x 5 mio U/week as initial therapy. In 7 patients dose modifications were necessary.

21/25 patients are evaluable for preliminary results up to now. In 9/21 a complete hematologic remission (CHR) was observed (median 4 mo; 1 - 8,5+ mo), in 5/21 a partial hematologic remission (PHR) (median 10 mo; 3 - 13 mo). In only 1 patient Philadelphia chromosome positive metaphases were reduced in bone marrow. No true cytogenetic remission was observed however. 5 patients developed antibodies to IFN ∝2 B which were confirmed by radioimmunoassay, elisa, and bioassay. Of these 1 had CHR for 2 mo, 1 had PHR for 1 mo and relapsed subsequently. The other 3 patients had no PHR or CHR. Side effects consisted in thrombopenia (1/21 WHO grade 1, 1 grade 2, 2 grade 3, 1 grade 4), anemia (6 grade 1, 1 grade 2, 1 grade 3), fever (21 grade 2), fatigue (15 grade 1), changes in EEG (6/21) and depression (1/21).

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IN VIVO SENSITIVITY OF HEMATOPOIETIC PRECURSOR CELLS TO RECOMBINANT INTERFERON ALPHA (rIFN-alpha), RECOMBINANT INTERFERON GAMMA (rIFN-gamma) AND RECOMBINANT TUMOR NECROSIS FACTOR ALPHA (rTNF-alpha) IN NORMAL CONTROLS AND IN PATIENTS WITH CML: RELATIONSHIP TO THE IN VIVO RESPONSE

D. Geissler, G. Gastl, W. Aulitzky, H. Tilg, G. Konwalinka, Ch. Huber

In an ongoing phase II trial we aimed to predict clinical responses to rIFN-alpha in vitro. Until now, 23 patients with Ph^{1+} CML were entered into this study: 13 presented with chronic-phase CML (group A), the remainder showed disease acceleration (group B). Bone marrow samples were taken before treatment from 12 of the patients (six each in group A and B) and tested for the antiproliferative activity of rIFN-alpha and r-IFN-gamma by using a soft agar colony-forming assay system. In healthy controls and group A patients, colony formation for CFU-GM, BFU-E, CFU-E and CFU-MEG was inhibited by rIFN-alpha and TNF-alpha in a dose-dependent manner. Inhibition of colony formation was only minimal, even at highest doses ($10x10^4$ U/ml) in some patients in the chronic phase of CML and in some normal controls. BFU-E and CFU-MEG proved to be the most sensitive cell lineages, whereas CFU-E and CFU-GM were about ten times less sensitive. In the responder group, 50 % growth inhibition was already obtained at rIFN-alpha concentrations between 10 and 100 U/ml. In contrast, group B patients exhibited a marked in vitro resistance to IFN-alpha. Even at IFN-concentrations of 1,000 - 10,000 U/ml, 30-50 % of residual colony formation of CFU-GM and CFU-MEG was observed. This interferon resistance occurred either in CFU-MEG and CFU-GM colony formation alone or in both cell lineages. Corresponding to the in vitro results, all of the CML patients in chronic phase of the disease responded to relatively low doses of IFN-alpha (2-4 mio. U/d), whereas only four of the group B patients showing in vitro resistance to rIFN-alpha were succesfully treated by the addition of single-agent chemotherapy to alpha IFN.

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IN VITRO INFLUENCE OF α- AND γ-IFN ON PH$^+$CELLS FROM CFU-GM

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Mononucleated cells from bone marrow (BM) and peripheral blood (PB) of 6 Philadelphia positive CML (chronic phase) patients were incubated in semisolid media with and without α- and γ-IFN (each 100 U/l). Colony formation was counted after 6 and 14 days. Parallel samples were resuspended and prepared for chromosome analysis following routine procedures.
Colony formation from BM-cells was suppressed by α-IFN to a median of 15% (day 6) or 20% (day 14) of the control and by γ-IFN to 67% on day 6. No inhibition in the CFU-GM growth by γ-IFN was detected on day 14 in these patients.
The median percentage of Ph$^+$ cells was higher in the BM than in the PB on day 6 (100% vs.68%) as well as on day 14 (74% vs.63%). Additional chromosome aberrations have not been detected in the metaphases of these short term in vitro cultures. Ph$^+$ cells from BM-CFU-GM were not suppressed under the in vitro influence of α-IFN on day 6 (88%,median) or on day 14 (73%,median). Similarly, γ-IFN did not reduce the percentage of Ph$^+$ cells (86% on day 6, 72% on day 14,median). In addition, neither α- nor γ-IFN influenced the percentage of Ph$^+$ cells from PB-CFU-GM (day 6:79% vs. 71%, day 14:56% vs. 53%).
Concluding our preliminary observations, α- and γ-interferon (100 U/ml) in vitro does not seem to suppress the proliferation of Philadelphia positive CFU-GM selectively.

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CYTO CONVERSION OF PLURIPOTENT STEM CELLS OF PATIENTS WITH Ph' CHROMOSOME POSITIVE CHRONIC MYELOGENOUS LEUKEMIA TREATED WITH RECOMBINANT INTERFERON

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The antiproliferative activity of human alpha interferon has been demonstrated in a number of human tumors as well as in normal and myeloid leukemic progenitor cells. We studied the effect of recombinant human interferon alpha (r-IFN α) on bone marrow derived hematopoietic stem cells of normal individuals and of patients with Ph' positive chronic myelogenous leukemia (CML), and evaluated the cytoconversion of stem cells grown in the presence of human recombinant interferon. We found that the inhibitory effect of interferon was more pronounced in multilineage colony forming cells (CFU-GEMMT) of marrow cells of patients with chronic myelogenous leukemia (CML) compared with stem cells of normal marrow. T-cell colony formation (CFU-TL) of Ph' positive (Ph') T-cells obtained from primary multilineage hematopoietic colonies was inhibited by rIFN α. Inhibition of T-cell colony formation of Ph T-cells was more profound by interferon compared with secondary T-cells of healthy individuals. Southern blot analysis of secondary T-cells from normal individual revealed a germ line pattern when examined for T-cell receptor (TcR) gene rearrangements using the cDNA for the ß chain. Subcloned Ph' T cells of a few patients with CML demonstrated gene rearrangements of the TcR. We also demonstrated the disappearance of Ph' positive multilineage hematopoietic colonies when marrow cells were preincubated with interferon and subsequently cultured in the presence of interferon. The cytoconversion of the Ph'chromosome in metaphases obtained from individually analyzed multilineage hematopoetic colonies suggests that interferon might allow the restoration of nonclonal hematopoiesis in patients with CML.

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INTENSIVE INDUCTION CHEMOTHERAPY FOR Tdt NEGATIVE, NON_LYMPHOID BLASTIC CRISES OF Ph-POSITIVE CML

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9 patients with a Tdt negative, non-lymphoid blastic crisis of Ph-positive CML received 1-3 courses of intensive induction chemotherapy with TAD (Adriamycin, Ara-C, 6-Thioguanin), or DAV (Daunomycin,Ara-C, VP-16). 1 patient died on day 7, the other 8 patients responded to therapy with complete clearing of the blasts from the peripheral blood giving a response rate of 89 %. However, bone marrow aplasia with less than 5 % blasts was seen in 2 patients only. These 2 patients subsequently received an allogenic bone marrow transplant and achieved complete remissions lasting 3 and 6 months. Only 1 patient achieved a second chronic phase of CML lasting 7 months. The osther 5 patients regenerated with CML-hemopoiesis (median time to granulocytes higher than $1x10^9/l$ = 33 days) and blasts (median time to blasts higher than $1x10^9/l$ = 21 days). All patients died due to progression of blastic crises. Median survival of the group was 155 days. These results were compared to a historical control group of 31 patients with myeloblastic crises of Ph-positive CML from our institution, treated with Vincristin/Prednison, in whom we observed 9 responses (29%), Despite a significantly better response rate to aggressive induction chemotherapy (8 of 9 versus 9 of 31, p=0.01) survival was only marginaly better and a logrank test of the survival curves showed no significant differences (p=0.10) between both groups.

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TREATMENT OF BLAST CRISIS IN CHRONIC GRANULOCYTIC LEUKEMIA WITH VINDESINE AND PREDNISONE

R. Grunewald, U. Jehn, J. Mezger and Ch. Clemm

Treatment of blast crisis (BC) in chronic granulocytic leukemia (CGL) remains a major problem after bone marrow transplantation, and other experimental treatment modalities have failed. Therefore, twenty patients (pts) in CGL-BC entered a phase II trial with vindesine and prednisone. Median duration of the chronic phase was 21 months in 17 pts, one pt had a preceding polycythemia vera, two pts presented with a primary BC. Twelve pts had myeloblastic features as evidenced by morphology, cytochemistry, and cell surface antigens; four had a mixture of myeloid and lymphoid blast cells, three with lymphoblastic and one with myeloid predominance; two had blast cells which displayed lymphoid characteristics; one was classified as undifferentiated and one showed basophilic differentiation.Three pts achieved a complete remission lasting 1 month (myeloid), 3 months (mixed) and 5 months (lymphoid) without further maintenance treatment. Thirteen pts had a minor response with reversion to a chronic type differential WBC having a median duration of 2 weeks (nine with myeloid, three with mixed, and one with undifferentiated leukemia). Four pts did not respond to vindesine including one with a basophilic differentiation. Leukopenia and thrombocytopenia were severe and prolonged independent of their morphologic or immunologic subtype.Individual dose and schedule modifications during induction therapy and combination with other drugs for early maintenance treatment should be attempted in future trials.

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PRIMARY (IDIOPATHIC) MYELOFIBROSIS-/OSTEOMYELOSCLEROSIS - SYMPTOMS, SIGNS, CLINICAL COURSE, AND BONE MARROW HISTOPATHOLOGY IN 110 CASES WITH SPECIAL EMPHASIS ON THE EARLY (HYPERPLASTIC) STAGE OF DISEASE

R. Zankovich, B. Mödder, J. Thiele, T. Steinberg, K.G. Simon, R. Fischer and V. Diehl

A clinicopathological study was performed on 110 patients with primary (idiopathic) myelofibrosis - osteomyelosclerosis (OMF) to reveal laboratory and histomorphological parameters of prognostic impact on survival. In addition to multiple correlations between various disease features, survival data suggest that a few related conditions excert an unfavourable influence on prognosis: marrow failure - dysfunction and / or hypersplenism (anemia, thrombocytopenia, reduction of hematopoiesis including megakaryocyte count in the bone marrow), extramedullary hematopoiesis (splenomegaly, immature blood cells, level of LDH) and resulting non-specific relevant prediagnostic symptoms. In trephine biopsies of the bone marrow reduction of hematopoiesis was assessed by evaluating the amount of fat cells plus the degree of osteosclerotic lesions. These combined features of histomorphology were correlated significantly with clinical signs and symptoms of marrow failure and thus with a poor prognosis.

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Agnogenic myeloid metaplasia with myelofibrosis (AMM/MF): Clinical course and prognosis of 103 patients.

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AMM/MF is a member of the chronic myeloproliferative syndromes characterized by progressive splenomegaly, marrow fibrosis and anemia, arising from a pluripotent hematopoietic stem cell. We analysed the clinical course of 103 patients with AMM/MF (50 males, 53 females, mean age 57±12 years), seen at our hospital between 1967 and 1986. Laboratory findings at the time of diagnosis: WBC-count 14,2±15,4 x 10^9/l; Hb 10,7±2,5 g/dl; platelets 343±292 x 10^9/l; peripheral blood blasts 1±1%; marrow blasts 2±3%; ANP-score 128±117 (normal or elevated: 90%); LDH 609±418 U (elevated: 86%); marrow fibrosis 96%; osteosclerosis 55%; spleen size 8,9±6,6 cm. Median survival of the patients was 4,3 years with 25% still living at 8,9 years. Of the 69 patients that died 5 (7%) developed acute leukemia. Survival of AMM/MF patients was significantly ($P<0.001$) longer than survival of a contemporaneous group of 230 CML patients (3.5 years) but shorter than in 141 P.vera patients (9.3) years and in 22 patients with essential thrombocythemia (10.4 years). The prognostic influence of several disease parameters at the time of diagnosis was tested: age over 45 years, sex, presence of osteosclerosis, spleen size > 5 cm and percentage of peripheral blood blasts + promyelocytes > 15% had no significant influence on the length of survival.

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RECOMBINANT ALPHA-2b INTERFERON (rIFNα2b) IN THE TREATMENT OF ESSENTIAL THROMBOCYTHAEMIA

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9 patients with essential thrombocythaemia received induction treatment with $5x10^6$ units rIFNα 2b s.c. daily until platelet counts were lower than 450G/l. Thereafter, the same dose of IFN was given every other day for 4-8 months followed by a further stepwise reduction to twice and once a week. In 8 pts. IFN was the primary treatment; 1 pt. had been pretreated with busulfan for 2 months without response. At present 7 pts. are evaluable (4m/3w;age 41-65a, med.54a;hb 120-175 g/l,med.151 g/l;WBC 6,6-18 G/l,med.10G/l; platelet count 780-2100 G/l,med.1600 g/l). 3/7 had bone marrow fibrosis, stage I. Before diagnosis disturbance of peripheral circulation was present in 3 patients (1 x thrombotic complication) ,haemorrhage occurred in 1 pt. Platelet counts were reduced to below 450 G/l in 6/7 pts. within 14-84 days (med. 50 d.),1pt. is still on a daily schedule (59+ d.). 3 pts. after 28 days of daily induction treatment have now been at normal platelet counts for 87+,259+ and 373+ days, whereas 3 pts. (induction treatment for 72-84 days) showed increasing platelet values 28-51 days after dose reduction, so that IFN was again administered daily. In two of them treatment had to be terminated on day 189 and 303 because of a generalized exanthema, which was reversible within a few days after withdrawal. Flu-like symptoms occurred in all patients (7 x increased temperature,1 x chill, 2 x malaise, 2 x vertigo, 3 x fatigue) but disappeared within 1-30 days (median 5 d.) during continuous treatment. Until now, three patients responding promptly to the daily treatment of IFN have had continuous benefit.

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TÜ300: A MURINE MONOCLONAL ANTIBODY AGAINST THROMBOXANE SYNTHASE

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Highly purified thromboxane synthase (E.C.5.3.99.5; 58,800 dalton MM) was isolated from pooled human platelets and injected into BALB/c mice, four times i.m. with 10, 5, 5 and 4 microgram in complete Freund's adjuvant. A fifth i.v. injection with 10 microgram of the purified enzyme was given 4 weeks later. Spleen cells were hybridized with the Ag 8 mouse myeloma line. After screening of several hundred of the primary hybridoma clones in an ELISA, a single culture supernatant revealed specific binding activity against thromboxane synthase at dilutions of up to 1:512. After recloning of this hybridoma, called TÜ300, the monoclonal antibody was used for the preparation of an immunoaffinity column, where it also reacted specifically. In immunoprecipitation TÜ300 bound to the 58,800 dalton enzyme molecule. In addition, the biological activity of thromboxane synthase was blocked by TÜ300. This reagent was then employed in indirect immunofluorescence on normal bone marrow as well as on blast cells from chronic granulocytic leukemias, whereby specific staining of megakaryocytes was documented. Thus TÜ300 appears to be a monoclonal antibody against human thromboxane synthase and may be of value to define defects of blood clotting in man.

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DEGRADATION OF PLATELET AGGREGATING ADP BY ENDOTHELIAL CELLS AND BLOOD

M. Böck*, S. Nees, H. Stiegler*, M. Klug, and E. Gerlach

Platelet aggregating ADP is known to be rapidly degraded within the vascular system. In order to evaluate to what extent vascular endothelial cells (EC) and blood contribute to this phenomenon, we have studied the metabolism of extracellularly applied ADP in a) endothelial cell cultures of macro- and microvascular origin: aorta (AEC), vena cava (VEC) and pulmonary artery (PEC) from pigs, coronary system (CEC) and liver sinus system (SEC) from guinea pigs; b) isolated perfused vena cava segments (IPVS) from rabbits and c) heparinized blood from pigs and rabbits.

Results: In all endothelial cell cultures ADP becomes rapidly dephosphorylated extracellularly to platelet inhibiting adenosine (AR) by means of ecto-ADPase and ecto-5'-nucleotidase. Degradation rates (pmoles/min · 10^6 EC) after addition of 10^{-5} M ADP were: AEC 943±59; VEC 1418±67; PEC 1041±60. While in cultures of microvascular endothelial cells (CEC, SEC) the formed AR is quickly taken up and preferentially degraded to uric acid, the nucleoside accumulates in the medium of all macrovascular endothelial cells (AEC, VEC, PEC). - ADP intraluminally applied to IPVS is also rapidly dephosphorylated (2240±20 pmoles/min · 10^6 EC at 10^{-4} M ADP) and AR accumulates as the main degradation product in the perfusate. - Degradation of ADP in heparinized whole blood proceeds at a rate about 100-1000 times slower than in the incubation medium of EC (0.83±0.2 pmoles/min · 10^6 blood cells at 10^{-5} M ADP).

Conclusions: The rapid elimination of platelet aggregating ADP within the vascular system is mainly due to an endothelium-mediated dephosphorylation. As proposed recently (Gerlach et al. in "Topics and perspectives in adenosine research", Springer Verlag 1987, p.309) the resulting formation of platelet inhibiting AR is considered to be an effective mechanism contributing substantially to the antithrombogenic and antiaggregatory properties of the vascular wall, thus limiting or preventing pathological thrombus formation.

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CORONARY THROMBOLYSIS WITH PROUROKINASE, COMBINATION THERAPY WITH UROKINASE, AND THE POSSIBLE EFFECT OF A "LYTIC STATE".

H. Engler, W. Kasper, S. Hohnloser, K. Hasler, G.W. Löhr

Prourokinase (scu-PA) is a fibrin specific precursor of urokinase (UK). We compared the thrombolytic properties and fibrinolytic side effects of scu-PA alone and in combination with UK. Scu-PA was given to patients with myocardial infarction alone and in combination with a bolus of UK. All Patients received a bolus of 5000 IU of heparin. Fibrinogen was measured with Clauss method, Plasminogen , Antiplasmin with chromogenic substrates. In group I , scu-PA was administred as infusion in a dose of 15 to 60 mg.Complete reperfusion was achieved in 3 patients (n=9) in 57 ± 6 min.,partial reperfusion in 2 patients. In group II, n=18 , scu-PA was given ina total dose of 48 mg and an initial bolus of 200 000 U of UK.Reperfusion was achieved in 15 patients after 38 ± 23 min.
In group II, fibrinogen levels decreased from 272 ± 83 mg% to 163 ± 122 mg% ($p<0.05$), antiplasmin levels from93 ± 18% to 22 ± 20%, plasminogen levels from 80 ± 21% to 42 ± 24%($p<0.05$), while they did not change in group I.
An initial bolus of UK improves thrombolysis in combination with scu-PA. This may be due to the different kinetics (lag - phase of scu-PA) of both or by initiating a thrombolytic state with combination therapy.

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PLATELET HETEROGENEITY FOLLOWING SPLENECTOMY IS NOT ASSOCIATED WITH DETECTABLE CHANGES OF PLATELET FUNCTION IN VITRO AND IN VIVO*

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Experimental evidence suggests that platelet heterogeneity (with respect to size, boyant density, organelle content, and functional capacity) increases after loss of the splenic platelet pool. To further evaluate platelet subpopulations in relation to platelet function, we determined platelet density distribution, platelet aggregation, and platelet secretion in vivo before and 7 to 14 days after selective splenectomy in 9 patients with Hodgkin's disease (HD, n=4), myeloproliferative disorders (MPD, n=5), and idiopathic thrombocytopenic purpura (ITP, n=1). In addition, analysis of mean platelet volumen (MPV) was performed. Mean modal platelet density before splenectomy was 1.061 ± 0.0020 g/cm^3 and did not differ from that after splenctomy (1.061 ± 0.0018 g/cm^3). In contrast, the number of larger platelets increased after splenectomy; MVP rose from 6.74 fl to 7.58 fl. Mean plasma levels of ß-thromboglobulin (ßTG) remained unchanged after splenectomy (ßTG, pre- vs post-splenectomy, HD: 40 vs 41 ng/ml; MPD 104 vs 117 ng/ml). In patients with MPD aggregation defects in response to adrenaline or ristocetin were still present after splenectomy. In patients with HD aggregation remained normal. These findings confirm that the number of larger platelets is increased after splenectomy. However, the presence of this circulating platelet subpopulation does not lead to detectable changes of platelet function in vitro and in vivo. Thus, the clinical relevance of platelet heterogeneity following splenectomy remains a matter of controversy at the present time.

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PLASMATIC COAGULATION AND FIBRONECTIN DURING TREATMENT OF AML WITH INTENSIVE CHEMOTHERAPY

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Parameters of the plasmatic coagulation and fibronectin were studied in 23 patients with AML (4 M1, 10 M2, 4 M4, 5 M5). Tests were done daily during induction chemotherapy and every other day afterwards for another 10 days. Statistically significant normalization occurred in the global test of prothrombin-time. Significantly increasing values were observed for fibrinogen, factor II, V, plasminogen and alpha-2-antiplasmin after some days of treatment. Factor VIII: AG levels rose immediately after maximum reduction of the tumor burden. Moderately elevated levels of FDP were found. Decreasing levels for factor XIII and fibronectin were observed in the later course. At III levels were without characteristic changes. All the observed changes were more pronounced in patients with initial leukocyte counts below 30 000/qmm. There were statistically significant differences in prothrombin-time, factor II, V, AT III, plasminogen and alpha-2-antiplasmin. Bleeding tendency was comparable in patients with high or low leukocyte counts however. Patients with initial fibronectin values below 30 mg/dl had a significant higher risk of subsequent infection. As thrombin-antithrombin-complex levels were elevated, we regard the observed changes partly as consequence of a disseminated intravascular coagulation with activation of thrombin.

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TREATMENT RESULTS OF IDIOPATHIC THROMBOCYTOPENIA (ITP)+

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To evaluate the best treatment of ITP we studied 27 patients, 9 men and 18 women, in the period 1980-1985. The cardinal symptoms were: purpura (74%), bleeding (70%), epistaxis (30%), hypermenorrhea (30%). The platelet count ranged from 1500 - 84000 /ul with a mean of 13250 /ul. Platelet bound IgG (PAIgG) was measured in 8 patients and was positive in 6 cases. Bone marrow megakaryocytes were increased in 82%.
68% of the patients responded to treatment with 100 - 250 mg prednisone per day after 10 respectively 20 days. Altogether, we observed a complete remission in 48% after maximal first treatment with cortisone, splenectomy, immunglobulins and chemotherapy. After splenectomy 5 from 10 patiente relapsed. 4 patients were treated with immunglobulins. All 4 responded, but showed no sustained remission. After each relapse we achieved a further remission of 50%. The concentration of PAIgG was a good parameter in the clinical course.

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+ Dedicated to Prof. Dr. G.W. Löhr on his 65th birthday

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CHRONIC IDIOPATHIC THROMBOCYTOPENIC PURPURA - 51 CR PLATELET KINETIC STUDIES - RESULTS OF SPLENECTOMY

M. Gietz, E. Hiller, W. Mempel, W. Wilmanns

51 Cr-platelet kinetic studies were performed in 78 patients with chronic ITP. The incidence of splenic, spleno-hepatic and hepatic sequestration site was 78%, 10% and 12% respectively. In 41% of the patients platelet survival was extremely shortened to 0 - 3 hours, whereas only 26% of the patients had a survival time of more than 24 hours. Those patients with low platelet counts also had a very short survival time, whereas patients with higher platelet counts ($>$ 50 000/mm^3) had longer survival times. 51 patients (65%) were splenectomized following the kinetic studies. 73% of the patients with splenic sequestration site had normalized platelet counts and 19% platelet counts between 80 000 - 149 000/mm^3 12 months after splenectomy (92% full and partial remission). Of the 13 patients with hepatic and hepatic/splenic sequestration site 5 patients had full remission, 4 partial remission, 2 patients had minimal improvement whereas 3 patients were treatment failures.
Thus determination of the sequestration site is definitely of prognostic value. However, this time consuming and expensive test should not be mandatory since most patients (78%) have a splenic sequestration site and of the remaining 25% half of the patients may reach a full or partial remission in spite of an unfavourable sequestration site.

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NEONATALE ALLOIMMUNE THROMBOCYTOPENIA (NAIT): STUDIES WITH A QUANTITATIVE COMPETITIVE IGG-ELISA (cELISA) AND IMMUNOBLOTTING

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A diagnosis of NAIT was established by demonstrating platelet specific antibodies (anti Pl^{A1}, n=2, yet unspecified, n=1) in the mother's sera in 3 different families. In cases with anti-Pl^{A1} antibodies HLA immunization was found. The cELISA (V. Kiefel et al, Vox Sang 1987) was modified to quantitate in-vitro antibody binding onto platelets. One of the fathers was homozygous for the Pl^{A1} antigen ,the other one was heterozygous. A complete antigen-pedigree was drawn in these two families. Western blot analysis showed strong reactive sera with a 90kD band on non-reduced platelets corresponding to glycoprotein (GP) IIIa. One of these sera stained a further band at 110kD. In the third case serum of the propositus' mother was reactive with the father's platelets, and with platelets of known HLA, $Pl^{A1/2}$ and Bak^{a} specificity and with thrombasthenia Glanzmann platelets. Immunoblotting demonstrated a band at 75kD, presumably GP V.
We conclude that cELISA is a feasible technique for drawing a pattern of inheritance of platelet specific antigens. Immunoblotting assists in the characterization of target platelet antigens and their identification.

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ANALYSIS OF CHILDHOOD ACUTE LEUKEMIA WITH MIXED-LINEAGE FEATURES BY A MULTIPARAMETER APPROACH

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Standard morphologic/cytochemical techniques and a large panel of monoclonal antibodies were used to analyze the incidence and prognosis of acute leukemia with mixed-lineage features in 580 children enrolled in the ALL- and AML 83 studies. Of 500 patients with ALL, 20 (4%) showed evidence of myeloid-related antigens, whereas blasts from 10 of 80 children (8%) with AML expressed lymphoid-associated antigens. In 16 of these patients, adequate cell numbers were available for determining the biologic phenotype of leukemic cells by more extensive studies, including Ig and T cell receptor gene rearrangement and DNA flow cytometric analyses. The immunologic and molecular-genetic findings suggest that in 10 children malignant transformation occurred in a progenitor cell with the potential for both lymphoid and myeloid differentiation. Cytogenetic studies, successfully carried out in 4 of these children, disclosed 11q23 translocation-associated leukemias [t(4;11), n=3; t(9;11), n=1]. The poor treatment outcome in these cases indicated that the future design of chemotherapeutic protocols should consider their mixed-lineage features. Ambiguous phenotypes in the remaining 6 patients probably resulted from aberrant gene expression or insufficient reagent specificity. In none of the 16 children did flow cytometry disclose DNA aneuploidies consistent with a common clonal origin of these cases.

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Adult acute leukemia with unusual phenotype: Clinical review of five cases

C. Tirier, J. Kraft, R. Becher, E. Thiel, F. Wendt

In recent publications discrepances between morphological/cytochemical, immunological and cytogenetical findings are reported more often than expected. This cases are associated with poor prognosis.
We are reporting about five cases with acute leukemia in adults with unusual morphological/cytochemical, immunological and cytogenetical laboratory features.
In one case L3 morphology was in contrast with immunological findings of monocytic antigens, cytogenetical studies showed t(8,14)!
Another case with L2 morphology showed monocytic surface antigens.
One case with L1 morphology couldn't be classified at all (Tdt+,CALLA+, My+). No cytogenetical abnormalities were found.
Blast cells of two patients with L1 morphology expressed HLA-DR and T-cell as well as myeloid antigens in one case, HLA-DR, CALLA, B- and T-cell surface antigens in the other case. Hyperdiploid metaphases were found in both cases.
Response to chemotherapy was poor in the first three patients: Remission couldn't be achieved neither with standard ALL/AUL chemotherapy, nor with TAD-9, HDAra-C/Mitoxantrone or /m-Amsacrine.
The latter patients achieved complete remission by treatment with ALL/AUL high risk protocol, ongoing 12+ respectively 15+ months.

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QUANTITATION OF THE mRNAs CODING FOR B- AND T-CELL SPECIFIC RECEPTORPROTEINS IN LEUKEMIAS OF DIFFERENT MATURATION STAGES BY IN SITU HYBRIDIZATION WITH FLUOROCHROME LABELED GENE PROBES
I. THE EXPRESSION OF /u mRNA INCREASES DURING B-CELL MATURATION

P. Mar***, K. Pachmann*, B. Doerken**, K. Reinecke***, B. Emmerich* and E. Thiel***

In order to determine quantitatively the expression of cellular mRNA gene probes were directly fluorochrome labeled and hybridized to cells attached to slides by cytocentrifugation. Determination of the fluorescence intensity of probe bound to cellular mRNA by microfluorimetry allowed to investigate the amount of mRNA during cell surface marker expression and thus to correlate the expression of specific mRNA to cell differentiation. With increasing expression of B-differentiation markers the amount of mRNA increased. There were populations which had very high values of expression, preceeding and correlated to the expression of IgM. With further maturation into expression of additional immunoglobulin chains /u mRNA faded lacking in CLLs which had completely switched from IgM to another immunoglobulin. The expression of the T-cell receptor genes was determined also, and some leukemias showed positive values in addition to the /u mRNA. - Investigations on the bearing of these results in leukemias to normal mRNA expression are in progress.
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DETERMINATION OF THE GROWTH FRACTION IN ACUTE LEUKEMIA USING THE MONOCLONAL ANTIBODY KI-67.

S. Schwartz, R. Schwarting, W.-D. Ludwig, J. Gerdes, and H. Stein

The growth fraction of untreated acute leukemias of 123 children (BFM-study patients) including 0-ALL (n=9), c-ALL (n=83), T-ALL (n=16) and AML (n=15) was determined in peripheral blood or bone marrow cells using the monoclonal antibody Ki-67 which recognizes a nuclear antigen associated with cell proliferation. The antigen was visualized applying the alkaline phosphatase anti-alkaline phosphatase (APAAP) staining procedure on cytopreparations or, in some selective cases, by flow cytometric analysis of fixed cells. The latter provides a rapid means to quantify the number of cycling cells and allows the assignment of cell surface markers to the proliferating cell population by double immunofluorescence. The mean value of Ki-67 positive cells of the ALL's was in the range from 28 (0-ALL's) to 39% (c-ALL's). The mean value of Ki-67 cells in AML's was 20%. Interestingly, within the immunologic subtypes there was a large variation of Ki-67 positive cells suggesting that leukemias of the same type might show significant differences in their biological behavior. As yet, the follow up is to short to evaluate the clinical significance of Ki-67 expression in acute leukemia. Extended observation period might reveal important information for future treatment protocol.

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SURFACE MARKER ANALYSIS IN CHILDHOOD ACUTE MYELOGENOUS LEUKEMIA (AML): DIAGNOSTIC VALUE AND PROGNOSTIC IMPACT ON THERAPY

W.D. Ludwig, U. Creutzig, J. Ritter, A. Gatzke and G. Schellong

The immunophenotype of blast cells in 127 children with AML was prospectively analyzed with a panel of monoclonal antibodies (MoAbs) recognizing differentiation-associated antigens of the myeloid lineage (CD11b, CD13, CD14, CD15, CD33, CDw41, Glycophorin A), T or B cell lineage (CD7, CD19) and non-lineage-restricted antigens (OKIa1, CD10). All children were included in the cooperative trial AML-BFM 83 and treated in a uniform fashion. In our study, it was possible to clearly discriminate FAB subtypes M1/2, M3, M4/M5b in about 65% of the cases by immunophenotyping; however, a substantial proportion of M4 and M5a showed little or no reaction to monocyte markers. The potential value of surface marker analysis was proved in 5 children originally unclassifiable by morphology. Three of the cases expressed early myeloid antigens (CD13, CD33) and 2 disclosed megakaryocyte differentiation by the presence of GP IIb/IIa. In our experience, only My7 expression was associated with lower CR rates (76% vs. 90% for My7 - cases), whereas no significant differences with regard to event-free survival (EFS) have as yet been observed among any of these antigens. TdT positivity (≥10%) occurred in 17% of the patients, mainly in FAB M1/M2 subtypes. In contrast to other reports, response to treatment and EFS was not worse within this subgroup. Analyses comparing the relationship of surface antigen expression to clinical prognostic factors such as hyperleukocytosis, CNS involvement, and extramedullary disease will be presented.

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AUTOCRINE GROWTH FACTORS IN CONDITIONED MEDIA (CM) OF PERMANENT CULTURES OF ACUTE MYELOMONOCYTIC LEUKEMIA

K.H. Pflüger, A. Grüber, H. Köppler, M. Klausmann and K. Havemann

Continuous proliferation of many leukemic cell lines is only maintained in serum containing culture media. We adapted three human myelomonocytic cell lines EW 2, LG 3 and MS 6 to serum free growth in media supplemented with transferrin, insulin and selenium (SIT). Proliferation inducing activity was tested in the CM of LG 3 and EW 2 on LG 3, EW 2, MS 6 and HL 60 as target cells employing 3H thymidin incorporation, cloning assay in soft agar and cell counting. The proliferation inducing activity in CM was further characterized by gel chromatography, DEAE chromatography and reversed phase HPLC (C4).

Four distinct fractions with proliferation inducing activity could be demonstrated. The most potent growth factor so far characterized was transferrin produced by the cell lines (in addition to transferrin used as a supplement) as shown by immunological methods including a radioimmuno-precipitation assay. In addition to these stimulating components we could demonstrate fractions showing proliferation inhibition. These data suggest that in vitro growth of human leukemic cells of myeloid or myelomonocytoid origin is regulated by growth stimulating and inhibitory activities in an autocrine manner.

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DIAGNOSTIC IMPLICATION OF ULTRASTRUCTURAL DEMONSTRATION OF MYELOPEROXIDASE IN ACUTE UNCLASSIFIED LEUKEMIAS.

G. Heil, E. Gunsilius, A. Raghavachar, E. Thiel, E. Kurrle, H. Heimpel

15 cases of acute leukemias, which remained unclassified after routine morphological and cytochemical analysis have been studied by electron microscopy using ultrastructural demonstration of myeloperoxidase (POEM). The immunological diagnosis of these cases was characterized by the absence of reactivity with the anti-T-cell antibodies (Leu 1, OKT 11) and CALLA and negativity for expression of cytoplasmatic Ig. 10/15 cases could be clearly identified as early myeloid leukemias by detection of myeloperoxidase activity in the membranous structures such as the nuclear envelope, the endoplastic reticulum and the Golgi complex or in small granules in more then 5 % of the blasts in the peripheral blood or in more than 10 % of the blasts in the marrow samples. 6 of these POEM-positive cases had been characterized as myeloid null-AL(L) by immunological analysis (HLA-Dr, TdT, VIM D5, VIM 2, My 9), whereas the immunological phenotype of 4 POEM positive cases was completely undefined. On the other hand 4 cases were classified as myeloid null-AL(L), while no myeloperoxidase was detectable so that definite lineage affinity of these cases remains unclear. These data indicate that ultrastructural analysis of myeloperoxidase activity is helpful to identify early myeloid leukemias among the subgroup of acute unclassified leukemias.

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QUANTITATION OF THE mRNAs CODING FOR B- AND T-CELL SPECIFIC RECEPTOR PROTEINS IN LEUKEMIAS OF DIFFERENT MATURATION STAGES BY IN SITU HYBRIDIZATION WITH FLUOROCHROME-LABELED GENE PROBES II. EXPRESSION OF RECEPTOR GENE RNAs IN T-DETERMINATED LEUKEMIAS AND ABERRANT EXPRESSION IN LEUKEMIAS CARRYING MARKERS OF NON-LYMPHOID LINEAGE AFFILIATION

K.Pachmann,P.Mar,K.Reinecke,B.Dörken,B.Emmerich,E.Thiel

26 leukemias expressing T-lineage markers and 20 leukemias expressing non-lymphoid markers were investigated for their quantitive expression of T- and B-cell antigen receptor gene mRNA. Whereas the mRNA for the Tα chain was regularly expressed in all T-leukemias,the mRNA for the Tβ chain was expressed variably. However,mRNA for the u heavy chain of the immunoglobulin was also found in some T-leukemias. Transcription of these genes was also found in immature leukemias carrying myeloid markers. In some very immature leukemias,expression was even higher than in mature leukemias of the respective lineage. Thus even though antigen receptor mRNA expression is correlated with maturation in the respective lineages,it cannot be regarded as line specific. Therefore only multiparameter studies may give conclusive results.

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ISOLATION AND PURIFICATION OF HEXOSAMINIDASE ISOENZYMES FROM HUMAN PLACENTAE AND LEUKEMIA CELL LINE REH

R. Schedel, J.R. Novotny, H.G. Drexler and G. Gaedicke

ß-Hexosaminidase (ß-N-acetylglucosaminidase, E.C. 3.2.1.30) occurs mainly in two forms: Hex A and B. A third isoenzyme, Hex I, is expressed in certain subtypes of acute leukemia (for example, most prominently in common ALL). This Hex I has been shown to be a homopolymer enzyme being composed of two ß-chains (as in the "physiological" form of Hex B). However, there are certain characteristic differences (i.e. molecular weight, isoelectric point, etc.) between Hex I and Hex B. Hex A is a heteropolymer enzyme of one alpha- and one ß-chain. The analysis of the processing of the ß-chain-precursor suggests a abnormal glycosylation of the peptide chains in acute leukemia cells. For further investigations we isolated the total Hex activity from human placenta tissue and from the leukemia cell line REH. Using established biochemical methods (Con A-sepharose column chromatography, affinity chromatography, DEAE-ion exchange chromatography, cation exchange chromatography and gel filtration), we were able to purify the respective isoenzymes some 6,000 fold.
This purified material will enable us a) to perform further studies on the processing of the Hex isoenzymes (which appears to be deranged in leukemic differentiation), and b) to generate antibodies specific for the alpha- and ß-subunit.

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PROGNOSTIC SIGNIFICANCE OF SURFACE MARKERS IN AML

M. Freund, J. Kemnitz, B. Menzel, H. Link, H. Diedrich, H.J. Wilke, and H. Poliwoda

We have studied bone marrow and peripheral blood smears in patients with AML with a panel of monoclonal antibodies including AML2-23, BI-3C5, FMC10, Glycophorin-A, GP IIb-IIIa, Ki67, MY4, MY7, MY9, Mo1, OKM1, OKM5, PM81, PMN6, PMN29. The immunohistochemical method of alkaline phosphatase in "sandwich" modification was applied. Though this study is retrospective, and though there is an imbalance in respect of age in some groups, some monocytic markers and the proliferation marker Ki67 may be of prognostic significance as summarized below in preliminary results:

marker		n	M1	M2	M3	M4	M5	M6	M7	med.age	CR%	med.surv.
MO1	+	41	3	19	1	13	5	0	0	51.7	56	9.2 mo
	-	8	3	2	1	1	1	0	0	53.4	62	34.4 mo
MY4	+	26	1	8	1	12	4	0	0	49.2	54	9.2 mo
	-	21	4	13	1	1	1	1	0	54.2	62	15.5 mo
OKM1	+	11	1	2	0	4	4	0	0	64.9	19	2.0 mo
	-	24	6	11	0	5	2	0	0	47.9	54	9.2 mo
OKM5	+	8	1	0	0	4	3	0	0	45.7	25	1.7 mo
	-	21	6	8	0	5	2	0	0	49.7	62	11.0 mo
Ki67	≥30%	7	0	6	0	1	0	0	0	54.2	57	8.7 mo
	<30%	7	1	2	2	0	2	0	0	52.7	71	n.r.

(+ = ≥20% positive cells; n.r. = not reached)

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INTERFERON ALPHA 2 IN THE TREATMENT OF ERYTHROLEUKEMIA

G. Steger, J.D. Schwarzmeier*, C. Dittrich and K. Moser

The clinical course of erythroleukemia (FAB M-6) is similar to other forms of AML. The predominant cells are proerythroblasts, but erythroid precursors in all stages of maturation may be seen in BM and PB. Since it is a rare disorder serial studies on the efficacy of different treatment schedules have not been performed and in general a chemotherapy according to AML protocols is recommended.

We report on a 43 years old male patient with acute erythroleukemia (AEL) who was initially treated with thioguanine, ara-C and daunomycin (TAD protocol) to which he responded with CR. After 10 months he relapsed and he was now put on ara-C and adriamycin ("7 & 3"). However, only a minor response was achieved. Now, a trial with Interferon alpha (IFN-α2) was started. The patient received 10 Mio Units IFN-α2b sc./day for three weeks. The treatment had to be discontinued because of pancytopenia (platelets < 15.000, wbc < 800/µl). At this time the spleen (10 cm below costal margin before IFN) had decreased considerably in size. During the following weeks a gradual improvement of pancytopenia was noted and the patient came into PR with no blasts in PB and less than 10 % in BM. - He is now in a stable remission for more than 4 month. - These data point to a possible role for IFN-α in the treatment of AEL.

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CALCITONIN-RELATED PEPTIDES IN ACUTE LEUKEMIA: PRODUCTION AND EVIDENCE FOR BIOLOGICAL EFFECTS

K.H. Pflüger, A. Grüber, P. Kiefer, H. Köppler and K. Havemann

An ectopic production and secretion of peptide hormones by several malignancies including acute leukemia is well established.
In 77 patients with acute leukemia a high incidence of elevated serum levels of calcitonin-related peptides was found: human calcitonin (h-CT) in 47 %, calcitonin gene-related peptide (CGRP) in 51 % and salmon calcitonin (s-CT) in 21 %. A coincidence between these three hormones could not be demonstrated. Elevated serum levels of these peptides were significantly connected with more immature forms of acute leukemia such as M1 and AUL. Increased h-CT but not CGRP and s-CT was significantly correlated with poor prognosis such as early death during the first 4 weeks after diagnosis. Using multivariate analysis age and serum level of h-CT were found to be the most important prognostic factors in patients with acute leukemia.
All three peptides were demonstrable in conditioned media (CM) and/or cell extracts of the permanent leukemic cell lines LG 3, MS 6, EW 2, HL 60, KG 1 and K 562. After induction of differentiation of HL 60 cells in presence of PMA a marked increase of specific mRNA coding for h-CT was observed after 15 h using northern blot techniques. Furthermore, in media with low calcium content calcitonin induced a moderate dose dependent stimulation of 3H thymidine incorporation of HL 60 cells. These data indicate that calcitonin-related peptides beside as markers for prognoses may serve as autocrine growth factors in acute leukemia.

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DETECTION OF Ph1-POSITIVE CHRONIC GRANULOCYTIC LEUKEMIA (CGL) 33 MONTHS AFTER DIAGNOSIS OF c-ALL-ANTIGEN POSITIVE ACUTE LYMPHOBLASTIC LEUKEMIA (c-ALL)

W.A. Flegel (1/2), J.U. Böhlke (1), W. Kern (1), B. Anger (1), An. Raghavachar (2), H. Heimpel (1)

A 40 year old male presented with c-ALL and was treated with the BMFT-regimen for ALL. During remission, 33 months after diagnosis of c-ALL a typical CGL with leucocytosis, Ph1-chromosome and c-abl/bcr translocation was diagnozed. At this time the initial findings at diagnosis of c-ALL were reevaluated. Features of CGL could be found up to one year before diagnosis of c-ALL (slight leukocytosis, myeloblasts in peripheral blood and anemia). However no cytogenetic studies were available at diagnosis of c-ALL.

It could be possible that the patient actually presented with ALL and CGL developed as a second malignancy. This seems unlikely because features of CGL were retrospectively found for a period of one year before diagnosis of c-ALL. We suggest, that the patients primary disease was CGL and that he developed a lymphatic blast crisis. Treatment according to the BMFT-protocoll for ALL was highly successful, a complete hematological remission was achived and lasted 33 months before CGL relapsed. The patient is alive 45+ months after blast crisis in chronic phase of CGL.

We conclude, that the intensive chemotherapy for ALL was a successful approach of lymphatic blast crisis of CGL in this patient. In our view, this could justify an ALL-adopted treatment in other patients with lymphatic blast crisis of CGL.

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EFFICACY OF α-INTERFERON TREATMENT IN AN UNUSUAL CASE OF PH-POSITIVE LEUKEMIA

O.A. Haas 1, W. Mor 1, C. Bartram 2, P. Ambros 1, G. Gastl 3, H. Gadner 1

α-interferon is an effective treatment for chronic phase CML. It commonly results in haematologic remissions accompanied by at least partial eradication of the Ph-positive cell population.
We report our experience with this therapy in an unusual case of Ph-positive leukemia occurring in a 13 8/12 year old girl. At onset it appeared as CALLA-positive ALL with 100 % Ph-positive mitoses in the bone marrow. Treatment was initiated according to the BFM 81-ALL medium risk protocol and included 18 Gy prophylactic CNS irradiation. She experienced two isolated CNS relapses II and 30 months after diagnosis, respectively, which were treated according to intensive reinduction protocols with 30 Gy CNS and 24 Gy spine irradiation. As commonly observed in Ph-positive ALL, partial cytogenetic normalization with up to 95 % normal bone marrow mitoses was achieved during this period. However, 48 months after diagnosis (i.e. 6 months after end of therapy) chronic phase CML developed. Following 7 months of therapy with intermittent doses of hydroxyurea, interferon treatment was started with 6 mcg interferon α-2c (Berofor 2, R; Boehringer Ingelheim) and consecutively adapted to the haematological values. At present, 68 months after diagnosis and 13 months after initiation of interferon therapy, the patient is in a persistent and complete haematological and partial cytogenetic (40 % normal bone marrow mitoses) remission. Molecular genetic studies, which contribute to the delineation of Ph-ALL from CML, are currently in progress and may lead to a more precise classification of this clinically and cytogenetically unusual leukemia.

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MYELOSARCOMATOSIS WITH GENERALIZED SKIN INVOLVEMENT PRECEEDING AMML

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Myelosarcoma is a rare precursor of acute myelocytic leukemia. Most common sites of involvement are bone, periosteum, soft tissue, lymph nodes and skin. We present the case of a 76 year old patient, who was admitted because of multiple skin nodules. Beginning on the left arm, the tender nodules generalized within a few weeks mainly in regions not exposed to light. The skin biopsy revealed an infiltration of blastic cells with a positive NASD-chloracetate-esterase reaction. This indicated a myeloproliferative disease. However, at the time of skin biopsy there was no evidence of acute leukemia. Six months after the first skin lesions, acute myelomonocytic leukemia (FAB M 4) developped.
To our knowledge this is the first case report of a generalized myelosarcomatosis of the skin preceeding acute leukemia.

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PALLIATIVE TREATMENT OF ACUTE LEUKEMIAS WITH LOW DOSE MITOXANTRONE

Th. Hecht, M. Henke, K. Bross, G.W. Löhr

Mitoxantrone has known activity in the treatment of acute leukemias. It is supposed to be less cardiotoxic than the anthracyclines but produces prolonged myelosuppression. We have used therefore this drug in patients with acute leukemia as palliative treatment for control of the leucocyte count, when remission induction therapy was not possible or indicated. Nine patients have been treated so far for eighteen treatment episodes with no severe non-hematologic side effects. Median age was 51 years(range 22-66), median leucocyte count 33700/ul (range 10.000-90.000) and median platelet count 79100/ul(range 14.000-180.000). Circulating blasts ranged between 10% and 90%. 10-20 mg mitoxantrone were daily administered for one to three days. The leucocyte count returned to normal values in all treatment episodes, reaching the nadir between three and ten days after initiation of therapy. Severe thrombocytopenia occured in three patients, who already presented with a low platelet count. When mitoxantrone was administered every two to three weeks fast rising leucocyte counts were consistently controlled in leukemia patients by moderate platelet toxicity.

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PNEUMONIA IN PATIENTS WITH MALIGNANT DISEASES.

B. Jany, P. Meyer

Patients (pts.) with immunedeficiency are subject to a wide variety of serious pulmonary infections with a high mortality. These pts. may also develop noninfectious pulmonary infiltrates related to their underlying diseases or secondary to radiotherapy and/or cytotoxic drugs. The different therapeutic approaches of infectious and noninfectious pulmonary infiltrates require the establishment of an etiologic diagnosis. We studied the value of the combined use of blood cultures, fiberoptic bronchoscopy, and sputum culture in 36 pts. with malignant diseases, which developed a radiologically documented pulmonary infiltrate with or without fever. 31 pts. had malignant hematologic diseases, 5 had solid tumors. In 21 pts. (58.3 %) an etiologic diagnosis could be established by combining the diagnostic procedures. In 21 pts. the infectious etiology of the infiltrate could be documented (20 bacterial, 1 fungal). 2 different etiologic microorganisms were identified in 6 pts.. 12 pts. (33.3 %) died of pneumonia. 13/15 blood cultures identified the etiologic microorganism. Fiberoptic bronchoscopy including biopsy, brushing, bronchoalveolar lavage, and transbronchial biopsy was performed in 9 pts.. All 9 procedures identified the etiologic agent. 11/19 sputum cultures gave positive results, in 3/19 cases the etiologic agent was identified by sputum culture solely. We conclude:

1. Mortality of pneumonia in immunedeficient pts. is high.
2. Combination of common diagnostic procedures will identify the etiologic infectious agent in about 60 % of pts..
3. In our pts. fiberoptic bronchoscopy was the most sensitive diagnostic method.

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INFECTION PREVENTION IN ACUTE LEUKEMIA PATIENTS - SUSCEPTIBILITY PATTERNS OF POTENTIAL PATHOGENS UNDER LONG TERM ADMINISTRATION OF CIPROFLOXACIN AND NORFLOXACIN

Maschmeyer, G., Hövelhaus, B., Wendt, F.

Fourty-four patients with acute leukemia undergoing aggressive remission induction chemotherapy were treated with ciprofloxacin or norfloxacin for infection prevention during severe granulocytopenia over at least six weeks. Faeces, urine and oral washings were cultured twice weekly for bacteriological and mycological surveillance. The susceptibility of documented potential pathogens to the 4-quinolones was observed from 1984 through 1987. Preliminary results show no significant emergence of gram-negative bacilli apart from primary resistant strains of Pseudomonas maltophilia, but selection of resistant enterococci and, in single cases, decreasing susceptibility of Staph.aureus under long term administration of the drugs. Clostridia and other anaerobic bacteria were not affected by the 4-quinolones.

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AUTOMATED RECOGNITION OF ABNORMAL GRANULOCYTE FUNCTION IN SEPSIS AND TRAUMA PATIENTS BY FLOW CYTOMETRIE

G. Rothe[1], W. Kellermann[2], G. Valet[1]

Sepsis- and trauma-related organ failure is intimately related to granulocyte function both through the protective role of granulocytes against bacteria and through the endothelial injury caused by oxygen radicals and proteases released by activated neutrophils. Phagocytosis and degradation of bacteria, intracellular pH, esterase activity, spontaneous and stimulated respiratory burst activity, amount of cells dying during phagocytosis and cell volume of human neutrophils were measured by flow cytometry as indicators of risk, course and prognosis of sepsis- and trauma-related pulmonary and cardiovascular organ failure with 206 blood samples from 48 patients of an intensive care unit. 42 Parameters were calculated from 3 different flow-cytometric list-mode measurement s per sample. The samples were classified into four categories: Septic failure, post-traumatic failure, no severe organ failure ans transition stages. As evaluated by Kruskal-Wallis one way analysis of variance 20 of these 42 flow-cytometric parameters showed significant differences between the groups (p 0.05). More than 76 % of the samples could be classified correctly by 3 canonical discrimant functions. The imminence of sepsis resp. posttraumatic organ failure was recognized three days in advance in more than 81 % of the samples. This is important for the early onset ot treatment.

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MOLECULAR CLONING AND EXPRESSION OF EPSTEIN-BARR VIRUS GENES IN E. COLI

G. Doelken and A.D. Riggs

Three cosmid clones covering about 70% of M-ABA-EBV-DNA were used to construct three libraries of EBV-DNA fragments in lambda gt11. By the nature of the lambda gt11 vector, the cloned DNA fragments are expressed as beta-galactosidase fusion proteins if inserted in the proper reading frame. The libraries were screened with a pool of high anti-EBV positive human sera. As the detection system we used biotin-labeled secondary antibodies and streptavidin-conjugated alkaline phosphatase. Four clones have been selected from these libraries for further characterization: two clones carry early EBV genes, one of them codes for the 98 kDa subunit of EBV-induced ribonucleotide reductase. The third clone expresses a fusion protein from a large 3.6 kb insert, which has not yet been shown to be transscriptionally active in EBV-transformed cell lines. The fourth clone carries a 1.9 kb fragment from the Bam HI R/f/K region of the EBV-DNA. 500 base pairs belonging to the BRRF-2 open reading frame are expressed as a beta-galactosidase fusion protein of 160 kDa. Sequencing of this lambda gt11 clone revealed the reading frame of BRRF-2, and the amino acid sequence of the fusion protein could be determined. Human antibodies bound and eluted from the partially purified fusion protein reacted with an EBV-induced protein of 68 kDa in the producer cell line B95-8.

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TREATMENT OF SEVERE CMV-INFECTIONS WITH GANCYCLOVIR WITH OR WITHOUT CMV-HYPERIMMUNOGLOBULIN(SANDOGLOBULIN CMV)

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We started a trial to test the clinical efficacy of Gancyclovir for treatment of severe CMV-infections in immunocompromised patients. Nine patients with life-or sightthreatening CMV-infections were treated either with Gancyclovir alone or Gancyclovir and a CMV hyperimmunoglobulin (HIG). Four patients had received a marrow graft,two were solid organ transplant recipients and three patients suffered from HIV infection. Major clinical manifestations were pneumonitis (6/9),chorioretinitis (3/9),hepatitis (1/9) and pericarditis (1/9).All patients were treated with 2.5mg/kg thrice daily.Five of the patients received a combination of Gancyclovir and 20 gCMV HIG every alternate day. Seven out of nine patients showed significant improvement of their clinical symptoms. Two patients with advanced interstitial pneumonitis after BMT died despite treatment. One patient, who showed a complete response to treatment, relapsed and died because of disseminated CMV disease.Two patients suffering from AIDS died from concomitant diseases(Pneumocystis carinii pneu., Kaposi's sarcoma).Four of the patients are still alive and well without evidence of CMV disease.Therapy had to be discontinued because of hematotoxicity in four patients. All of them had shown significant cytopenia at the beginning of therapy. These preliminary results suggest that Gancyclovir is effective for treatment of severe CMV disease. No obvious additional beneficial effect of HIG was observed.

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IN VITRO IMMUNOLOGICAL STUDIES IN PATIENTS SUFFERING FROM CHRONIC LYMPHOCYTIC LEUKEMIA (B-CLL) AND FREQUENT INFECTIONS

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The frequency of infections in patients suffering from B-CLL is known to increase with falling levels of immunoglobulins. Increasing duration of the disease results in diminution of antibody production which correlates to the amount of monoclonal B-lymphocytes which do not mature into immunglobuline (Ig) secreting plasma-cells. The lack of regulation by T-cells is not fully understood. Therefore we measured in 79 patients in different stages of B-CLL the Ig levels and determined the lymphocyte subpopulations either of the malignant monoclonal B-cells as well as the polycolonal T-cells. The frequency of infections, mostly being in the upper and lower respiratory tract, was documented in the period of october 1984 until april 1987. In about 70 % of the patients low Ig levels were shown decreasing with duration of the disease. The ratio of CD4 + and CD8 + cells was lower than in a age and sex matched normal population (median values: 1.7 vs. 2.6) Following values were measured: HLA-DR + cells: 83 %, CD 5 + cells: 94 %, pan-B-cells: 74 %. The subpopulations of the polyclonal T-lymphocytes were: CD 3 + cells: 18 %, CD 4 + cells: 12 %, CD 8 + cells: 7 %.
Our study shows that the helper-suppressor-ratio, diminution of T-cells and antibody levels correlate with the frequency of infections in patients with B-CLL.

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SEVERE EPSTEIN-BARR VIRUS INFECTION WITH LYMPHADENOPATHY MORPHOLOGICALLY INDISTINGUISHABLE FROM HODGKIN'S DISEASE.

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The increased incidence of Hodgkin's disease (HD) in persons with a history of infectious mononucleosis (IM) and the frequent finding of elevated antibodies to Epstein-Barr virus (EBV) capsid antigens in patients with HD suggest a possible relationship between both disorders. In this report a 18-year-old boy is described who was admitted to the hospital because of fever,general lympha - denopathy,necrotizing tonsillitis and hepatosplenomegaly.The clinical course was complicated by involvement of the lungs requiring respirator therapy.Although the hematological findings where not characteristic for IM,this diagnosis could be confirmed by a positive Paul-Bunnell test and elevated EBV-associated antibodies(EBV-IgG 1:5120,EBV-IgM 1:80).Enlarged lymph nodes were removed for histological examination which showed a mixed cellular reaction with presence of numerous Sternberg-Reed cells, suggesting the diagnosis of HD.The specimens were examined by a number of reference pathologists.Additional immunoperoxidase stains with various antibodies did not allow a definite diagnosis.After treatment with glucocorticoids, vincristine and cyclophosphamide in reduced dosage, the lymphadenopathy,hepatosplenomegaly and the pulmonary infiltrates disappeared rapidly.Three months after onset of the disease the boy is in good condition and all examinations including CT scans are normal. In conclusion this case illustrates that EBV infection may cause morphological changes similar to those seen in HD.In regard of the rapid and stable remission after one course of chemotherapy,the diagnosis of HD is unlikely,although follow-up studies are required for definite exclusion of malignant lymphoma.

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CHEMILUMINESCENCE (CL) FROM HUMAN GRANULOCYTES (PMN) AFTER BONE MARROW TRANSPLANTATION (BMT)*

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Monitoring CL from stimulated PMN can predict the microbicidal potential of these cells. Since functional defects may explain the increased susceptibility of patients (pts) to infections following BMT we measured phorbolester (PMA) induced and luminol enhanced CL to study PMN function in pts (n=18) after allogeneic and autologous (n=4) BMT.

Blood samples (n=68) were taken from adult volunteers (n=13) to monitor inter- and intraindividual variation of absolute CL-values longitudinal over a mean period of 16 weeks (8 - 22 weeks) within intervalls of about 3 weeks. PMNs separated from whole blood were stimulated with PMA and luminol enhanced CL registrated over a period of 30 min. In controls the interindividual value of CL was $11.8 \pm 2.9 \times 10^5$ counts/1000 cells x min ($\bar{X} \pm SD$), while intraindividual longitudinal variation ranged from minimally $11.5 - 12.5 \times 10^5$ to maximally $6.7 - 20.0 \times 10^5$ counts/1000 cells x min. CL-values of pts before BMT (n=10) were within the range of controls but rose to 1.5 to 10 fold during the first 120 days (n=36) following marrow engraftment. CL was not influenced by graft-versus-host-disease (GVHD) as this rise in CL was seen in pts after allogeneic as well as after autologous BMT. CL-values were not affected by infections. CL fell to normal range again after day +120 following BMT and was not altered by chronic GVHD. But one patient showed markedly depressed CL-values during a period of herpes zoster infection.

We conclude that measuring CL shortly after BMT shows higher values in most pts but is severely depressed during periods of viral infections.

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PREVENTION OF CYTOMEGALOVIRUS INDUCED INTERSTITIAL PNEUMONITIS IN MARROW TRANSPLANT RECIPIENTS BY INTRAVENOUS ADMINISTRATION OF A CMV HYPERIMMUNE GLOBULIN WITH A HIGH TITER OF NEUTRALIZING ANTIBODIES

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83 patients undergoing bone marrow transplantation for different underlying diseases received intravenous hyperimmune globulin ("Cytotect^R", 100 mg/kg body weight) twice before and every third week following BMT until day +100 to prevent CMV infections. Blood products for cell substitution were obtained from donors unscreened for CMV serostatus, ten of the patients received therapeutic granulocyte transfusions. Inspite of this only 8 patients, 4 of them during the time they received hyperimmune globulin prophylaxis were found to develop primary or reactivation of a latent CMV infection by serological and cultivation techniques performed routinely once a week or at the onset of symptoms. Only two patients, one when receiving passive immunization, developed symptomatic CMV infection. Both patients suffered from gastroenteritis histologically and by cultivation techniques found to be caused by cytomegalovirus. None of the 83 patients developed CMV pneumonitis or died due to complications of CMV infections.

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DISTRIBUTION OF IMMUNOGLOBULIN G SUBCLASSES IN TUMOURPATIENTS AND PATIENTS WITH HUMORAL IMMUNDEFICIENCY SYNDROMES

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IgG subclass patterns were analyzed in 23 patients with malignous lymphomas and 13 patients with solid tumours during polychemotherapy. The same assay was also applied to 6 patients with known IgA, IgG or combined immune deficiencies. IgG subclasses were detected by sandwich enzyme immunosorbent assays with monoclonal antibodies against the four IgG subclasses (1st layer) and a HRPO-labeled monoclonal antibody against total IgG (2nd layer). Immunoglobulin concentration was decreased in about 90 % of the Hodgkin- and Non-Hodgkin lymphomas: total IgG to 78 %, IgG1 to 74 %, IgG2 to 84 %, IgG3 to 86 %, IgG4 to 66 %, that suggests a defect in the humoral immune system. Serum IgG concentrations in patients with solid tumours were within normal range. Infectious complications during chemotherapy (7 patients) did not significantly change serum IgG levels.

Subclass patterns found in patients with humoral immune defects: normal IgA, IgG, IgM levels combined with reduced IgG2 and IgG 4 (2 patients); diminished IgA, IgG and IgM as well as IgG1, IgG2 and IgG4 concentrations associated with normal IgG3 (2 patients); isolated IgA deficiency with normal IgG subclasses (1 patient); IgA deficiency with no detectable IgG2 and IgG4 (1 patient). The most common infection observed in these patients was recurrent pulmonary infection.

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IMMUNOLOGICAL PROFILE IN HIV-INFECTED CHILDREN WITH HEMOPHILIA

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The present study was performed to analyse the distribution pattern of lymphocte subsets and lymphocyte function in 7 HIV-seropositive hemophiliacs. Fourty seronegative hemophiliacs and 21 normal children served as controls. Surface markers were tested using a panel of monoclonal antibodies (MAB) and proliferative responses of lymphocytes were measured after in-vitro stimulation with different mitogens (PHA, ConA, PWM and OKT3). Quantitative serum immunoglobulin concentrations were also determined and compared with the number of peripheral blood (PBL) B-cells. Our results demonstrate, that the percentage as well as the total number of T4 are markedly decreased in our group of HIV-seropositive patients. The total number of T8-cells were found to be normal, thus proportional increase of T8 is due to T4 decrease. This leads to an abnormal T4/T8 ratio (0,66 +/- 0,3) in 6 of our 7 children, as compared to the seronegative hemophiliacs (1,54 +/- 0,46) and our normal controls (1,94 +/-0,3). There was no correlation between the number of PBL B-cells (15% +/- 7,61%) which are in normal range and hypergammaglobulinaemia (1520-4620mg/dl). A distinct decreased response of PBL to mitogens as measured by 3H-thymidine uptake was detected in 3 of 7 patients. In one child with full blown AIDS no proliferation could be detected in response to the mitogens used. In addition of high purified IL-2 (hpIl-2) the cultures the 3H-thymidine uptake increased slightly. During that observation period (since 1984) the immunological parameters show a continuous deterioration. We found here a better correlation between T4-cells and the clinical picture than with the T4/T8 ratio, so that we prefere the absolute T4 number for evaluation of the immunological status.

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EPSTEIN-BARR VIRUS NEGATIVE BURKITT CELL LINES FROM A PATIENT WITH AIDS AND B-CELL LYMPHOMA

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To analyze the pathogenesis of B-cell lymphoma in patients with the acquired immunodeficiency syndrome (AIDS) we studied two cell lines, Es I and Es III, established from one such lymphoma for the presence of the Epstein-Barr virus (EBV) and the human immunodeficiency virus (HIV) as well as for the presence of cytogenetic abnormalities and monoclonal rearrangements of immunoglobulin and T-cell receptor genes. Both cell lines expressed the same IgM-kappa phenotype as the original lymphoma. The karyotype of Es I was 46,XY,t(8;14),2p+,inv(6p),17p- and the cells of Es III had in addition an i(7q). Immunoglobulin gene studies demonstrated the identical monoclonal rearrangements in both cell lines. Neither EBV nor HIV sequences were detectable in the malignant B-cells leading to the conclusion that mechanisms other than transformation by EBV or HIV may have contributed to the development of B-cell lymphoma in this patient and possibly also to the generally increased frequency in patients with AIDS.

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MALIGNANCIES IN THE COURSE OF HIV INFECTION

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Kaposi's sarcoma was diagnosed in our institution in 50 HIV antibody positive men. In addition, Non-Hodgkin's lymphoma, multiple myeloma, malignant melanoma and acute myelomonocytic leukemia was found in 6 patients. We report the clinical course and management of those patients who developed malignancies. Three patients had high-grade Non-Hodgkin's lymphoma including a case of Burkitt-type lymphoma. Rapid tumour progression and involvement of extranodal sites determined the further outcome. Plasma cell dyscrasia occurred in a further patient who developed subsequently multiple myelomas. Malignant melanoma was found in a single lymph node of a HIV patient. Radical neck dissection was performed, however, a few weeks later metastases were found which disseminated rapidly throughout the bone. In another patient with fever, bone-marrow examinations were diagnostic for acute myelomonocytic leukemia. Interferon treatment led to a stabilization for 2 1/2 months, thereafter progress was noted.
The rapid course of malignancies developing under conditions of cellular immunodeficiency is a particular feature. The frequency of malignancies in our patients with AIDS was convincing to assume a causative correlation between HIV infection and the development of malignant diseases.

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FUNGAL INFECTIONS IN AIDS

F.Staib.

Among the opportunistic infections in AIDS, a distinction must be made between the endogenous and localized Candida infection (oral and esophagal) and the exogenous airborne and disseminating infection by Cryptococcus neoformans, in endemic areas, there are also histoplasmosis and coccidioidomycosis. In Germany so far, cryptococcosis has been the most frequent disseminating fungal infection. Ist precise and early diagnosis by microscopy, a special culture medium and detection of antigen is possible; its therapy consists in a combination of amphotericin B + flucytosine. Mycological control of AIDS patients and prophylaxis are needed.

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FOLLOW UP OF HIV STATUS AND CELLULARY IMMUNITY IN 36 PATIENTS WITH HEMOPHILIA A FROM1983-1987.

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36 hemophiliacs, 27 of them with serious form of disease, were studied between 1983 and 1987 in regard of their HIV serology and helper- suppressor cell ratios.
HIV status: 3 of 28 patients were positive in 1983/1984, 12 in 1985 and 14 in 1986/87 (33 tested). There was one seroconversion from 1985 to 1986 despite the use of heat treated products. The three patients and no other , who were already seropositive in 1984 developed lymphadenopathy , one of them recurrent herpes zoster infections, one other fever , reactivation of hepatitis and weight loss. In the 1987 HIV positive group OKT4/8 ratio was 0.86 ± 0.26 in 1984;0.77 ± 0.33 in 1986/87. In the negative group the ratio was 1.08 ± 0.48 in 1984 and 1.4 ± 0.74 in1987.
Impairment of cellulary immunity is a common phenomenon in hemophilia, due to patients overload with foreign proteins and various virus infections.While helper- suppessor cell ratio in later seropositive patients was over a 3year period markedly depressed, the ratio improved in the same time in seronegative patients to subnormal levels. This may have a cause in more restricted use of factor VIII concentrates but also in less virus loaden preparations of factor VIII.

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IMPROVEMENT OF VISION IN A DHPG-TREATED PATIENT WITH AIDS AND CYTOMEGALOVIRUS (CMV) RETINOPATHY

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CMV-retinopathy is one of the most common (25-46%) opportinistic infections in patients with AIDS. Without treatment it results in permanent blindness. Therapy with DHPG (Dihydroxypropoxymethylguanin) only in a few patients seemed to improve visions (D'Amico, D.J. et al.: Arch.Ophthalmol 104: 1794-1800, 1986).
Cytomegalovirus retinopathy was diagnosed in a 26-year-old homosexual man with AIDS whose visual acuity rapidly decreased. Because of worsening of vision from '1.0' to '1/35' in 6 days, treatment with DHPG was started in a dosage of 5 mg/kg-bodyweight two times a day. During therapy visual acuity increased to '0,3 p' within 8 days. Prior to treatment only discrete maculopathy but exsudative and hemorrhagic papillitis were seen by ophtalmoscopy and documented by photography. The visual loss in this patient probably was caused by inflammation of the optic nerve from CMV-infection and not by maculopathy as in most cases published so far.
We suggest early treatment with DHPG if CMV-papillitis is presumed to cause visual loss in patients with AIDS to prevent develeopment of irreversible maculopathy.

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CLONAL ANALYSIS OF AUTOREACTIVE T4/T8 DOUBLE POSITIVE T CELLS MEDIATING IN VITRO SUPPRESSION OF HEMATOPOIESIS IN APLASTIC ANEMIA

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A T cell line was established from peripheral blood mononuclear cells (PBMC) of a patient with severe aplastic anemia (SAA) following stimulation with allogeneic B-lymphoblastoid cells. Cloning of this line by limiting dilution resulted in 18 T cell clones, whose clonality was confirmed by Southern analysis. Among these clones, 8 coexpressed T4 and T8, a phenotype known for common thymocytes, but not for PBMC. T4/T8 positive T cell clones exhibited cytotoxic activity towards an allogeneic B-lymphoblastoid cell line, that could be inhibited by monoclonal anti-T3, -T4, -HLA-DP, but not anti-T8, -HLA-DR or -HLA-DQ antibodies. Importantly, a representative T4/T8 clone (WM2), suppressed clonal growth of autologous hematopoietic cells in semi-solid media suggesting an autoaggressive function. To further investigate this possibility an autologous EBV-transformed B cell line was established and used as targets for WM2 in cytotoxicity experiments. WM2 killed these autologous B cells efficiently that was blocked by anti-HLA-DP antibodies. These data suggest that autoreactive T cells expressing an immature T cell phenotype circulate in this patient that may be involved in hematopoietic failure.

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LYMPHOKINES AND APLASTIC ANEMIA

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There are sufficient laboratory data to suggest that lymphocytes and their lymphokine products are linked to hematopoiesis. Hematopoietic proliferation in vitro may be regarded as dependent on a balance between positive and negative growth factors. Efforts to demonstrate a physiologic role for lymphokines in the regulation of hematopoiesis in vivo have been disappointing. Recently, several observations suggested a possible pathogenic role for interferon (IFN) in the mediation of bone marrow failure in aplastic anemia (AA). This paper, based on in vitro studies of lymphokine abnormalities in more than 20 patients with severe AA, tries to further define the role of IFN and other regulatory factors in hematopoietic suppression associated with AA. In summary, our data do not support the hypothesis that IFN is the direct effector of immune-mediated aplasia. The interaction of IFN with other cells and factors appears to be very complex. Within this intricate regulatory network, hematopoietic suppression may be amplified by IFN in several ways: (a) Interaction with positive growth factors (b) Activation of other cells which in turn may inhibit the proliferation of hematopoietic cells (c) Synergistic suppressive interactions between IFN and other molecules. At present, it seems questionable whether further studies of lymphokines in AA will yield a clue to etiology.

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TREATMENT OF APLASTIC ANEMIA WITH CYCLOSPORIN A

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Cyclosporin A (CyA) has been widely used as an immunesuppressive agent in bone marrow transplantation. In contrast its application in autoimmunehemolytic anemia, immune thrombocytopenia and aplastic anemia (AA) is pretty rare. We report 4 cases with AA who were ineligible for bone marrow transplantation in which CyA treatment has been initiated. All patients were females aged 19 to 74 years with a prior, history of AA for 6 to 40 months. Three patients were transfusion dependent, one patient highly thrombocytopenic. While patient one and two did not met the criteria for severe AA, patient three was found to have pure red cell aplasia (PRCA) in combination with malignant thymoma. Thymomectomy and corticoid treatment proved to be without any effect on PRCA. Patient four had severe AA and was initially treated with ATG. She relapsed 38 months later and CyA treatment was started. CyA treatment induced amelioration of anemia with striking reduction of transfusion requirements in two patients and gaining transfusion independency in the other patient. In the severe thrombocytopenic case thrombocyte counts reached normal value. CyA blood concentrationes were adjusted between 100 and 250 ng/ml. (HPLC) Daily CyA-doses ranged between 4 and 12 mg/kg body weight. All four patients are on treatment for more than two, six, seven and twelve months without any therapy induced side effects.

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DEFECTIVE TERMINAL DIFFERENTIATION OF PERIPHERAL BLOOD MONOCYTES FROM PATIENTS WITH APLASTIC ANEMIA

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Macrophages (MO) are important effector cells of the immune system but also play an essential role as regulator cells in hematopoiesis. They originate from circulating blood monocytes (mo) as immature precursor cells which undergo terminal differentiation to mature cells upon migration from the capillary bed into the various tissues. MO maturation also occurs in vitro and is accompanied by a typical change in cell morphology, cytochemistry and function as well as surface phenotype. We measured the capacity of blood mo to differentiate into MO in teflon culture by their expression of late differentiation antigens (5 MAX antigens, transferrin (TF) receptor, 13C2) as evaluated with a cell-ELISA. In addition to newly expressed antigens normal MO increase their content of CD14, beta2micro-globulin (b2m) and HLA-DR antigens during maturation in vitro. When blood mo from 6 patients with hematopoietic failure consistent with the diagnosis of aplastic anemia were tested they appeared to differentiate to normal MO as judged by morphology and cytochemistry. However, these mo-derived MO either consistently failed to express a 64 kD molecule defined by MAX.1/.11 antibodies or expressed only minute amounts of this antigen on a small percentage of the MO. Whereas b2m, HLA-DR, TF receptor and MAX.26 expression was normal the defective maturation resulted also in a reduced expression of CD14, MAX.3 and the osteoclast specific antigen 13C2. In conclusion, our description of a loss of specific MO differentiation antigens in patients with aplastic anemia may indicate a MO involvement in the pathogenesis of this disease. Investigations into possible functional impairments of mo-derived MO which may correlate to the defective phenotype are in progress.

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ACQUIRED IDIOPATHIC SIDEROBLASTIC ANEMIA: EVIDENCE OF TWO SUBTYPES OF THE DISEASE

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73 patients diagnosed as having acquired idiopathic sideroblastic anemia (AISA) were devided into two groups. 40 patients had characteristic findings of dyserythropoiesis on cytologic bone marrow examination; these patients were categorized as having "pure" sideroblastic anemia (PSA). 33 patients showing dysplastic features of granulopoiesis and/or megakaryopoiesis in addition to dyserythropoiesis and were considered to have a myelodysplastic syndrome (refractory anemia with ringed sideroblasts, RARS).
Cumulative survival rates at 1 year were 95 % for PSA and 52 % for RARS.
5 year survival was 60 % and 17 %, respectively. Cumulative rates of leukemic transformation were 2,5 % for PSA and 55 % for RARS. Infections (15,2 vs. 5 %) and hemorrhages (9 vs. 0 %) were more frequent causes of death in the RARS group. Bone marrow culture studies (CFU-GM) were performed on 20 consecutive patients with PSA and RARS. RARS patients showed grossly impaired colony formation with features of the so-called leukemic growth pattern, whereas patients with PSA numbers of CFU-GM colonies in PSA were shown to be negatively correlated with the degree of erythroid hyperplasia in the bone marrow.
We conclude that on cytomorphological examination AISA can be subdevided into pure sideroblastic anemia (PSA) and a myelodysplastic form (RARS), with both types differing considerably in terms of survival, risk of leukemic transformation and findings on bone marrow culture (CFU-GM).

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DAILY LOW DOSE ALPHA-2-INTERFERON IN MYELODYSPLASTIC SYNDROMES WITH POOR PROGNOSTIC CRITERIA

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Myelodysplastic syndromes (MDS) with criteria, i.e. excess of blasts or marked cytopenia, have a poor prognosis. Between June 1986 and April 1987 8 patients (pts) with MDS with clear-cut poor prognosis fullfilled the criteria to enter this study with daily low dose alpha-2-Interferon (IF). Characteristics of the 7 evaluable pts: 4 males, 3 females, median age 68 yrs. (range, 56-73), 3 RAEB, 2 RAEB-t, 1 RA and 1 RA-S, both with marked cytopenia and high frequency of RC-transfusions.
Treatment plan: Induction phase with 0.5 M. I.U. IF sc daily for 12 weeks; subsequently depending on the efficacy either mainten - ance with 0.5 M. I.U. sc 3x/week or dose escalation to 1 M. I.U. sc daily.
Results: No pt fullfilled our criteria for response or improve - ment. 1 pt with RAEB-t showed disease progression after 14 weeks of therapy. All pts demonstrated some degree of myelotoxicity, with mild to severe thrombo- and/or granulocytopenia, fully reversible after interruption of IF administration. No other side effects were registered. Subjective tolerance was excellent.

Despite the small patient number these data suggest, that daily low dose IF is unlikely to yield a beneficial effect in MDS.

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SERUM DEOXYTHIMIDINE KINASE IN PATIENTS WITH MYELODYSPLASIA

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Deoxythimidine kinase (dTK) represents an important "salvage-pathway" enzyme in the synthesis of DNA. Elevated levels of serum dTK occur in a number of hematological malignancies and it has been suggested that the enzyme activity correlates with tumor cell mass, grade of malignancy and prognosis of the disease. The aim of the present study was to evaluate the behavior of serum dTK activity in patients with myelodysplastic syndromes (MDS).
Using a commercial radioenzyme assay (Sangtec Medical, Bromma, Sweden) serum dTK was measured in 43 untreated patients with MDS (RA 6, RARS 9, RAEB 10, RAEB-T 9, CMML 9). In a large series of normal probands of different ages and sexes an enzyme activity of less than 5 U/µl was found. Most MDS patients (65 %) presented with normal or slightly elevated (5-15 U/µl) levels, although in rare cases (RAEB-T and CMML) markedly increased activities (up to 300 U/µl) were found. dTK levels were shown to be significantly correlated with erythrocyte sedimentation rate ($r=0.44$; $p=0.002$), LDH ($r=0.65$; $p=0.001$) and bone marrow blast count ($r=0.48$; $p=0.009$). From these data we conclude that the serum dTK widely reflects the increase of bone marrow cellularity and blast cell count in patients with MDS. The close relationship between enzyme levels and conventional laboratory and morphological parameters suggests that the measurement of dTK will not provide additional information for this patient group.

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MORPHOLOGICAL CHARACTERISTICS OF MYELODYSPLASTIC SYNDROME (MDS) IN PERIPHERAL BLOOD (PB) AND BONE MARROW (BM) SMEARS.

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PB and BM smears of 261 MDS patients (RA 56, RARS 33, sideroblastic anaemia 40, RAEB 60, RAEB-T 37 and CMML 35) were examined with conventional staining technics. All haematopoetic cell lines showed qualitative and quantitative morphological abnormalities specified in the following lists:
Granulopoiesis: BM: shift to immature precursors 77 %, hypogranular cells 56 %, hyperplasia 47 %, partial defect of forms 35 %, Pseudo pelger cells 32 %, hypersegmentation of neutrophils 26 %, hypogranular neutrophils 20 %. CMML was characterized by an excess of monocytoid cells in BM (94 %) and PB (100%).
Erythropoiesis: BM: shift to immature precursors 91 %, sideroblastosis 61 % (more than 15 % ring sideroblasts 38 %), hyperplasia 49 %, nuclear fragments 36 %, positive PAS reaction 29 %, multinuclearity 21 % PB; anisocytosis 98 %, poikilocytosis 76 %, makrocytosis 56 %, polychromasia 44 %, nucleated precursors 29 %.
Megakaryocytopoiesis: BM:mikromegakaryocytes 35%, mononuclear megakaryocytes 33 %, hyperplasia 29 %, multinuclear megakaryocytes 23 % PB: anisometry of platelets 69 %,hypogranulated platelets 29 %, giant platelets 28 %. Our data indicate, that a specific diagnostic marker for MDS is lacking, whereas the concidence of a various number of morphological criteria is highly suggestive of the diagnosis.

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A MYELODYSPLASTIC SYNDROME TRANSFORMING TO ACUTE LYMPHOBLASTIC LEUKEMIA

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We present a 77 year old female patient with a myelodysplastic syndrome (MDS), subtype RAEB-t, transforming to acute lymphoblastic leucemia (ALL) after a period of 4 months. Under treatment with vincristine and prednisone marked subjective improvement was achieved, although no objective hematological response could bei documented.
To our kowledge, this is the sixth case reported in the literature with MDS progressing to pure ALL.
We suggests, that the routine use of immunological surface marker studies in all patients with RAEB, RAEB-t and acute leucemia emerging from MDS, could demonstrate a substantial number of cases with lymphatic blasts and ALL, respectively Obviously, this would bei of great importance for the therapeutic approach and consequently the prognosis of these patients.

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INTERFERONS IN THE TREATMENT OF MALIGNANT MASTOCYTOSIS (MM) AND CMMoL DEVELOPING IN MM

F. Griesinger, L. Bergmann, P.S. Mitrou, D. Hoelzer

Malignant mastocytosis (MM) is a hematologic disorder with poor prognosis and so far no effective therapy. As to the authors' knowledge, no data concerning immunologic parameters in this disease is available. Based on immunomodulatory and cytotoxic effects of interferon gamma (IFN-γ) and on therapeutic trials with IFN-γ in hematologic malignancies, a therapeutic attempt with IFN-γ was made in two patients with MM. Stable disease was achieved with IFN-γ 100 µg/die s.c. for 16 and 10 months respectively. With the exeption of the CD4/8 ratio, which returned from 5.0 at diagnosis to normal levels, in vitro and in vivo immunological abnormalities present at diagnosis, persisted. During further clinical course, CMMoL developed in both patients, refractory to even escalated doses of IFN-γ. Based on favourable results in CML with IFN-alpha, therapy with IFN-alpha 10×10^6 IE/die s.c. was started. One patient remained in partial hematologic remission for 7 months and is alive for 32 months after diagnosis of MM. The other patient developed severe infectious complications under IFN alpha and died of progression of disease after discontinuation of IFN-alpha. We conclude that IFN-γ and -alpha may be effective in MM, however, so far, IFN-alpha should be considered as first line therapy.

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CHRONIC MYELOMONOCYTIC LEUKEMIA EVOLVING TO ACUTE MIXED-LINEAGE LEUKEMIA

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According to the FAB group, chronic myelomonocytic leukemia (CMML) represents a myelodysplastic disorder involving both granulocytic and monocytic cell lines. As in other myelodysplastic syndromes, a substantial proportion of patients progresses to acute myeloid leukemia. In this report, we describe 2 cases of CMML which transformed to mixed-lineage leukemia carrying both myeloid and lymphoid features.
Diagnosis of CMML was confirmed by morphological, cytochemical and laboratory (lysozyme) studies 6 and 48 months, respectively before blastic transformation. On cytogenetic analysis, the Ph^1 chromosome was found to be absent. One patient was treated with low dose cytosine arabinoside during the chronic phase of the disease. At the time of transformation, bone marrow aspirates showed a marked increase of blasts (70 a. 95 %) which were positive in the myeloperoxidase (22 a. 37 %) and non-specific esterase reaction (10 a. 20 %). In one patient, PAS staining was positive with coarse granules in 40 % of the blasts. Additional surface marker studies showed that a considerable percentage of the leukemic cells was also reactive with lymphoid monoclonal antibodies. In both cases, the blasts were positive with CALLA (30 a. 27 %), TdT (35 a. 32 %) and DR-related antigens (53 a. 47 %). No chemotherapy was started, and both patients died from infectious and homorrhagic complications.
In summary, these cases illustrate that CMML can evolve to mixed acute myeloid and lymphoid leukemia. Our findings suggest that the malignant proliferation in CMML is not restricted to the level of the granulocyte-macrophage progenitor cell, but involves a more primitive pluripotent stem cell common to both myeloid and lymphoid lineages.

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DIFFERENTIAL EFFECTS OF THE REPEATED INJECTIONS OF CCNU, MITOMYCIN-C, CYCLOPHOSPHAMIDE OR CISPLATIN ON HEMATOPOIESIS IN MICE

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Cumulative toxicities and long term effects of CCNU, Mitomycin-C (MMC), Cyclophosphamide (Cy) and Cisplatin (DDP) on hematopoiesis were evaluated in mice given equitoxic (1/2 LD10) doses of the drugs (22.4 mg/kg of CCNU, 3.58 mg/kg of MMC, 200 mg/kg of Cy or 4 mg/kg of DDP) weekly for 4 wks. WBC count and bone marrow concentrations of pluripotential (CFU-S) and granulocytic (CFU-C) progenitor cells were determined 7 days after each injection and 15-22 wks after the last injection. During 4 wks of treatment, marrow CFU-S and CFU-C content showed a continuous decrease in all 4 groups, but the cells appeared to be suppressed more severly by MMC than by the other 3 drugs. WBC count seemed to recover and steadily increase from injection to injection in Cy-treated mice but an almost continuous decrease in those treated with CCNU. The increase of the WBC count in Cy-treated mice and the decrease in CCNU-treated animals was mainly due to a rise in the granulocyte number and a fall in the lymphocyte number. At 15-22 wks after drug cessation, there were no marked changes in WBC count or marrow CFU-S and CFU-C content in animals treated with Cy or DDP. In contrast to this, in CCNU-treated mice, the marrow CFU-S content was reduced to 77 %, the CFU-C content to 63 % and the WBC count to 45 % of those in the controls, and in the MMC-treated mice, the marrow CFU-S content to 46 %, the CFU-C content to 38 % and the WBC count to 66 %. In addition, the proliferative potential of CFU-S (R_S) measured in Cy-treated mice was reduced to 72 %, in MMC-treated mice to 44 % and in CCNU-treated mice to 15 % of the control values. On the basis of these results, CCNU and MMC appear to have greater cumulative toxicities and particularly long lasting effects on hematopoiesis in mice than Cy or DDP have. - Supported by BMFT in Bonn (PTB 03 8720).

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REPRODUCTIVE AND ENDOCRINE GONADAL CAPACITY IN PATIENTS TREATED FOR GERM CELL TUMORS (GCT): RESULTS OF A 5-YEAR PROSPECTIVE STUDY.

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Introduction: In recent years it has become apparent that 70%-100% of patients (pts) with disseminated GCT are cured of their disease by chemotherapy (CT) and surgery. Most pts are young adults and concerned about the degree, duration and reversibility of endocrine and reproductive dysfunctions induced by CT. This prospective study was conducted to evaluate the impact of CT on acute and chronic gonadal toxicity in pts with GCT. Patients and Methods: Starting 1982, 44 pts were studied. They were treated with cisplatin, bleomycin, vinblastine ± ifosfamide and etoposide. Diagnostic procedures consisted of hormone analyses, sperm evaluation, testicular histology and a questionnaire. Results: 37/44 (84%) pts showed oligo- or azoospermia before CT, but only 3/44 (7%) had elevated FSH serum levels. In contrast, all pts revealed elevated FSH levels and azoospermia 1-24 months (m) after CT. 10/10 pts showed germinal cell aplasia with severe spermatogenic stem cell depletion immediately after CT. In 34/44 (77%) pts recovery of spermatogenesis occurred with normalization of FSH levels 25-60 m after CT. In pts receiving 2 cycles of adjuvant CT median recovery time was 24 m vs 43 m in pts treated with 4 cycles. Testosterone and LH levels were mostly within normal limits before and after CT due to resistance of Leydig cells. 5 pts fathered 7 healthy children after CT. Conclusions: Our data suggest 1) drug-induced germ cell aplasia with azoospermia and elevated FSH levels 1-24 m after CT in all pts, 2) recovery of spermatogenesis in 77% of pts, 3) median recovery time was 24 m in pts receiving 2 cycles vs 43 m with 4 cycles, 4) no increased risk of fetal malformations, 5) FSH serum levels seems to be a feasible marker to assess degree and duration of drug-induced germ cell aplasia.

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CYTOTOXIC EFFECT OF U-46619 ON ERYTHROCYTES

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A membranolytic activity of thromboxane A_2 (TxA_2) has been suggested in experiments performed with liver and cardiac lysosomes. Furthermore, eicosanoids like TxA_2 may act in the state of a free radical thus initiating lipid peroxidation. In the present study erythrocytes were used as a model and were incubated with a synthetic TxA_2 analog (U-46619) under various conditions. The hemolytic effect of U-46619 was studied. Alkames like pentane and ethane known to be released during fatty acid breakdown following lipid peroxidation were measured by gas chromatography in a head space vial system (Clemens et al, Biochem Pharmacol 32:3877,1983). In addition, the fatty acid composition of erythrocytes was gas chromatographically analyzed before and following the incubation procedures. Incubation of erythrocytes (hematocrit 2.5%) with U-46619 concentrated more than 1 mmol/l for 2 h at 37°C in the presence of synthetic air resulted in an enormous degree of hemolysis up to 95% and in a methemoglobin formation up to 10%. Experiments performed in the presence of nitrogen or CO-loaded erythrocytes exhibited the same hemolytic action of U-46619. In all experiments lipid peroxidation has not been detected. Thromboxane receptor antagonist were not able to block the hemolytic activity of U-46619. The study shows that the hemolytic potency of U-46619 in this model was not oxygen-dependent, thromboxane receptor-mediated nor a result of free radical induced membrane lipid peroxidation. Further studies are needed to clarify the cytotoxic mechanism of the TxA_2 analog U-46619.

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DOES DEXAMETHASONE USED AS AN ANTIEMETIC DRUG ALTER THE ANTITUMOR ACTIVITY OF CISPLATINUM/IFOSFAMIDE?

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High dose glucocorticoid steroids, particularly dexamethasone, are often part of an antiemetic program concomittant cisplatin or ifosfamide chemotherapy. To roule out a possible dexamethasone effect on the antitumor activity of cisplatin or ifosfamide in pts treated for testicular tumor, the proliferation of heterotransplanted human testis tumor cell lines (H12.1 and H12.7) growing subcutaneously on nude mice was studied under treatment with ifosfamide or cisplatin given with or without concurrent dexamthasone. Results: Concomittant administration of dexamethasone did not significantly influence the antitumor activity of cisplatin and ifosfamide. Dexamethason itself induced a slight but statistically not significant retardation of tumor growth in both cell lines. Conclusion: Dexamethasone itself might slightly alter the growth of testicular tumors (they mostly have glucocorticoid steroid receptors); however, its concomittant administration as antiemetic drug patients treated with cisplatinum and/or ifosfamide does not inflence the antitumor activity of both drugs in this nude mice model and probably not in man.

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LACK OF THERAPEUTIC ACTIVITY OF THE LIPOIDAL AMINE CP-46,665 IN RODENT TUMORS AND HUMAN NON-SEMINOMATOUS GERM CELL TUMORS GROWING IN NUDE MICE.

W. E. Berdel, M. R. Berger, H. Falk, A. Harstrick, S. Danhauser, H. D. Schick, H.-J. Schmoll, D. Schmähl, W. R. Vogler, and J. Rastetter

The alkyl-linked lipoidal amine 4-aminomethyl-1-[2,3-(di-n-decyloxy)-n-propyl]-4-phenylpiperidine (CP-46,665) has shown promising in vitro cytotoxic activity in neoplastic cells from humans (Cancer Res. 45:1206-1213, 1985), but did not produce differential cytotoxicity comparing human leukemic with non-neoplastic bone marrow cells (Blut 53:196, 1986). The compound was then tested for therapeutic activity in 2 rodent tumor models and 2 human non-seminomatous germ cell tumors growing in nude mice.

CP-46,665 failed to show therapeutic efficacy in 3-Lewis lung carcinoma growing in syngeneic C57Bl6-mice, in MNU-induced rat mammary carcinomas and in 2 human non-seminomatous germ cell tumor cell lines (H 12.1, H 12.7) growing in nu/nu NMRI-mice when given in a dose range including non-toxic doses and doses higher than the LD_{10}.

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ANTITUMORAL ACTIVITY OF XANTHATE COMPOUNDS IN VITRO

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Xanthate derivatives were shown previously to display antitumor activity against transformed fibroblasts and lymphomas in combination with monocarboxylic acids. In order to explore their range of antitumoral activity various malignant cell lines of human origin (LiA, DHL-4, K-562, HTB-38, HTB-47, WiDr, N 59, N 63, N 64, N 65) were exposed to the drugs in vitro in ^3H - thymidine uptake, trypan blue dye exclusion and clonogenic assay. In addition, several tumor cell lines displaying resistance against commonly used anticancer drugs (adriamycin, methotrexate) were included in this study. The combination of tricyclodecan-9-yl-xanthogenate (D 609) with undecanoic acid (C_{11}) exerted clear dose dependent cytotoxic and antiproliferative effects on cell lines both from solid tumors and leukemias, after an incubation time of 24, 48 and 72 hrs. The effective concentration of D 609 was 10 µg/ml, if C_{11} was added in a concentration of $\geq$ 20 µg/ml. These concentrations were shown previously to leave normal human fibroblasts mitotically active. The same dose while leaving the growth of normal mouse keratinocytes almost unimpaired led to quantitative killing of chemically transformed carcinoma derivatives thereof. Moreover, the D 609/C_{11} combination was capable of killing both methotrexate- and adriamycin-resistant L 1210 and S 180 cells, indicating that there exists no cross resistance between these drugs and D 609/C_{11} in vitro. (DFG Be 822/2-4)

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PHASE I STUDY OF THE POLYELECTROLYTE CARBETIMER ADMINISTERED I.V.

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Antitumor activity of the synthetic low molecular weight polyelectrolyte Carbetimer was demonstrated in vitro (Proc. ASCO 4:29,1985) and in several animal tumor models (J.Med. Chem. 25:1060,1982). It was brought to a clinical phase I trial in patients (pts) with advanced malignant disease. The schedule was a one hour i.v. infusion once every four weeks with a starting dose of 180 mg/m^2. Dose escalations proceeded up to 16,690 mg/m^2. At least 3 pts were treated at each dose level and each pt was eligible to receive repeat courses at the same dose, until progressive disease or dose-limiting toxicity intervened. 48 pts received 92 evaluable courses. Pt characteristics include: Median age 53 (range 13-69), male 30, female 18, prior therapy CT only 13, RT only 8, CT+RT 12,none 15. Adverse effects such as reversible proteinuria, hypercalcaemia, pain at infusion site and nausea and vomiting were seen partly in a dose-related manner, but did not represent the maximum tolerated dose (MTD). No hematological toxicity was observed. Limiting toxicity was encountered at the 16,690 mg/m^2 dose level, consisting of ocular symptoms ("light flashes"), a moderate decrease of blood pressure and nausea or vomiting. 16,690 mg/m^2 was considered the MTD, when infused over one hour. There were 4 deaths on study, all due to tumor progression. 14 pts had stable disease for more than 2 courses, which, however, could also be attributed to the natural course of disease. No clear-cut antitumor responses were noted in our study population. We are currently conducting a phase II study with this drug in patients with malignant melanoma.

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HIGH-DOSE ARA-C TREATMENT OF PATIENTS WITH ACUTE LEUKEMIA

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Twenty patients (12 m; 8 f) with acute leukemia were treated with high-dose ARA-C. Mean age was 29.6 years. 9 patients received consolidation therapy and 11 were reinduced (6x refractory leukemia; 5x relapse). ARA-C (3.4 g/m²/day) was given for a mean of 4.5 days. Additional drugs were mitoxantrone (7x), VP-16 (5x), daunorubicine (2x) and mitoxantrone/VP-16 (4x). 17 treatment episodes are evaluable. Almost all patients complained vomiting. Skin reactions and conjunctivitis (2x), discrete impairement of liver (11x) and kidney studies (5x) were seen. Severe neurologic symptoms occured two times and therapy was stopped. 19 patients experienced thrombocytopenia (28 d) and leukopenia (23 d). 16 patients required i.v.-antibiotics for 12.5 days (4 to 27) and one patient died of fungal pneumonia. Two of 7 patients in remission recovered from cytopenia with leukemia, three relapsed 4.5 months following hematopoietic recovery and two are still in unmaintained remission for 5.5 and 11 months. One of 3 patients with relapsed leukemia attained remission lasting now 3.5 months. Two of 6 patients with refractory leukemia experienced a remission of 2.3 months.

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MYOCARDIAL INFARCTION AFTER VINCRISTINE-CHEMOTHERAPY FOR HODGKIN DISEASE

H.J. Lenz, U. Schuler, W. Voelker, G. Ehninger

Alternating chemotherapy with COPP and ABVD has been reported to have a complete remission rate of 92 % in patients with advanced Hodgkin disease. Consolidation irradiation will be delivered to sites of initial involvement following the chemotherapy. Complications of mediastinal irradiation are radiation pneumonitis, less commonly pericarditis and rarely coronary artery disease. There is only one case report of a myocardial infarction in 377 patients with advanced Hodgkin disease, who underwent mantle field irradiation. In the literature there only 6 case reports of myocardial infarction or myocardial ischemia following chemotherapy with vinca-alcaloids. We present the case of an 18 year old patient with a mediastinal relapse of Hodgkin disease stage III B, who developed an acute myocardial infarction 3 hours after application of 2 mg vincristine. He had already undergone a mediastinal irradiation following an alternating chemotherapy of 4 cycles COPP and ABVD 3o months ago.

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PULMONARY EDEMA AS A COMPLICATION OF THERAPY WITH CYTOSINE ARABINOSIDE (ARA-C).

K. Brandstetter, R. Haas, S. Kiesel, A. Deboben, B. Dörken, A. Ho, W. Hunstein

Recently, a number of reports on pulmonary edema as a result of Ara-C therapy especially in high doses (> 3 g/m^2) have been published. We describe two patients who received Ara-C in a lower dose (700 mg resp. 1520 mg total dose) and developed pulmonary failure. Patient 1 (57 years old) received 140 mg/day Alexan for 5 days and 150 mg/day Thioguanin as a treatment for necrotic arteriitis. Seven days after starting the treatment severe dyspnoe developed. Chest X-ray examination revealed discrete patchy shadows; O_2 partial pressure was 20 mm Hg. Normalization of the pulmonary function and of the chest X-ray was achieved by high dose prednisone therapy (100 mg/day) and forced diuresis. Patient 2 (52 years old) diagnosed as acute myelocytic leukemia was treated with Thioguanin (100 mg/m^2), Daunorubicin (60 mg/m^2) and Ara-C (100 mg/m^2). Three days after starting therapy dyspnoe and a decrease of PO_2 preceeding lethal pulmonary failure developed within hours.

Our observations indicate that pulmonary edema can be a complication not only in high dose Ara-C therapy but also in a polychemotherapy containing lower doses of Ara-C. This demonstrates the importance of detecting early signs of pulmonary failure such as dyspnoe and fall of PO_2 arising under Ara-C therapy. Immediate treatment with high dose corticosteroids and forced diuresis can be life-saving.

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THE ACTION OF CYCLOPHOSPHAMID (CYT) AND CIS-PLATIN (DDP) ON ACUTE AND CHRONIC TOXICITY OF TLI (TOTAL LYMPHOID IRRADIATION) IN RATS,

C. Schöber[1], A.C. den Hertog[2], V. Bendel[2], Kh. Renner[2]

Interactions between cytostatics and radiation offers certain advantages but includes the risk of serious acute and chronic side effects. The aim of this study was to define the toxicity of CYT and DDP in addition to single dose irradiation involving large treatment volumes.
Methods: In dose-finding studies equitoxic dosages of single dose CYT (60 mg/kg), DDP (2 mg/kg) and TLI (7 Gy) were established. 60 Lewis female rats were separated in 6 treatment groups as follows: CYT or DDP injected i.p. alone or prior to TLI, TLI alone and untreated controls. All animals received a skin graft of DA-female donors the day after radiation.
Results: 1. CYT increases graft survival in combination to radiation (13,2 vs 10,4d) but added only hematotoxicity. 2. DDP increases hematologic and gastrointestinal toxicity without any prolongation in graft survival (11.2 vs 10,4d). 3. The prevalence of spontaneous tumors was not increased with the radiation chemotherapy combination (3/13 vs 6/10 in the only irradiated control) but the evaluable number of 13 animals are indicative for treatment related deaths during the first year not attributable to acute toxicity.
Conclusions: Side effects of drug-radiation combinations need further investigations, especially long term toxicity is often neglected. Optimal scheduling may increase tumour response without adverse reactions.

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SURVIVAL OF AN EXTREMELY HIGH-DOSE OF MITOXANTRONE IN A 73-YEARS-OLD FEMALE WITH SMALL CELL BRONCHIAL CARCINOMA

L. Marosi, R. Koppensteiner, R. Lenzhofer, E. Minar.

Mitoxantrone, a synthetic anthracendione, is characterized by an antitumor action with is comparable to that of doxorubicin, while its cardiotoxicity is said to be less pronounced. Bone marrow suppression is the dose-limiting factor, so that single doses generally do not exceed 12-14 mg/m^2 body surface area.

This report concerns a 73-years-old female patient suffering from small cell bronchial carcinoma of central type without evidence of extrathoracal manifestation and without any overt heart failure. This patient inadvertantly received a single dose of 275 mg of mitoxantrone (equivalent to 183 mg/m^2), together with 300 mg cyclophosphamide (=200 mg/m2) and 1.5 mg vincristine (1 mg/m2). During the drug infusion she developed shaking chills, nausea and vomiting, which was adequately controlled by antiemetics. Immediately after the accidental overdosage had been realized, the patient was isolated, shieled under sterile conditions and treated with digitoxin. On day 7 following the administration of the toxic mitoxantrone dose the white cell count dropped to below 200/µl and the platelet count to below 30.000/µl. An unexplained temperature peak on day 9 with normal blood urinary cultures and non-contributory chest x-ray prompted antibiotic and antimycotic treatment with cefotaxim, piperacillin, gentamycin and ketoconazole, respectively. In the further course the patient was afebrile. Hemorrhage was prevented by continous platelet replacement. The red cell count as well as a number of other parameters (SGOT, SGPT, LDH, CPK, alkaline phosphatase, BUN, creatinine) showed no serious abnormalities. Bone marrow suppression persisted for about 20 days with gradual return of the leucocyte and thrombocyte count to normal. In particular, there were no signs of stomatitis, mucositis or diarrhea. On radionuclide ventriculography done after cessation of the bone marrow suppression LVEF was within the normal range (76%).

The case of this patient shows that the accidental administration of an extremely high mitoxantrone dose, i.e. 14 times the recommended therapeutic dose, while causing sustained massive bone marrow suppression with pronounced thrombo- and leucopenia, is compatible with life provided intensive treatment is established; the patient did not develop cardiac decompensation. On the other hand this extremely high single dose of mitoxantrone did not produce any clinically demonstrable antitumor effect in this particular patient.

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ERYTHROLEUKEMIA AFTER MELPHALAN THERAPY OF MULTIPLE MYELOMA

H.U. Krueger, H.J. Lenz, G. Ehninger

After cytotoxic therapy with alkylating agents the incidence of secondary acute non-lymphocytic leukemia (ANLL) is up to 100 times higher. The characteristics of secondary ANLL are: manifestation mostly in the 4-6th year after beginning of the chemotherapy, often preceeding myelodysplastic changes, higher frequency of chromosomal abnormalities, lower therapeutic response. Standard treatment of the multiple myeloma uses a combination of melphalan and prednisolone. The median survival of myeloma patients is now 24-37 months, but markedly longer survival is possible. The risk of secondary ANLL depends on the cumulative dose, an argument for stopping chemotherapy in some cases of good remission under a consequent follow-up. We report a case of an 84 year old man, who developed abdominal pain in 1971. He was found to have multiple myeloma, and received 37 cycles of a melphalan/prednisolon pulse therapy until 1985 (cumulative melphalan dose 2630 mg). In 1986 the patient was admitted because of pancytopenia. An erythroleukemia (FAB: AML-M6) was diagnosed. There are only few further cases of secondary erythroleukemia reported.

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ENRICHMENT OF LYMPHOCYTES WITH SPECIFIC NK-ACTIVITY FOR ESTABLISHED TUMOR CELL LINES

U.Reichmann, E.Heidemann, H.Schmidt

In order to isolate tumor specific natural killer cells (NK-cells), lymphocytes from normal donors were incubated with different tumor cell lines or tumor cells isolated from patients. Cells attached to tumor cells were separated from non adherent lymphocytes by centrifugation through percoll density gradients. Tumor cell adherent lymphocytes could be shown to have an up to 6.5 times higher NK-activity than lymphocytes before separation (20 experiments).
Using 4 different target cells in the NK-assay the augmentation of NK-activity could be shown for each target cell, but the highest NK-activity was always detected for the cells used to separate the tumor cell adherent lymphocytes (augmentation index: 6.5 versus 3.5; 2.0 and 4.0). A selective augmentation due to interferon production could be excluded. We conclude that the natural killer cell activity of these tumor adherent lymphocytes is partly specific for a certain tumour.

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EVALUATION OF RETICULOENDOTHELIAL SYSTEM (RES) FUNCTION: STUDIES WITH 111-IN-PLATELETS AND WITH IGG-COATED 99M-TC-RED CELLS

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We compared kinetics of 111-In-platelets with IgG-coated 99m-Tc-red cells in non-splenectomized patients (pt) with normal or impaired (Howell-Jolly bodies-positive -HJB) spleen function (n=23). In pt without HJB the rate constants of liver uptake of both isotops was similar. The rate constants of splenic uptake of these two particles was also similar up to 30 min after injection; thereafter, 111-In remained constant while a further increase of 99m-Tc was recorded over the splenic site. In 5/5 HJB-pt 111-In did not accumulate at the splenic site. In 4/5 HJB-pt the sequestration of 99m-Tc-red cells was impaired; in 1 pt sequestration was compensated by the liver. In 3/18 pt with normal 111-In-platelet kinetics impaired 99m-Tc-red cell sequestration was found. Subsequent investigation with 111-In-platelets 6 months later showed failure of accumulation at the splenic site.
Thus, splenic pooling capacity of 111-In-platelets and extraction of IgG-coated red cells accomplish evaluation of RES function.

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COLOUR-CONTRAST STAIN OF TWO DIFFERENT LYMPHOCYTE SUBPOPULATIONS: A TWO-COLOUR MODIFICATION OF ALKALINE PHOSPHATASE MONOCLONAL ANTI-ALKALINE PHOSPHATASE (APAAP) COMPLEX TECHNIQUE

L. Wagner*, C.P. Worman+, J. Schwarzmeier*

A dual staining method for different human lymphocyte subpopulations with non-overlapping antigen distribution pattern is described. Cytocentrifuge slide preparations of peripheral blood non-adherent mononuclear cells (NAMNC) or buffy-coat smears were fixed in acetone and incubated with a primary mouse monoclonal antibody (MAb) against a lymphocyte antigen (CD8, Ig-light chain, CD19, CD4) followed by rabbit anti-mouse immunoglobulin (Ig) and the alkaline phosphatase monoclonal anti-alkaline phosphatase (APAAP) complex. After repeating the "bridge" antibody and the APAAP, a red colour product was developed with Fast Red TR-naphthol ASBI phosphate. This one-colour-stain was then followed by repeating the process using a different primary mouse MAb against another lymphocyte antigen (CD4, Ig-light chain, CD3, MHCII DR, CD5) and substituting Fast Blue BB-naphthol ASMX phosphate at the last step to yield a blue colour product. Control slides stained by the standard one colour APAAP method with the relevant primary MAb showed that there was no non-specific labelling and percentage positive cells in a given test were almost identical. This technique allows sensitive two-colour staining without being limited to the few antigens against which heteroantibodies are available.

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MYELOPEROXIDASE (MPO) - DEFICIENT NEUTROPHILS: CLINICAL PICTURE AND CORRELATION TO OTHER GRANULAR ENZYMES

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MPO, a glycoprotein present in the azurophilic granules of polymorphonuclear leukocytes (PMNs), plays an important role in PMN microbicidal activity. Using an automated cytochemical analyser for routine hematology work - subjects with complete or partial MPO-deficiency were detected. Patients suffering from hematological diseases and sepsis were excluded. Using a flow cytometer concentrations of elastase and lactoferrin being markers for azurophilic and specific granules, respectively, were evaluated in PMNs of these MPO-deficient persons. Up to now 65 subjects with MPO-deficiency have been analysed according to this protocol. Arterosclerotic vascular disease, malignant tumors and infections were most frequent diagnoses. Concentration of lactoferrin in PMN of MPO-deficient persons was not found to be significantly different as compared to the controls. In contrast evaluation of elastase differentiated two groups of persons. The first one showed a decreasing concentration of elastase in parallel to the MPO content. The second subgroup was characterized to have an elevated amount of elastase. In this subgroup a more pronounced MPO-deficiency was present. Mostly these persons have been detected on the occasion of routine controls without any clinical symptoms or findings. In conclusion using an automated cytochemical analyser MPO-deficiency was found more often as currently suggested and was more often present in patients with vascular, malignant and infectious disease.

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NUCLEOLAR PATTERNS IN NORMAL GRANULOPOIESIS AND MYELOBLASTIC LEUCEMIA

K. Gruber, W. Linkesch, R. Kain

Consecutive silver (Howell & Black, Experientia 36:1014, 1980)-panoptic staining and electron optic methods were applied to leucemic and healthy individuals in order to determine the nucleolar pattern (NP) of granulopoietic and transformed cells. 3 parameters were used to describe of individual pattern of a single cell or a cell type: number and size of nucleoli, number of nucleolus organizer condensates.
It turned out that, during granulopoiesis, cell of each differentiation level showed a characteristic NP clearly visible by Ag-staining.
The blood smears and bone marrow aspirates of 18 patients suffering from leucemia (8 AML, 10 CML) also showed NPs which were constant and characteristic for each patient ans remained unchanged during and after cytostatic chemotherapy. Two patients with CML blast crisis in relapse also preserved their leucemic NP during and after the CML-BCs.
In three patients having undergrone heterologous bone marrow transplantation because of leucemia (1 AML, 1 AL-biphenotype, 1 CML) ist could be demonstrated that the pathological NPs disappeared after transplantation.
In summary, it seems that nucleolar morphology is not only determined by the cell-cycle and by chemical and metabolic influences, but also by a rather strong and conservative clonal component.

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FANCONI ANEMIA MUTATION CAUSES CELLULAR SUSCEPTIBILITY TO AMBIENT OXYGEN

D.Schindler, M.Kubbies, H.Höhn, A.Schinzel and P.S.Rabinovitch

Fanconi anemia (FA) is an inherited condition of growth retardation, congenital anomalies, and pancytopenia (G.Fanconi, Semin Hematol 4:233, 1967). The presumptive single gene defect is expressed at the cellular level since FA fibroblasts show poor proliferation as clones and mass cultures (R.Weksberg et al, J Cell Physiol 101:311, 1979). The in vitro growth deficit is caused by highly specific endogenous transit delay and complete arrest during the G2/M segment of the cell cycle, both in lymphocytes and in fibroblasts (M.Kubbies et al, Am J Hum Genet 37:1022, 1985).

It is shown that FA fibroblasts are hyper-sensitive to oxygen. Cultivation at 5%, 20%, and 35% (V/V) oxygen reveals that they benefit from long and short-term exposure to low oxygen to a much greater extend than do normal cells to restore their in vitro growth deficit by a reduction of G2/M phase blockage. The genetic lesion in FA may thus consist of increased susceptibility to, or deficient repair of, oxygen related cell damage.

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Enrichment procedure for electron microscopic investigation of human megakaryocytes

M. Winkelmann, U. Losch, A. Behrens, W. Schneider

Megakaryocytes amount to only 0.05% of all nucleated cells in human bone marrow aspirates. Therefore, electron microscopic studies of human megakaryocytes are practicable only with enrichment procedures, especially in bone marrow with hypoplasia of megakaryocytopoiesis. We investigated bone marrow specimen of 24 patients with different haematological disorders with concomitant hypo- or hyperplasia of megakaryocytopoiesis, respectively. 6 ml of routinely gained citrated bone marrow were incubated with glutaraldehyde 0.8% for one hour. After washing in modified catch medium, the fixated bone marrow specimen were given on a discontinous percoll gradient (40%/55%). Megakaryocytes were concentrated in the first layer of the percoll gradient. A 20±10-fold purification from 0.39±0.24‰ to 8.41±7.01‰ was achieved. This enrichment was sufficient for electron microscopic investigations without artefacts even in a patient with hypomegakaryocytic bone marrow (radius aplasia syndrom). In the sediments and the other layers even less than 0.1‰ megakaryocytes were still identified by immunoperoxydase method. By scanning cytophotometry no significant alteration of ploidy distribution patterns between native and enriched bone marrow specimens were found.

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THE EFFECT OF NONSTEROIDAL ANTIINFLAMMATORY DRUGS, GLUCOCORTICOIDS, cAMP, cGMP, AND AMINOPHYLLINE ON THE MATURATION OF SKIN WINDOW MACROPHAGES.

B. Hölzl, N. Sepp and F. Schmalzl

We investigated the effect of several nonsteroidal antiinflammatory drugs (NSAID) as well as of cAMP, cGMP, and aminophylline on the transformation of monocytes into skin window macrophages. Skin window tests according to Rebuck and Crowley (Ann.NY Acad. Sci.59:757,1955) have been performed on ten healthy students. The mentioned substances and 6-methylprednisolone (6MP) were dissolved in varying concentrations in 0.9 % saline, which served also as blanc control. Because of the known inhibitory effect of 6MP on macrophage maturation (Int.J.clin.Pharmacol. 5: 206,1971) it was used as a positive control. The solutions were applied topically on the skin window lesions initially and at each change of the coverslips. The maturation of the macrophages was assessed by evaluating the morphological changes and the cytochemical reaction product of acid phosphatase.
Whereas the inhibitory effect of 6MP was confirmed, acetylsalicylic acid, indomethacine, and other NSAID induced no detectable changes of macrophage development. cAMP and aminophylline had clearcut inhibitory effects, whereas cGMP seemed to improve macrophage maturation. Thus, our results indicate clearcut differences in the effect of NSAID and 6MP on inflammatory cells.

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ABILITY OF CLINICAL AND BIOCHEMICAL PARAMETERS TO PREDICT BONE MARROW METASTASES

K.H. Pflüger, R. Holle and K. Havemann

In patients with solid tumors bone marrow infiltration (BMI) is a relatively rare site of metastatic spread. Only in patients with small cell lung cancer (SCLC) BMI is found more frequently. This investigation was initiated to evaluate a number of clinical and biochemical parameters to predict bone marrow metastases. 172 patients suffering from SCLC (n=73), non-SCLC (n=27), breast cancer (n=40), and other tumors (n=32) were included in this study. Sixty-five patients showing BMI were compared with 107 patients without BMI but otherwise comparable parameters such as tumor burden, age, performance status and treatment. The following clinical and biochemical parameter were found to be significantly correlated to BMI: leukoerythroblastosis, thrombocytopenia, thrombocytosis, anemia, elevation of serum LDH, alkaline phosphatase, GOT, γ-GT, alpha-1- and alpha-2-globulins as well as a decrease of serum albumin and thromboplastin time. WBC, number of granulocytes and eosinophiles, calcium level, GPT, urea, uric acid, proteine, pain, and tumor markers showed no correlations to BMI. Closer inspection of the data of well matched subgroups revealed only severe anemia, thrombocytopenia and leukoerythroblastosis to have high predictive value for BMI. Patients with SCLC and BMI rarely show normal serum levels of LDH. On the other hand patients without one of these parameters and without signs for distant metastases are highly unlikely to have BMI.

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AUTOMATED ABRIDGED (3-MODAL) WBC HISTOGRAM DIFFERENTIAL COUNTS PROVIDED BY COULTER COUNTER

J. Anagnou, H.J. Avenarius, and H. Poliwoda

The Coulter Counters of the latest generation now provide three part differential WBC counts in means of percentages of lymphocytes (size 45-99 fl), mononuclear cells (size 100-150 fl, including monocytes, basophils, eosinophils), and segmented neutrophilic granulocytes (size 150-350 fl). With the widespread use of the Coulter Counter three part differentials are now readily available. Accuracy and precision have proven to be excellent. As in 90 % of the cases, in which a physician requests a blood count to be done, the main indication is the measurement of the granulocytes, the importance of a quick estimation of the percentage and the absolute number of granulocytes, especially in leukopenic patients during and after chemotherapy, and the savings in labor are obvious. We now routinely print out the WBC histogram the appearance of which may provide besides the three part differential valuable clues to the presence of blasts, normoblasts and other abnormal cell populations. Interference due to the presence of platelet clumps, erythrocyte fragments, or unlysed erythrocytes are evident on examination of the histograms. The differential diagnostic gain of the routine use of the WBC histograms, provided adequate interpretation, will be demonstrated on the basis of serial WBC histograms obtained since 1983 from patients with different haematological conditions. In our experience, the partial differential information can finally suffice when patients are repeatedly studied for changes in cell populations, such as during and after chemotherapy. All measurements were performed with the Coulter Counter Model 5 Plus IV and confirmed manually.

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A COMBINED SIMPLE METHOD OF IMMUNOCYTOCHEMISTRY AND METHYLMETHACRYLATE EMBEDDING OF BONE MARROW BIOPSIES

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Marrow smears and imprints of biopsies of 12 patients (4 normals, 2 HCL,2 CLL, 2 ALL, 2 MM) were air dried and then divided into two groups:1) frozen immediately by -20°C, and 2) frozen by -20°C after 3 days air drying at room temperature. The biopsies were embedded in methylmethacrylate routinely without decalcification. All smears and imprints were incubated with the following monoclonal antibodies; CD 19,CD 22, T4 helper, T8 suppressor, T 11 Ki67 and leucocyte common antigen using the immuno-alkaline phosphatase labelling technique (APAAP). All samples allowed a reliable identification of granulo-, erythro- and megakaryocytopenies and of the lymphoid cells, according to the nuclear structure and the immunochemical reaction. No difference in the intensity of staining was seen between the groups. However, morphological details were better preserved in the imprints than in the smears. Summarizing it is possible to make a combined histological and immunocytological diagnosis even when the smears and imprints were dried at room temperature for three days.

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COMBINED UTILISATION OF CRYOSTAT AND PLASTIC SECTIONS FROM BONE MARROW BIOSPIES

R.Bartl *)**), W.Hill **), S.Madsen **), B.Buchenrieder *), H. Muthmann *), R.Burkhardt *)**)

Bone marrow biopsies were taken as part of the initial investigation of 100 patients (haematologic malignancies n=40, solid tumours n=30, myelopathies n=10, osteopathies n=20). The biopsies were taken by means of an electric drill (60 %) or with a manual trephine(40%) and immediately halved longitudinally with the aid of an especially designed plastic device. One biopsy half was then fixed for embedding into pastic, and the other half frozen for cryostat sectioning. The narrow biopsies (diameter of 2mm) were cut into segments. For rapid diagnosis crystat sections were examined within 1h of obtaining the biopsy. Furthermore, cryostat sections and imprints enabled the application of a wide range of specialized techniques used in modern haematopathology, in this report particularly applied to immunohistology (APAAP, PAP and FITC) . This study demonstrates the possibilities of the selective application of a variety of techniques to single bone marrow biopsies.

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BIOAVAILABILITY OF ORAL IRON - EVALUATION IN EXPERIMENTAL IRON DEFICIENCY

J.P.Kaltwasser, E.Werner, K.P.Schalk, M.Niechzial

Evaluation of the bioavailability of oral iron by measurement of intestinal iron absorption only has shown striking differences for various commercial iron preparations (MMW 121,(1979),1104;DMW 104,(1979),742).Studies of that kind do however not consider the therapeutical efficacy of a particular iron prepara - tion ,which is the final objective of any iron treatment.We have developed a test model, which allows to study intestinal absorption as well as therapeuti - cal efficacy in a suitable population under controlled conditions.Healthy male volunteers were phlebotomized in weekly intervals until complete exhaustion of body iron stores (ferritin<5µg/l) and developement of an iron deficiency anae - mia(9,0<Hb<11,0g/dl). On a total population of 48 male volunteers we studied the influence of valency, physical state and galenic properties of different oral iron preparations.A highly significant difference was observed intraindividually for intestinal iron absorption of Fe-III as compared to Fe-II in li - quid preparations (1,2$\pm$0,1% and 43,7$\pm$7,1% ($\bar{X}\pm$SD) respectively).In contrats to published data, based on intestinal absorption only (MMW 121,(1979),1104), no significant differences in Hb-increase were measured for oral iron,different in galenic properties (slow/quick-release).Hb-increase after 4 weeks of treatment of equivalent doses of Fe-II (100mg/d) amounted to 38$\pm$ 11g/dl for a quick-release preparation as compared to 34$\pm$ 12g/dl for a corresponding slow release preparation.
We conclude that beside valency,differences in bioavailability of oral iron preparations as measured by intestinal absorption are due to differences in experimental conditions rather than due to differences in bioavailability.
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FERRITIN CONTENT OF ERYTHROCYTES AS A CRITICAL INDEX OF IRON-DEFIENCY ANEMIA IN POLYARTHRITIS

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In its circulating form,the soluble iron-storage protein ferritin is regarded as an important parameter for diagnosis and differentiation of disturbed iron metabolism and hypochromic anemia in rheumatoid arthritis(RA).The study aimed at evaluating the question as to the clinical significance to be accorded to the ferritin content of the erythrocytes(RBC)in anemic patients with active RA(n=17).The basic RBC ferritin was assayed immuno-enzymatically using a newly developed sensitive ELISA test.In all patients,a significant correlation could be detected between the degree of normocytic or microcytic anemia(Hb,96$\pm$17 g/l)and the high systemic inflammatory activity of RA.Owing to decreased RBC ferritin values ($<$ 5 ag/RBC),iron deficiency could be diagnosed with certainty even with normal serum ferritin concentrations.The results indicate the diagnostic significance of RBC ferritin in active RA.
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A SIMPLE METHOD TO CONSTANTLY OBTAIN SUFFICIENT AMOUNTS OF GASTRIC CANCER TUMOR CELLS FOR SHORT TIME IN VITRO TESTS

F.Friedrich, D. Schnalke, D. Reile, H.-J. Meyer, H.-J. Schmoll and H. Wilke

The quality of most short time essays for predicting chemotherapy sensitivity of tumors depends on the quantity of viable tumor cells available. Mechanical mincing of tumor material reveals besides isolated cells considerable amounts of cell aggregates. Enzymatic isolation by trypsin and/or collagenase often produces irreversible cell damage. Cell aggregates and cell damage make proper evaluation of predictive tests difficult. In order to establish a reproducable predictive short time essay in gastric cancer we developed a simple method to isolate sufficient numbers of gastric cancer cells by combining mechanical and enzymatic procedures with subsequent short time culturing.
Material and methods: Freshly gained gastric cancer specimens(1-3cm^3) were cut to small pieces (1mm^3) and then incubated with collagenase 0,5% at 37° Celsius for 15 to 45 minutes until cell aggregates of 10-20 cells were obtained. Duration of incubation time depended on consistence of tumor material (stroma, cellularity). After a 24 hours period of restoring in culture medium (RPMI+DMEM, 37°) a second enzymatic step with dispase 0,5% was done to obtain single cell suspension with subsequent second 24 hours culturing as described. Vitality was tested by trypan-blue exclusion. Percentage of fibroblasts, lymphocytes and tumor cells was stated by conventional cytology and immunocytochemistry. In case of high amounts of lymphocytes a dense centrifugation with Ficoll was performed. Results: If sufficient primary tumor material was available, this procedure constantly revealed 20mio. to 40mio. tumor cells. Percentage of living and dead cells was 10:1 to 15:1. The combination of mechanical and enzymatic procedures with short time culturing to overcome acquired cell damage offers a simple method to obtain single cell suspensions of viable gastric cancer cells.
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CEA AND TPA CONCENTRATIONS IN BRONCHOALVEOLAR LAVAGE FLUID

U. Loos and P. Oehr

In bronchial carcinoma, tumor marker levels in bronchoalveolar lavage fluid may be more reliable than in plasma because it is obtained from a compartment which is nearer to the disease.

We determined CEA and TPA concentrations in plasma and lavage fluid of 57 patients who were classified in the following groups: (1) 10 healthy volunteers, (2) 13 patients with benign lung diseases, such as pneumonic infiltrates or lung fibrosis, (3) 9 patients with pulmonary metastases of different origin (e.g. breast cancer, hypernephroma, and M. Hodgkin), (4) 25 patients with bronchial carcinoma (11 squamous cell, 3 adeno-, 1 large cell, 6 oat cell, and 4 undifferentiated carcinoma).

All subjects underwent lavage with 2 x 50 ml sterile physiologic saline. CEA and TPA plasma levels were significantly increased in patients with bronchial carcinoma (group 4). Additionally, TPA was raised in patients with pulmonary metastases (group 3).

Compared to these results in plasma, there was nearly the same picture for the CEA and TPA lavage values. However, if the lavage fluid was obtained from a lung region close to tumor, the CEA and TPA concentrations were even higher; the values were lower when the procedure was performed in a lung area distant to tumor.

Thus, CEA and TPA tumor marker values are useful in diagnostics of bronchial carcinoma, especially in tumors, that are poorly accessible to bronchoscopy.

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LIPID PEROXIDATION AND OXIDATIVE DEFENCE IN RED BLOOD CELLS OF DIFFERENT AGES

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There is much current interest in the biological production of free radicals and their involvement in the development and ageing process. This is particulary true of the red cell, which is highly susceptible to oxidative damage. In our auto-oxidation test isolated erythrocytes of calves and adult cattles were incubated in glucose-free buffered salt solution/pH 7,2/ at 37°C in air atmosphere for 24 hours.

The superoxide radical produced by autoxidation of oxyhaemoglobin has induced a remarkable lipid peroxidation/involving the progressive intracellular accumulation of MDA and fluorescent chromolipids/, the degree of which reflected the dynamic balance between the pro- and antioxidant factors and depended on the content of unsaturated fatty acids in the cell membrane.

The limited lipid peroxidation capacity of calf red cells possibly can be explained by the high activity of superoxide dysmutase enzyme and the relative deficiency of polyunsaturated fatty acids.

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PURIFICATION OF A BILOGICAL ACTIVITY IN SPLEEN CELL CONDITIONED MEDIUM WHICH ENHANCES THE PROLIFERATION OF INTERLEUKIN § DEPENDENT MAST CELL LINES

J. Moeller, L. Hültner, and P. Dörmer

Permanent Interleukin 3 (IL-3) dependent "mucosal type" mast cell lines have been established from mouse bone marrow using pokeweed mitogen stimulated spleen cell conditioned medium (SCM) or WEHI-3B conditioned medium (WCM) as a source of IL-3. In the presence of SCM proliferation of these cell lines significantly exceeded the level obtained with either crude WCM or purified IL-3, suggesting the presence of an additional mast cell groth enhancing activity (MEA) in SCM. MEA can be destroyed by heat inactivation ($70^{o}C$, 20min) or by digestion with pronase. Proliferation tests with L167, one of our mast cell lines, show that MEA is different from GM-CSF, G-CSF, M-CSF, IL-2 and IFN-y. 32D-cl.23, another IL-3 dependent cell line, does not respond to MEA. These two cell lines are used to screen for IL-3 and MEA during chromatographical separation methods. Both activities do not bind to DEAE-Sephacel at pH 7.0, but bind to S-Sepharose coloumns at pH 4.5 and cofractionate with increasing NaCl concentration from 0-1 M. MEA can be separated from IL-3 with affinity chromatography. Both proteins bind to Procion Red ligands, coupled to agarose (Red A, Amicon). The elution conditions for IL-3 and MEA are 0.5 M NaCl and 2 M NaCl with 2 M urea respectively. Recently a similar mast cell stimulating effect has been reported from IL-4/BSF-1. Whether MEA and IL-4/BSF-1 are identical molecules is subject of further investigation.

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PARTIAL PURIFICATION OF A M-CSF DERIVED FROM EBV-POSITIVE B-CELL LINES

J. Sindermann, L. Hültner and G. Reisbach

Spontaneously developed EBV-positive B-cell lines established in our laboratory from the peripheral blood of healthy donors produce constitutively macrophage-CSF, as tested in the murine bone marrow cell assay (G. Reisbach et al., Cell. Imm., in press). For biochemical characterization of this M-CSF cell culture supernatants of our cell line BLY 9.84 containing 1% FCS were conditioned for 5 days and concentrated by ultrafiltation. Seguent purification steps by ion exchange and hydrophobic interaction chromatography on Q- and Phenyl-Sepharose were done. The active fractions showed weakly hydrophobic interactions at pH 6.8. On HPLC gel filtration with phosphate-buffered saline on a Bio Sil TSK 250 column they eluted with a broad peak between 100-300 kD. Specific activity was raised from 50 to 10^5 col/mg protein, overall yield was about 10%. M-CSF from another EBV-B-cell line derived from human bone marrow cells eluted in the same way on Q-Sepharose. Gel filtration of these active fractions under dissociating conditions (6M guanidine-HCl) on Sepharose CL 6B 200 revealed an apparent molecular weight of about 70 kD. Possible identity with CSF-1 will be further investigated.

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HUMAN BONE MARROW LONG TERM CULTURES
EXPERIENCES IN ESTABLISHING AND IMPROVING CULTURE CONDITIONS

J. Greher, B. Völkers, A. Ganser, D. Hoelzer

The human bone marrow long term culture represents a complex in vitro system to study the regulation of hemopoiesis and as recently reported can be used for autologous bone marrow transplantation in acute leukemia.
The aim of this investigation was to establish this culture system with optimal conditions. After a test series involving twelve normal bone marrow samples and four samples from patients with chronic myelogenous leukemia the use of alphamedium containing 5 x 10^{-6} M Hydrocortison, 12.5 % of each preselected fetal calf serum and horse serum in selected plastic tissue culture flasks showed the best results; pluripotent (CFU-GEMM) and megakaryocytic progenitors (CFU-Mk) could be detected up to three weeks, erythroid bursts (BFU-E) up to five weeks and granulocyte-macrophage progenitors (CFU-GM) up to seven weeks from the onset of the culture with a single bone marrow inoculum.

Another possible way to improve culture conditions concerns the adherent layer of bone marrow derived stromal cells, which is essential to support the growth of hemopoietic progenitors. Therefore preincubation of tissue culture flasks with fibronectin (1-5 µg/cm^2 growth area), a major part of the extracellular matrix in the adherent layer, was done, but did not show an improvement.

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LEUKOCYTE CONCENTRATES AND CELL DISTRIBUTION CURVES FOR EARLY RECOGNITION OF GRANULOCYTE REGENERATION AFTER APLASIOGENIC CHEMOTHERAPY OR BONE MARROW TRANSPLANTATION IN PATIENTS WITH LEUKEMIAS

R.Kuse [1], S.Schuster[1] and R.Thom[2]

The examination of smears in extremely leukocytopenic (0.5/ml) patients after aplasiogenic chemotherapy or bone marrow transplantation is time consuming, inaccurate, and not very informative. But the evidence of granulocytic regeneration as soon as possible gains increasing clinical importance.
In routine cell counts curves of fictitious cell volumes in the particle distribution analyzer PDA-410 have been evaluated in respect to the appearance of mature myeloid cells in every patient with very low leukocyte count. Leukocytes of these EDTA blood samples were concentrated by centrifugation. The sediment was distributed for smears and for counting with a Sysmex® CC-800 counter linked to a PDA-410. The smears were examined in the routine way (100 cells). The analysis by instrument included leukocyte count and white cell distribution curves. By this preparation technique leukocyte counts lower than 0.5/nl could be enhanced 10-30 fold. Also the time for the manual differential could be about 15 minutes. While at levels of 0.0-0.5 leucocytes/nl mostly no clear myeloid cell population could be seen at the PDA-410 at normal concentration surprisingly often a slight indication or clear myeloid "hump" could be detected in the concentrates. This corresponded well with the conventionelly enumerated 10-60 percent of bands and granulocytes.
Thus a quicker and substantially improved statement concerning myeloid cell regeneration could be made.

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NUMBER DENSITY OF ERYTHROCYTES AND MEAN CELL VOLUME AS A TOOL FOR DIFFERENTIATION OF ACQUIRED HAEMOLYTIC ANAEMIAS (AHA)

R. Kuse (1), E. Arnold (1), V. Müller (2) and R. Thom (3)

Results of red cell count and MCV determined with electronic counters in the routine way can be falsified by agglutination or swelling and are generally not usable for clinical purposes. Therefore we have developed a program to get most exact values by using a water bath adjusted to 37°, isotonic solution, and inactivation of complement. This may be emphasized by two examples:

1. AHA due to unspecified autoantibodies of immune type with complement activation. At room temperatur a continuous decrease of erythrocytes (3.88 to 3.07 /pl) and an increase of MCV (104 to 128 fl) have been seen already shortly after venipuncture and also during the next hours. Storing the sample at 37° or exchanging the plasma for a specimen with identical blood group led to the same alterations. The complement inactivation of the patient's plasma at 60° stabilized red cell counts (about 4.22/pl) and MCV (about 99 fl) for several hours however.
2. Complement mediated AHA due to warm autohaemolysins and cold agglutinins. At room temperature and after plasma substitution by CellentR the above mentioned alterations could be observed (E: 0.12/pl; MCV: 86-118 fl). In contrast no stabilization was found by complement inactivation, whereas interruption of cold agglutination by warming the three samples at 37° showed corresponding and stable date (E: about 2.60/pl; MCV: 100-109 fl).

These results demonstrate that errors of electronic cell counting can be overcome and conclusions concerning the pathomechanism of AHA can be drawn by simple means in the haematological laboratory.

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INFLUENCE OF PH, BUFFER SYSTEM AND DI-K-EDTA ON STANDARD-ROMANOWSKY-GIEMSA STAINING AND THE EFFECT IN BOTH CELLULAR ARCHITECTURE AND COLOR MEASURED PERIPHERAL BLOOD CELLS

I. Baumann, U. Gunzer*, H. Harms, H.M. Aus, V. ter Meulen

Commercial Romanowsky-Giemsa stain solutions produce unreliable results in cellular image quality because the components of the stain have not been standardized. The lack of reproducible image quality presents problems in imaging cytometry. In routine hematological laboratory, variations in pH of the staining solution, the use of different buffer systems and the addition of coagulation-inhibitors also influence the staining results. The influence of these factors was investigated. Blood smears were stained using a standardized Azur B/Eosin Y solution, according to Wittekind. The blood smears were prepared with and without Di-K-EDTA. The staining-solutions were mixed with two common buffer-systems and diluted at 5 pH-levels. Stained mono- and polymorphonucleated leucocytes were scanned with a color-TV-high-resolution image system (12,5 pixel/micrometer). 24 cellular features were measured and analysed. In the myelogenous cell line the selection of the "wrong" buffer even at the prescribed pH 6,9 level leads to large degregation of the nuclear features. No optimal solution was found for all the cellular features. Most blood cell features are better in specimens prepared with HEPES-buffer without Di-K-EDTA in a pH spectrum between 6,4 and 7,4. The complete results are described in I. Baumann's medical thesis.

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PAPPENHEIM STAIN COMPARED TO STANDARD-ROMANOWSKY-GIEMSA STAIN BY COMPUTER AIDED CELL IMAGE ANALYSIS

I. Baumann, H. Harms, H.M. Aus, U. Gunzer*, V. ter Meulen

Romanowsky-type stains such as Pappenheim's stain have been indispensable in routine hematology for about 70 years. It is however well known, that dye solution providing the Romanowsky-Giemsa-effect are non standardized solutions of varying contents of the specific compounds. In consequence routinely stained smears show a high variability in staining pattern and in most cases no reproducible staining effect can be obtained. In our laboratory a computer aided cell analysis system was developed using Pappenheims stain. A standardized Romanowsky-Giemsa stain using purified Azur B and Eosin Y according to the Wittekind's procedure and a routinely used Pappenheim stain have been compared on blood smears obtained from patients with acute myelogenous leukemia and infectious mononucleosis. A high spatial resolution color image TV system (13,3 pixel/micrometer) was used for the analysis of cell nuclear and cytoplasmic features. The cell analysis algorithms run on a Vax 11/750 computer. 24 cell features were calculated for the nucleus and the cytoplasma. The comparison shows differences in color values and texture features for the same cell types. Wittekind's stain provides more pronounced contrasts in nuclear features while Pappenheim's solution (Fa. Merck, Darmstadt, FRG) show a more intense contrast in cytoplasma. Dependent on the cell species the investigation shows advantages and disadvantages of the standardized Azur B/Eosin Y stain.

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MITOXANTRONE IN COMBINATION WITH PREDNIMUSTINE IN TREATMENT OF UNFAVORABLE NON-HODGKIN LYMPHOMA

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Mitoxantrone (Novantrone®) and prednimustine (Sterecyt®) are both active as single agents in the treatment of unfavorable non-Hodgkin lymphoma (UNHL). The efficacy and toxicity of the combination of these agents (NOSTE) was evaluated in 28 patients with advanced histopathologically proven UNHL who were not eligible for aggressive conventional chemotherapy. The median age was 68, range 45-84. Sixteen patients were previously untreated. Eleven patients had received doxorubicin or epidoxorubicin containing regimens and 1 patient had received CVP as first line therapy. MUGA scan was used in monitoring cardiac function in patients with cardiac risk. Novantrone® was administered at a dose of 8 mg/m^2 IV on days 1 and 2 and Sterecyt® 100-150 mg on days 1 through 5. The regimen was repeated every 4th week. The number of courses per patient ranged from 2 to 10. Objective response was obtained in 22 (78%) patients (20 CR and 2 PR). No response occurred in 6 patients (4 SD, 2 PD). Decreased left ventricular ejection fraction was recorded in 1 patient who suffered from asthma and ischemic heart disease. Hematological toxicity was tolerable. Gastrointestinal toxicity was rare. No hair loss was observed. Over a median follow-up the survival was 53%. Fourteen of twenty complete responders are still in remission, the median duration of remission is 24 months, range 12-36.

NOSTE reduced the discomfort associated with conventional dose anthracycline-containing regimens and could be safely used in the treatment of elderly patients and some patients with cardiac risk. Controlled studies are necessary for further evaluation of this promising combination chemotherapy.

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FIRST RESULTS OF THE CHRONIC LYMPHOCYTIC LEUKEMIA TREATMENT BY EXTRACORPOREAL PHOTOPHERESIS

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The progress of chronic lymphocytic leukemia, usually of B-cell type (B-CLL), can not be influenced by leukapheresis only. Extracorporeal photopheresis was successfully applied in patients suffering from cutaneous T-cell-lymphomas (N Engl J Med 1987; 316:297-303), i.e. a similar lymphocyte related malignancy. We tested this therapy in patients with B-CLL, using the effect of UVA activated 8-methoxypsoralen (8-MOP) which leads to irreversible bridgings of the pyrimidine bases of DNA. All patients received 0.6 mg/kg body weight 8-MOP to achieve plasma levels of 50 ng/ml or above. 240 ml of leukocytes obtained by leukapheresis where suspended in 300 ml of patient's plasma, extracorporeally exposed to UVA light (200 J/cm^2) and retransfused. This regimen was executed on 2 successive days monthly. The procedure was well tolerated, no side effects occured. There was a sustained cell reduction with diminution of the pan B^+ lymphocytes and reappearance of granulocytes and CD 3^+ lymphocytes. Platelets and red blood cells remained unchanged. Extracorporeal photopheresis caused the de-novo appearance of normal peripheral blood cells suggesting its superiority in comparison with leukapheresis alone. The underlying mechanism remains not yet clear.

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PLEOMORPHIC T-HELPER CELL NON-HODGKIN LYMPHOMA OF THE SKIN DEVELOPING AFTER TRANSIENT DRUG INDUCED BONE MARROW APLASIA

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A 51 year old previously healthy man developed bone marrow aplasia after the consecutive administration of Doxicyclin and Cefaclor. On admission WBC were 300/µl, platelets 35000/µl, Hb 7.8 g%. Bone marrow was hypoplastic with a moderate relative increase of mature lymphocytes. Physical examination revealed a generalized affection of the skin with small macular pruritic lesions. Lymphnodes were not enlarged. The skin rapidly deteriorated towards a generalized erythroderma with nodular infiltrates and a complete desquamation of superficial epidermal layers. Bone marrow function normalized 10 days after the commission of Cefaclor. Skin histology revealed an infiltration with medium to large sized lymphatic cells with characteristics of T-helper cells, the growth fraction was high (50 % Ki67+). During the rapid further course enlarged lymph nodes and an infiltration of the bone marrow with blastic T4 positive cells developed. The patient responded transiently to COPBLAM and died 3 months after admission due to pneumonia.
These observations are interesting for two reasons: First, this is a rare case of a T4 lymphoma of the skin initially presenting with the biological and histological signs of a high grade NHL. Slowly progressing transitions from mycosis f. to T-immunoblastic or anaplastic lymphomas have been reported. Secondly, allergic reactions to Cefaclor have been observed, which mimicked serum sickness and erythema exsudativum multiforme. We speculate that a hyperimmune reaction to this drug primarily affecting the skin may have evolved to this high grade T-helper cell lymphoma. This situation reminds us of the postulated origin and the clinical behaviour of angioimmunoblastic lymphoma in which according to recent gene rearrangement data T cells constitute the malignant cell clone.

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T GAMMA LYMPHOPROLIFERATIVE DISEASE:
STUDIES OF PHENOTYPIC, FUNCTIONAL, AND MOLECULAR GENETIC CHARACTERISTICS AND MODULATION OF IMMUNE FUNCTIONS BY BRM

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We report on a patient with chronic T gamma lymphoproliferative disease. The expanded cell population was CD2, CD3, CD8, Leu7, CD11a-c and FcIgG-receptor positive but lacked B, CD4, CD25 and HLA-DR antigens. The studies of immunological functions in vitro showed marked reduction of PWM-induced immunoglobulin (Ig) synthesis as well as decreased lymphocyte proliferation capacity to mitogens. NK-cell activity was barely detectable despite the expression of NK-related antigens. Co-culture experiments with lymphocytes from the patient and from healthy volunteers resulted in reduced Ig synthesis by the normal cells and inhibition of the lymphocyte proliferative responses, indicating suppressive activity of the patient's T cells. Treatment of the lymphocytes with biological response modifiers (IFNalpha, IFNgamma, IL-2) in vitro showed that IL-2 is able to induce proliferative responses and to normalize the Con A-driven proliferation; IFNs had a suppressive effect on mitogen-induced lymphocyte proliferation. The severely reduced NK-cell function could be moderately augmented by IFNs, whereas IL-2 stimulated NK activity most remarkably. DNA analysis revealed clonal rearrangement of β-TCR gene, demonstrating the monoclonal evolution of the disease. Our studies provide some insights into the biology of the T gamma-LPD and the potential of immunomodulatory agents as a possible therapeutic approach.

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ANTI-IDIOTYPE MONOCLONAL ANTIBODIES IN B-CELL MALIGNANCIES

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B-cell malignancies often express immunoglobulins on the surface of the malignant cell. Anti-idiotype monoclonal antibodies are therefore specific tools for monitoring (1) therapeutic effects and (2) depletion of blood (in vivo) or bone marrow (in vitro) from residual malignant cells after chemotherapy. We have produced 4 MoAbs directed to the idiotype of two non-Hodgkin lymphomas. Specificity was proven by reactivity exclusively with the patients B-cells and non-reactivity with T-cells in the PAP-slide technique.
In addition, the reaction could not be blocked by a polyvalent anti-human immunoglobulin.

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IMMUNGLOBULIN AND T CELL RECEPTOR GENE REARRANGEMENTS IN HUMAN LYMPHOMAS AND LEUKEMIAS

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DNA samples from blood leukocytes of patients with various leukemias [chronic lymphocytic leukemia (20); CALLA positive acute lymphoblastic leukemia (2); hairy cell leukemia (1); acute nonlymphocytic leukemia (2); blast crisis of chronic myelogenous leukemia (1)] and 11 lymphnode biopsies [Non-Hodgkin lymphoma (9); Hodgkin lymphoma with mixed cellularity (2)] were analyzed for rearrangements of the immunglobulin (Ig) and T-cell receptor (TCR) genes by the Southern blot hybridisation method.

19 of 20 chronic lymphocytic leukemias, the hairy cell leukemia, and both of the acute lymphoblastic leukemias (CALLA positive) showed rearrangements in the Ig heavy chain joining region genes (J_H). One of the CLLs was of biclonal B cell pattern, the otherone of the TCR-genes pattern. Germline prints were obtained with both probes in acute nonlymphocytic leukemias and in blast crisis of CML. One Hodgkin lymphoma with mixed cellularity had a TCR-gene rearrangement; one unclassified Non-Hodgkin lymphoma showed deletion of the 8 Kb-constant TCR-band after Hind III digestion. The Non-Hodgkin lymphomas: Burkitt lymphoma (2), centroblastic lymphoma (2), centrocytic-centroblastic lymphoma (2), centrocytic lymphoma (1), unclassified high-grade lymphoma (1) showed J_H rearrangements.

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PRELIMINARY RESULTS OF THE MACOP-B REGIMEN IN PATIENTS WITH HIGH-GRADE MALIGNANT LYMPHOMA

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18 patients (15 male and 3 female) with high-grade malignant lymphoma have been treated with the MACOP-B chemotherapy according to Klimo and Conners (Ann. Intern. Med. 102: 596, 1985). Pretherapeutic diagnostic procedures revealed stage I in 1 case, stage II in 6 cases, stage III in 3 cases and stage IV in 8 cases. In 6 patients there were constitutional (B) symptoms. The original histology was reclassified by one of us (K.D.) according to the Rappaport classification. Up to now, 8 patients achieved a complete remission and 10 patients a partial remission. Of 8 patients with diffuse histiocytic lymphoma, 4 showed complete remissions. Only one of three patients with lymphoblastic lymphoma achieved a short-term complete remission. The other two patients with lymphoblastic lymphoma showed progression on week 10 and 11 of the scheduled treatment after having achieved a transient partial remission. As major toxicity we observed mucositis in 5 cases. Other side effects were minor. We conclude that the 45 % complete remission rate observed by us does not yet allow confirmation of the encouraging results previously reported by others. Furthermore, we would caution against the use of this regimen in lymphoblastic lymphoma.

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"IDIOPATHIC" (IMMUNE) THROMBOCYTOPENIA (ITP) AND LOW GRADE NHL (LP-IMMUNOCYTOMA)

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In NHL thrombocytopenia is primary a result of bone marrow infiltration. The coincidence of NHL with ITP is a rare event. Two cases of lymphoplasmocytoid immunocytoma (LPIC) are reported in which ITP preceded the diagnosis of lymphoma.
Case one, male, 42 years, was splenectomized 12 months after diagnosis of ITP because of unsatisfactory corticoid treatment. The patient gained continuous complete remission of ITP. The spleen was found to be discretely infiltrated with LPIC. 39 months after onset of disease the patient fell ill with generalization of lymphoma.
Case two, female, aged 41 was treated because of ITP with corticoids and splenectomized 13 months after onset of disease. Spleen histology revealed discrete infiltration by NHL. Patient did not obtain continuous remission of ITP and went on to be treated with corticoids and azathioprine intermittently. Bone marrow histology revealed infiltration by LPIC 41 months after diagnosis of ITP. Despite a varying degree of thrombocytopenia she does well now 3 years after splenectomy and no other signs of LPIC are present.
In both cases ITP was found to be the first symptom of low grade NHL neither detected by clinical methods nor bone marrow biopsy at this time.

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MACOP-B CHEMOTHERAPY AND CONSOLIDATION RADIOTHERAPY IN PATIENTS WITH ADVANCED HIGH GRADE NON-HODGKIN LYMPHOMA

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Twenty-two patients (pts) with advanced high grade NHL were treated with MACOP-B followed by involved field radiation in an ongoing study. Median follow up at this time is 12 months (2-24 months). Out of 15 pts receiving this regimen as first line therapy, 13 are still in complete remission (CR), 1 patient is alive with relapse 4 months after CR, 1 patient died after PR. The main toxicity was mucositis. The myelosuppression was well tolerated without dose reduction. On the contrary, myelotoxicity was severe in 6 pretreated pts with the MACOP-B regimen as second line therapy. Although only 2 pts received the full dose, the regimen was effective in all pts: 4x CR, 2x PR.
From this preliminary data it is concluded that MACOP-B with consolidation radiotherapy is highly effective and well tolerated as first line therapy in advanced high grade NHL. In high risk pts further intensification of chemotherapy and/or radiotherapy seems to be possible. In low risk pts dose reduction might be reasonable, as MACOP-B at reduced dose was still effective.

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TREATMENT OF MYCOSIS FUNGOIDES BY RECOMBINANT LEUKOCYTE ALPHA-A INTERFERON AND VINBLASTINE

M.E. Scheulen, S. Öhl and M. Bamberg

The results of the treatment of mycosis fungoides by alpha-interferon are inconsistent. Synergistic effects of interferon with vinca alkaloids against human tumors have been suggested in clonogenic assay systems. Thus, we have performed a phase I/II study with Hoffmann-La Roche recombinant leukocyte alpha-A interferon (INF) and vinblastine (BVL) in patients with mycosis fungoides to test antitumor effects and tolerance.
Six patients received INF i.m. from a starting dose of $3x10^6$ IU on day 1 to 3, $9x10^6$ IU on day 4 to 6 up to $10x10^6$ IU/m^2 on the following days for 12 weeks and the same dose three times weekly thereafter. In addition, VBL was administered i.v. once every three weeks from 0.05 to a maximum of 0.15 mg/kg.
Responses were seen in 5/6 patients: one complete response for 17 weeks which could not be maintained because of patient's rejection, three partial responses (5, 14, 49 weeks), and one minor response (5 weeks), respectively.
Except one patient who developed hypotonia and an acute toxic state with coma causing discontinuation of therapy, side effects were mild or moderate. Flu like symptoms were seen in all patients during initiation of therapy and could effectively be suppressed by acetaminophen. One patient was found to have elevated transaminases. After recovery he refused further treatment in complete remission.
In conclusion, combined treatment by IFN and VBL appears to be an effective regimen in patients with mycosis fungoides.

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TREATMENT OF REFRACTORY OR RECURRENT MALIGNANT LYMPHOMAS WITH A COMBINATION OF ETOPOSIDE (VP-16), IFOSFAMIDE, METHOTREXATE, AND BLEOMYCIN (VIMB)

M.R. Nowrousian, B. Schoetensack, C. Anders, N. Niederle, R. Pfeiffer, S. Seeber and C.G. Schmidt

Patients (pts) with refractory or relapsed malignant lymphomas are known to have a poor prognosis. To improve the results in these pts, we have used a therapeutic regimen consisting of VP-16 (90 mg/m^2/day, Days 1,3,5), Ifosfamide (1200 mg/m^2/day + Mesna, Days 1-5) and Methotrexate (30 mg/m^2/day, Days 1,5) with or without Bleomycin (15 mg/day sc, days 1,5,12) (VIMB). From March 1984 to October 1985, 34 pts (26 males, 8 females), ranging in age from 17 to 66 (median 39) years, were treated. Of the 34 pts, 10 pts had relapsed following a complete response (CR) to first-line chemotherapy, 22 pts had failed to achieve CR to front-line therapy and 2 pts had failed to respond to multiple salvage regimens given after relapses of their disease. All pts had received extensive prior chemotherapies, with combinations containing Adriamycin in 24 of 34 pts. Histological types of lymphomas (Kiel classification) were: lymphoblastic 2, immunoblastic 2, immunoblastic-centroblastic 1, centroblastic 6, undifferentiated large cell 4, pleomorphic T-cell 2, centrocytic 2, centrocytic-centroblastic 3, lymphoplasmocytoid 1 and Hodgkin's disease 11. The overall response rate was 85 % with 35 % CR and 50 % partial remission (PR) or minor response (MR). The median relapse-free interval was 10 months in pts with CR and 6 months in those with PR or MR. Forty six percent of pts with CR are predicted to have continued CR at 2 years. The projected survival at 2 years is 63 % in pts with CR and 17 % in those with PR or MR. On the basis of these results, VIMB combination appears to provide long-term disease-free survival and possibly a cure in a small but significant number of pts with refractory or relapsed lymphomas.

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ALTERNATING PROMACE/MOPP CHEMOTHERAPY IN ADVANCED DIFFUSE POOR PROGNOSIS NON HODGKIN LYMPHOMA (NHL). A PRELIMINARY REPORT OF THE NON-HD-LYMPHOMA COOPERATIVE STUDY GROUP (NHDLCSG), ITALY.

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Third generation chemotherapy (CT) regimens have recently increased complete remission (CR) rate and disease free survival (DFS) in advanced stages of aggressive lymphoma. Up to April '87 eighty-four patients (p), 57 males and 27 females, median age 49 ys., with diffuse, intermediate and high-grade malignancy NHL were treated with the PROMACE/MOPP protocol. Criteria for entry into the study included: no prior therapy, III (24 p) and IV (60 p) stage, histological diagnosis of diffuse centroblastic-centrocytic (19 p), centroblastic (32 p), immunoblastic (23 p), T-zone (7 p) and histiocytic (3 p) lymphoma. All p received six courses of chemotherapy at full dose, plus radiotherapy on bulky disease. B symptoms were present in 28 p (33%), bulky disease (>10 cm) in 23 (27%), and splenic involvement in 20 (24%). The most common localizations for extranodal disease were bone marrow (19%), gut (16,5%) and liver (15,5%). 8 p (9,5%) died during therapy and at present 72 p are evaluable for response. 48 out of 72 p achieved CR (66,5%), 11 PR (15%), 9 were NR (12,5%) and 4 went into PD (5,5%). At a median of six months of remission follow-up (range 2-23 months), DFS was 79%, since 10 out of 48 p (21%) had a relapse in a median time of 5 months (range 2-15) from CR.

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CLONAL EVOLUTION AND RESPONSE TO 2'DEOXYCOFORMYCIN (PENTOSTATIN) IN ATYPICAL, REFRACTORY HAIRY CELL LEUKEMIA (HCL)

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A 51-year-old male presented with fatigue, pancytopenia, and splenomegaly in June 1985. Based on typical bone marrow histology, dry tap, and monocytopenia a diagnosis of HCL was made. Rearrangement of the T_β chain gene in blood and spleen cell DNA suggested a clonal T cell disorder. Monoclonal B cell markers were absent. After a short, effective course of recombinant interferon alpha 2c (IFN-alpha), splenectomy was performed in August 1985 with subsequent restoration of normal blood cell counts. In March 1986, the patient relapsed and IFN-alpha was reinstituted. Despite dose escalation, the HCL proved refractory and a generalized maculopapular rash developed accompanied by rising serum levels of polyclonal IgG. Therefore, in August 1986, 2'deoxycoformycin treatment was initiated (4 mg/m^2 in weekly to monthly intervals for nine months) resulting in normalization of platelet, granulocyte, and erythrocyte counts after two, and five months, respectively, and marked reduction in bone marrow infiltration. In October 1986, clonal rearrangement of the T_β chain gene was no longer detectable and skin alterations had resolved. Serum IgG levels, however, continued to be elevated and in February 1987, IgG-kappa paraprotein appeared. Clonal expansion of B lineage cells was confirmed by the demonstration of rearranged c_μ bands in blood cell DNA. Thus, 2'deoxycoformycin proved effective in atypical HCL refractory to IFN-alpha after splenectomy. Mechanisms to be elucidated, however, may permit or even favour clonal B cell expansion and maturation during low-dose maintenance treatment.

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THERAPY AND PROGNOSIS OF LOW-GRADE MALIGNANT NON-HODGKIN'S LYMPHOMAS (NHL) WITH PRIMARY EXTRANODAL STAGES I AND II

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In 46 out of 407 (11,3 %) patients of the period 1976-1985 with centroblastic-centrocytic (CB-CC; 19/173), centrocytic (CC; 7/53) and immunocytic (IC; 20/181) NHL an extranodal manifestation of stage I and II was observed. 26 were in stage IEA, 12 in IIEA, and 8 in I/IIEB. Median age was 60,5 years. Predominant extranodal sites were stomach and gut (16), skin (8), and salivary glands (5). Other sites were thyroid, mamma, testis, lung, kidney, bone/soft-tissue, oral/nasal/pharyngeal region. There was a gastrointestinal predilection for germinal cener NHL (7 CB-CC, 3 CC), whereas IC NHL had some affinity to the skin (5/8) and the salivary glands (3/5). Treatment consisted of operation in 17 cases followed by chemotherapy and/or radiotherapy in 11 of them. This was also given to further 28 patients that were not operated or inoperable. A complete remission was achieved in all stages IEA and I/IIEB, and in 73 % of stages IIEA. Relapses occured in 33 % of all operated and in 61 % of non-operated cases. This difference may be biased as many operated cases had a relatively smaller tumor mass. No specific localization or histology predisposed to relapses. Overall 10-year-survival probability was 68 %, for stage IEA 71 %, and 50 % for stage IIEA with a continuous plateau from the third year on. No survival difference was found compared to the corresponding nodal stages.

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The Primary Malignant Non-Hodgkin Lymphoma of the Stomach. Pathology and Prognosis.

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In 52 patients with primary non-Hodgkin lymphoma of the stomach, from the archives of the Institute of Pathology of Hannover Medical School, the tumors were re-classified and the pathological tumor stage at the time of surgical operation was determined retrospectively. With recourse to hospital reports, the clinical stage as well as the kind of therapy administered in addition to the surgical operation were researched. Information concerning the history of the disease were obtained from the physicians responsible for the out-patient management and, partly, from the patients themselves. The tumor stage was found to be the most important prognostic factor. In lower tumor stages, with confinement of the neoplastic disease to the gastric region, a five-year disease-free survival was achieved in over 80%. Opposite to this, in the more advanced tumor stages, the prognosis was rather poor.
The centroblastic-centrocytic and the centroblastic type were found most frequently. The histological tumor type, however, proved to be essential for the further course of the disease only in the earlier tumor stages. Lymphomas with a low grade of malignancy could be cured in practically all cases in which the tumor was confined to the gastric region. The centrocytic type showed an intermediate course.
The combination of resection and radiotherapy, in more advanced stages supported by adjuvant chemotherapy, yielded the best results with respect to complete remission.

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Therapeutical Evaluations of Primer Gastrointestinalis lymphomas

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Some new diagnostic and therapeutical possibilities of Hodgkin and Non-Hodgkin lymphomas were developed in the last decade. Previously the patients recovery was dependent on the surgical treatment; recently cytostatic and radiological therapy - applied in due time - has beneficial effects like complete or partial remission. The authors analyse their cases from the clinical point of wiew. Their cases are three Hodgkin and fifteen Non-Hodgkin lymphomas: the latter divided in high grade (10 cases) and low grade (5 cases). The gastro-intestinal localisations of Non-Hodgkin lymphomas are the following; 10 in the stomach, and 5 in the large intestines. The Hodgkin lymphomas was localisated in the stomach. Most of the patients received cytostatic therapy immediately after surgical resection; two of them received it 10-14 months later. The diagnosis was made postoperatively according to the histological examination. The effect of the cytostatic therapy was followed by ultrasound, CT, scintigraphy and the clinical symptoms. 13 patients were cured, partial remission was registered in three cases; two patients died. Authors compare their results with the data of the international literature.

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PRIMARY EXTRANODAL DISEASE IN IMMUNOCYTIC LYMPHOMA (IC)

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The Kiel classification separates CLL and IC because of their different prognosis and clinical behaviour. One striking difference is the occurrence of primary extranodal disease in IC. We investigated 380 CLL patients and found no primary extranodal manifestation, but 19/147 IC (13%) presented with localized disease in various organs (skin:10, gastrointestinal tract:6, brain:2, orbita:1). Histologic subtypes were available in 17 cases (IC-oid:8, IC-cytic:5, IC-polymorphic:4 patients respectively).3 patients (2 polymorphic and 1 IC-oid) showed conversion to secondary IB with a poor prognosis despite aggressive treatment. Therapy in the other patients consisted of either surgery alone or combined with irradiation. 12 patients received chemo-therapy in addition. All but one patient responded. 5 patients died during observation, 3 of them because of progressive disease. Folow up of the 11 patients with complete remissions showed that only 3 patients relapsed localy, the latest recurrence occured after 24 months. Survival time in extranodal IC was 65 months compared to 52 months in IC with advanced disease. According to the long term follow up in our patients our paper should contribute to the question of the possibility of cure in localized extranodal IC.

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DEFINITIVE RADIOTHERAPY IN LOCALIZED ORBITAL NHL

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The records of 41 pts irradiated for orbital NHL from 1958 to 1986 were reviewed. All were histologically verified. According to the Kiel classification there were 15 low malignant and 9 high malignant lymphomas. The 17 pts resting had been classified according to the old German classification as reticulum cell sarcoma (15 pts) and lymphosarcoma (2 pts). Stage distribution showed 14 pts in stage I, 19 in stage II, 8 in stage III/IV, all E.
Megavoltage irradiation was given in conventional fractionation from 26 Gy up to a total dose of 46 Gy using different techniques. Since 1978 treatment has been based upon CT scans and corresponding computed treatment planning (8 pts). Whenever possible, the lens was shielded.
Clinically complete remission was achieved for stage I in 13/14 pts (93%), for stage II in 17/19 pts (90%) and for stage III/IV in 5/8 pts (63%). There were 9 pts with local recurrence: within the radiation field 3 pts, on the field edge 6 pts. Dissemination was seen in 6 cases. Recurrence resp. dissemination occured only twice beyond two years. Only 2 pts out of this group survived more than 3 years. The freedom from relapse rate is 65 % (10/14) for stage I, 75% (13/17) for stage II after a median follow-up of 36 months. As to low/high malignant lymphomas the freedom from relapse rate is 89% and 43% respectively. The rate of side effects was low. 2 pts showed slightly dry eye; twice the lens had to be removed because of progressive cataract.
We conclude, radiotherapy is the treatment of choice for localized orbital NHL. Freedom from relapse rate is high, morbidity low.

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PRIMARY AND SECONDARY AILD/LGR-X-LIKE LESIONS IN THE BONE MARROW

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Between 1966 and 1987 129 bone marrow biopsies (B.M.) of 114 patients were studied. Granuloma-like changes containing a variety of lymphoid infiltrations and a variety of "reactive" cells, similar to angio-immunoblastic lymphadenopathy/lymphogranulomatosis X (AILD/LGR-X) type infiltration in lymph nodes, were observed. Correlating these B.M. infiltrations with clinical diagnoses and pathologic changes in lymph nodes the following groups were distinguished: A) AILD/LGR-X or AILD/LGR-X-type of peripheral T-cell lymphoma (PTL) and Lennert's lymphoma with lymphadenopathy (n=27); B) similar focal infiltrations in the B.M. without peripheral, thoracical and abdominal lymphadenopathy (myelo-PTL-like lesions) (n=64); C) similar focal infiltrations in the B.M. associated with AIDS (n=3); D) granulomatous lesions of the B.M. associated with various reactive and neoplastic lymphadenopathy (e.g.SLE, Hodgkin's disease) (n =20). For the subtyping of LGR-X 5 histologic subtypes of the focal infiltrations were distinguished previously described: 1) epithelioid cell type (15%); 2) lymphocytic type (40%); 3) plasma cell type (7%); 4) mixed cell type (25%); and 5) blast cell type (13%). The focal infiltrations of our first three groups (A, B, C) were similar in all cases irrespective of the diagnosis in lymph node biopsies. The different histologic subgroups (1-5) could not be allied to the different well distinguished pathologic entities. Myelo-PTL-like malignant lymphoma without lymph node enlargement was identified and compared with AILD/LGR-X, AILD/LGR-X-type, PTL and Lennert's lymphoma. Therefore we propose the existence of a primary multifocal PTL-like lesion in the B.M. without simultaneous involvement of lymph nodes.

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STAGING AND RESPONSE CRITERIA IN HAIRY CELL LEUKEMIA (HCL): AN ANALYSIS OF THREE CLASSIFICATIONS (C').

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The 2nd Int. Conf. on HCL, Leeds, 1986, recommended to classify the responses in the treatment of HCL. This c' focusses on CR which is rather rare in HCL and is difficult to confirm by bone marrow (BM) biopsies unless immunohistology is included. After treatment of HCL many of our pts were classified as MR according to Leeds-c' (LC) but were in a non-symptomatic and stable phase of the disease (Karnofsky 90-100). Since the LC may neither be sensitive nor discriminative enough to distinguish responses, we compared the LC with 2 other c's, Jansen's Stages (JS) and with a newly proposed c' (NC) which focusses on non-symptomatic stable disease (nsSD). 36 pts, 29 males and 7 females, age 55.2±11.5 yr, survival rate 0.83 after 13 yrs, were evaluated according to these 3 c's during 3 periods of disease (POD): POD splenectomy (Sx), interferon therapy before Sx, and interferon after Sx. The responses were classified after 1,3,6,12,24,48,96 mos during each POD. The responses according to LC were scored: CR=4, PR=3, MR=2, NR=1, JS scores were A=4, B=2.5, C=1. In the NC we scored nsSD=4, sSD or ns PD =2.5, and symptomatic progressive disease =1. Mean values ±SD were calculated from 3-57 single scores of each c' in each POD before, after 1-6 mos, and after more than 6 mos of therapy. The analysis of 594 assessments showed: The mean scores of the LC were lower than the mean scores of JS or NC. The SD of the scores from LC were smaller than from JS or NC. It is demonstrated that the JS and NC scores increase faster than the LC scores although BM biopsies were not considered for assessment of responses. Since LC can not be used for c' before therapy and LC is neither as sensitive (mean values) nor as discriminative (SD values) as JS or NC, it might be useful to describe the staging and the responses to treatment of HCL according to JS or NC.

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IMMUNOHISTOLOGICAL EXAMINATION OF BONE MARROW BIOPSIES FROM PATIENTS WITH HAIRY CELL LEUKEMIA: CHANGES FOLLOWING TREATMENT WITH INTERFERON OR 2-DEOXYCOFORMYCIN

J. Thaler, H. Denz, G. Gastl, C. Gattringer, M. Lechleitner and H. Huber

Bone marrow biopsies from 19 patients with hairy cell leukemia (HCL) were investigated using cryostat sections and an immunoperoxidase method. Interferon (IFN)-alpha-2 produced a significant reduction of bone marrow infiltration after a median treatment period 12 months ($p < 0,001$). Nevertheless a complete disappearance of hairy cells could not be observed.
Bone marrow evaluation of 3 patients treated with 2-deoxycoformycin (dCF) showed a more rapid and pronounced reduction of hairy cell infiltration. The immunological phenotype of hairy cells remained unchanged following IFN-treatment; similar results were seen in the few patients treated with dCF.
Within the infiltrated bone marrow a considerable number of "reactive" T lymphocytes was identified with a prevalence of CD4+ subtype in untreated cases. IFN induced a reduction of T helper cells ($p < 0,05$). The potential meaning of these findings is discussed.

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A BICLONAL LYMPHOMA: HAIRY CELL LEUKEMIA AND PRO-LYMPHOCYTIC LEUKEMIA

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We describe a patient who presented with the clinical picture of hairy cell leukemia (HCL). Bone marrow and peripherial blood lymphoma cells showed morphologic and immunologic features of HCL. Under recombinant &-2-interferon (&-2-IF) therapy lymphoma cell morphology converted to prolymphocytic leukemia (PLL). At diagnosis the lymphoma cells expressed CD 24 and FMC 7 surface antigen, but stained negative for surface immunoglobulins, light chains and anti-CD 5. During &-2-IF treatment surface antigen expression changed to CD 24, CD 5 and FMC 7. Surface Ig-D and lambda light chains became strongly positive. Southern Blot analysis of peripherial blood mononuclear cells showed two rearranged immunoglobulin bands at diagnosis but only one upon &-2-IF therapy. These data suggest, that this case suffered from a biclonal lymphoma, HCL and PLL. While undergoing &-2-IF treatment the HCL came into remission, wheras the PLL clone proved to be poorly sensitive to &-2-IF therapy.

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VALUE OF SERUM THYMIDINE KINASE (TK) IN STAGING OF LOW GRADE MALIGNANT NON-HODGKIN LYMPHOMA (NHL)

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The value of serum TK for the staging of NHL as compared with serum β_2-mikroglobulin (β_2MG) and serum lactat dehydrogenase (LDH) was investigated in 73 patients. In addition, the performance status was determined by the Karnofsky index (KI). Patients with chronic lymphocytic leukemia (CLL; n=34) and immunocytoma (IC; n=17) were staged according to the Binet classification, and the other low grade NHL (n=22) according to the Ann Arbor classification. Patients who had received chemotherapy within two weeks before the blood sampling were excluded.

The analysis (Kruskal-Wallis one-way ANOVA) of all CLL and IC patients revealed that only TK values increased significantly with Binet stages (p=0.04; n=41), but neither LDH (p=0.26; n=33) nor β_2MG (p=0.26; n=14) did so. The TK values of these patients in Binet stage A were 8.5 $\pm$ 2.1 U/µl (mean $\pm$ S.E.M.; n=22; range 1.9-39.6), in stage B 13.7 $\pm$ 6.4 U/µl (n=5; range 3.8-38.8), in stage C 13.3 $\pm$ 2.6 U/µl (n=14; range 3.7-31.7) and of 22 healthy controls 3.8 $\pm$ 0.2 U/µl (range 2.2-6.0). The comparison of TK, LDH and β_2MG values in CLL and IC patients with identical Binet stages revealed no difference (U Mann-Whitney-Wilcoxon rank sum test). No correlation was found between the serum TK, LDH, β_2MG and the KI (n=73). The results indicate that TK might be a better parameter to estimate NHL progression than LDH or β_2MG.

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BONE MARROW SPARING LEUKEMIC B-CELL LYMPHOMA WITH A PHENOTYPE BETWEEN HCL AND PLL: A NEW LYMPHOMA ENTITY?

E. Thiel, R. Zankovich, V. Diehl and R. Schlag

We observed the consistent following features in nine patients (7 males, 2 females; age range from 54 to 72 years):

(1) Overt leukemia with a WBC between 20.000 and 60.000 at diagnosis.
(2) Unchanged bone marrow as analysed by several biopsies in every patient and by NMR.
(3) Gigantosplenomegaly without any or with only moderate lymph node enlargements.
(4) A cellular phenotype of large lymphoid cells having PLL-like nuclei with one or two prominent nucleoli, a broad cytoplasm often with short hairy-like protrusions, a strong tartrate-sensitive acid phosphatase, and the following membrane antigens: monoclonal surface IgM; B antigens CD 19, 24, 20 and 22; CD 5 (Leu 1); Leu M 5.

Since the composite cellular phenotype combines elements of both hairy cells and prolymphocytic leukemia cells, it may be misdiagnosed as a tartrate-sensitive HCL-variant or a Leu M5-positive PLL-variant. Clinical course and response to therapy, however, also differ from PLL and HCL being long-lasting and having a good response to alkylating drugs, but no response to interferon therapy.

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MALIGNANT TRANSFORMATION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) OCCURENCE IN EXTRANODAL ORGANS

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Malignant transformation to high grade NHL is a relatively rare event in CLL. We investigated 380 patients and found 3 cases with primary extranodal localisation of the secondary high grade NHL. 2 cases represent true Richter's syndrom, i.e. the development of histologic conversion in the gastrointestinal tract (stomach). One patient, a 59 year old man had centroblastic lymphoma (CB) PS IE. Lymph nodes removed during surgery revealed the picture of CLL. Survival was 57 months from the diagnosis of CLL and he died 5 months after surgery. The other case is a 81 year old male with CB involving the stomach and the abdominal nodes, diagnosed by surgery 12 months after establishing the diagnosis of CLL. It seems remarkable that despite mild treatment leukocyte counts and differential counts normalized gradually in the months preceeding the diagnosis of CB. The patient ist still under treatment (CHOP) and alive. The third case is a 47 year old female treated from the time of diagnosis with different forms of therapy because of the unfavorable course of the disease. 60 months after the initial diagnosis she developed skin infiltration suggesting the histologic conversion. Despite repeated lymph node biopsies in our CLL patients showing an unfavorable course we did not see histologic transformation in the removed nodes. So the fact that the development of the secondary high grade NHL in CLL shoes a prevalence to extranodal localization seems to be of interest and will be discussed with regard to the literature.

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B-CELL LYMPHOMA/LEUKEMIA DEVELOPING A NEW CYTOGENETIC MARKER

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A cell line has been established in vitro from the bone marrow of a male patient with malignant lymphoma having the same morphological features as its in vivo counterpart. The cells were cultured in IMDM (Iscove's Modified Dulbecco's Medium) with 20% of fetal calf serum. They grew vigorously in culture immediately after their explantation and have been propagated in this medium for 14 months. After this period of growth the cell line was able to grow continuously in serum-free IMDM supplemented with bovine albumin, iron-saturated transferrin and soy-bean lipids. Immunological analyses of the cell line confirmed its B-cell phenotype and identity with the in vivo original malignant cell population i.e. IgM kappa. The cells in vivo have been shown to have the karyotype 46, XY, t (8, 14), dup (1q). The cultured cells retained the same karyotype but in addition acquired del (18q). Our studies confirm that the cultured cells are not substantially differrent from the original malignant cell population, but that deletion at chromosome 18 might provide a growth advantage. Thus, the cell line provides an appropriate model for further studies of B-cell malignancy.

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SPONTANEOUS REMISSION IN HIGH GRADE NON-HODGKIN LYMPHOMA STAGE IV

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Spontaneous remission in high grade non-Hodgkin lymphoma has been reported only rarely. We describe the case of a 60-year-old patient who underwent emergency laparotomy for an acute abdomen. Excision of a perforated stomach ulcer was performed and biopsies of metastatic liver lesions were taken. Histologically immunoblastic lymphoma of the stomach with involvement of the liver was diagnosed. Postoperative staging revealed no other site of manifestation. Sonography and computed tomography confirmed liver lesions. Immunoblastic lymphoma stage IV A was diagnosed and chemotherapy (CHOP) planned.

Endoscopically a persistant stomach ulcer was found. Therefore gastrectomy was performed to prevent perforation during chemotherapy, 4 weeks after the emergency laparatomy. At this time, no more liver lesions could be detected peroperatively. Histologically, no signs of lymphoma were found. Sonography and computed tomography of the liver also became normal postoperatively. Chemotherapy was withheld, and the patient remains in remission four years after diagnosis.

Possible mechanisms of spontaneous remission in non-Hodgkin lymphoma as viral or bacterial disease, host defense factors or trauma like an operation are still unclear, but may provide important clues for understanding causes and developing concepts in cancer therapy.

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CYTOLOGIC DIAGNOSIS OF MALIGNANT LYMPHOMA IN 80 PATIENTS USING IMMUNOCYTOCHEMICAL STUDIES

J. Oertel, B. Oertel, D. Huhn

Fine needle aspiration of lymphnodes has gained acceptance as a valuable diagnostic technique. The purpose of this study was to investigate if accuracy can be improved in the cytologic diagnosis of malignant lymphoma by combination of cytologic smears and immunologic marker studies. An indirect immunoperoxydase technique and the immunoalkaline phosphatase (APAAP) technique were used.
Of 42 low grade non-Hodgkin's lymphomas (Kiel-classification) 39 expressed CD-24. Surface immunoglobulin with a predominant light chain type was identified in 38 of the 39 cases. 3 patients showed antigens of T cells ($CD3^+$ $CD4^+$). The percentage of T9 (transferrin receptor) positive cells exceeded 15 % in 3 cases with short survival.
17 cases of high-grade lymphomas (centroblastic 10, immunoblastic 6, Burkitt 1) were studied. All lymphomas reacted with CD24 and showed light chain class restriction. The percentage of T9 positive cells exceeded 45 % in all cases.
Two lymphoblastic lymphomas (one of the T cell type, one of the pre B cell type) expressed terminal desoxynucleotidyl transferase. 3 patients with pleomorphic T cell lymphoma showed a predominant population of $CD4^+$ (> 80 %) and $T9^+$ (> 45 %) cells.
16 cases of Hodgkin's disease were included in this series. We were able to make a diagnosis by cytology alone in 11 cases. The presence of $BerH2^+$ cells with the morphology of Sternberg-Reed cells confirmed the diagnosis in 2 additional cases.
Our findings lead to a simple schema for the immunologic diagnosis of malignant lymphoma in cytological preparations.

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DEOXYCOFORMYCIN FOR TREATMENT OF LYMPHOID MALIGNANCIES - BIOCHEMICAL PREREQUISITES AND SEQUELAE.

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Deoxycoformycin (DCF) is an adenosine deaminase (ADA) inhibitor and has been shown to be effective in lymphoid malignancies. Our previous studies have indicated that sensitivity of the lymphoid cells depends on their enzymatic profils. In a prospective phase II trial conducted by our group, the efficacy of this drug in chronic T cell malignancies, in hairy cell leukemia and in B-CLL is being investigated. 32 patients are now evaluable for response. Simultaneously, we have studied the enzymatic make-up (ADA, 5NT, AdR-kinase) of the leukemic cells, the in vitro and in vivo effects of the drug on DNA strand breaks, on ADA activity, dATP and NAD levels of the leukemic cells from the patients. The constellation of low ADA, high 5NT and high AdR-kinase activities seemed to correlate with clinical response. Incubation of the leukemic cells with DCF invariably caused a prompt suppression (already at 4h) of ADA activity, subsequent dATP accumulation and NAD depletion after 24h. DNA strand breaks of > 50% was found in the responders and not in the non-responders. Studies of the ADA, dATP and NAD levels in the leukemic cells taken from the patients at 0h, 24h, 48h, and 5 days after the first administration of DCF also showed that ADA was suppressed, and dATP accumulated up to 5 days after application of DCF but the extent of these changes did not correlate with response. On the other hand, DNA strand breaks in the leukemic cells in vivo correlated with clinical response. Thus NAD depletion and DNA strand breaks are involved in response to DCF therapy. The knowledge of these cytotoxic mechanisms is important for the development of further specific enzyme inhibitors for treatment of lymphoid neoplasms.

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HISTOLOGIC CLASSIFICATION AND STAGING OF CLL

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Bone marrow biopsies (BMB) of 610 untreated and 250 treated patients with CLL were assessed for diagnostic evaluation. 14 histologic variables were correlated with the clinical findings to determine factors of value in predicting prognosis. According to the predominant lymphoid cell 3 histologic types were distinguished: 1) small round, 2) notched and 3) mixed, with median survivals of 53, 26 and 28 months respectively. Three growth patterns were observed in the BMB: nodular, interstitial and packed marrow, and these patterns had outstanding prognostic and clinical significance at all clinical stages (Rai) (median survivals of 90, 46 and 28 months respectively). The consistancy of these patterns was established by serial sections in 5 cases of each pattern, while a change from nodular to diffuse signified progression of disease. In addition, the quantity of lymphoid cell burden in the BMB served as a useful criterion for histologic staging of CLL, supplementing any clinical staging system in use. Biopsies taken after therapy showed reduction in tumour cell burden, but frequently also in haematopoietic tissue. Furthermore histologic features of indolent (smouldering) CLL with survivals of more than 10 years are described.

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STUDIES ON SIGNAL TRANSDUCTION IN B-CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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The phorbol ester 12-0-tetradecanoylphorbol 13-acetate (TPA) and the calcium ionophore A23187 bypass the initial steps in the signal transduction pathway by activating directly protein kinase C. TPA and A23187 act synergistically in inducing differentiation of B-CLL suggesting that the second messenger pathway downstream from protein kinase C is intact in B-CLL.
We studied the response of B-CLL cells to external signals: B-cell differentiation factor (BCDF) and IL-2. B-CLL cells from 17 patients were incubated in the presence of TPA, A23187, IL-2, or BCDF for 6 days. Parameters of induced changes were morphology, 3H-uridine incorporation (RNA synthesis), expression of Tac antigen (IL-2 receptor) and production of monotypic immunoglobulin. TPA or TPA + A23187 were effective in all cases. Although Tac antigen was induced, IL-2 alone or in combination with TPA ± A23187 did not induce differentiation. Similarily, BCDF (effective on normal peripheral blood B-cells) did not cause any apparent changes in B-CLL cells nor had the combination of BCDF and TPA ± A23187 any further influence on immunoglobulin synthesis. These data indicate that whereas the second messenger pathway can be stimulated effectively by direct action of TPA + A23187, B-CLL cells appear not to be able to respond to external signals. It is important to differentiate between non-responding B-CLL cells and responsive residual normal B-cells suggesting falsely a differentiation inducing effect of BCDF or IL-2 on B-CLL cells.

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HUMAN RECOMBINANT INTERFERON-ALPHA IN THE TREATMENT OF PATIENTS WITH HAIRY CELL LEUKEMIA

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During the last 2 yrs we have treated 14 hairy cell leukemia /HCL/ patients with human recombinant interferon-alpha-2c /Boehringer Ingelheim/. The above group consisted of 8 non-splenectomized and 6 previously splenectomized progressive HCL cases. The patients received daily doses of 5 x 10^6 units of IFN for 3 mths and 2 x per week for the next 3 mths thereafter by i.m.route. The therapy resulted in the complete /9 cases = 64%/ or partial /2 cases = 14%/ clinical and haematologic remission /response rate = 78%/ with disappearance or marked reduction in circulating and bone marrow hairy cells, decreased spleen size and recovery of normal haemopoiesis. The above results lasted for about 1 yr when in the majority of cases the gradual reappearance of hairy cells with deterioration of haematologic values developed. Administration of low doses of IFN again caused marked improvement and maintains the remission state until now. Apart from a transient flu-like syndrome during the first 2 weeks of the therapy no other side effects were observed.

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DEFICIENCY IN INTERFERON RESPONSE OF PERIPHERAL BLOOD LEUKOCYTES FROM PATIENTS WITH NON-HODGKIN-LYMPHOMA:

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In search for a rationale for the use of interferons in treatment of non-Hodgkin-lymphoma (NHL), we have investigated the interferon systems of 13 patients with low grade NHL, 15 patients with high grade NHL, and 20 patients with chronic lymphocytic leukemia or leukemic immunocytoma (CLL/IC) at the time of diagnosis. Productions of interferon induced by phytohemagglutinin (PHA), concanavalin A (Con A), pokeweed mitogen (PWM), corynebacterium parvum (C. par.), Herpes simplex virus (HSV), Newcastle disease virus (NDV) and interleukin 2 (IL2) were studied in the peripheral leukocytes from the patients and from 21 control persons by means of a whole blood technique. All 3 groups of patients with NHL had significantly reduced production upon stimulation by NDV (p ranged between 0.0038 and <0.0001) compared to controls. Similarly, C. par also induced lower titers of interferon in the leukocytes of patients with non-leukemic NHL ($p=0.0015$ for low-grade NHL and $p=0.0038$ for high grade NHL). When stimulated by PHA, the interferon response of all groups of patients was within normal range. With the exception in low-grade NHL, Con A also induced normal titers of interferon in the patients. The levels of interferon induced by PWM, HSV and IL2 were very low and no differences between controls and patients could be found. As NDV and C.par induce mainly interferon alpha and the mitogens PHA and Con A interferon gamma, our results suggest that there is a deficiency in interferon alpha response but normal response in interferon gamma in the patients with NHL. The implications of this study are: (1) Interferon therapy in patients with NHL represents substitution therapy, (2) Interferon alpha is more promising than interferon gamma in patients with NHL.

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PROGNOSTIC FACTORS IN ADVANCED HODGKIN'S DISEASE

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The prognostic value of several clinical parameters was tested by means of regression models in a group of 78 patients with Hodgkin's disease stages IIIB and IV treated with a COPP chemotherapy. Some patients had recieved additional radiotherapy. Biochemical parameters could not be included in the analyses, since they were abnormal only in a small minority of patients.

The propability to reach a complete remission (CR) was negatively correlated to the presence of infiltrations of the liver. Other parameters such as age, sex, stage, presence of B-symptoms, bulky disease, infiltrations of bone marrow or lungs were of no influence to the remission rate. In patients with CR, relapse-free survival was negatively correlated to the presence of bone marrow infiltrations at the time of diagnosis. Overal survival was mainly correlated to the age of the patient. However, this correlation based on the bad course of older patients not reaching a CR. In older patients in CR overal survival did not differ significantly from that of younger patients. Besides of age, presence of liver infiltrations was the only factor with a correlation to overal survival.

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FAST ALTERNATING COP/ABV/IMEP CHEMOTHERAPY IN PATIENTS WITH ADVANCED HODGKIN'S LYMPHOMA .

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In a pilot study of the German Hodgkin Study Group pts. in stages IIA-IIIA with risk factors and IIIB/IV receive a fast alternating chemotherapy consisting of COP (Cyclophosphamide 800mg/m^2 d1, Vincristin 1.4 mg/m^2 d1, Prednisone 40 mg/m^2 d1-15), ABV (Doxorubicin 40 mg/m^2 d15, Bleomycin 10 mg/m^2 d15, Vinblastin 6 mg/m^2 d15), and IMEP (Ifosfamide 1000 mg/m^2 d29-33, Etoposid 100 mg/m^2 d29-31, Methotrexate 30 mg/m^2 d31, Prednisone 40 mg/m^2 d29-35).

To date, 27 pts. (12 untreated, 15 previously treated) are evaluable. 19/27 (70%) pts. are in CR after 2-4 cycles of chemotherapy, CR after additional radiotherapy is 22/27 (81%). COP/ABV/IEMP is well tolerated, the main toxicities being leukopenia, slight nausea/vomiting and alopecia. Even though therapy was continued as soon as leukocytes were > 2.5 x 10^3 / mm^3, no serious infections were observed. COP/ABV/IMEP is an effective and well tolerated chemotherapy protocol which should be tested against standard protocols in a prospective randomized trial. The recruitment continues and updated results will be presented.

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CHEMOTHERAPY (CTX) OF HODGKIN'S DISEASE (HD) WITH A NEW REGIME: ADRIAMYCIN/ CYTOXAN/ETOPOSIDE/DEXAMETHASONE/VINCRISTIN (ACEDO)

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Advanced stages of HD especially with high tumor burden have only a moderate prognosis with conventional CTx (MOPP;COPP-ABVD) requiring more effective first line induction regimes. Therefore the ACEDO-regime was developed combining the most active agents in HD. Treatment schedule: Adriamycin 40mg/m² i.v. d 1, Cyclophosphamide 650mg/m² i.v. d 1, Etoposide 100mg/m² i.v. d 3,4,5, Dexamethasone 15mg/m² p.o. d 1-4, 8-11, Vincristine 1,4mg/m² i.v. d 1,8; q d 22, 4-6 cycles (depending on tumor bulk + response). 23 pts entered the protocol. Pts-characteristics: 13 male, 10 female, mean age 36 yrs (21-64); mean Karnofsky PS 90%; clinical stages IIB 3, IIIA/B 9, IVA/B 11; bulky disease (EORTC-criteria) 9/23; 13 pts untreated, 10 pretreated (CTx 6, RTx 1, CTx+RTx 3; refractory disease 2, relapsing 8). Results: 1 early toxic death occurred during the first cycle leaving 22 pts evaluable for response: CR 13/22(59%), PR 9/22 (41%), total response (CR +PR) 100%. Pts with no prior treatment (13):CR 8(62%) PR 5(38%); pretreated pts (9): CR 5(56%), PR 4(44%). Median remission duration for CR is 11+ mos (4-14+) and for PR 10+ mos (2-13+); the median survival for all pts. is 13+ mos (2-21+). Toxicity (23 pts.,112 eval. cycles) was predominantly related to the bone marrow with leucopenia WHO °2(20%), °3(61%), °4(19%) and thrombocytopenia °4(10%), fever and infection °1(25%), °3(5%), °4(10%) incl. 2 lethal septicemias. Nausea + vomiting were tolerable with 33% °3. One pt with high anthracycline pretreatment suffered from cardiomyopathy. Neurophathy °1 and °2(33%). Conclusions: The ACEDO-regime shows therapeutic results comparable to other aggressive regimes in advanced stages HD in untreated as well as pretreated pts and has excellent subjective tolerability but high bone marrow toxicity.Further investigations with this type of regimes are justified.

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IMMUNOHISTOCHEMICAL INVESTIGATION OF HODGKIN'S DISEASE LYMPHOCYTIC PREDOMINANCE TYPE IN COMPARISON WITH THE CLINICAL STAGE OF DISEASE

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Nodular paragranuloma (NP; n=104), diffuse paragranuloma (DP; n=16) and mixed type of Hodgkin's disease with lymphocytic predominance (MTLP; n=22) were studied with a panel of monoclonal antibodies (mAb). Cells positive with mAb detecting B-cell antigen (Ki-B3) and NK-cells/ T cell subset (leu-7) were quantified by morphometry and compared with the clinical stage. The significantly largest B-cell areas were found in NP (stage I). Regardless of clinical stage the B cell areas were prominent but smaller in DP; they were smallest in MTLP. In NP stage I significantly higer numbers of Leu-7^+ cells could be detected than in any other stage and subtype. Hodgkin, Sternberg-Reed, and L&H cells were positive with a mAb detecting X-hapten (3C4) in only a few cases of NP (14/96) and DP (2/15); in nearly all cases of MTLP, by contrast, these cells reacted positively (16/18). A similar reaction pattern was found for the mAb Leu-M1.
In summary: The B-cell content in Hodgkin's disease lymphocytic predominance type shows a significant correlation with the subtype, but not with the clinical stage. A high content of Leu7^+ cells appears to be characteristic for NP stage I.

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DIFFERENT IMMUNE FUNCTION IN HODGKIN'S DISEASE (HD)

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We studied lymphocyte reactivity by 14 different mito- and antigens,suppressor cell activity and using monoclonal antibodies T-cell subpopulations in 27 patients with HD and 88 healthy controls.According to histological criterias HD-patients could be divided into 2 groups,1(n=17)with nodular sclerosis and 2(n=10) with mixed cellularity.Cluster and discrimination analyses (F-test;T-test)were done according to the programs of the UCLA.Controls could be clustered into 3 groups with significantly different mito- and antigen responses($F>4-20; p<0.01$),group 1(n=18) showing medium,2(n=49)low and 3(n=21)high reactivities in all 14 systems tested,but normal suppressor activity and T-cell subpopulations.Suppressor activity was normal,T-cell subpopulations and T4/8-ratio were significantly reduced in both HD-groups($p<0.01$). HD-group 1 showed a lymphocyte reactivity as control group 1,HD-group 2 as control group 2 a significantly lower reactivity.Those control clusters then well could be reservoirs for different forms of HD.On the other hand lower lymphocyte reactivity in mixed cellularity may be due to the disease itself and indicative for a heavier lymphocyte impairment compared to nodular sclerosis.Lymphocyte reactivity well could be an important parameter for prognosis and course control in HD,therefore.

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CYTOSTATIC TREATMENT OF HETEROTRANSPLANTED HUMAN HODGKIN CELL LINES IN NUDE MICE.

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Treatment results of refractory or recurrent Hodgkin's disease are rather disappointing. A preclinical model was established for the testing of various substances and schemes for usefulness in relapse therapy of Hogkin's disease. The investigations were performed in two long term in vitro Hodgkin cultures (L428, L540). The L540 induces both intramuscular (i.m.) and intracerebral (i.c.) tumors in the nude mouse, the L 428 produces i.c. tumors only. Response to therapy was judged by the reduction in tumor volume (i.m.-system) or prolongation of survival time compared to a control group (i.c.-system). 9 different cytostatic drugs were tested as monotherapy in a variety of doses. Results: With the cell line L 540 similar results were obtained with regard to sensitivity and resistance in both the i.m. and i.c.-systems. DTIC and procarbazine proved to be the most effective drugs, both produced complete remission of all i.m. tumors, no relapses occurred within the observation period of a hundred days. In the i.c. system both drugs induced significant prolongation of survival, but the animals then died due to tumor relapse. CCNU, Bleomycin and MTX were found to be fairly effective in tumor reduction. No effect was seen after treatment with Vinblastine, Ifosfamide or Cisplatin. The L428 line proved to be very resistant to treatment attempts. Only Cisplatin produced a response with intracerebral tumors (median survival 22 days vs. 12 days in the control group). Conclusions: The effectiveness of DTIC and Procarbazine in the L 540 line is in contrast to the tumor progress of the patient under COPP/ABVD therapy. The problem of giving high effective substances in too small a dosage in multi-drug regimes will be discussed.

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ANTIBODIES TO A NORMAL CELLULAR PROTEIN (P-65) IN PATIENTS WITH HODGKIN'S DISEASE (HD)

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Samples from the L-428 Hodgkin cell line and from several other lymphoma cell lines were lysed and the soluble proteins were subjected to one- or two-dimensional gel electrophoresis. The separated proteins were transferred to nitrocellulose by the 'Western Blot' technique; antibody reactivity was detected by an immunoperoxidase reaction.

26 of 152 (17%) sera from patients with HD reacted with protein P-65 whereas only one serum was reactive in the control group consisting of 35 healthy persons and 20 patients with other malignant diseases. Thus, the difference between the two groups was highly significant ($p<0.01$). These antibodies were most common in stage III of HD disease. Splenectomy had no effect on the incidence of these antibodies, and there seemed to be no correlation with B-symptoms or with the histological subtype.

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AGGLUTINATION AND CO-AGGLUTINATION PHENOMENA OF HODGKIN CELLS

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New agglutination and co-agglutination phenomena are described in Hodgkin culture cell lines. 1) "Specific" agglutination by a lectin of the Sambucus nigra plant without neuraminidase treatment (NT), on the contrary T- and B-lymphocytes are only agglutinated after NT. 2) Reversed (antigen) agglutination (RA) of Hodgkin cells by arabinogalactan and certain galactans due to galactose specific lectins of the "liver family type". 3) They are also responsible for the co-agglutination of Hodgkin cells after NT with Chang liver cells. 4) The rosette formation with NT Streptococci B is also due to the Hodgkin cell lectins: it is inhibited by tunicamycin and pronase treatment of these cells. 5) The RA with mannans and fucans (sponges) demonstrates the presence of mannose and fucose specific lectins on Hodgkin cells. 6) Co-agglutination with yeast and candida confirm the occurrence of mannose-specific lectins. 7) Co-agglutination of NT lymphocytes by L 540 as a primer of mitogeneity of HD/SR cells and sialylation of galactose residues on the partner cell. In summary, the Hodgkin cells show a similar lectin pattern as most macrophages do and may show similar adherence phenomena with bacteria, viruses and other organ cells. Blocking of these lectins, by galactose infusions, for instance, may influence the pathogenesis of this disease.

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HODGKIN`S DISEASE WITH PRIMARY BONE MARROW (BM) INVOLVEMENT: AN ANALYSIS OF 55 CASES.

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Primary bone marrow (BM) involvement by Hodgkin`s disease (HD) is relatively rare but deserves special considerations for therapeutic strategies. During a 5 year period 55 cases of HD with primary BM involvement were reported to the German Hodgkin study group (6.7% of all documented cases) 43 of whom qualified for the study protocol (HD-3, stages IIIB and IV). Most of these patients had B symptoms (80%) and an extension of the disease over 3 or more lymphatic regions (78%). Additional extralymphatic sites or the spleen were involved in 27 and 29%, respectively. The distribution of histological subtypes was somewhat different to the whole population with a predominance of mixed cellularity (42%) and nodular sclerosis (21%). A large mediastinal mass was significantly less frequent (5%) than in the whole population. Patients qualifying for HD-3 had a similar mean age as all patients in that protocol (36 vs. 34 years) and a similar response rate (70 vs. 75%). So far, 3 relapses have occured. Therefore, patients with primary BM involvement, despite representing a bad prognostic group, have a very good chance of obtaining complete remission when treated by combination chemotherapy.

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CYTOSPINS OF PERIPHERAL BLOOD LYMPHOCYTES (PBL) IN THE FOLLOW-UP OF MALIGNANT NON-HODGKIN-LYMPHOMAS (NHL)

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During the course of disease leukemic conditions in NHLs of either low or high grade malignancy are characterized by PBLs expressing the same monoclonal surface marker pattern as do the malignant cells in the appropriate lymphnode biopsies. Could immunocytology on PBLs be helpful in confirming the diagnosis or monitoring the therapy?
13 patients were observed suffering from malignant NHLs (12 white males, 1 white female, mean age 66, from 16-78 y.). Histology according to the Kiel classification of NHLs: 8/13 cases low grade malignancy (4/8 immunocytoma, 2/8 mycosis fungoides, 1/8 centrocytoma, 1/8 T-zone-lymphoma); 5/13 high grade malignancy (2/5 centrocytoma, 1/5 T-lymphoblastic, 1/5 immunoblastic, 1/5 unclassified B-type); clinical stage according to the Ann Arbor classification: 1/13 stage I, 5/13 stage II, 7/13 stage IV. Cytospins from density gradient purified PBLs were stained with B1, Ia1, IgG, IgM, IgD, kappa & lambda light chain, cALLA, OKT3, OKT4, OKT8, OKT9 and OKT10 monoclonal antibodies.
Results: PBLs of stage I and II did not exhibit monoclonality unless the total lymphocyte count was more than 3,800-4,000/µl blood while all stage IV cases did. Adequate chemotherapy reduced the neoplastic cell clone and gave rise to the non-neoplastic clone of either T or B cell origin. In poor and non-responders cell reduction, as could be judged by the cytospin method, was only moderate.

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EFFECTS OF rTNF-α OR rIFN-γ ALONE AND IN COMBINATION ON HUMAN GASTROINTESTINAL CARCINOMAS (GICA) IN VITRO AND IN NUDE MICE.

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Basing on encouraging pilot trials (Klapdor et al.Dig.Dis.Sci. 31:1137,1986,abstract) we now tested a panel of gica in vitro and/or in vivo for their sensitivity to rTNF-α and rIFN-γ.Tumors:7 cell lines (4 paca,2 CRC,1BC) and xenografts of 7 different gica (6 paca,1 stomach ca),identical to the human ca with regard to histology,grading,IH and tumor marker secretion.Assays: monolayer proliferation assay (in vitro),nu/nu Balb-C mice (in vivo),Substances:rTNF-α (BASF) and rIFN-γ (Bioferon) in doses up to 10^4 U/ml (in vitro) and 164000 U (rTNF-α) or 400000 U/d i.p. (rIFN-).Parameters of efficacy in nude mice:tumor volume,serum CA 19-9 as well as tumor uptake and tumor/blood relation on day 4 after i.v. injection of I-131-labeled anti-CA 19-9.Duration of in vivo therapy 3 weeks. Results: 4/7 cell lines responded to rIFN-γ and 4/6 to rTNF-α; rTNF-α resulted in vivo in 6/6 in a significant dose dependent growth inhibition and rIFN-γ in 5/7 during treatment period,without correlation to grading or expression /secretion of CA19-9.Combination of both drugs resulted in a significant enhencement of the efficacy of the single agents, in 1/4 xenografted tumors in partial remission. Serum CA19-9 and tumor/blood ratio reflected a therapy efficacy more sensitive than tumor volume.Systematically different results for both assay systems in the case of direct comparison indicate the importance of testing in both models in parallel.

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INTERLEUKIN 3 MODULATES LEVELS IN HEMOPOIETIC CELL LINES (32Dcl-3 AND NSF-60)

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Tetrahydrobiopterin is known to represent the electron donating cofactor for hydroxylation of aromatic amino acids. Thus, it is essentially involved in phenylalanine catabolism and in catecholamine and serotonine biosynthesis. Proliferation of the murine cell lines 32D cl3 and NSF-60 is dependent on IL-3. Other than in IL-2-dependent T cell proliferation, IL-3 is additionally needed for maintaining the metabolism of the target cells. In the following we have begun to study the modulating effect of IL-3 on pterin synthesis. Total biopterin levels (tetrahydrobiopterin + dihydrobiopterin) were determined by reverse phase HPLC. Upon addition of IL-3 to the cells, depleted from the lymphokine for 1-3 hrs, levels of basophil-like 32D cl3 cells increased from 1.5 to 3.3 pmol/10^6 cells within 10 min and returned gradually to initial levels during the next 2 hours. On the contrary, in myelocytic NSF-60, addition of IL-3 caused a decrease from initially 3.0 to 1.6 pmol/10^6 cells within the same time. No significant restoring occurred within 2 hours. From these data intracellular biopterin concentrations are calculated to range between 1-4 x 10^{-6} M. In contrary to T cells, phorbol ester could not replace interleukin 3 in modulating pterin synthesis.

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PHASE-I-STUDY OF INTRATUMORAL (i.t.) AND INTRAVENOUS (i.v.) APPLICATION OF RECOMBINANT TUMOR NECROSIS FACTOR (rHuTNF).

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To date, 16 patients with malignant diseases resistant to standard treatment received a continuous 24-hours i.v.-infusion. 21 patients received an intratumoral injection. Main side effects were fever, chills, tachycardia, and changes in blood pressure. The side effects after 24h-infusion were more severe and took more time for resolution than after intratumoral injection, despite of the fact that maximal plasma levels reached were much higher (60 U/ml) after i.t. injection than after24-hours infusion (5 U/ml). Dose-limiting side effects after continuous infusion application occurred at the maximal tolerable dose of 7.5 x 10^5 U/m^2 (0.34 mg/m^2) when applied without prophylactic treatment. They consisted of severe hypotension, CNS effects and general prostration due to the sum of all side effects together. In contrast, for i.t. injection, the maximal tolerable dose has not yet been reached with 12 x 10^5 U/m^2 (0.340 mg/m^2). As effects on the tumor have only been observed after i.t. injection, intratumoral application of rHuTNF seems to be better tolerated and more efficient than continuous 24-hours infusion.

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NEPHROTOXIC EFFECTS OF HUMAN INTERFERON ALPHA-2A (Hu-Ifn-alpha-2A) IN PATIENTS WITH MYCOSIS FUNGOIDES

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Clinical data dealing with nephrotoxic properties of human interferons are still very scanty. During a study with Hu-Ifn-alpha-2A in patients (pts.) with mycosis fungoides we studied the effects on renal function, enzyme and protein excretion.

8 pts. (male 5, female 3) with mycosis fungoides refractory to standard therapies received 3 x 10^6 (day 1 - 3), 9 x 10^6 (day 4 - 6) and 10 x 10^6 (from day 7 for 12 weeks) units/m^2 Hu-Ifn-alpha-2A subcutaneously either alone or in combination with vinblastine (6 pts.) 0,1 mg/kg body weight every 3 weeks.

To detect renal injury we analysed the excretion of 5 urinary enzymes (Lactate dehydrogenase=LDH, alanine aminopeptidase=AAP, gamma-glutamyltransferase=GGT, ß-galactosidase=GAL and N-acetyl-ß-glucosaminidase=NAG), protein excretion and S-creatinine before and weekly during treatment.

Five pts. had a significant increase of LDH, AAP, GGT and NAG and developed mild proteinuria up to 2,2 g/24h. S-creatinine increased in 3 pts. up to 1,5 mg/dl. SDS-gel-electrophoresis of the urine in one patient with proteinuria showed complete tubular proteinuria.

Our data show that in a considerable portion of pts. treated with Hu-Ifn-alpha-2A ± vinblastine nephrotoxic effects could be observed. Tubular and glomerular damage was detectable. These results are consistent with our findings in more than 60 pts. with various solid tumors and hematological neoplasia treated with human interferon alpha-2b monotherapy.

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THE EFFECT OF ANTITHROMBIN III, HIRUDIN AND TUMOR NECROSIS FACTOR (TNF) ON THROMBIN-INDUCED CELL-GROWTH

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Thrombin does not only act as coagulation enzyme catalyzing the change from fibrinogen in fibrin but also as tissue hormone stimulating the proliferation of cells of different tissues (fibroblasts, vessel wall cells, malignant cells) as is shown in own experiments (increase in cell count and thymidine uptake). In further own investigations the hormone-like action of thrombin is documented by an intracellular increase of the "second messenger" cGMP and by the existence of specific thrombin receptors that are sensitive to neuraminidase and beta-galactosidase incubation. Hirudin blocks the mitogenic effect of thrombin upon cells completely. On the contrary antithrombin III is unable to inhibit the mitogenic effect of thrombin. Tumor necrosis factor (TNF) does inhibit the proliferation of certain cell lines. However, it does not interfere with the thrombin-induced proliferation effect directly.

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RECOMBINANT HUMAN GM-CSF DIRECTLY INDUCES RESPIRATORY BURST IN POLYMORPHONUCLEAR LEUCOCYTES

D.W. Dennig, G. Fischer and W. Knapp

Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been reported, not only to stimulate clonogenic growth of myeloid precursor cells, but also to prime mature granulocytes for enhanced responses to chemotactic peptides (Weisbart 1987). In order to further define the effects of GM-CSF on human granulocytes we performed single cell analysis by flow cytometry. For demonstration of oxygen metabolites granulocytes were loaded with 2',7'-dichlorofluorescin (DCFH), a non-fluorescent dye which is converted to the fluorescent dye DCF by oxygen metabolites (Bass 1983). Preincubation of DCFH-loaded granulocytes with recombinant human GM-CSF of different origin using varying concentrations led to a significant increase of the mean fluorescence intensity which was further increased by the addition of chemotactic agents. This finding demonstrates that recombinant human GM-CSF not only primes, but also directly causes respiratory burst in polymorphonuclear leukocytes.

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THE ABILITY OF RESTING B CELLS TO PROCESS AND PRESENT ANTIGEN AND ITS REQUIREMENT FOR INDUCTION AND RECEPTION OF COGNATE T CELL HELP

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We studied the ability of resting B cells to present antigen and receive the MHC restricted T helper signal. We present evidence that
1.) efficient antigen presentation of resting B cells depends upon the interaction of antigen with the mIg receptor. This requires no activating signal for the resting B cell. Rather resting B cells are fully able to process antigen defind by a time dependent process that involves an acidic compartment and results in a fixation and irradiation resistant state. During antigen presentation antigen is no longer bound to the mIg receptor.
2.) The MHC restricted interaction between Ag presenting resting B cells and a T helper cell line results in MHC restricted T cell dependent B cell activation, whereby the actual helper event is not MHC restricted and does neither involve the MHC class II nor the CD 4 molecule.

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NATURAL KILLER (NK) CELLS CAN BE INDUCED TO LYSE RESISTANT TARGETS BY MONOCLONAL ANTIBODIES AGAINST THE FC RECEPTOR FCR_{10} (CD16)

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In contrast to initial reports lymphokine activated killer (LAK) ciller cells now under study in clinical cancer trials have recently been identified as activated NK cells. Detailed insight in activation mechanisms of NK cells may therefore provide a ratio for future concepts in tumor therapy. In the present studies we demonstrate that monoclonal antibodies against the CD16 antigen expressed on NK lymphocytes but not on B or T cells both mediate the effector target binding process and trigger cytotoxicity against otherwise resistent tumor cell lines such as Daudi, U937, HL-60 and L1210. Different cloned NK cell lines but also peripheral blood NK lymphocytes were used as effector cells. Since complete CD16 antibodies only but neither isotype controls reactive with NK cells (e.g. NKH1) nor $F(ab')_2$ preparations of CD16 antibodies induce cytotoxity two steps appear to be critical.
1. Specific binding of CD16 antibodies to FcR_{10} at the effector cell level
2. Crosslinking of CD16 antigens by bound antibodies via Fc receptors of the corresponding target cells.
We conclude that FcR_{10} is not only the ligand for the Fc part of IgG but also involved in the induction of cytotoxicity of NK cells.

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BIOCHEMICAL CHARACTERISATION OF INTERLEUKIN-2 RECEPTORS ON HUMAN BASOPHILS

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We could previously demonstrate, that human basophilic granulocytes express surface structures reacting with the monoclonal anti Il-2 receptor antibody anti-Tac. The present study describes the biochemical and functional characterisation of this surface structure on human basophils.
In order to analyse the reactive component we first performed immunoprezipitation experiments with anti-Tac antiboby and could demonstrate a protein of molecular weight of 55 kd equivalent to the ß-chain of the Il-2 receptor on human T blasts. Binding studies with four other antibodies to different epitopes on the Il-2 receptor molecule also gave positive results. The functional activity of these receptors in terms of their Il-2 binding capacity was indicated by the observed binding of Il-2 to intact basophils. The surface bound Il-2 receptors on human basophil seem to be activly synthesized. In Northern blot analyses we could demonstrate two mRNA bands of 3.5 and 1.5 kb.

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A NEW CELL LINE (MONO-MAC-6) WITH CHARACTERISTICS OF MATURE MONOCYTES

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A cell line was established from peripheral blood of a patient with monoblastic leukemia.One clone (Mono-Mac-6) was selected based on its reactivity with the monocyte-specific monoclonal antibody (MAB) 63D3. The assignement to the monocyte lineage was further supported by the absence of markers of T cells, B cells and granulocytes and by the presence of several features that are characteristic of monocytes. Mono-Mac-6 is positive for staining with the MABs My-4,Leu M3,Mo-2,M 42 and UCHM 1 and the cell line expresses Fc-receptors,complement receptors and Class II antigens.
In morphology the cells exhibit a round or indented nucleus and a light blue cytoplasm with many granules. In phagocytosis of antibody coated erythrocytes 79 % of the Mono-Mac-6 cells are positive. Compared to the monoblastic cell lines U 937 and THP 1 the Mono-Mac-6 cell line appears to be the only one with characteristics of mature monocytes.

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HUMAN MACROPHAGE MATURATION AND HETEROGENEITY. RESTRICTED EXPRESSION OF LATE DIFFERENTIATION ANTIGENS IN SITU

R. Andreesen, H.G. Leser, U. Costabel, G.W. Löhr, and R.C. Atkins

Terminal maturation of human macrophages (MO) is an important step for creation of cell diversity amongst site-specific subpopulations and determining their functional competence in situ. As monocytes (mo) undergo differentiation in vitro, they express lineage-restricted antigens specific for differentiation stages beyond the blood mo level. We have analyzed the expression of MAX.1, MAX.2, MAX.3 and MAX.11 on exsudate-type MO from pleural and peritoneal cavity and the alveolar space as well as on resident and activated tissue MO in sections of spleen, lymph node, tonsil, liver, gut mucosa, skin, placenta, kidney and bone. "Free" MO in serous cavities expressed MAX antigens in a heterogenous patterns whereas none of the organ-specific tissue MO subsets did so. Only during allograft rejection were infiltrating MO found to express MAX antigens but not at sites of "non-specific" inflammation or granuloma formation. However, Cyclosporin A treatment seems to suppress the induction of MAX antigen expression on intragraft MO. Freshly harvested MAX^- exudate MO converted to the complete MAX^+ phenotype on further cultivation. Isolated Kupffer cells were able to express only the MAX.2 antigen in culture but did not react with the MAX.1 and MAX.3 mAbs. Normal alveolar MO which are $MAX.1^-$ convert to $MAX.1^+$ phenotype during active sarcoidosis and exogen allergic alveolitis. Interestingly, the MAX.1/.11-gp64 antigen is co-expressed on glomerular mesangial cells whereas the MAX.2-gp200 is found also on capillary endothelium but restricted to tissues of active immune response. These results demonstrate a phenotypic heterogeneity within the macrophage system as a result of site-specific influences and modulation during a cellular immune response.

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HUMAN MACROPHAGE MATURATION IN VITRO: EXPRESSION OF FUNCTIONAL TRANSFERRIN BINDUNG SITES OF HIGH AFFINITY

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Human blood monocytes (mo) when cultured in suspension on hydrophobic teflon membranes undergo terminal maturation to macrophages (MO). Together with the appearance of new lineage-restricted differentiation antigens of the MAX series mature MO but not blood mo express transferrin (TF) receptor molecules as detected by immunostaining methods (1,2). Simultaneously they increase their content of intracellular ferritin by a factor of 100. Here we report that radio- and fluorescein-labelled TF binds to a single class of high affinity binding sites on MO but not on mo. As mo mature in vitro an increase in TF receptor numbers (on the average about 10^6 per matures MO) is observed. TF binding by these non-proliferating MO was characterized by a K_d of 5.1 + 2 nM and thus of consistently lower affinity than by the proliferating K562 tumor cells (K_d of 2 + 0.4 nM) taken as a standard reference. These data refer to the binding of Fe_2TF whereas the very low binding of apoTF to either MO or tumor cells suggested a K_d of > 1 µM. Treatment of MO with human recombinant interferon-gamma which resulted in activated tumorcytotoxic effector cell function did not substantially alter TF receptor expression. In addition, mo-derived MO take up ^{59}Fe-TF in amounts at least comparable with the amount measured for the K562 tumor cells. Similarily, apoTF-promoted uptake of ^{67}Ga by mature MO was noted. These results emphasize the importance and vital role of MO in the storage and distribution of iron. It appears that they fill their iron stores not only from the hemoglobin of ingested senescent erythrocytes but also from iron-carrier proteins in the circulating blood.

(1) Andreesen et al., Blut 49:195, 1984
(2) Andreesen et al., Blood 67:1257, 1986

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DIFFERENT REGULATION OF HLA B ANTIGEN EXPRESSION IN TRANSFECTED MOUSE L CELLS BY INTERFERON

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Genes coding for different HLA class I antigens were integrated in mouse L(tk$^-$) cells by DNA mediated gene transfer. In earlier experiments we could show that the expression of the HLA-B7 antigen in these cells can be enhanced by mouse interferon at least two times stronger than the expression of the HLA-A2 or HLA-Cw3 antigen. In further experiments three different genes coding for HLA-B locus antigens (HLA-B7, HLA-B27, HLA-B51) were used for transfection experiments. Using monoclonal antibodies against sub- and supertypic HLA determinants, transfected cells were shown to express HLA molecules with serologically defined epitopes corresponding to HLA antigens of the same specificities on human cells. The exposure of the transfected cells to 10^4 IU of mouse interferon per ml medium resulted in the accumulation of the HLA specific mRNA after 24 hours and the enhanced expression of the antigen at the cell surface after 36 hours. Comparing the induction pattern of all three different HLA-B genes used, we could show that the HLA-B7 gene was 2- to 3-times more inducible than the other HLA-B genes. This finding may indicate specific regulatory mechanisms of HLA class I antigen expression possibly influencing T-cell recognition in immune response.

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INHIBITION OF HOMOTYPIC AGGREGATION OF A HUMAN BURKITT LYMPHOMA CELL LINE

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Homotypic cell aggregation has been demonstrated for normal and transformed cells. Several lines of evidence indicate, that the molecules involved in these interactions carry carbohydrate determinants. It also has been shown, that the metastatic potential of tumor cells seems to be positively correlated with increased homotypic aggregation. We examined the ability of a human Burkitt lymphoma cell line (Raji) to reaggregate in the presence of several carbohydrate and glycoconjugate inhibitors. Complete inhibition was seen with bovine submaxillary mucin. Asialomucin was not effective. Also, complete inhibition of aggregation was found with the ganglioside GM 1. The common structure of these molecules is the determinant NeuNAc-(gal)-galNAc. The involvment of high mannose type, sialyllactosamines, or Thomsen-Friedenreich structures could be excluded. Aggregation was resistant to trypsin, but sensitive to neuraminidase and EDTA treatment. Tunicamycin inhibited the aggregation, indicating the necessity of N-linked carbohydrate chains. Homotypic aggregation was not effected by methylornithine and, therefore, independent of proliferation. From these results we conclude, that two molecules are involved in homotypic aggregation of Raji cells. One molecule carries the carbohydrate determinant NeuNAc-(gal)-galNAc, which is sensitive to neuraminidase treatment. The other molecule is thought to be a lectin-like glycoprotein with N-glycosidically linked carbohydrate chains, which is not synthesized or inactivated in the presence of tunicamycin. This conclusion is supported by the fact, that sialyllactosamine carrying glycoproteins are not inhibitory in the aggregation assay. (Supported by the Robert-Bosch-Stiftung)

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HUMAN NATURAL KILLER CELLS ENHANCE B CELL IMMUNOGLOBULIN PRODUCTION

J. Becker, Th. Werfel, C. Schreiber, H. Deicher and R.E. Schmidt

There is increasing evidence for the immunoregulatory role of natural killer (NK) cells, e.g. in immunreconstitution after allogeneic BMT. Several studies with NK enriched preparations from normal peripheral blood suggest a role of NK cells in B cell differentiation. Enhancement and suppression have been described. Using human NK clones, both NKH1+, T11+, T3- (JT_B18, CNK6, NKC4) and NKH1+, T11+, T3+ (JT9, JT10) cells, we studied the regulatory effects in a Staphylococcus Aureus Cowan strain I/rIL-2 activated B cell system. After coculture of highly purified B cells with NK clones IgG and IgM were determined in an ELISA. Both IgG and IgM production were significantly increased in the presence of NK clones. Optimal enhancement was found at NK/B cell ratios between .1 to 1. Kinetic studies revealed optimal antibody production, when NK clones were added to day 2 preactivated B cells. For studying the mechanisms involved in B cell differentiation by NK cells we performed co-cultures in the presence of monoclonal antibodies blocking cell-cell adhesion (anti LFA-1, CD 11a). Since such antibodies inhibited B cell differentiation by NK cells, cell-cell interaction via specific cell surface structures appears to be necessary. Whether soluble factors are released during this interaction and involved in mediation of B cell differentiation by these effector cells is addressed in further studies.

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ANALYSIS AND APPLICATION OF LINEAR IMMUNOGENIC DETERMINANTS

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Molecular biology can provide sequence data of proteins that are not yet purified. Immunogenic determinants of a protein are surface-oriented. They are in or close to areas of increased hydrophilicity, reversed β-turns and increased flexibility and thus can be predicted when analyzing the amino-acid sequence of proteins by appropriate means. Using a combination of hydrophilicity and flexibility calculations we are able to predict the immunogenic regions of known proteins. We additionally determined immunogenic areas of thus far not purified oncogene products. Synthetic peptides corresponding to these areas are used as immunogens to produce antibodies to the native protein. The purification and further characterization of these proteins should be possible. Further, synthetic peptides may be usefull as highly specific vaccines.

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SIGNIFICANT DIFFERENCES IN THE HLA ANTIGEN FREQUENCY OF PATIENTS WITH SEMINOMA AND NONSEMINOMATOUS TESTICULAR TUMOURS

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Several studies conducted to determine a possible relationship between the major histocompatibility complex HLA and testicular cancer have reported conflicting results in pts. with nonseminomatous tumours (NST; DeWolf 1977, Aiginger 1980, Pollack 1982, Oliver 1986) and no significant differences in seminoma pts. We have studied 143 pts. between 1979 and 1986 for HLA A, B, C and DR antigens (49 sem., 40 MTI+TD, 43 MTU, 11 MTT). The frequency of A, B, C and DR antigens was not different after correction for the number of comparisons (p_c) when the testis tumour group as a whole was compared with 450 healthy controls. However very substantial differences within the subgroups could be observed. The frequency of DR1 was significantly higher in the seminoma group than in the NST group (33vs13%, $p_c < 0,03$). The joint calculation of the results of Pollack, Oliver and our study (n= 362) confirms the increase of DR1 in seminoma pts. ($p_c < 0,02$) and furthermore reveals an increase of DR5 (35vs20%, $p_c < 0,015$) in seminoma pts. The frequency of DR5 was increased also in pts. with haematogenous metastases (our study: all pts. 36vs 23%, NST 38vs14%; pooled data: all pts. 33vs20%, $p_c < 0,05$) and in pts. with seminoma+MTU+MTT when compared both with MTI pts.(30vs17%, $p_c < 0,05$) and the control group (30vs20%, $p_c < 0,01$). This study extends the list of cancers possibly connected with increas.of DR5.

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BIPHENOTYPIC EXPRESSION OF MYELOID AND B CELL ANTIGENS ON MONOCLONAL EBV POSITIVE CELL LINES DERIVED FROM NORMAL DONORS.

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Cell lines grown from 5 normal donors either spontaneously or induced by B95 cell culture supernatant containing Epstein-Barr virus (EBV) were studied with respect to surface markers and immunoglobulin gene rearrangements. Antigens were determined repeatedly by an indirect enzyme-immunoassay using an alkaline phosphatase conjugated secondary antibody on adhesive slides. 70-90% of the cells were positive with B1- and B2-antibody (CD20) wherease the proportion of VIM-D5 positive cells (CD 15) varied between 3 and 90% among the cell lines. Both markers were found on the same cells by means of a double marker technique employing consecutive stains with fast blue BB- and fast red TR- salt and interposed blocking of the enzyme by HCl (2 M) which allowed the simultaneous detection of 2 determinants without destruction of antigens. Clonality was assesed by Southern blot technique: DNA of the cell lines was digested by Hind III and hybridized to a μ-specific probe, demonstrating monoclonal rearrangements of the μ chain immunoglobulin gene. These data indicate that immortalization of normal B cells by EBV produces clones which, besides B antigens, express myeloid antigens at a constant individual degree. It can only be speculated if clones with high proportion of biphenotypic antigen expression represent cells with disturbed differentiation or transformed precursor cells of earlier progeny.

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MONOCLONAL ANTIBODIES OF CLASS II TRANSPLANTATION ANTIGENS (HLA-D)

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From the human lymphoblastoid B cell line H2LCL, homozygous at the HLA loci(HLA A3,3;B7,7;Dw2,2;MT1,1;DQ1,1;MB1,1;DP4,4) class II antigens were isolated by an exclusively chemical procedure. They consist of 2 noncovalently associated subunits: an α-chain of M_r = 34 kd (229 amino acid residues) and a ß-chain of 29 kd (237 amino acid residues).The determination of the complete amino acid sequence of the DQ1 α-chain as well as the characterization of at least 7 class II ß-chains provides an insight into the isotypic complexity of human class II antigens. The α- and ß-chains were characterized by polyacrylamid gel electrophoresis (PAGE) and isoelectric focusing (IEF). By two dimensional gel analysis (PAGE/NEPHGE) the α-chain fraction revealed 4 equidistant spots of identical M_r, the ß-chain pool, however resulted in a more complex pattern indicating at least 7 spots of varying intensity, distance and M_r = 29 kd - 27 kd. The complex subunits of the α- and ß-chains were separated into single pure peptides by high performance liquid chromatography (HPLC) and used for immunization. 4 monoclonal antibodies (moAb) were prepared and characterized. MoAb 35.12 and moAb 43.7 specific for p 34 were shown to be different after 2D separation of the heavy chain. In the immunoblot reaction moAb 43.7 was shown to react with all α-chains, whereas moAb 35.12 reacted only with a single α-chain with an acidic IP = 4.1. MoAb 11.10 and 44.23 were shown to be specific for p 29; moAb 11.10 reacted with a single band of p 29, moAb 44.23 reacted with 2 spots after 2D PAGE. MoAb 44.23 also reacts with a well defined peptide of the DR 2 $ß_1$ chain in position 23-36 (RVRFLDRYFYNQEE). Comparison of sequences of other ß-chains of human class II antigens showed distinct differences in their isotypes especially in this region. These moAb may be used for the isolation of specific mRNA of the H2LCL cell. These procedures are under investigation.

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ANTIBODY TC12 DETECTS A RARE LEUKEMIA ASSOCIATED ANTIGEN

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Monoclonal antibody TC12 was developed after initial immunization with T-cell acute lymphoblastic leukemia (ALL) cell line CEM. Testing for reactivity on peripheral blood cells revealed no staining with TC12. In addition, no significant reactivity was found on bone marrow preparations of patients without malignant hematological diseases. From a wide variety of different human cell lines considered equivalents of hematopoietic differentiation only few were found TC12 positive, namely CEM and RPMI 8402, both T-cell ALL lines, and promyelocytic line HL-60. When more than 70 freshly isolated preparations of leukemias or advanced lymphomas were immunophenotyped, only 6 were found bearing the antigen detected by TC12. Two of these were T-cell ALL, and one case each was classified M3, M4, M5, and M6 acute myelocytic leukemia (AML) according to FAB criteria. Sequential studies on cells from a patient with promyelocytic leukemia demonstrated loss of staining with TC12 when remission was achieved, but reoccurence in relapse. Thus, TC12 appears to detect a rare leukemia associated antigen and may even have therapeutic applications in certain cases of T-cell ALL or AML.

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THE PHENOTYPE OF HUMAN CONNECTIVE TISSUE TYPE (CTMC) AND MUCOSAL MAST CELLS (MMC)

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CTMC (foreskins,n=2; ascites,n=5) and MMC (lungs,n=5) were characterized using a combined toluidine/immunofluorescence staining procedure. Although both MMC and CTMC express only a small number of hemopoietic surface markers (60 moabs tested) the T200 antigen clearly defines them as leucocytes. In all patients, both CTMC and MMC are reactive with the moab BA-2 (p24/CD 9), with moabs recognizing surface bound IgE and with the moab YB5.B8 (otherwise only positive with a subset of myeloid blast cells). The p67 surface structure detected by the moab MY 9 (CD 33) is expressed only on CTMC but not on MMC. The common surface characteristics of mast cells with basophils (Stain et al.) are restricted to the T200 antigen (CD 45), the p24 structure (CD 9) and the surface IgE binding sites. Our results clearly demonstrate that mast cells represent a distinct cell population in terms of their immunological surface characteristics and that human MMC and CTMC can also be distinguished by their own surface marker profile.

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THE ANTIGENIC PHENOTYPE OF MULTIPLE MYELOMA CELLS AS DEFINED BY A PANEL OF MONOCLONAL ANTIBODIES

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In contrast to other B-cell neoplasias the immunologic phenotype of myeloma cells is only poorly defined. Therefore we have performed an extensive immunephenotypic analysis of 3 human myeloma cell lines established in vitro in our laboratory (LOPRA-1 (IgA_2/kappa), LOPRA-2 (IgG_1/lambda), LOPRA-3 (IgG_3/kappa)) by flow cytometry (FACS 440) and immunocytochemical staining (APAP). In addition, 10 bone marrow samples of multiple myeloma patients were phenotyped by APAP technique. The myeloma cells were consistently negative for surface immunoglobulin and HLA class II (HLA-DR/DP, HLA-DQ) antigens. Moreover, HLA class II antigens were not inducible by IFN_{alpha} or IFN_{gamma}. The myeloma cells did not stain for most B-cell differentiation antigens (CD10 (CALLA), CD19 (B4), CD20 (B1), CD21 (B2), CD22 (HD39), CD23 (MHM6), CD37 (BL14), CD39 (G28-8)) except PCA-1 and CD38 (OKT10). Monoclonal antibodies of the CD24 cluster gave a heterogeneous staining pattern: Some monoclonal antibodies were consistently negative, whereas others showed a broad reactivity peak which may be due to posttranslational modifications of the CD24 molecule synthesized in myeloma cells. In addition, we have employed hybridoma technology to produce monoclonal antibodies to cell surface antigens restricted to myeloma/plasma cells. Six antibodies detected antigens on the immunizing myeloma cells; two of them displayed a staining pattern restricted to late B-cell differentiation stages as analysed on a panel of normal and neoplastic tissues.

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IMMUNOTHERAPY OF EXOCRINE PANCREATIC CARCINOMA XENOGRAFTS IN NUDE MICE BY THE MONOCLONAL ANTIBODY 17 - 1A

R.Klapdor, M.Bahlo, O.Schwarzenberg, G.Riethmüller

In order to look for further therapeutical modalities of paca we studied antitumor effects of the Mab 17-1A in nude mice after s.c. injection of colorectal(CR) or paca cell lines,after transplantation of tumor pieces of CR and paca and in established xenografts(HT29 as CR,ISCH84 and ASCH84 as paca).The tumors expressed the antigen 17-1A to a different degree.Treatment: 400 - 600 ug/animal/die i.p. for 11 or 21 days. Results: We found a dose dependent effect of Mab 17-1A on the growth of transplanted tumor slices and cell suspensions of HT 29 and ASCH84 when treatment was started at time of transplantation(complete inhibition by 600 ug/animal/die for 21 days). However, no effects were seen in studies with ISCH 84, in nude mice bearing already established tumors of HT 29 and ASCH84 and in comparative studies with Mab without ADCC, as BW431/31(antiCEA) or BW 494/32 F(ab)$_2$ fragments. The results a) support the concept to try "cold" immunotherapy with Mab with ADCC as 17-1A as an adjuvant therapy in human pancreatic cancer and b) demonstrate that human pancreatic carcinomas will also show a heterogenous behaviour against treatment with Mab with ADCC - as already known for cytostatics.

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IMMUNOHISTOLOGICAL ASSESSMENT OF REACTIVE CELLS INFILTRATING HUMAN BREAST CANCER TISSUE

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Using an indirect immunoperoxidase technique and a panel of monoclonal antibodies we studied the nature of infiltrating lymphocytic cells on frozen sections of 59 breast cancer specimens. 35 patients (59 %) showed a moderate or extensive lymphocytic infiltration. The majority of these cells were of T cell origin with a predominance of CD4+ cells in most cases. CD8+ cells were superior in number only in biopsies with slight infiltration.
There was no significant correlation between the strength of infiltration and T- or N-stage of the disease. Patients with a low content of receptors for extrogen (ER) or progesterone (PR) showed a strong lymphocytic reaction more frequently than those with high ER- and PR-values ($p=0,1$ and $<0,05$, respectively). Furthermore we examined the presence of receptors for the epidermal growth factor (EGF) on the tumor cells. Positive results ($>$ 10 % of cancer cells positive) were found in 17/54 cases (31 %).
Regarding the clinical course of patients (evaluable in 54 cases, mean observation time 19 months) no single parameter was of clinical relevance comparable to that of N-stage of the disease. Nevertheless, it remains remarkable, that both patients without positive lymph nodes suffering from local recurrence or metastases (2/21) showed positive EGF-receptor stage.

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A PHASE-II-STUDY: COMBINATION CHEMOTHERAPY FOR ADVANCED BREAST CANCER WITH MITOXANTRONE, 5-FLUOROURACIL AND PREDNIMUSTINE

H.Samonigg, H.Stöger and A.K.Kasparek

Forty-one patients with metastatic breast cancer were treated with a combination of mitoxantrone, 5-fluorouracil and prednimustine. Seventeen patients had previously received hormonal treatment (8), chemotherapy (5) or a combination of both (4). Treatment courses were given every three weeks and consisted of mitoxantrone (12 mg/m² on day 1), 5-fluorouracil (500 mg/m² on day 1) and prednimustine (110 mg/m² on days 1 through 5). A minimum of 3 and a maximum of 13 treatment courses were given (median: 5). Complete remission (CR) was obtained in 3 patients and partial remission (PR) in 15 (CR + PR = 44 %). No change (NC) was seen in 17 (41 %) and in 6 (15 %) there was progressive disease (PD). In the 18 patients who responded to therapy (CR + PR), duration of the response averaged 5 months (range 1,5 to 11,5 months). Leukopenia was the limiting factor for continuation of therapy with 21 patients (51 %) revealing a WBC of less than 3000/mm³ (WHO-Grade II - IV). Nausea and vomiting was observed in 33 (80 %) and 4 (12 %) required antiemetic therapy (WHO-Grade III). Minimal hair loss was found in 20 patients (49 %). When patients with previous chemotherapy and newly treated patients were analyzed seperately the response rates (CR + PR) were 33 and 47 % respectively. When compared to other treatment regimens currently used our results show a comparable response rate. So far the duration of response is not satisfactory.

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CMIF-P TREATMENT IN PATIENTS WITH POOR-PROGNOSIS ADVANCED BREAST CANCER

I.Kührer, H.Ludwig, W.Scheithauer, Ch.Zielinski

The objektive of the present study was to evaluate the clinical efficacy of the CMIF-P (cyclophosphamide, mitoxantrone, 5-fluorouracil and prednisolone) treatment in patients with poor-prognosis breast cancer.

70 patients (median age=58, range 28-79 years) have been entered into the study. 36 patients were ctegorized as showing poor-prognosis, 34 patients could be assigned to the good-prognosis group. All of the latter were resistant to hormone treatment. Chemotherapy consisited of cyclophosphamide 500mg/m², p.inf., mitoxantrone 10mg/m², p.inf., 5-fluorouracil 650mg/m² p.inf. and prednisolone 40mg/m² days 1-14 orally.

Of the 62 patients presently evaluable, 6 (9.7%) have achieved complete remission and 6 (9.7%) partial remission. 13 patients (21%) showed stable disease. Progressive disease was noted in 37 cases (59.6%). The overall response rate (CR+PR) was 19.4%

Side effects were tolerable in all cases. 21% showed mild to moderate (WHO grade I+II) and 14% severe (WHO grade III+IV) hematological toxicity. Inappetence was seen in 18%, nausea in 18% and alopecia in 9% of the patients.

In conclusion, the CMIF-P therapyeffected an overall response rate of 19.4% after at least two treatment cycles. The regimen was well tolerated and seems to be a pratical and efficient treatment protocol for poor-prognosis patients with metastatic breast cancer.

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PREOPERATIVE POLYCHEMOTHERAPY WITH MITOXANTRONE, 5-FLUOROURACIL AND PREDNIMUSTINE FOR INFLAMMATORY BREAST CANCER

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Thirteen patients with inflammatory breast cancer were treated preoperatively with a combination of mitoxantrone (M), 5-fluorouracil (F) and prednimustine (P). Every three weeks patients received a course of M (12 mg/m^2 on day 1), F (500 mg/m^2 on day 1) and P (110 mg/m^2 on days 1 through 5). A minimum of 1 and a maximum of 7 treatment courses were given (median: 4). The response to treatment could be evaluated in 12 patients: A decrease of edema and redness of the skin and of warmth and induration of the underlying breast was obtained in 7 patients (58 %). Four showed a reduction of tumor mass by more than 50 %. No change was seen in 4 patients (33 %) and in one there was tumor progression. So far 6 patients have been treated with radical mastectomy following the polychemotherapy. Extensive tumor cell necrosis was seen on histological examination in 3 patients. The dose limiting side effect was leukopenia. WBC was decreased to less than 3000/mm^3 (WHO-Grade II) in 3 patients. Therapy was stopped in one patient with persistent of less than leukopenia $\geq$ 1000 (WHO-Grade IV). Nausea and vomiting was observed in 9 patients within WHO-Grades I - II. Six patients developed alopecia WHO-Grade I- II. We conclude that preoperative combinationstherapy (M, F + P) may benefit patients with inflammatory breast cancer.

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RECOMBINANT INTERFERON ALFA 2A (rIFN-Alfa-A) IN PATIENTS WITH METASTATIC RENAL CELL CARCINOMA

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Seventeen patients (pts) with advanced renal cell carcinoma were treated with rIFN-Alfa-A (18 million U s.c. three times a week). 8 pts had received prior hormonal therapy (tamoxifen). On entering the therapeutic schedule with rIFN-Alfa-A all pts had a progressive disease with multiple metastases. Flu like symptoms occurred in general - they were severe in 8 cases. An impairment of the liver was shown by a rise of SGOT/SGPT in 2 pts, and the kidney function worsened in one. A Fall in blood cell counts was seen in 5 cases. 4 pts developed a transient stomatitis.

Five patients (5/17) showed minor responses, and one is still in partial remission after 5 months. Both, the marginal benefit from this treatment as well as some severe side effects indicate the need for further critical evaluation and possible improvements of this therapeutic approach.

rIFN-Alfa-A was a generous gift from Hoffmann-La Roche.

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FREE GASTRIC CANCER CELLS IN PREOPERATIVE INTRAABDOMINAL LAVAGE- A POSSIBLE RISK FOR INTRAABDOMINAL RECURRENT DISEASE.

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About 50% of potentially curative resected patients with gastric cancer will develop local recurrent disease or peritoneal carcinosis. It is well known from other tumors(pancreas, ovary) that free tumor cells are the main reason for recurrent intraabdominal disease.
Peritoneal lavage was performed in 62 patients shortly after laparotomy in order to look for free gastric cancer cells.
Material and methods: male 34; female 28; mean age 59 yrs.(31-80); resection rate 89%. Peritoneal lavage was done with 100 ml of normal saline(37°C) infracolic(small pelvis) and supracolic(close to the tumor). After centrifugation of 10 ml lavage fluid smear preparations of the sediment were prepared for immediate cytology. Cytological diagnosis and tumor classification were done according to WHO criteria.
Results: 11 pts.(18%) had free intraabdominal tumor cells. 7 of these 11 pts had macroscopical peritoneal carcinosis. 4 pts. with local advanced disease (T_2 - T_4, N_1 - N_2) had an unsuspicious lavage infracolic, but free tumor cells supracolic(located to the tumor).
Conclusions: Free intraabdominal tumor cells reflect the high risk of intraabdominal relapse of gastrointestinal carcinomas. Whether surgical procedures (gastrectomy, lymphadenectomy) increases the rate of free tumor cells,has to be investigated in a further trial.

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LEUKOVORIN(L)/ETOPOSIDE(E)/5-FLUOROURACIL(F) IN ADVANCED GASTRIC CANCER - PHASE I/II STUDY IN ELDERLY PTS. OR PTS. WITH CARDIAC RISQUES

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E and F are active in gastric cancer and act synergistic in vitro and in vivo. The enhanced cytotoxicity of F in combination with L is well known. A phase I/II study was carried out to determine the recommended dose for phase II studies. In addition first data on antineoplastic activity were ascertained. L 300 mg/m² 10 min inf. followed at once by E 100 mg/m² 50 min inf. followed at once by F 500 mg/m² 15 min inf. were given on day 1, 2, 3; repeat day 22-28. E was escalated in doses of 20 mg/m² per dose level until intolerable toxicity occured in 2 out of 5 pts.. 5 pts. received 100 mg/m² of E. Dose limiting toxicity was seen with 140 mg/m² E. Thus the recommended dose of Etoposide in this schedule was 120 mg/m². So far 17 pts. were treated with this dose in an ongoing phase II study. Pts. were older than 65 yrs. or had underlying cardiac disease. 17 pts. are evaluable for toxicity ($\geq$1 cycle), 15 pts. are evaluable for response ($\geq$2 cycles). 2 pts. are too early to evaluate. All pts. had measurable disease. Characteristics: Male 11; female 6; mean age 66 yrs.(46-75); mean Karnofsky PS 75%(60-100); local advanced disease 4; M1-disease 13.
Results: CR 2(1pCR proven by second look gastrectomy), PR 6; CR+PR8(53%), PD 7. Toxicity WHO grade: leukopenia 1^0(5), 2^0(6), 3^0(4), 4^0(1); thrombocytopenja 1^0(6), 2^0(2); mucositis/stomatitis 1^0(4), 2^0(2); nausea/vomiting 1^0(6), 2^0(4); alopecia 3^0(14); median remission duration and median survival too early. Conclusions: This regimen is well tolerable in elderly pts. and pts. with cardiac risques. The response rates in this ongoing study seem to be at least as good as treatment results being reported with chemotherapy programs including anthracyclines.

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FAST NEUTRON IRRADIATION, CHEMOTHERAPY AND SURGERY IN SOFT TISSUE SARCOMAS - RESULTS OF MUILTIMODALITY TREATMENT

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The consideration of multimodality approaches to the local and systemic treatment of soft tissue sarcomas is an important aspect of the modern management of this disease. Between 1984 and 1987 83 patients with soft tissue sarcomas of different location and different histologic types are reviewed.
All of these patients were treated with fast neutrons (DT, 14 MeV). The majority of them was treated in combination with other treatment modalities.
Two or more neutron-fields were applied. Most of the patients received a moulage for the neutron irradiation. Different doses were delivered in relation to the individual situation (location, radiotherapy before the neutron irradiation, neutron irradiation as preoperative irradiation etc.).
Most of the 83 patients received surgery in the management of primary tumour, local recurrence or distant metastases.
15 patients received chemotherapy, 5 of them with the intention of prophylaxis of dissemination, 10 patients in managing distant metastases. The most often employed regimens were CYVADIC, ADM-DTIC or combinations of various agents with Holoxan.
Principles and results of fast neutron irradiation and chemotherapy of soft tissue sarcomas will be presented.

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MALIGNANT FIBROUS HISTIOCYTOMA (MFH): HOTCHPOTCH OR ENTITY?

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We investigated seventy surgical specimens of MFH in order to assess to what extent these tumors show interindividual and intraindividual heterogeneity . Most of the cases presented the morphology of well known subtypes, i.g. storiform-pleomorphic, myxoid, giant cell, inflammatory and angiomatoid subtype with moderate interindividual diversities . Some of the cases presented with histologic criteria of a myogenic differentiation which could be demonstrated ultrastructurally as well as immunohistochemically.

More importantly a considerable number of cases revealed intraindividual heterogeneity: not rarely there were histologies of two or more subtypes within an individual tumor.

The findings are discussed with regard to the justification of a strict histogenetic classification of MFH.

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MALIGNANT SOFT TISSUE TUMORS - HISTOLOGICAL RECLASSIFICATION AND THERAPEUTIC PRINCIPLES

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Cooperative multi-disciplinary treatment of soft tissue sarcomas is based on exact pathological evaluation and classification.
One hundred thirty-five patients with malignant soft tissue tumors were treated over a 12 year period at our institution. Retrospective data analysis reveals, that only 43 % of the patients were seen with a primary lesion. Fourty-seven percent were referred after excisional biopsy, insufficient resection or the first local recurrence. Ten percent of the patients were admitted with the second or multiple local recurrence. Precise histological staging and grading was performed in only 5 % of the patients referred to our department. Until 1980 15 amputations and 58 local resections with "safety margins" were performed. In 1980 limb saving resections with functional restoration of the extremities by defect coverage with local or free myocutaneous flaps have been introduced into the therapeutic regimen. Only 2 amputations were performed ever since. These treatment principles allow for local disease control in more than 90 % of all cases. The histological slides of 60 patients were reevaluated. Fourty percent of soft tissue sarcomas had previously been classified as liposarcomas, 20 % as fibrosarcomas. Reclassification of the slides demonstrated a completely different distribution of soft tissue sarcomas. In only 31 % of the cases liposarcomas were confirmed, whereas malignant Fibro-Histiocytomas (MFH) were found in 26 %. Only 3 % of the tumors were classified as fibrosarcomas. This shifting of pathologic classification in our experience might have considerable impact to therapeutic regimen. Radicality as well as surgical proceeding and adjuvant radiation or chemotherapy depend on correct histological diagnosis. Exact staging and grading and evaluation by an experienced pathologist is urgently required.
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CYTOSTATIC TREATMENT OF ADVANCED SOFT TISSUE SARCOMAS (STS).
CLINICAL REPORT ON 64 PATIENTS

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From Jan. 1978 to May 1987 sixty-four pts with advanced STS were treated with CYVADIC (n=31) or with a combination of Ifosfamide (Ifo) in various dosages (5 g/m² d_1; 2,5 g/m² d_{1-2}; 1,5 g/m² d_{1-5}) together with ADM 50 mg/m² d_1 (n=13) or Ifo combined with other cytostatics (n=15). Five pts received different schedules (VACA; DDP, Theprubicin or high-dose MTX). In case of failure the pts were crossed over from CYVADIC to an Ifo-containing regimen or vice versa. The pts aged between 14 - 71 yrs. Histologically we met: fibrosa. 11, leiomyosa. 10, liposa. 9, malignant fibrous histiocytoma 9, rhabdomyosa. 7, angiosa. 5, synovialsa. 3, neuro-fibrosa. 3, others 7. Eleven pts presented an inoperable local recurrence without distant metastases. Eight pts had a local mass concurrent with metastases. In 45 pts a metastatic disease was found. Twenty-four pts had metastatic lesions in one organ (lungs 14/24; liver 4/24; bone 3/24). Twenty-nine pts (43 %) had a tumor spread to two or more organs. For all 64 pts the response rate was 21/64 (33 %) with CR: 2 (3 %). Median survival for responders was 16 (6-72) mos. NC 11/64 (17 %). PD 32/64 (50 %) with median survival of 6 (1-20) mos. For the different treatment regimens we achieved the following response rates: CYVADIC: 10/31 (32 %) with CR 2 (6 %), ADM-Ifo: 3/13 (23 %), Ifo combined with others: 3/15 (20 %). There were no striking differences between CYVADIC and Ifo-containing schedules. Five pts of the CYVADIC-group who responded primarily to CYVADIC ha a second response to an Ifosfamide regimen. In contrary 4 primarily resistant pts showed a response to second line chemotherapy. Toxicity was tolerable, however, two elderly pts suffered from transitory severe encephalopathy related to Ifosfamide. One pt died from septicemia due to leucocytopenia.
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INTRAVESICAL APPLICATION OF MITOMYCIN C WITH AND WITHOUT HYALURONIDASE (H.) IN PATIENTS WITH SUPERFICIAL BLADDER CANCER

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In 64 patients with superficial bladder cancer 20 mg Mitomycin C were applicated intravesically after transurethral resection. In a randomised trial 33 received only Mitomycin C and 31 patients received additional 200.000 u H. (Biochemie Sanabo, Austria). In 20 (10/10) of these 64 patients Mitomycin C plasma levels were compared after intravesical application with or without H.. No differences could be observed.
In the remaining 44 patients the recurrance rate was compared after intravesical Mitomycin C with and without H.. In 23 patients receiving only Mitomycin C 5 local relapses developed up to now, in 21 patients receiving additionally H. only 1 local recurrance was observed. The difference is not yet statistically significant.

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LONG TERM TOXICITY OF TREATMENT OF NONSEMINOMATOUS TESTICULAR GERM CELL TUMOR (NSGCT)

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From 1980 until 1986 100 patients (pts) with NSGCT were followed up in our outpatient clinic after successful therapy with cisplatin. Chemotherapy consisted of 4 cycles of PVB (cisplatin,vinblastine,bleomycin) only in 62 pts (group I), 8 pts had intensified 4-drug chemotherapy (group II) and the remaining 30 pts had additional treatment or pretreatment with other regimens (group III). All 100 pts were observed for more than 1 year, 68 pts more than 3 years and 36 pts more than 5 years. The observed toxic side effects were : 1. Paresthesia in 66%,with areflexia in 50% of group I during the first year, mild paresthesia in 50% after 5 years, areflexia persisting in only 10%. Group II was similar, but in group III 76% of the pts had paresthesia in the first year, 70% after 3 years, and also 50% after 5 years. 2. The Raynaud phenomenon was observed in about 30% of all 3 groups, rather constant over a period of 5 years. 1 pt had cerebral apoplexy. 3. Lung toxicity of clinical relevance occurred in only 5%, but in these pts bleomycin dose exceeded 400mg. Radiological signs of up to 30% in the first year declined to 10% after 3 years. 4.Mild serum creatinine elevation up to 1,8 mg/dl was consistent in about 50% in all groups over a 5 year period. 5. Bone marrow toxicity showed only mild thrombopenia of 50.000-100.000 in about 20% of the cases. Since most of the observed side effects are only lightly or not at all reversible, it is important to exclude chronic side effects by dose limitation or elimination of drugs (vinblastine substituted by etoposide) and possibly reconsidering the indication for secondary surgery.

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CLASSIFICATION OF MELANOMA BASED ON THE HISTOLOGICAL GROWTH PATTERN

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For the classification of melanoma Clark's proposal is generally recommended. As opposed to this, Ackerman developed a unifying concept, showing that all melanomas have a tendency to grow horizontally. His concept accounts for the difficulties in melanoma classification. In order to learn more about the significance of Clark's classification, one of us classified the melanomas of the years 1976-79 (University Hospital for Dermatology, Freiburg). Our reclassification was assumed to be correct, if it was in accordance with the diagnosis of the clinical pathologist. In the case of differences the classification was repeated and a diagnosis accepted if it was the same as a previous one. In the case of three different diagnoses, a third classification was performed. All classifications were carried out without knowledge of previous results. Alterations of connective tissue, signs of inflammation as well as clinical observations were not considered. According to these rules, 146 cases were classified in the first or second, 36 in the third run while 5 cases remained unclassified.
Difficulties in discrimination arise especially between superficial spreading melanomas and lentigo maligna melanomas and likewise between the latter and nodular melanomas. Moreover, differentiation between atypical melanocytes and just crowded normal melanocytes is somewhat arbitrary. This problem can be considered a so-called subvisual one and can be solved with automated methods only.

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SIGNIFICANTLY LOWER INCIDENCE OF INTERSTITIAL PNEUMONIA (IP) AFTER BMT FOR LEUKAEMIA POSSIBLY DUE TO IMMUNOGLOBULINE PROPHYLAXIS EXCLUSION OF CMV-POSITIVE PLATELET DONORS AND A MODIFIED RADIATION REGIMEN

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The IBMTR evaluated 6 risk factors for interstitial pneumonitis (IP): MTX for GvHD-prophylaxis, age 21 years, acute severe GvHD, 6 months interval from diagnosis to BMT, performance score pretransplant 90 %, dose rate 5 rad/min together with MTX for GvHD-prophylaxis). The effects of CMV immunoglobuline prophylaxis and the use of only CMV-negative platelet donors were not evaluated. We started CMV-Immunoglobuline prophylaxis (Cytotect* 1 ml/kg b.w., every 2 weeks up to day 100) and excluded related and unrelated platelet donors (except the marrow donor) with antibodies agains CMV. TBI was adjusted as follows: Non-fractionated irradiation (1000 rad, lungs 800 rad), delivered by a Co^{60} source at 3-4 rad/min. Between IX/83 and XII/86 none of 29 consecutive allogeneic leukaemic marrow graft recipients acquired IP. Based on the risk factors defined in the IBMTR analysis, the individual risk to acquire IP would have been between 9 % and 61 % (median: 43 % = 9/23 patients) in our series. The probability to remain free of IP only by chance was less than 0,0005 % (Binomial test) in our patients. This incidence is significantly lower than that seen in the 932 reported IBMTR patients ($p < 0,01$, chi-square test). We conclude that in addition to the described low dose rate radiation procedure CMV-immunoglobuline prophylaxis and the exclusion of CMV positive platelet donors reduce the incidence of IP after allogeneic BMT for leukaemia.

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BONE MARROW TRANSPLANTATION: DOES PRIOR IRRADIATION INFLUENCE CYCLOPHOSPHAMIDE (CP) PHARMACOKINETICS ?

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Radiation induced inhibition of mixed functional oxidases (MFO) has been reported to be the cause of altered elimination of mitomycin-C after total body irradiation (TBI). CP is an inactive prodrug metabolized as well by hepatic MFO forming the active alkylating agent 4-hydroxycylophosphamide (4-HOCP). Therefore a reduced exposition to cytotoxic compounds might occur after prior irradiation of patients.

Aim of our study was to compare the elimination and activation of CP in 7 patients who had TBI prior to chemotherapy with data of 11 patients who had chemotherapy first. CP was given in a dose of 50-60 mg/kg B.W. IV over 60 minutes over 2-4 days. CP levels were measured by N/P-flame ionization gas chromatography, 4-HOCP by liberation of acrolein and its fluorometric determination.

As in patients without pretreatment (), we found a decrease in the terminal half-lives of CP from 4.3 (7.1) hrs on the first day to 2.3 (5.5) hrs on the second and 2.2 (4.3) on the fourth day of treatment. At the same time AUC values of activated metabolites after hydroxylation increased from 11.4 (10.5) to 23.28 (18.2) and 16.7 (26.1) hrs*nmol/ml respectively (differences n.s.).

The activation and elimination of CP is not significantly altered by prior TBI. There may be an insignificant decrease of half-lifes of CP, however there is no difference in the exposition to the alkylating metabolite 4-HOCP. The results are in accordance with animal studies done in NMRI mice.

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PURGING OF BONE MARROW WITH IMMUNOMAGNETIC TECHNIQUES

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Autologous bone marrow transplantation (ABMT) is increasingly performed in leukemia patients for consolidation when a complete remission (CR) is reached by standard or high dose remission induction chemotherapy. However, even in CR the bone marrow may be contaminated with malignant cells, that eventually cause leukemic relapses. We have used an immunomagnetic technique for cleansing bone marrow from antigen positive cells with monoclonal antibodies, polystyrene microspheres and a magnetic field (Lea et al., Scand. J. Immunol. 23, 509, 1986). Bone marrow was aspirated from the iliac crest from healthy volunteers. The mononuclear cells were separated by density gradient centrifugation and contaminated with 1o%, 15% and 20% of cultured cell lines, expressing the Leu-1 antigen (Molt-4) and the B-1 antigen (Daudi). After incubation with Leu-1 and B-1 monoclonal antibodies for 3o min at 4 0C, the cells were rosetted with the microspheres for half an hour at 4 0C and finally separated with a magnetic field. The fractions obtained were studied for purity by the PAP-slide technique (Hecht et al., Blood 58, 856, 1981) or-in some experiments- with a fluorescence microscope, when the contaminating cells were labelled with the Hoechst 33352 nuclear dye. Less than 0.1% of residual contamination was seen upon examination. This technique may be usefull in purging bone marrow prior to ABMT.

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HAEMOPOIETIC RECONSTITUTION AFTER BONE MARROW TRANSPLANTATION FOR TREATMENT OF CHRONIC MYELOID LEUKAEMIA (CML)

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25 patients with CML (chronic phase n=18, accelerated phase n=6, blast crisis n=1) received an allogeneic bone marrow transplant from a HLA identical sibling. Haemopoietic reconstitution was studied by analysis of the transplanted cells (MNC's, CFUc, BFUe, CFUe) and the peripheral blood counts (granulocytes, platelets, reticulocytes) every second day. Statistical analysis was done by Spearman rank correlation coefficients and Wilcoxon testing. Between the transplanted MNC's and the time to reticulocyte recovery a statistically significant correlation was found, but not between transplanted progenitors and reticulocytes. Neither a significant correlation between transplanted cells and time to granulocyte recovery was found. This was probably due to a wide range of granulocyte and reticulocyte recovery. In median 500 granulocytes/µl p.b. were reached on day 25 (13-110), 1000 granulocytes/µl p.b. on day 29 (13-139), platelets $\geq$ 50000/µl p.b. on day 23 (13-110), reticulocytes $\geq$ 40000/µl on day 26 (13-104). Analysis of single factors (e.g. age of patient, age of donor, duration of disease, splenectomy (n=4), GvHD (n=8), relapse (n=4), interstitial pneumonitis (n=6), graft rejection (n=5)) showed that time to granulocyte and reticulocyte recovery is prolonged in patients with later graft rejection and relapse occuring after BMT. The difference in time to granulocyte and reticulocyte recovery between patients with and without relapse after BMT was statistically significant ($p < 0.05$).
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AUTOLOGOUS BONE MARROW TRANSPLANTATION IN PATIENTS WITH METASTATIC BREAST CANCER. A CLINICAL PHASE II TRIAL.

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Conventional chemotherapy and/or hormonal therapy does not offer a cure for patients with metastatic breast cancer. However in most patients with recurrent disease some response to high dose chemotherapy can be achieved. The time period of tumor regression and control of the disease is rather short. We treated 5 patients with metastatic breast cancer with high dose combined chemotherapy followed by autologous marrow transplantation. The conditioning regimen consists of 4 agents i.e. cisplatinum 200 mg/m^2 (total dose) given day 1-5, cyclophosphamide 150 mg/kg on 3 days, mitoxantrone 30 mg/m^2 in 3 doses each of 10 mg/m^2, and melphalan 80 mg/m^2 in 2 doses escalated to 100 mg/m^2 at present time. The cryopreserved marrow, which has been purged with mafosfamide was reinfused 24 hrs after the last dose of chemotherapy was given. The conditioning was fairly well tolerated by all patients. The major complains were nausea and vomiting. All patients demonstrated prompt hematological recovery. WBC was > 1000 within 21 days, at day 26 all patients became independent of platelets transfusions. Bony pain, a chief complain of 3 of the transplanted patients resolved completely. Although the follow up on these patients is rather short (3-9 months) this approach might offer long term remissions or even a cure for metastatic breast cancer patients.

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INCIDENCE, PROGNOSIS AND CONSEQUENCES OF ENDOCRINE AND REPRODUCTIVE DYSFUNCTIONS IN PATIENTS (PTS) AFTER BONE MARROW TRANSPLANTATION (BMT).

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BMT for treatment of pts with aplastic anemia and leukemia is resulting in an increasing number of long term survivors. Since alkylating agents and irradiation are known to cause irreversible gonadal toxicity in men and women (J. Cancer Res. Clin. Oncol. 113: 260, 1987) we conducted a study to evaluate the impact of conditioning therapy on endocrine and reproductive functions in pts after BMT. 20 women and 26 men successfully transplanted for AUL/ALL (n= 14), AML/AMML (n=13) CGL (n=13), and SAA (n=6) were studied. Diagnostic procedures included hormone analyses, interviews and sperm evaluations. In 20/20 women therapy-induced primary ovarian failure occurred 1-3 months after BMT resulting in severe endocrine and reproductive dysfunctions. All women except those using oral contraceptives showed FSH-, LH-, estradiol- and progesterone values within postmenopausal range. 16/20 (80%) women suffered from premature menopausal symptoms. No woman showed recovery of ovarian functions after BMT. 26/26 men showed elevated FSH-levels and azoospermia without evidence of restitution of reproductive capacity after BMT. In contrast testosterone and LH-levels were within normal limits in all men. Our results suggest 1) Irreversible sterility but normal gonadal steroid synthesis in all men after BMT. 2) Irreversible infertility and chronic hormone deficiency in all women resulting in premature menopausal symptoms in 80%. 3) Effective estrogen replacement is strongly indicated in women after BMT to prevent late metabolic and psychic sequelae due to chronic hormonal deficiency.

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HLA-"DY", NOVEL CLASS II DETERMINANTS FOR THE INDUCTION OF AUTOREACTIVE T CELLS AND THEIR APPARENT ROLE IN IMMUNOREGULATION AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT)

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Thus far four lines of evidence have allowed the definition of a novel HLA-class II-like determinants called HLA-"DY":
1.) Selective expression on defined leukemic blast cells lacking HLA-DR, -DQ and -DP antigens. 2.) Sequential immunoprecipitations and 2-dimensional gel-electrophoresis employing HLA-DR, -DQ, -DP-specific moab as well as moAb defined HLA-"DY" as molecules of HLA-class II nature. 3.) Specific inhibition of autoreactive inducer-T cell clones with either suppressor or helper function for T cell proliferation by "DY"-reactive monoclonal antibodies (moAb) and not by moAb specific for -DR, -DQ and DP. 4.) Preferential expression of "DY"-determinants on antigen-presenting cells as well as on activated T cells in peripheral blood from BMT patients during the early phase of hematopoetic reconstitution and selectively prolonged expression in patients with chronic virus infections. From these results it was concluded that an autostimulatory network of T cell subpopulations is apparently regulated by novel HLA-class II molecules, designated "DY". "DY" may be apparently transiently necessary during T-T interaction circuits involved in tolerance induction. Moreover, "chronic DY-hyperexpression" may indicate a persistent disequilibrium in T cell reactivity which in turn conditions immunodeficiency in these latter patients.

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EFFECTS OF NONFROZEN PRESERVATION OF HUMAN BONE MARROW ON THE SUBSEQUENT GENERATION OF MICRO LONG-TERM BONE MARROW CULTURES*

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Previous studies have shown that human pluripotent stem cells capable of regenerating bone marrow aplasia may be cryopreserved in liquid nitrogen. However, the freezing procedure is time consuming, expensive and requires technological investment. Moreover, indications exist that there is a certain degree of cryoinjury. In special cases, Hartmann et al. (Blut 1981, 42: 209-220), showed that cryopreservation of bone marrow cells may be replaced by nonfrozen preservation techniques. We, therefore, analyzed the effects of marrow storage at 4°C on both hemopoietic progenitor cells and bone marrow cells responsible for the induction and maintenance of hemopoiesis in vitro. The culture of the latter was performed by using a new micro long-term bone marrow culture technique. Storage of marrow cells for up to 72 h at 4°C resulted in a recovery of GM-CFC not significantly different from the control. Micro long-term cultures also initiated by bone marrow cells stored for up to 72 h at 4°C generated nucleated cells and GM-CFC for 4 weeks at least. Furthermore, these cells established confluent and morphologically normal adherent layers. Thus, nonfrozen storage of human bone marrow cells for at least 72 h will preserve hemopoietic progenitor cells and both stromal and immature hemopoietic cells being capable of inducing and maintaining hemopoiesis in vitro.

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EFFECTS OF RECHARGING WITH ALLOGENEIC HUMAN BONE MARROW CELLS OF MICRO LONG-TERM BONE MARROW CULTURES CONTAINING PREFORMED MARROW-DERIVED STROMAL LAYERS*

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Recently, we have established a miniaturized human long-term bone marrow culture (LTBMC) technique. This was shown to be as effective as conventional LTBMCs in terms of maximum proliferation period and progenitor cell production. In order to establish two-step allogeneic micro LTBMCs we analzyed the effects of recharging the cultures by fresh allogeneic bone marrow cells (BMC). Using normal human BMC many identical micro LTBMCs were established and fed by weekly removal of half the non-adherent cell suspension and addition of an equal volume of fresh medium. After confluency of the adherent stromal layers the non-adherent cell suspensions were taken off and the cultures were recharged with fresh allogeneic mononuclear BMC. The latter was performed either directly or after irradiation of the adherent layers. Thereafter, the cultures were fed as above and the cells removed were counted and assayed for granulocyte-macrophage colony-forming cells. Normally, one would expect that putative stem cells responsible for induction and maintenance of in vitro hematopoiesis should have an enhanced survival potential when seeded onto a preformed stromal layer. Interestingly, however, there was no obvious difference between sham-recharged cultures, cultures which have been recharged directly, and cultures recharged after irradiation.

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CAMPATH-1 KILLS ADDITIONAL BONE MARROW CELL POPULATIONS WHEN EMPLOYED WITH HIGHER LYTIC ACTIVITY: IMPLICATIONS FOR CLINICAL T-DEPLETION

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T-depletion of human bone marrow (BM) grafts is now mostly carried out with the rat anti-lymphocyte monoclonal antibody CAMPATH-1 (CP-1) + human complement (HC). BM purging with CP-1 + HC lowers graft-versus-host-reaction (GvHR) rate to 6% in fully MHC-matched transplantat recipients but increases incidence of host-versus-graft-reaction (HvGR) from 1 to 11%. We recently tested CP-1 with a rabbit complement (RC), which was almost completely detoxified by absorption with the human lymphoid cell line KM-3, and observed nearly 2 log enhancement of specific lytic activity of CP-1 for peripheral mononucleated cells when using RC instead of HC. Based on this finding CP-1 + RC was compared with CP-1 + HC for use in BM T-depletion. The following results were obtained: (1) Significant increase of the amount of specifically lysed BM cells at saturating CP-1 concentrations (26% versus 12% as tested in specific Cr-release assay); (2) a lower number of BM cells still bearing CP-1 on surface after lytic treatment (4% versus 9% positive cells as assayed by immunocytochemical methods); (3) a stronger reduction of both lymphatic and monocytic BM cell numbers, while BM stem cells are not affected. Conclusions and suggestions: CP-1 + RC eliminates a broader target cell spectrum in BM. This phenomenon may be due to the fact that BM cells (GvHR-effectors?) with low Campath-1-antigen density are solely lysed by the much more effective RC. On the other hand, insufficient lysis by HC results in a significant proportion of CP-1 coated cells remaining in the BM graft. This may facilitate recognition of graft cells by the host's immune system and thus promote HvGR.

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A T-DEPLETING MONOCLONAL ANTIBODY STRONGLY REDUCES BONE MARROW IMMUNOGENICITY AND THUS MAY FACILITATE THE PREVENTION OF GRAFT REJECTION

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In clinical bone marrow (BM) transplantation, T-depletion of the graft by use of monoclonal antibodies (MoAb) has proven to prevent graft-versus-host-reaction. However, this kind of graft manipulation has increased the otherwise rare incidence of graft rejection. In this context, earlier findings from our laboratory revealed that the immunogenicity of a T-depleted BM graft depends on the antibody employed for depletion. Therefore we studied our newly developed anti-pan-human-lymphocyte MoAb K31 for reduction of BM immunogenicity and for suitability in clinical T-depletion. Results: (1) When tested in a MLC-like assay, T-depletion with K31 + complement reduces BM immunogenicity nearly to background levels, while other clinically established MoAb like CAMPATH-1 or CT-2 reduce BM immunogenicity only insignificantly. (2) The reduction of immunogenicity caused by K31 correlates with K31-mediated lysis of BM cells of monocytic appearance. (3) All known properties of K31 are compatible with its use for clinical T-depletion: K31 is a mouse IgM that fixes rabbit complement. Though binding to all human mature lymphocytes and monocytes, it does not affect the majority of nucleated BM cells including stem cells; it does not bind to tissues which are not of hematopoetic origin (as far as tested); it displays no intrinsinc mitogenic activity. Conclusions: (1) Reduction of BM immunogenicity without eliminating hematopoetic progenitor cells is possible by performing T-depletion with K31 + complement. (2) With regard to graft rejection, K31 or other T-depleting MoAb(-cocktails) that strongly reduce immunogenicity appear to be superior to anti-T-cell MoAb like CT-2 and to CAMPATH-1.

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R I A - H P L C - RATIO OF CICLSPORINE AFTER BONE MARROW TRANSPLANTATION

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Monitoring of ciclosporine (CS) after bone marrow transplantation (BMT) is necessary to achieve therapeutic but not toxic blood levels. In radioimmunoassay (RIA) the value of CS is overestimated due to crossreacting metabolites, while the pure substance can be measured by high performance liquid chromatography (HPLC). Therefore we measured CS in whole blood with HPLC using a modification of the extraction procedure described by Annesley et al. (Clin Chem 32:1407, 1986). It consisted in using EDTA-blood, centrifugation at 1200 x g and 4°C, reconstitution after evaporation with 150 µl mobile phase and 250 µl n-heptane, and injection of 90 µl of the bottom layer after the last centrifugation step. We used a 250 x 4.6 mm Octyl-Daltosil column maintained at 72°C. Mobile phase was acetonitrile/methanol/water (7:1:2) at a flow rate of 1,2 ml/min. The effluent was monitored at 213 nm. With this method we achieved a linear calibration curve from 25 to 2000 ng/ml.
Comparison of CS values measured by HPLC and ^{125}J-CS-RIA (IBL, Hamburg, FRG) showed a mean RIA-HPLC-ratio of 1,8 in patients after BMT. Patients after renal transplantation showed a significantly higher ($p<0.01$) RIA-HPLC-ratio of 3.1. This may be due to a lower hepatic metabolism in patients after BMT or to a diminished renal elimination of metabolites after renal transplantation. Thus, CS blood levels have to be interpreted in both assays in context of the underlying disease respectively the therapeutical regimens.

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HIGH-DOSE BUSULFAN (BU) AND CYCLOPHOSPHAMIDE (CY) AS CONDITIONING REGIMEN IN BONE MARROW TRANSPLANTATION (BMT)

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Twenty-one of 203 patients (pts) who received marrow grafts at the West German Tumor Center Essen as of May 1987 were pretreated with BU (16 mg/kg p.o.) and CY (120-200 mg/kg i.v.) as conditioning regimen. The primary disease was AML in 1st (n=10) or 2nd (n=1) remission or in relapse (n=2);ALL in 2nd remission (n=1) or in relapse (n=2);CML in chronic phase (n=1) or in blast crisis (n=1); CMML (n=1);myeloblastoma (n=1) and medulloblastoma with marrow infiltration (n=1). Sex ratio M/F was 11/10;median age was 33 yrs.(16-45 yrs.). The pts received allogeneic (n=10),syngeneic (n=1) or unpurged autologous (n=10) grafts. - Mucositis occured in 19/21 pts (90%). Following BU/CY-administration a characteristic elevation of transaminases and gamma-GT/alkaline phosphatase, resolving after a median of 10 days and 4 weeks,respectively,was found in 17/21 pts (81%). Three pts showed persistent changes of liver function tests;in two of them biopsies revealed severe liver damage. Within the first 4 weeks post BMT 2 pts developed acute cholecystitis and one patient developed acute cholangitis. Following antibiotic treatment symptoms resolved over a period of 1 week in all cases.Three pts suffered from severe hoarseness lasting 1-10 weeks. In 10/13 pts surviving more than 3 months prolonged alopecia occured,which appears to be irreversible in some long term survivors.Prolonged alopecia,hoarseness,biliary system toxicity and liver enzyme patterns as described above were not seen in the pts grafted during the same time period following total body irradiation (TBI) and CY. We assume these signs to be due to BU,in combination with CY. - Eight pts (38%) died 7-133 days post BMT of transplantation-related causes,one of them of interstitial pneumonia. Four pts (19%) relapsed with leukemia. Nine pts (43%) are alive and disease-free 3-20 months post BMT. - Our experience confirms that BU plus CY is a potent myeloablative drug combination in the BMT-setting,presumably equivalent to TBI plus CY in eradicating the malignant clone. However, liver and biliary system toxicity may be pronounced and must be taken into account in pts with previous liver disease.

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SECRETION OF HEMATOPOETIC GROWTH FACTORS BY T CELLS FROM PATIENTS WITH GRAFT-VERSUS-HOST DISEASE (GvHD) AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT)

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In vivo activated T cells from patients suffering from acute Graft-versus-Host disease (GvHD) can be enriched by in vitro cultures using purified interleukin 2 (IL 2). The majority of these activated T cell populations as well as a number of T cell clones expressed the T4 antigen and exhibited inducer function. Since activated T cells represent important producers of hematopoetic growth factors, T cell derived supernatants were tested for stimulatory and inhibitory activity in bone marrow colony assay. Interestingly, the majority of T cell lines isolated during aGvHD from bone marrow transplant patients secreted factors which stimulated colony formation. These results were confirmed by proliferative assays using mouse indicator lines sensitive for GM-CSF and IL 3 as well as by Northern Blots.

It is concluded that the majority of in vivo activated T cells from patients with acute GvHD account for a substantial stimulation of hematopoetic recovery rather than its suppression.

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ERYTHROPOIETIC, GRANULOPOIETIC AND MEGAKARYOCYTIC COLONY STIMULATING ACTIVITY IN SERUM FROM PATIENTS UNDERGOIND BONE MARROW TRANSPLANTATION: ENHANCING EFFECT OF RECOMBINANT GM-CSF

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To define the role of various colony stimulating activities during recovery after bone marrow transplantation (BMT) sera from 8 patients undergoing BMT were tested in a microagar culture system for committed hematopoietic precursor cells. The content of megakaryocytic, granulocytic and erythropoietic colony stimulating activity was defined as the number of colonies per plate induced by 30 % test serum using monocyte and T cell depleted bone marrow from healthy volunteers as target cells. Monocyte and T cell depletion was performed to reduce the influence of endogenously produced lymphokines or monokines known to modify precursor cell proliferation. Serum of healthy individuals without colony stimulating activity, pretested serum batches from patients with aplastic anemia, Erythropoietin and recombinant GM-CSF were used as controls. Only low levels of granulocytic, megakaryocytic and erythrocytic colony stimulating activities were observed during the first 5 days after BMT, then colony formation incrased constantly reaching a peak level between day 10 to day 17. Thereafter CSF levels rapidly declined. The peak of colony stimulating activity was followed constantly by a rise of peripheral blood leukocytes. In another experiment we tested the capability of recombinant GM-CSF to further stimulate colony formations by adding rGM-CSF in pretested optimal concentrations to 30 % post-transplant serum. Both the number and terminal differentiation of granulocytic colony forming units was enhanced by GM-CSF up to day 14, while the number of BFU-E and CFU-Meg remained unchanged. These results suggest, that treatment with rGMCSF might be beneficial for bone marrow transplantation patients by accelerating hematopoietic recovery.

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FRACTIONATED VERSUS SINGLE DOSE TOTAL BODY IRRADIATION (TBI) IN THE CONDITIONING FOR ALLOGENEIC T-CELL-DEPLETED BMT

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The mode of radiotherapy used in the conditioning regimen for allogeneic BMT is thought to have a major influence on the rate of complications such as IP, graft rejection and leukemic relapse especially if T-cell depletion is used for GvHD-Prophylaxis.
We retrospectively analysed 42 patients with leukemia, which received single dose TBI (10 Gy, lungs shielded after 8 Gy) (n=26) or fractionated TBI (12 Gy, 6 x 2 Gy)(n=16). The overall survival was 38% in the single dose group compared to 77% in the group that received fractionated TBI. The rejection rate was 19% versus 25% respectively. However, the relapse rate was 39% in the single dose group whereas no patient who received fractionated TBI relapsed. If only low risk patients are compared the relapse rate was 19% in the group with single dose TBI and the survival rate was 50% versus 80% in the single versus fractionated TBI group. Fatal IP occured in 3 (12%) of the single dose patients and in 1 (6%) patient of the fractionated group.
Thus, fractionated TBI seems to improve survival by reducing the relapse rate with no impact on the rejection rate.

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T-CELL DEPLETED ALLOGENEIC BONE MARROW TRANSPLANTATION: FUNCTIONAL T-CELL RECONSTITUTION IN THE EARLY PHASE AFTER ALLOGENEIC BONE MARROW TRANSPLANTATOIN MEASURED BY LIMITING DILUTION ANALYSIS

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We investigated the first four months of immune reconstitution after T-cell depleted allogeneic bone marrow transplantation (aBMT) of leukaemic patients. T-cell purging was performed by incubating HLA identical, MLC negative sibling grafts with the monoclonal antibody (MoAb) Campath 1 and autologous complement. Peripheral blood mononuclear cells (PBMNC) were obtained at several times within the first four months after aBMT. Frequencies of Il2 secreting T-cells (HTL) and cytotoxic T-cells (CTL) after polyclonal or alloantigen specific stimulation were assessed using limiting dilution methodology. We detected in most patients remarkably high frequencies for both, nonspecific HTL and CTL even in the phase of not yet normalized peripheral blood lymphocyte counts. Moreover we found alloantigen specific CTL-reactivity which in comparison to healthy subjects ranges on a much lower level than do nonspecific CTL-frequencies in the observed period.
Our data suggest that functional T-cell reconstitution occurs much earlier than supposed. Besides the predominance of CD8-positive cells in the early period after aBMT our results indicate the existence of a relevant CD8-positive Il2 producing subpopulation.

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ANTI-HOST AND -DONOR CYTOTOXIC T CELL PRECURSOR FREQUENCIES AFTER HAPLOIDENTICAL BONE MARROW TRANSPLANTATION:SUPPRESSOR CELLS MAINTAIN TOLERANCE.

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Severe combined immunodeficiency(SCID)has been successfully treated by T-cell-depleted HLA-haploidentical bone marrow transplantation(BMT).Donor-derived T cell precursors are able to mature within the HLA-different host environment into functionaly normal T cells;Graft versus Host disease does not develop.To examine the mechanism which generate this state of tolerance,we have used the limiting dilution assay(LDA)to determine allo- and autocytotoxic T cell precursor frequencies after BMT against lymphoblastoid cell lines of donor and host origin.
LDA revealed different response patterns for donor-and host- LCL-targets.Whereas a bi-or triphasic pattern was observed against host cells,a linear pattern with levelling off was seen against donor cells.Split well limiting dilution experiments revealed significantly increased reactivity to K562 as compared to control responder cells.This lends support to the hypothesis that NK activity,which is known to be increased after BMT,might be responsible for the observed triphasic pattern against host cells.Apart from this,the observed biphasic pattern at low responder cell doses suggests that anti- host reactivity is under the control of suppressor cells.

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IMMUNORECONSTITUTION AFTER BONE MARROW TRANSPLANTATION (BMT): AN ANALYSIS OF PERIPHERAL LYMPHOCYTE RECOVERY AND IMMUNOGLOBULIN LEVELS

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The regeneration kinetics of lymphocytes and immunoglobulins (IgG, IgA, IgM) were analyzed in 79 patients treated with BMT for severe aplastic anaemia (SAA, n=12) or haematological malignancies (n=67). The immunefunction was reduced in the vast majority of BMT-recipients for about one year after BMT: lymphocytes and IgA-levels reached normal levels one year after BMT, the IgG-levels were near the lower range up to one year, the IgM-levels were reduced for about half a year.
During the first two years after BMT patients with SAA showed markedly reduced levels of lymphocytes, IgG and IgA whereas no difference occurred in IgM-levels as compared with patients transplanted for haematological malignancies. Patients with graft-versus-host disease (GvHD) had decreased IgG- and IgA-levels but no differences in lymphocyte and IgM-levels compared with patients without GvHD. The use of T-cell-depletion for GvHD-prophylaxis induced a more rapid increase of IgA-levels to the normal range as compared with patients with conventional GvHD-prophylaxis. Lymphocyte-, IgG and IgM-levels were not affected by the method of GvHD-prophylaxis.

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LYMPHOHAEMOPOIETIC REGENERATION AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION USING EX VIVO MARROW TREATMENT WITH ANTIBODIES AND COMPLEMENT

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Six patients with acute lymphoblastic leukaemia (4 C-ALL, 2 T-ALL) received autologous bone marrow transplantation (ABMT). Before cryopreservation the marrow was incubated with anti-B (VIL-A1+VIB-C5+VIB-E3) or anti-T-cell antibodies (Campath 1+2) and human complement to purge the graft of residual leukaemic cells.
The median period to peripheral blood recovery is day 29 (18-61) for lymphocytes >500/ul, day 30 (22-37) for granulocytes >1000/ul, day 28 (17-36) for reticulocytes >40 000/ul and day 34 (27-46) for platelets >50 000/ul. Only the time for reticulocyte recovery correlates significantly ($p < 0.05$) with the number of nucleated cells and erythroid progenitors (CFU-E) of the graft. The CD4/CD8 ratio of the peripheral blood lymphocytes is reduced for about one year postgrafting due to a decreased incidence of CD4-positive cells. B4 (CD 19)- positive cells are consistently found about two months after ABMT with an increased proportion of HLA-DR-positive cells during this time. Serum IgM and IgA levels return to normal in a median 3 (1-4) and 8 (3-14) months postgrafting respectively.
In conclusion, lymphohaemopoietic regeneration after ABMT is not impaired by ex vivo marrow treatment with antibodies and complement. The data will be discussed in comparison to our experience in T-cell depleted allogeneic BMT.

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HUMAN LONG TERM BONE MARROW CULTURES USING INTERLEUKIN 2 (IL 2) AND INTERLEUKIN 3 (IL 3) WERE APPLIED TO ASSESS THE HEMATOPOETIC POTENTIAL OF TRANSPLANTED BONE MARROW

I. Lorenz, P. Wernet and E.M. Schneider

A novel culture-protocol was established in order to qualify and quantify the capacity of human bone marrow (BM) harvested for allogeneic bone marrow transplantation (BMT). Mononuclear cells from BMT material were cultured in purified natural IL 2 and IL 3 as well as small amounts of granulocyte-macrophage colony stimulating factor (GM-CSF). In contrast to cultures performed with IL 2 alone or without exogeneous growth factors, the addition of IL 2 and IL 3 firstly inhibited the spontaneous maturation of myeloid progenitors, but allowed the continuous proliferation of stem cells. Secondly, the proliferation of BM-contaminating T cells was abrogated, and thirdly, the formation of a mainly monocytoid stromal layer was induced. The endogeneous production of additional GM-CSF was apparently responsible for the maturation of a fraction of myeloid precursors exhibiting phagocytic and cytotoxic function comparable to that displayed by mature monocytes and granulocytes. Although anamnestic parameters of patient himself play a major role in the regenerative phase after BMT, primary analyses regarding culture kinetics of the transplanted BM as well as its potential to continuously regenerate monocytic and granulocytic cells in this in vitro system revealed striking correlations to the course of early hematopoetic recovery in vivo. This was in contrast to the poor predicitive value of classical parameters such as colony assays as well as the total amount of transplanted BM mononuclear cells. This novel BM culture system appears suitable to estimate the potential hematopoetic recovery by donor bone marrow samples.

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ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) IN PATIENTS AGED OVER 40 YEARS

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BMT is usually not performed on recipients aged over 40 years because of the expectation that such patients would experience unacceptable conditioning toxicities and increased severity of GvHD. Evidence is presented here that this expectation may be unfounded.
Thus eight patients (age range 40-49 years, median 46 years) were transplanted from HLA identical siblings. The diagnoses were: AML 1st CR (N=3), AML 2 nd CR (N=1), ALL 2nd CR (N=1), and CML (N=3). Ciclosporin plus methotrexate was usually given as GvHD prophylaxis. Engraftment was documented in all patients. Four out of 7 evaluable patients developed aGvHD (grade II, N=3; grade III, N=1). Two patients died from relapse, two from infectious complications. Four patients are alive with a median follow up of 13 months (range 4-24 months).
In this small series of older patients the incidence of severe GvHD and the transplantation related morbidity and mortality was not appreciably greater than in younger patients.

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CYTOGENETIC ANALYSIS OF T-CELL COLONIES EARLY AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Radioresistant T lymphocytes play a critical role for the rejection of the graft seen after allogeneic bone marrow transplantation (BMT). In this context we sought to find out the origin of T-cell colony-forming cells (T-CFC) grown from the peripheral blood of 12 consecutive BMT patients. In order to test the validity of our approach mononuclear cells of transplant candidates and their HLA-identical siblings were mixed (50:50, 80:20, 90:10, 95:5) and cultured. Analysis of 50 mitoses allowed the identification of the minority of plated cells in all cases.
Addition of 0.5 % PHA, 5 % PHA-LCM, 10 µg/ml TPA, and 10 U/ml Interleukin 2 (Biogen, Geneva) to support colony formation resulted in the growth of a sufficient number of colonies in all patients as early as day 15 after BMT (range: day 14-56). After a conditioning regimen including total body irradiation (12 Gy) followed by cyclophosphamide (120 mg/kg) or VP 16 (60-70 mg/kg) 9/12 patients showed exclusively donor mitoses at the time of first investigation after BMT. Two patients exhibited 1/27 or 1/59 host mitoses, respectively, on day +15. Later investigations gave only donor cells. A single patient who had been transplanted for ALL in second remission showed 8/82, 1/70, and 3/64 host mitoses, two, four and eight weeks after BMT without recurrence of ALL. Another patient with Philadelphia chromosome-positive CML showed a single Ph1-negative host mitosis out of 58 mitoses analyzed on day 106.
Our results show that in patients transplanted from HLA-identical siblings circulating T-CFC are of donor origin as early as day 15 after BMT in the majority of cases. T-CFC of host type are rarely found and are largely outnumbered by donor cells. If the same pattern of chimerism applies to HLA-nonidentical BMT is currently under investigation.
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FUNCTIONAL ASPLENIA - A LATE COMPLICATION OF BONE MARROW TRANSPLANTATION

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In order to investigate the high susceptibility to bacterial infections in long term survivors after bone marrow transplantation, we evaluated splenic function in 15 patients (7 females, 8 males, median age 32 years) transplanted for chronic myelogenous leukemia, acute myelogenous leukemia, severe aplastic anemia and Non-Hodgkin's lymphoma. 13 patients received allogeneicgrafts, 2 were autografted. 6 out of 15 patients had Howell-Jolly bodies in peripheral blood smears. These 6 patients had higher platelet counts ($p < 0.01$), higher recovery of Indium-111 labelled autologous platelets ($p < 0.05$), decreased splenic blood flow ($p < 0.001$) and reduced or absent accumulation of radioactivity at the splenic site ($p < 0.001$). Spleen size of these patients was significantly smaller (p 0.001). These findings are indicative for loss of splenic function. The incidence of bacterial infections was 4 times higher in patients with functional asplenia. Howell-Jolly bodies were detected only in allogeneic graft recipients. They were found in 5/11 patients who had received total body irradiation (TBI) but also in 1/4 without irradiation. 5 patients with Howell-Jolly bodies suffer from extensive and 1 from limited chronic graft-versus-host disease, respectively. Our data suggest that functional asplenia, a hitherto underestimated complication of allogeneic BMT, may be a major cause for the high susceptibility to bacterial infections in the late posttransplant period.

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PSYCHOSOCIAL LONG-TERM EFFECTS IN MARROW TRANSPLANT RECIPIENTS

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Psychosocial long-term effects in adult BMT recipient survivors have received little study.
23 (=88%) out of 26 patients surviving in complete remission (all transplanted before 10/85) participated in a retrospective analysis between 5/86 and 5/87. The aim of the study is to evaluate psychosocial consequences, coping strategies, and personal experiences with the disease and the therapeutic interventions.
Our analysis included semistructured interviews, psychometric and projective tests (such as the Freiburg Personality Inventory or FPI-R, WAIS, Rorschach test) as well as physical findings. The 23 patients (7women, 16men) participating in the study 18 to 89 months after BMT (mean, 38months) were 18 to 48 years old (mean, 25yrs) at the time of examination. All donors were HLA-identical siblings (aged 18-50yrs;mean, 26yrs). 12 donor-recipient pairs were of same sex (men only). The underlying diseases the patients were transplanted for were: 6 SAAs (severe aplastic anemia) and 17 leukemias. 2 out of the 3 women transplanted for SAA became pregnant and delivered healthy babies. Our findings indicate that from a medical point of view about 80% of the long-term survivors are doing well (Karnofsky-Index more than 90%), psychosocial also most of the patients are content with their life after BMT, but reported different changes in self-perception.

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EVIDENCE FOR EFFECTS OF $CD8^+$ T CELL SUBPOPULATIONS AND IFN-Y ACCOUNTING FOR REDUCED BFU-E FROM BLOOD AND BONE MARROW IN BONE MARROW TRANSPLANT (BMT) PATIENTS

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Despite the fact that patients after BMT recovered their peripheral hematological parameters, stem cell assays revealed significantly decreased numbers of colony formation. The present study addressed the problem of a reduced capability to grow erythroid burst forming units (BFU-e). This decreased stem cell growth might be explained by either reduced production of helper factors or by inhibitory factors secreted by contaminating T cells or monocytes. A possible inhibitory factor is Interferon-gamma (IFN-y) which reduced BFU-e from PBMC. On the other hand low concentrations of IFN-y mediated stimulatory effects on BFU-e from BM. Transplant BM-MNC contained roughly equal amounts of $CD4^+$ to $CD8^+$ population. BM from BMT-patients contained significantly more CD 8^+ cells. Interestingly a major population of these $CD8^+$ cells were also Leu 7 positive. In these cases BFU-e grown from PBMC and BM-MNC were most significantly reduced. Results are discussed on the basis of reactivation of latent virus-infections such as Cytomegalovirus (CMV), Epstein Barr virus (EBV) and Herpes simplex type I (HSV I).

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DOES RECIPIENT TYPE BLOOD CELLS AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION PREDICT LEUKEMIC RELAPSE ?

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The incidence of mixed chimeras after allogeneic bone marrow transplantation (bmt) increases with more and more sensitive methods testing chimerism. Opposed to use of blood group markers, cytogenetic methods and restriction fragment length polymorphism isoenzyme typing has the advantage of testing each cell lineage independent of proliferating status or DNA content. Mixed chimerism seems to be more frequent among patients (pts) with T-depleted marrow grafts, who show a less severe graft versus host disease (GvHD) and an higher incidence of leukemic relapses and rejections. To estimate the risk of leukemic relapse after allogeneic bmt we examined repeatedly 33 pts postgrafting. 29/33 pts were complete chimeras, 5 of these relapsed. 3/33 pts showed recipient type cells among mature blood cells (one pt among PMN, lymphocytes, monocytes and platelets, one pt among PMN, MNC and platelets, one pt among PMN). 1/33 pt showed host type cells among MNC of morphologically normal bone marrow with cytogenetically donor type cells exclusively; this pt relapsed, whereas two of the other mixed chimeras changed from mixed to complete chimerism; the third mixed chimera continues to be a mixed chimera. The change of chimerism was accompanied by development of acute GvHD grade IV in one pt, in the other pt chronic GvHD remained to be mild. The stable mixed chimera as well as the relapsing pt didn't show any sign of GvHD.
We conclude, that evidence of mature recipient type blood cells doesn't suggest impending leukemic relapse. GvH reaction might favour complete chimerism, whereas complete chimerism doesn't reject leukemic relapse.

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KINETICS OF NEOPTERINE INCREASE AND ITS DIAGNOSTIC VALUE IN PATIENTS FOLLOWING ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT)

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Increased serum neopterine levels are generally accepted to mirror immune-stimulation by T cells which in turn activate macrophages to secrete neopterine. Although BMT patients remain immunodeficient for a prolonged period of time we detected high levels of neopterine simultaneously with the onset of acute graft-versus-host disease (GvHD) in 10 patients who were followed at daily intervals after transplantation. Moreover, neopterine in the sera rose significantly during bacterial infections. Reactivation of latent as well as acute virus infections resulted in variable, mainly lower, neopterine increases. The latter phenomenon may be due to a lack of immunocompetent T cells producing interferon-γ which is known as the most potent inducer of neopterine release. However, a more direct pathway to induce neopterine by monocytes is postulated, because activation of non-specific effector cells represented by monocytes and granulocytes alone, coincided with neopterine increases. In addition, a peak release of neopterine was observed in some cases, although activated T lymphocytes were identified only some days later. Neopterine determinations in our patients after BMT are quamtitatively and qualitatively related to the "extent" of non-specific and T cellular specific immuneresponsiveness and may be of value to estimate the course of acute GvHD already at its onset.

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IMMUNOLOGICAL RECONSTITUTION AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION OF PATIENTS WITH MYELOGENOUS LEUKEMIA

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The long term outcome of allogeneic bone marrow transplantation (BMT) depends widely on immunological mechanisms like graft versus host disease (gvhd), host versus graft reaction (hvg), immunity against infectious agents and control of residual disease. Therefore immunological reconstitution was retrospectively analyzed in a group of 10 patients with myelogenous leukemia being at least two years (37.3 + 15 (SD) months) after BMT still in complete remission and without immunosuppressive therapy or signs of chronic gvhd. The posttransplantation period was characterized by persistently low percentages of T-helper cells (OKT4+/Leu3+) without strong correlation to immunosuppressive therapy with CSA or MTX. T-suppressor cells (OKT3+/Leu2+), however, were slightly increased only in the first months after BMT. B-lymphocytes (B1+) and NK-cells (Leu7+) remained normal. The discrepancy between normal B-cell numbers and impaired immunoglobulin synthesis in vitro could be explained by defective T-helper cell function. Whereas regular substitution of standard immunoglobulin allowed maintainance of normal concentrations of serum IgG, the IgA-concentration after BMT needed 36 months for normalization. Specific humoral immune responses after proved viral infections with either CMV or HSV were detected after 12 months in CSA- and after 4 months in MTX-treated patients. When Anti-HSV-antibodies were demonstrated in pretransplant sera the same titers could be measured in sera of the early posttransplantation period.

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CELL KINETIC INVESTIGATIONS ON BONE MARROW FIBROBLASTS FROM PATIENTS WITH FANCONI ANEMIA BEFORE AND AFTER ALLOGENEIC BONE MARROW TRANSPLANTATION

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Cells from patients with Fanconi anemia (FA) are unusually sensitive to agents like Mitomycin C (MMC) which are capable of cross-linking DNA. Bone marrow buffy coat was cultivated in α - MEM with 20 % fetal calf serum until confluence was reached. After the first trypsinization cells were grown with 10 % FCS. After the second or third passage the cells were exclusively fibroblasts. The cells were exposed to 0.05 mg/l MMC for two hours. After further 24 or 48 hours in culture the fibroblast were stained with propidium iodide and analyzed by DNA flow cytometry. After 24 hours there was no significant difference between FA and the controls. After 48 hours following the release from MMC treatment fibroblasts from three healthy donors and five patients with other hematological disorders form a cluster in a G1 - G2 Diagramm ($G2/G1 < 0.5$) which is well separated from the fibroblasts of three patients with FA before bone marrow transplantation (BMT) and two patients 97 and 480 days after BMT ($G2/G1 > 0.9$). Two patients 14 and 46 days after BMT are in an intermediate region. We conclude that cell kinetics can help to define FA status. In the early post transplant phase stroma cells show a behaviour of the donor type, whereas in the later phase the typical predominant disturbance of the host could be demonstrated.

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CLONOGENIC ASSAYS AND ENGRAFTMENT IN ALLOGENEIC BONE MARROW TRANSPLANTATION.

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The significance of clonogenic assays for determining the hematopoietic potential of grafts is controversial. We determined the numbers of myeloid (GM-CFU), early erythroid (BFUe) and mixed myeloid/erythroid (CFU-GEM) clones in 33 consecutive allogeneic bone marrow grafts. The growth of GM-CFU was stimulated by placental conditioned medium, whereas both phytohemagglutinin-stimulated leucocyte conditioned medium and supernatant from the 5637 bladder carcinoma cell line served as equally efficient stimulators for BFUe and CFU-GEM growth. The plating efficiencies of GM-CFU and the numbers of myeloid progenitors transplanted per kg body weight (bw) were significantly lower in those patients who died from infections whithout signs of leucocyte recovery (n=3, 160 vs 390×10^3/kg bw, P=0.001). The same was true if two further fatal cases whith beginning leucocyte recovery were taken into consideration (n=5, 160 vs 409×10^3/ kg bw, P=0.001). No significant differences existed with respect to the numbers of grafted nucleated cells, mononuclear cells, BFUe, and CFU-GEM, respectively. These data indicate that patients with low numbers of grafted GM-CFU run a higher risk of death from infection following bone marrow transplantation. They argue for a contribution of GM-CFU in the seeding of an aplastic bone marrow.

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CULTURE OF MONOCLONAL RECIPIENT TYPE B CELLS FROM AN ALLOGENEICALLY GRAFTED PATIENT WITH "COMPLETE CHIMERISM"

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The development of secondary B cell neoplasms has been observed following bone marrow transplantation (BMT). Such tumors usually carry Epstein Barr virus (EBV) genomes in contrast to relapses of primary leukemias. We have established a B cell line from bone marrow cells of a patient in complete remission following allogeneic BMT for aplastic anemia 18 months postgrafting. Differences in sex and phosphoglucomutase 1 (PGM-1) - as well as in acid phosphatase isoenzymes allowed the exact determination of chimerism in this case. While the patient showed persisting complete chimerism of all cell lineages the cells grown in culture were of recipient type. Cytologically and cytochemically these cells were undifferentiated but expressed B cell markers. Hybridization of the DNA with a μ-specific probe showed a monoclonal rearrangement of the immunoglobulin heavy chain gene. The cells carried virus capsid antigen (VCA) and EBV nuclear antigen (EBNA). These observations show that EBV transformed B cells of the recipient can survive in patients who are "complete chimeras" as determined by methods detecting 1 % of foreign cells. The immortalized cells can be amplified in long term cultures in vitro. For the development of B cell neoplasias in vivo additional pathophysiological steps like severe GvHD or T cell suppression are obviously needed, because the patient continues to be in good health 32 months postgrafting.

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PLATELET AUTOANTIBODIES FOLLOWING ALLOGENEIC BMT: STUDIES WITH A COMPETITIVE PLATELET ELISA AND IMMUNOBLOTTING

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Autoimmune thrombocytopenia (ITP) was diagnosed in an AML patient (2.CR) after successful engraftment from a HLA A,B,C,DR (MLC nonreactive) identical sibling. ITP occured on day 59 when CMV infection was diagnosed. The cELISA (Kiefel V. et al, Vox Sang 1987) was modified to quantitate in-vitro antibody binding onto platelets (plt). Serum equally reacted with 30 panel plt of known HLA, $Pl^{A1/2}$ and Bak^{a} specificity as well as with plt of all family members including the bone-marrow donor. Antibody binding to plt proteins immobilized on nitrocellulose was detected using a biotin-avidin-peroxidase system. Serum antibody bound to nonreduced plt glycoproteins (GP) of 75kD (GPV), 90kD (GPIIIa) and 150kD(GPIIb).
We conclude that this antibody reacted with common target antigens.

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SUBSEQUENT EXPOSURE TO METHYLPREDNISOLONE (MP) ENHANCES CYTOTOXICITY OF 4-HYDROPEROXYCYCLOPHOSPHAMIDE (4-HC) FOR K562 BUT NOT FOR HUMAN CFU-GM. M. Zühlsdorf, S.D. Rowley, J. Hilton, H.G. Braine, O.M. Colvin and G.W. Santos

Combinations of cytotoxic drugs might increase the therapeutic ratio (kill of leukemic cells vs. normal human bone marrow cells (HBMC) for ex vivo purging in autologous bone marrow transplantation. A human leukemic cell line relatively resistant to 4HC, K562, was compared to HBMC for combined treatment with 4HC and MP. K562 and, separately, HBMC were incubated with graded doses of 4HC (0-250 µM) alone or in sequence with 10 mM MP in either order (MP→4HC, 4HC→MP) with 1h between incubations. Cell survival was quantified in clonogenic assays for K562 and CFU-GM. Synergism of the drugs was shown by their cooperative index (CI; fractional survival (fs) for drug combination/(fs for 4HC alone x fs for MP alone); CI=1, summation; CI<1, synergism; CI>1, antagonism).

drugs and concentrations	logs kill K562	CFU-GM	ratio K562/CFU-GM	CI K562	CFU-GM
4HC 100 µM	1.57	0.60	2.62	-	-
MP 10 mM	0.39	0.00	-	-	-
10mM MP→100µM 4HC	3.09	1.49	2.07	0.07	0.13
100µM 4HC→10mM MP	2.68	0.58	4.62	0.19	1.05

Sequential treatment with **4HC→MP** increased 4HC toxicity only for K562 and not for CFU-GM. Further studies combined graded MP doses (10-40 mM) with 50 µM 4HC. Synergism of 4HC and MP increased with 4HC doses but not with MP doses (>10 mM). An alkaline elution assay showed increased DNA crosslinking in K562 by 4HC with additional exposure to MP.

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Clq HIGH AND LOW AFFINE ANTIBODIES FOR SUPPRESSION OF GVHD IN FULLY MISMATCHED MICE

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One of the major immunological complications for bone marrow transplantation is graft-versus-host-disease (GVHD). We studied in a well established mouse model the effect of rat monoclonal antibodies of the same specificity (anti-Thy-1) but different isotype (IgM, IgG2b and IgG2c) on suppression of GVHD.

Spleen and bone marrow cells from mice previously treated with each antibody were transplanted to lethaly irradiated H-2 incompatible mice ($H\text{-}2^b \longrightarrow H\text{-}2^k$). Only the antibody of IgG2b isotype which has a high affinity for Clq suppressed acute and chronic mortality of GVHD.

Immunocytochemical and immunohistochemical examination of the lymphatic organs after a single injection of each antibody showed an extensive depletion of T-lymphocytes. However, only the high Clq affine rat IgG2b monoclonal antibody depleted 97% spleen T-lymphocytes whereas the Clq low affine IgG2c and IgM isotypes were able to eliminate 83% and 78% T-cells respectively. Interestingly, the lymph nodes could not be totally depleted of T-cells but only to 84%. The thymus did not appear depleted at all.

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RETROSPECTIVE STUDY OF THE SUBJECTIVE EXPERIENCE OF BONE MARROW TRANSPLANTATION

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After undergoing BMT (median of the elapsed time 21 months) 35 patients participated in a semistructured interview covering subjective status at time of study, retrospective status at time of falling ill, illness and treatment experiences and changes attributed by patients to the experience of illness and treatment. Most patients decided to have BMT following the recommendations of the treating physician. This might lend support to the hypothesis that these patients achieved a successful identification with the treatment procedure and the doctor. A number of recipients expressed the wish that the donor should be similar as to their attitudes, habits and physical appearances. This might indicate their need for "constancy of identity" in a traumatic situation. Experiencing BMT does not seem to be different from experiencing chemotherapy from a subjective perspective. Our impression is that the more time elapse since BMT peculiarities of the BMT procedure (e.g. total body irradiation, bone marrow transfusion, waiting for the take) don't seem to influence the subjective treatment experience in any special way. The majority of our patients reported changes in regard to physical and psychic status. Psychic changes, e.g. intensified life experience were mostly reported by patients with an otherwise unobstrusive physical status.

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EFFECT OF RECOMBINANT INTERFERON ALPHA-2 ON THE GROWTH OF HAEMATOPOETIC PROGENITOR CELLS IN CHRONIC MYELOGENOUS LEUKEMIA AND ITS RELATIONSHIP TO THE CLINICAL EFFICACY.

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In an ongoing phase-II trial we aimed to relate clinical responsiveness of chronic myelogenous leukemia (CML) to rIFN-alpha to in vitro sensitivity. From five normal controls and 13 CML patients bone marrow samples were taken before treatment and tested for the antiproliferative activity of rIFN-alpha, using a microagarsystem for CFU-GM, BFU-E and CFU-Meg. In normal controls CFU-GM, BFU-E, CFU-E and CFU-Meg were inhibited by rIFN-alpha in a dose dependent manner. BFU-E and CFU-Meg proved to be the most sensitive cell lineages, whereas CFU-E and CFU-Meg were about 10 times less sensitive. A rather heterogenous in vitro response, however, was obtained in CML-patients. Some patients even exhibited an in vitro resistance of CFU-GM derived colony formation to the highest doses ($10x10^4$ units/ml)used for in vitro testing. In the responder group, however, 50% growth inhibition for CFU-GM was obtained already at rIFN-alpha concentrations between 10-100 units per ml. The sensitivity of BFU-E and CFU-Meg in CML patients did not differ from that of normal controls. When patients were subsequently treated in vivo with rIFN-alpha-2 a striking correlation between these in vivo data and their subsequent response was observed.

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RECOMBINANT IFN-ALPHA-2 (rIFN-ALPHA-2) FOR TREATMENT OF CHRONIC MYELOGENOUS LEUKEMIA IN BENIGN AND ACCELERATED PHASE

G. Gastl, W. Aulitzky, H. Tilg, I. Lüttichau, G. Michlmayr, H. Gadner, C. Coser, H.L. Seewann, H. Huber, Ch. Huber

We have treated 27 CML patients with rIFN-alpha-2. Except one all the patients had previously been treated with conventional cytostatic agents. Eighteen patients (group I) with benign phase CML received 1-8x10^6 U rIFNalpha/d subcutaneously. Nine patients (group II) with accelerated disease were treated with a combination of rIFN-alpha-2 (1-8x10^6 U/d) and busulfan (2-4 mg/d) or hydroxyurea (500-1500 mg/d). The median follow up period is presently 36 weeks (range 6-63 weeks). According to the criteria of Talpaz et al. (ref.) 10 of the 18 patients in group I responded to IFN-treatment of whom five had a hematologic remission and five a partial hematologic remission. In three of the patients with hematologic remission a suppression of Ph positive metaphases was observed. During IFN-treatment, none of the 18 patients in group I showed disease acceleration or progression into blastic crisis. Within a median follow up period of 27 weeks (range 3-64 weeks), only two of the group II patients responded to a combination of IFN and busulfan or hydroxyurea respectively. Blastic transformation occured in three of the nine cases after three, 15 and 30 weks respectively. These results demonstrate that rIFN-alpha is effective in inducing hematologic remission in the majority of patients with benign phase CML but is mostly ineffective in advanced stage CML.

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Ref.: Talpaz et al., New Engl. J. Med., 1986

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TREATMENT WITH rIFN-alpha-2c CAN NORMALIZE ABNORMAL CONVERSION OF 14C-ARACHIDONIC ACID (AA) IN MYELOPROLIFERATIVE SYNDROME (MPS)

W. Linkesch, H. Sinzinger, H. Ludwig, H. Gisslinger

Our previous studies (Linkesch et al. 1985) demonstrated an excellent therapeutic efficacy of rIFN-alpha-2c (Boehringer Ingelheim International) in patients with MPS and excessive thrombocytosis. Therefore we investigated in 30 patients with MPS (5 CML, 13 polycythemia vera, 12 essential thrombocythemia; 8 male, 22 female; mean age: 63 years) parameters of platelet function (beta-thromboglobulin, PF_4, thromboxane (TXB_2) in plasma and serum, malondialdehyd, ADP induced aggregation and metabolism of AA. In two male patients (1 ET, 1 PV) we found an abnormal conversion of exogenous 14C-AA: after radio thin layer chromatography no TXB_2 was formed from exogenous 14C-AA. In parallel a shift to PGE_2 (cyclooxygenase) and HETE (lipoxygenase) or only to HETE (1 patient) occurred. After 8 weeks and 1 year of rIFN-alpha-2c therapy investigations in both patients revealed a normal pattern of AA-conversion with 44,7 and 42,4 % TXB_2. In vitro study using up to 500 I.E. rIFN-alpha-2c did not show any significant influence on AA conversion either on the 2 patients platelets or to healthy controls.

A pathological population of platelets in MPS with a different membrance composition therapeutically removed by rIFN-alpha-2c therapy may be responsible for our findings.

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ESSENTIAL THROMBOCYTHEMIA (ET): CORRELATION OF CLINICAL DATA WITH BONE MARROW HISTOLOGY IN 45 CASES

R. Hehlmann (1), M. Jahn (1), and R. Burkhardt

The clinical significance of ET lies in the tendency of the patients to bleed and in their predisposition to thromboembolic complications which are not or only rarely observed in patients with reactive thrombocytoses (RT). Since the clinical diagnosis of ET is primarily established by exclusion, the recognition of positive diagnostic criteria such as bone marrow histology or platelet aggregation test is of importance in cases in which the diagnoses of ET cannot be made readily. This is particularly relevant for the differentiation between ET and cases of persistent RT. Of 58 consecutive patients with ET followed from 1975 to 1987 45 were evaluated by one or several bone marrow histologies. Jamshidi biopsies were processed with the acrylate technique. The evaluation included megakaryocyte size and morphology, location of megakaryocytes in the marrow, grouping of the megakaryocytes in clusters, fiber content, and interstitial iron. In 37 cases (82%) the histologic diagnosis of megakaryocytic myelosis (mature subtype) was made, in 9 of these the diagnosis was megakaryocytoc granulocytic myelosis. In 4 cases each the histological diagnosis was polycythemia vera (PV) and myeloproliferative syndrom without specification. In the latter cases the correct diagnosis was made clinically by exclusion. The four cases histologically diagnosed as PV showed clinical features identical to the other cases with ET and different from those of 23 patients with clinically verified PV. In no case the presence of a myeloproliferative disorder was missed by histology in our series; in few other cases the histologic suspicion of megakaryocytic myelosis could not be verified by retrospection. It is concluded that the bone marrow histology is a valuable diagnostic help for the differentiation of ET from RT in unclear cases of thrombocytosis and also for the differentiation of the various myeloproliferative disorders.

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PRIMARY (ESSENTIAL) THROMBOCYTHEMIA - A CLINICO-PATHOLOGICAL STUDY OF 30 CASES

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A clinicopathological study was performed on 30 patients with primary (essential) thrombocythemia (PTH) showing a thrombocythemia in excess of 1000 x 10 9/l, a conspicuous megakaryocytic proliferation in the bone marrow without marked alterations of the megakaryocyte morphology or erythro- and granulocytopoiesis, no Philadelphia-Chromosome, no elevated red blood cell mass, no evidence for a reactive cause of the thrombocytosis. Comparison with the other entities of CMPD revealed a sustained elevation of the platelet count observable over a period of 1.5 - 8 years in individual patients with PTH; no leukocytosis or abnormalities of the differential blood count and a normal score of the leukocyte alkaline phosphatase. Numeric or structural chromosome abnormalites were only sporadic. In PTH survival time was significantly increased contrary to CGL with accompanying thrombocythemia. Episodes of hemorrhage and thrombosis as well as Raynaud's phenomenon and neurological symptoms were more frequently encountered in PTH (about 60%) than in the other CMPD.

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CYTAPHAIRESES (CA) AND PLASMADEPLETION (PD), A METHOD TO ACTIVATE AUTO-AGGRESSION (AAG) OR MYELOPROLIFERATION (MPS): SOME OBSERVATIONS TO CLINICAL USE

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CAs could rapidly reduce increased cell numbers from the peripheral blood and hence deminish the peril arising from a hyperviscosity syndrome. PDs on the other hand are increasingly performed in various diseases said to be of autoaggressive origin. Both methods are followed by a rebound phenomenon resulting in a marked increase of depleted cells in MPS or antibody production in AAG. This procedure seems to sensitize the disorders providing a better response to drugs. 75 patients (55 white males, 20 white females, mean age 48,5y., 26-65 y.) suffering from various subtypes of MPS (25/75 PCV, 18/75 CML, 15/75 megakaryocytic myelosis, 17/75 OMS) were treated with CAs and azathioprine ± allopurinol orally. CAs alone effected the a.m. rebound phenomenon for either platelets, white or red blood cells indicating that even in MPS stem cells are still subject to physiologic feed-back mechanisms. 10 pts. with idiopathic thrombocytopenia (ITP) were treated with intermittent fractionated PDs using a single-needle membrane-type plasmapheresis monitor. 500 ml PDs were performed daily, 5x the 1st week, 4x the 2nd, 3x the 3rd, 2x the 4th and 1x the 5th week without replacement of albumine. Antibody depletion of the 1st week was followed by a marked increase of platelets at onset of the 2nd week. Fewer PDs from 2nd week, however, could not inhibit recovery of antiplatelets antibodies resulting in a decrease of platelets, which could be inhibited by simultaneous administration of azathioprine and/or corticosteroids. Withdrawal of the products of myeloproliferation or autoaggression from the peripheral blood by CAs or PDs are apt to improve the response to immunsuppressives with cytotoxic effects.

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AUTOIMMUNE-HEMOLYTIC ANEMIA (AIHA) UNDER INTERFERON-ALPHA-2 (IFN-ALPHA-2) THERAPY IN A PATIENT WITH CHRONIC GRANULOCYTIC LEUKEMIA (CGL).

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The CGL is a clonal hemopathy characterized by a malignant transformation of an early hemopoietic precursor cell. IFN-alpha-2 has recently been used in the treatment of CGL. A sufficient cytoreduction could be achieved in patients during the chronic phase. We report of a 16 year old patient with a Ph^+ CGL diagnosed in May 1985. After several courses of busulfan, in January '86 a therapy with IFN-alpha-2 ($5x10^6$ I.U.) every second day was initiated. The treatment was well tolerated resulting in a satisfactory cytoreduction. In July '86 the patient complained of fatigue and breathlessness. The laboratory findings were: hemoglobin 5.4 g/dl, LDH 745 U/l, hyperbilirubinaemia with 13 g/dl. The Coombs test was positive with an autoantibody titer of 1:2000. We diagnosed an AIHA, a condition occuring in conjunction with malignancies of the lymphatic system. So far we know of no report about AIHA in a patient with CGL. The autoantibodies were directed against epitopes associated with antigens of the Rhesus family. There were no deposits of C3 and C4 on erythrocytes. The mechanism of the hemolysis is still controversial. It could be explained by the stimulatory effect of IFN-alpha-2 on mature B cells with an increased Ig production of an autoreactive clone. Erythrocytes coated with Ig bind to the surface of macrophages via their Fc-receptors. In vitro studies have demonstrated that IFN-alpha-2 is capable of inducing an increased expression of Fc-receptors on monocytes and macrophages, thereby inducing an augmented clearance in liver and spleen. After the termination of the IFN-alpha-2 therapy and under corticosteroid treatment within 2 weeks the hemoglobin was normal and an autoantibody titre of 1:20 was reached after 4 months. In conclusion, our data suggest that IFN-alpha-2 was causative in the development of an AIHA in a patient with CGL.

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RESULTS OF RECOMBINANT ALPHA 2-C INTERFERON THERAPY IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA (CML)

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Recombinant alpha 2-c Interferon (rec-IFN) seems to be effective in the treatment of various hematological malignancies.
In a clinical phase-II study 11 patients (4 female, 7 male, median age = 47.5, range 22-65 years) with chronic myelogenous leukemia (CML) in the chronic phase of disease were treated with rec-IFN. The initial dose was 21 Mill. Units IFN/week. After 8.5 weeks (median) rec-IFN was reduced to a maintenance therapy of 9 Mill. units/week. As criterion of treatment efficiacy decrease of leucocyte count, decrease of myeloid precursors, improvement of peripheral blood count, decrease of lactat dehydrogenase was used. Further parameters were ferritin and spleen size.
Up to now eight of eleven patients are evaluable. Two patients have less than 8 weeks rec-IFN therapy. One patient requests withdrawal from the study after 4 weeks because of intolerable side effects. Seven patients (87.5 %) achieved remission after a median time of 8.5 weeks of IFN-treatment. Progressive disease was noted in one case (12.5 %).
In conclusion, these data confirm the efficiacy of recombinant alpha 2-c Interferon in the therapy of patients with CML in the benign phase of disease.

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T-LYMPHOCYTIC BLAST CRISIS IN POLYCYTHEMIA VERA - A CASE REPORT

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Patients with polycythemia vera (p.v.) develop the picture of marrow fibrosis in about 15 %, transformation into acute leukemia occurs in another 15 %. We report on a 54-year old female with p.v. diagnosed in July 1986. After a short period of symptomatic treatment (phlebotomy) blood findings and bone marrow indicated a blast crisis in March 1987. The nature of the blast cells could be defined by immuncytological methods as T-ALL (LEU9>90, TdT pos. VILA1 neg., VIEG4 neg., myeloid marker profile neg.). Cytogenetic analysis showed a clonal abnormality (Trisomy8). After 3 courses of chemotherapy complete remission was achieved.
Our observations confirm that blast crisis in p.v. may arise without previous chemotherapy. The fact that the nature of blasts showed a T-cell phenotype indicates a clonal aberration at the level of pluripotent stem cells. After all a de novo development of acute leukemia as a second malignancy in p.v. cannot be excluded.

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PROGNOSTIC FEATURES IN CHRONIC MYELOID LEUKEMIA - THE IMPACT OF CLINICAL AS WELL AS HISTOMORPHOLOGICAL PARAMETERS

J. Thiele, R. Zankovich, C. Thienel and R. Fischer

On 115 patients (56 males, 59 females; median age 48 years) with chronic myeloid leukemia (CML) a clinico-pathological study was performed to reveal initial hematological, but particularly histomorphological features of predictive value for survival. All patients had a trephine biopsy of the bone marrow and entered this study without prior selection. Overall survival was 36 ± 27 months. In addition to multiple interactions between various disease features, multivariate regression analysis showed, that of the clinical parameters age, liver size and level of LDH were primarily and most closely associated with prognosis. Of several histomorphological variables (megakaryocytes > 60 per mm2 bone marrow area, fibrosis and presence of pseudo-GAUCHER cells), only pseudo-GAUCHER cells remained significant, i.e. excert an independent and favourable influence on survival.

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PROGNOSTIC RELEVANCE OF CYTOGENETIC ABERRATIONS IN ACCELERATED PHASE AND BLASTIC CRISIS OF CHRONIC MYELOCYTIC LEUKEMIA (CML).

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Between 1980 and 1986, 120 patients (pts) with CML were cytogenetically investigated. In 91/109 evaluable pts a typical Philadelphia (Ph) chromosome with 9/22 translocation was identified, 18 pts were Ph-negative. In 25 of the Ph-positive pts additional chromosome changes could be found. 4 of these 25 pts were investigated before bone marrow transplantation (BMT). This was also the case in 24 pts with Ph-chromosome only. These 28 pts with BMT were excluded from life table analysis. Of the remaining 63 pts, 42 had Ph-chromosome alone, 21 had additional changes such as isochromosome 17q, trisomy 8, "missing Y", double Ph-chromosome or hyperdiploidy. The life table analysis of these conventionally treated pts showed that pts with additional changes had a significantly shorter median survival time of 5 months as compared to the 40 months of the Ph-positive pts. The 18 Ph-negative CML pts had a median survival time of 15 months.
On the other hand, 25/109 evaluable pts had clinical criteria of blast crisis at the time of cytogenetic analysis : 2 were Ph-negative, 8 had Ph-chromosome only, and 15 pts had additional cytogenetic aberrations. The median survival time of these 25 blast crisis pts was 3,5 months which did not differ significantly from the 5 months of all pts with cytogenetically documented additional aberrations which can also occur during the chronic phase.
In conclusion we found that additional cytogenetic aberrations are an early sign of acceleration and may be correlated with poor prognosis and shorter survival time (with the exception of "missing Y"). They can be helpful criteria for the indication of treatment intensification such as BMT.

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THE SIGNIFICANCE OF PROMEGAKARYOBLASTS AND MICROMEGAKARYOCYTES FOR THE PROLIFERATION KINETICS IN CHRONIC MYELOID LEUKEMIA (CML)

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The bone marrow of 10 patients with CML at the age of 24 to 82 (average 50) years was examined. Megakaryocytes (mks) were identified by a monoclonal antibody (C 17, anti-glycoprotein IIIa) by immunofluorescent staining to include early precursors (promegakaryoblasts) not identifiable by morphological means only. For relocation of each individual cell for evaluation of cell morphology (Pappenheim stain) and DNA content by Feulgen cytophotometry, its position on the slides was automatically stored on tape by a computerassisted device connected to the microscope table.

Compared with normal persons the patients with CML showed the following changes of polyploidization pattern in the DNA-histograms: A regular shift to the left with a maximum at 8c (normal at 16c); high polyploid cells at 32c were largely impaired. Different numbers of diploid and tetraploid mks were found, most of them were mature cells and appeared as micromegakaryocytes, while others did not resemble a megakaryocytic feature. These cells are regarded as immature, differentiated mks. Their relative number in CML is impaired.

It is concluded, that the proportion of promegakaryoblasts in CML is not increased. The polyploidization capacity of mks is decreased as seen in the reduced number of high polyploid cells and the differentiation of mks with a low ploidy to mature cells, appearing as micromegakaryocytes. Some of them were even diploid while the majority was tetraploid and octoploid.

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GAUCHER-LIKE CELLS ARE CHARACTERISTIC ONLY FOR CML AND NOT FOR OTHER CMPD'S.

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Occurrence and prognostic significance of Gaucher-like cells - GLC - as well as of Seablue Histiocytes - SBH - were reported in CML formerly. A frequency of about 50 % before and about 70 % after busulfan application has been found in our resin-embedded bone marrow biopsies in some 500 CML patients provided a polarization microscopy was applied; SBH were seen in 20 % before and 16 % after therapy (DELVENTHAL et al., 1987). Comparison with 300 bone marrow biopsies of patients with other CMPD's has revealed no GLC, but SBH, using identical methods of embedding, staining and polarizing microscopy. - Since histologic distinction between CML and other CMPD is rather diffcult in some cases, the presence of GLC represents an interesting diagnostic indication.

CMPD	GLC	SBH
CML.CT	++++	++
CML.M	++++	++
P. VERA	0	+
PTH	0	0
CMGM	0	+

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SPONTANEOUS AND INDUCED SISTER CHROMATID EXCHANGE IN PH-POSITIVE CHRONIC MYELOCYTIC LEUKEMIA - A POSSIBLE APPROACH TO MONITOR CHEMOTHERAPY RESISTANCE

R. Becher

Sister chromatid exchange (SCE) occurrs spontaneously and can be visualized after incubation of cells for two cell cycles in BrdU containing medium. We evaluated the frequency of spontaneous SCE in normal bone marrow (38 cases) derived from donors for bone marrow transplantation and Ph-positive cells of untreated CML patients (40 cases) who were in the chronic phase of the disease. Significantly lower SCE was found in the Ph-positive leukemic cells. In addition, the SCE frequency was studied after in vitro exposure of normal and leukemic bone marrow to busulfan in a final concentration of 1.0µg, 3.0µg and 5.0µg/ml medium. At time of this report we have analysed 7 cases of normal marrow and Ph-positive cells of 8 patients with CML. Results show a significantly less increase of SCE in Ph-positive leukemic cells after exposure to equal busulfan doses.
Others have shown that SCE correlates closely with the degree of DNA damage caused by alkylating drugs and the inhibitory effect on cell proliferation. Therefore, due to the lower SCE in leukemic cells it can be concluded that busulfan effects the normal hematopoiesis more severely than Ph-positive CML. This finding might explain the failure of busulfan to eradicate the Ph chromosome or even to reduce it and provides an experimental basis for its purely cytoreductive effect in CML. It had been shown that residual normal hematopoiesis is always present in CML patients. According to our findings, the remaining population of normal hematopoietic stem cells in Ph-positive CML may be more severely damaged, if not eliminated by long term busulfan treatment.
Our data suggest the possible application of SCE studies for evaluation of the sensitivity of leukemic cells to DNA damaging antineoplastic drugs.

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CHROMOSOMAL ANOMALIES IN THE BLAST CRISIS OF PH-POSITIVE CHRONIC MYELOCYTIC LEUKEMIA

R. Becher

We report on cytogenetic analyses of 51 cases of Ph-positive chronic myelocytic leukemia CML in blast crisis using G-banding after short term trypsin treatment. Studies during the chronic phase of disease were also done in 32 of these cases and revealed a standard t(9;22). During blast crisis chromosomal anomalies in addition to the Ph chromosome were found in 40 cases (78 %). 11 cases had no evidence for clonal evolution. One of these cases presented with an atypical Ph translocation, t(12;22). Most cases observed were pseudodiploid or near diploid, only three within the triploid range.
The most frequent chromosomal rearrangement was an isochromosome of the long arm of chromosome 17, i(17q) which was observed in 15 cases representing 42% of the abnormal cases. It was observed as the sole anomaly in addition to the Ph chromosome in only 3 cases. More frequently it was associated with a trisomy 8 (9 cases). In other cases it was associated with trisomy 8 and a second Ph chromosome or found within a triploid karyotype.
Frequently observed numerical anomalies were a duplication of the Ph chromosome without further anomalies in 6 cases combined with trisomy 8 in one case. Clonal evolution by trisomy 8 only was found in two cases. Trisomy 8 was frequently also associated with trisomy 19. An additional chromosome 8 alone or together with other numerical or structural changes thus was the most frequent numerical change observed in 21 cases (52% of abnormal cases). 8 cases showed rare translocations involving chromosomes 2,6,11,14,17, 18 and 20. Our study confirms the nonrandom occurrence of clonal evolution in CML suggesting a percentage of cases with additional anomalies in CML blast crisis than reported in most other studies.

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PLATELET MORPHOMETRIC ANALYSIS BEFORE AND AFTER SPLENECTOMY

A. Wehmeier, R.E. Scharf, W. Schneider

We analysed platelet volume, platelet density, and platelet volumen from density subfractions in 4 patients with Hodgkin's disease (HD), 4 patients with myeloproliferative disorders (MPD) and 1 patient with idiopathic thrombocytopenic purpura (ITP) before and 7-14 days after splenectomy. The platelet volume distribution curve was determined of the total platelet population recovered from a continuous Percoll gradient, and after density equilibrium centrifugation of 21 subfractions covering the density range between 1.030-1.080 g/cm^3. Platelet density centrifugation was performed as described by Martin et al. (Brit J Hematol 1983,54,337). Apparent platelet volume was determined using the impedance method.
Mean platelet count was 291,000/µl before and 467,000/µl after splenectomy. Whereas mean modal platelet density was unchanged, mean platelet volume (MPV) rose from 6.74 to 7.58 fl after splenectomy. This is an increase of 16 % in MPD pts. and 13 % in HD pts. Before splenectomy, MPV showed a positive correlation with platelet density in all HD pts., the ITP pt. and 2/4 PMD pts. After splenectomy, platelet volume was independent from density subfractions in 7 pts. Thus, the ciruclating platelet population after splenectomy is characterized by large platelets with no apparent loss of granule constituents compared to pre-splenectomy levels both in HD and in MPD pts. The changes observed in the relation of platelet density to platelet volumen may result from a loss of splenic platelet pooling which consequently prevents the elimination of certain platelet subpopulations, or may result from an altered platelet production pattern in the bone marrow soon after splenctomy.

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EFFECTS OF VARIOUS DOSES OF SP 54 ON FIBRINOLYTIC ACTIVITY IN PATIENTS WITH THROMBOTIC DISEASES:

H.Losonczy, I.Nagy, M.Dávid

SP 54 was synthetized more than 30 years ago. But the proper oral dosage has not been established yet. Therefore we gave two doses, 150 and 500 mg to patients suffering from various arterial and venous thrombotic diseases, for investigating the dose-response effect. 16 patients received 150 mg, 14 patients 500 mg and 10 in each group both doses.
We examined the ELT, occlusion test, WBL, plasminogen level, alfa-2 antiplasmin, alfa-1 antitripsin, alfa-2 macroglobulin, ethanol gelation test, FDP, AT III and PTI, before and 1,2,4 and 24 hours after giving these doses.
We could observe significant changes in ELT,WBL and plasminogen level, but the increase of fibrinolytic activity was not dose--dependent in most cases. It indicates that an individual dosage is needed. Therefore we suggest an "SP 54 loading test" before prescribing the drug.

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FUNCTIONAL AND IMMUNOLOGICAL PROTEIN C EVALUATION IN DISSEMINATED TUMORS, LIVER CIRRHOSIS AND COLLAGENOSIS

K.H. Zurborn, W. Kirch and H.D. Bruhn

In a new practicable and precise evaluation method Protein C (PC) is activated by a snake venom activator. Then protein C activity (PC act) was measured by its property to prolong aPTT in a clotting assay (VK = 1,9 % and 4 % for intra- and interassay variance resp.) and compared to an immunological method (Pc ag; ELISA). 168 patients in 9 collectives and 40 normals were investigated. Decompensated liver cirrhosis led to pronounced significant decrease of PC act to 24 % (PC ag; 30 %) and Antithrombin III activity (AT III act) to 44 % (AT III ag: 46 %) with a quotient (act/ag) for PC and AT III of about 0.8. PC act was not significantly reduced to 66 % in compensated liver cirrhosis.
Liver metastases in disseminated tumors were associated with significant decreased activity of PC (48.5 %) and AT III (80 %) and of quotient (act/ag) for PC of 0.67. This was suggested to be caused by DIC as evidenced by elevated fibrinopeptide A levels (3.7 ng/ml) and accentuated by altered liver function. Decrease of PC and AT III was not significant in disseminated tumors without liver metastases, in localized tumors, in malignant lymphomas and plasmocytomas.
Patients with collagenosis showed normal or high PC levels.

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SUPPRESSION OF LUPUS-TYPE CIRCULATING ANTIBODIES WITH HIGH DOSE I.V. GAMMAGLOBULIN

L. Kanz, H. Engler and G.W. Löhr

Lupus-type circulating antibody is seen with a variety of disorders, and sometimes in patients without apparent underlying disease. Its presence may be associated with thrombotic events, pulmonary embolism or spontaneous abortion.
We report about a young woman with a spontaneously acquired anticoagulans, recurrent thrombosis and pulmonary embolism, which was successfully treated with high dose i.v. immunoglobulin. None of the known miscellaneous diseases that might be associated with the lupuslike anticoagulans could be diagnosed. The anticoagulans was assessed by prolonged APTT, which was correctable by high concentrations of rabbit brain phospholipid but not by normal plasma. In addition anti-phospholipid-antibody activities were demonstrated by positive anti-cardiolipin-antibodies, anti-platelet-antibodies and thrombocytopenia. Because infusion of high dose gammaglobulin is known to result in a decrease of antibody titers in some autoimmune diseases, possibly by idiotype/anti-idiotype interactions, we decided to treat the patient with 7S-immunoglobulin (Sandoglobin) intravenously for 5 days (0.4 g/kg/d). On day 2 platelet counts normalized and normalisation of the other parameters was observed within 1-3 weeks. Three months later there was again evidence of recurrent disease; as the patient refused rehospitalization for immunoglobulin therapy, she was set on azathioprine and corticosteroids.
Our observation suggests that patients with an anti-phospholipid-antibody syndrome might be successfully treated with high dose immunoglobulin. A role for this expensive - yet untoxic - treatment in the management of these patients remains to be defined, and its value has to be proven in controlled studies.

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POSSIBLE REGULATORY MECHANISMS OF MEGAKARYOPOIESIS IN CYCLIC THROMBOCYTOPENIA/-CYTOSIS (CTPTC)

F. Griesinger, A. Ganser, D. Hoelzer

The aim of the present investigation was to elucidate the mechanisms involved in the regulation of megakaryopoiesis in CTPTC. This rare platelet disorder was documented in a 50 year old male patient for 26 months with a cycle duration of 6 weeks and platelet counts between 1.500.000 and 10.000/µl. CTPTC was associated with an expansion of large granular lymphocytes type A, which exerted normal NK-activity. Using the clonal stem cell assay method, the incidence of CFU-Mk in bone marrow obtained at different time points and the effects of accessory cells and plasma on colony formation by CFU-Mk were investigated: 1) In unseparated bone marrow, the number of CFU-Mk varied considerably in time paralleling the shifts of platelet counts. 2) These differences were abolished after adherent cell depletion. 3) In adherent cell (obtained during the nadir of platelet count) conditioned medium a soluble heat instable factor was present stimulating CFU-Mk dose-dependently. 4) Strong Mk-colony stimulating activity was detected in plasma obtained during the nadir of platelet count. In conclusion, plasma factor(s) and accessory cells, by humoral factors and probably by direct cellular interaction may regulate megakaryopoiesis in CTPTC. However, the primary cause of the disease and the role of LGL's remains obscure.

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PLASMAPHERESIS AND IMMUNOSUPPRESSION IN IDIOPATHIC THROMBOCYTOPNIC PURPURA (ITP)

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Current concepts in therapy of autoimmune thrombocytopenia (ITP) include 1. immunosupp. (corticosteroids, azathioprine, etc.), 2. reduction of autoantibody production by splenectomy or, 3. blockage of autoantibody production by intravenous high dose immunoglobulin administration. Approx. 30% of all patients, however, do not respond to these approaches. Withdrawal of circulating antibodies from the bloodstream using plasmaphereses (PP), has been performed in various other disorders which as a single procedure cause a rebound phenomenon in the antibody production. In 10 patients suffering from ITP, previously treated with either corticosteroids and/or azathioprine (3 male whites, mean age 39,5, 17-74 y., 7 female whites, mean age 36,5 y., 20-53 y.), were treated with intermittent fractionated PP's using a single-needle membrane-type plasmapheresis monitor (Plasmapur(R), Organon Technica, Belgium), max. pore size, 0,65 µm, sieving coefficient 0,95 (IgG, IgM, IgA, alubmine, fibrinogen, F VIII, LDL, VLDL, HLDL). 500 ml daily PP has been performed 5x the 1st week, 4x the 2nd week, 3x the 3rd, 2x the 4th and 1x the 5th week without replacement of albumine. At the beginning of the 2nd week a marked increase of platelets could be observed which lasted for approx. 10 days and was followed by a slowly beginning decrease from 3rd to 5th week possibly due to the recovery of the depleted autoantibodies. Simultaneous application of immunosuppressives inhibited the marked early increase of platelets but yielded a longer lasting recovery of platelets counts.

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EFFECT OF RECOMBINANT HUMAN INTERFERON ALPHA ON PLATELET FUNCTION IN CHRONIC MYELOGENOUS LEUKEMIA*

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Recently it was demonstrated that recombinant interferon-alpha (rINF-alpha) is able to inhibit the in vitro growth of megakaryocytic progenitors (CFU-Mk) and in vitro differentiation of pluripotent progenitors (CFU-GEMM) along the megakaryocytic lineage (A. Ganser et al., Blut 53: 228, 1986). We examined the effect of rINF-alpha on platelet function in a 78-year-old patient with Ph'-positive chronic myelogenous leukemia. CML was diagnosed in 1981. In April 1986, the patient presented with a leukocyte count of $44.3 \times 10^3/\mu l$ (immature myeloid cells 24%) and a markedly elevated platelet count of $2.930 \times 10^3/\mu l$. The simplate bleeding time was significantly prolonged to 13 min (normal range 2.5 to 8 min). Platelet dysfunction was confirmed by additional testing: ADP (10^{-6} M), and ristocetin (0.9 mg/ml) failed to induce aggregation in platelet-rich plasma. Since treatment with melphalan was unsuccessful, rINF-alpha$_a$ (Roferon^R, Hoffmann-La Roche) was administered 5×10^6 IU/m^2/day s.c.). Within 21 days the leukocyte and platelet counts decreased to $14.0 \times 10^3/\mu l$ (immature myeloid cells 13%) and $900 \times 10^3/\mu l$. Reevaluation of platelet function in vitro revealed a normal aggregation in response to ADP, collagen, epinephrine, and ristocetin. In addition, bleeding time returned to normal (5 min). In conclusion, this observation illustrates that treatment with recombinant interferon-alpha was not only effective in suppressing the autonomous thrombocytopoiesis but also in restoring a normal platelet function. Our data suggest that rINF-alpha may be a promising agent to proliferative disorders.

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CHANGES OF BLOOD COAGULATION IN DIABETIC CHILDREN SUBSTITUTED WITH HUMAN INSULIN - ONSET AND REMISSION PERIOD

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Changes in the haemostaseological system in diabetics have mainly been described in adults and insulin dependent children substituted with porcine insulin. We observed 37 insulin dependent children, treated with human insulin only, with no clinical signs of diabetic vasculopathy. During onset of diabetes mellitus (group A n = 16), remission (group B n= 16), c - peptid > o.5ng/ml, and partial remission (group C n = 21), c peptid > 0.3ng/ml the following parameters were assayed: Factor VIIIC, von Willebrand factor (vWF), fibrinogen, plasminogen (PLASM), antithrombin III (AT III), protein C (PC), α2 macroglobulin (α2MG), α1 antitrypsin (α1AT), α1 antichymotrypsin (α1 ACHT), C 1 inactivator (C1), α2 antiplasmin (α2AP), platelet count (P), spontaneous platelet aggregation (SPA), PAT III (Breddin) and ß -thromboglobulin. Compared to an age matched control (n = 32) significant signs of hypercoagulability (FVIII C↑, vWF↑, SPA↑, P↑) was seen in the onset period as well as signs of counter-regulation (C1↑, AT III↑, α2 MG↓). During remission (investigation was performed 3, 6 and 12 months after diabetic onset) normalisation of the increased parameters could be seen with persistence of counter-regulation (PC↓, α2MG↓, α1ATR ↓, α2AP↓). In group C, partial remisson, an increase of FVIIIC, vWF, P and enhanced SPA was found as well as decreased levels of α2MG, α1ATR and α2AP. To the mean metabolic equilibrium (HBA1) significant correlations could be found for FVIIIC, PLASM and SPA. PLASM and SPA were significantly related to the duration of diabetes. We believe coagulation disorders in the early diabetic period are due to poor metabolic control whereas later measured haemostaseological changes could be signs of beginning diabetic angiopathy.

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IMPROVED RHEOLOGICAL PROPERTIES OF RED CELLS IN SICKLE CELL ANEMIA: EFFECT OF PENTOXIFYLLINE AND NIFEDIPINE

E. Friederichs*, M. Scharnetzky** and W. Tillmann**

The red cells from 12 patients suffering from sickle cell disease was investigated by cell filtration through polycarbonate filters and determination of hemoglobin solubility. Solubility was taken as hemoglobin concentration in the supernatant after sedimentation of the polymers by ultracentrifugation. Erythrocytes from the patients passed through the filter pores more slowly than cells from healthy controls. After treatment with pentoxifylline (8 weeks, 30-36 mg/kg), filtration rate was significantly improved. The extracorporal addition (1/2h, 37°C) of pentoxifylline (10 µg/ml) and nifedipine (1 µg/ml), respectively caused an increase in hemoglobin solubility of up to 30%, if the intracellular Ca^{++} concentration was lowered from 3.5 ± 0.5 µmol/l to nearly normal values (2.0 ± 0.2 µmol/l).
We present a model that accounts for the intracellular Ca^{++} concentration as a determining factor of the polymerisation of hemoglobin leading to an altered deformability of the red cells.

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ALTERATIONS OF THE HAEMOSTATIC SYSTEM IN CEREBRAL CIRCULATORY DISORDERS

U. Kloster, F. Nebel and H.D. Bruhn

50 patients with acute cerebral circulatory disorders were compared with 50 healthy subjects with regard to hypercoagulability, hypofibrinolysis and altered thrombocyte function in order to receive more precise information about thrombotic diathesis in patients with cerebral circulatory disorders. Thereby significantly shortened thromboplastin time, enhanced factor VIII activity and fibrinogen levels and a lowering of antithrombin III were established in these disorders. These parameters must be regarded as signs of hypercoagulability. Simultaneously elevated plasminogen values as well as elevated α_1-antitrypsin values, together with prolonged euglobulin lysis times document the existance of hypofibrinolysis. Furthermore, pathologically elevated thrombocyte aggregation was registered (according to Breddin, 1975). The examined patients with cerebral circulatory disorders suffered from a thrombotic diathesis whereby hypofibrinolysis and enhanced thrombocyte aggregation were measured in hypercoagulability.

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FIBRIN DEGRADATION PRODUCTS (FbDP's) AS A MEASURE OF INTERACTION OF TISSUE TYPE PLASMINOGEN ACTIVATOR (rt-PA) AND SYSTEMIC FIBRIN IN THE VASCULAR SYSTEM.

E.Seifried[1,2], D.C.Rijken[2], C.Kluft[2] and W.Nieuwenhuizen[2]

Increased levels of t-PA are measured in blood of subjects during physical training, stress, hypoxemia, after endothelium stimulation by DDAVP and in patients undergoing thrombolytic therapy with rt-PA. To elucidate the interaction of high t-PA levels and systemic fibrin pools in the vascular system recombinant t-PA was infused in healthy male volunteers. Over a period of 60 min, three groups (n=6 each) were given i.v. 0.25 mg rt-PA/kg (group I), 0.50 mg rt-PA/kg (group II) and a placebo infusion (group III), respectively. As a measure of lysed fibrin we analysed FbDP's in plasma taken on citrate/aprotinin using a new enzyme immunoassay (EIA), based on monoclonals and developed by us. Before infusion all volunteers had FbDP levels << 0.5 µg/ml. Upon infusion FbDP levels in group I and II increased to average values of 1 µg/ml and 0.8 µg/ml, respectively. FbDP levels in group III remained << 0.5 ug/ml. The results show that fibrin degradation products appear at high levels of rt-PA suggesting lysis of systemic fibrin. Formation of FbDP's in plasma was not dose-dependent. This suggests that a dose of 0.25 mg rt-PA/kg is sufficient for lysis of at least one easily accessible vascular fibrin pool in man.

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ALLOIMMUNIZATION TO HLA-ANTIGENS IN MULTITRANSFUSED PATIENTS

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Sera collected at various times from 19 multitransfused patients were tested by 1073 lymphocytotoxicity-crossmatches (LCT), using 27 different blood donors. Alloimmunization could be demonstrated in 17 patients (89%).- Additionally 200 of these sera were also tested by platelet immunofluorescence test (PIFT): 76 sera were found to be positive. In contrast, the LCT of these sera revealed only 43 positive results.- A positive correspondence between both methods was obtained in 67% (133 sera, $p<0,001$), whereas 50 sera were only positive in the PIFT and 17 only in the LCT.- Comparing the results of LCT and PIFT with the respective transfusion require, a positive correlation between crossmatch and transfusion frequency can be concluded if not-filtered red cell concentrates were used. Repeated transfusion of leucocyte-free-filtered red cells, however, did not cause any leucocyte antibody formation; in some cases, it was even accompanied by the disappearence of preformed antibodies.- Alloimmunization was found to be different in leucaemic and chronically anaemic patients: patients with leucaemia developed less antibodies than patients with anaemia (at similar transfusion frequency). Conclusions: The combination of LCT and PIFT seems to be a sufficient method for the detection of alloimmunization.- In order to inhibit antibody formation in multitransfused patients, the use of (leucocyte-free-)filtered red cells can be recommended, above all in patients with chronic anaemia.

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MODULATION OF HLA DR ANTIGEN EXPRESSION OF HUMAN MONOCYTES BY PROSTAGLANDIN E IN VITRO

J.S. Schwamborn, I. Bochynek, and P.G. Scheurlen

Human monocytes express HLA DR antigens and secrete Prostaglandins of the E series. We evaluated the effects of exogenous Prostaglandin E (PGE) and prostaglandin synthetase inhibitor indomethacin on HLA DR antigen expression of human peripheral blood monocytes. Monocytes were isolated by adherence to plastic surfaces. Purity as determined by α-Naphtyl acetate esterase and immunofluorescence with anti-My 4 was always greater than 90 %. Subsequently cells were incubated several days without or with PGE or indomethacin. HLA DR antigen expression was assessed by immunofluorescence with monoclonal antibody L 243 and FITC-conjugated goat anti mouse IgG. At the time of isolation 86 ± 5 % (± S.D.) of monocytes were HLA DR positive. After 24 h incubation we observed an increase in HLA DR expression with 91 ± 4 % positive monocytes. In contrast addition of PGE (10^{-5}- 10^{-12} M) at the time of the culture resulted in a dose dependent suppression of HLA DR expression with 47 ± 6 % positive (L 243) monocytes after 24 h in vitro (PGE 10^{-5} M). Incubation of monocytes up to 5 days led to a continuous loss of HLA DR antigen expression. Enclosure of indomethacin (10^{-3} - 10^{-8} M)inhibited the loss of HLA DR antigens during the time of culture in a dose dependent manner. We conclude (a) Prostaglandin E serves as a suppressor of HLA DR antigens on peripheral blood derived human monocytes, (b) the wellknown loss of HLA DR antigen expression of peripheral blood monocytes during in vitro culture is probably due to secretion of Prostaglandin E by these monocytes.

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DIFFERENTIATION INDUCTION BY CHEMOTHERAPEUTIC AGENTS IN HL-60 CELLS: DISSOCIATION OF MORPHOLOGICAL DIFFERENTIATION AND CHANGES IN SELFREPLICATIVE CAPACITY

P. Meyer, M. Jähnel

HL-60 cells can be differentiated into the granulocytic pathway by a variety of chemotherapeutic agents. Differentiation is assayed as nitrobluetetrazolium reduction, viability as trypanblue exclusion and selfreplicative capacity as colony formation in semisolid medium after incubation with the inducer in liquid culture. Cytosinarabinoside (ARA-C), 5-Fluorouracil (FU), Methotrexate (MTX), Hydroxyurea (HU), Doxorubincin (DXR), Daunorubicin (DNR) and Actinomycin-D (ACT) were used as inducers. With ARA-C, MTX, HU, DNR, and DXR either no differentiation could be induced or differentiation occured with clearly toxic concentrations of the inducer. Increasing concentrations of FU and ACT first only decreased self replicative capacity, with higher concentrations increasing NBT positive cells and later decreasing viability. This suggests that only with FU and ACT differentiation induction is achieved at non toxic concentrations and that selfreplicative capacity is the first and most sensitive indicator of differentiation induction with FU and ACT.

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DMSO INDUCED DIFFERENTIATION OF HL-60 CELLS: EVIDENCE FOR A MULTISTEP PROCESS

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The human promyelocytic cell line HL-60 is a convenient model to study differentiation induction. DMSO induces granulocytic differentiation in HL-60 cells as measured by nitro blue tetrazolium (NBT) reduction. The transferrin receptor is thought to be present on all proliferating hematopoetic cells. We have studied the DMSO induced differentiation of HL-60 cells. After one day exposure transferrin receptor expression is reduced from 83.7 % to 2.23 % as measured by immunefluorescence. NBT reduction and cloning capacity was not different from controls. With five days exposure 68 % of the cells were NBT positive and cloning capacity was reduced to 12 % of control, transferrin receptor is positive on 1.5 % of the cells. FCS starvation for two days decreased G_1-fraction to 40 % of control with no significant change in transferrin receptor expression suggestig that growth arrest and transferrin receptor expression are regulated in two different ways. Loss of transferrin receptor seems to be an early step in DMSO induced differentiation followed by decrease in cloning capacity and increase of NBT reduction.

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ENDOTOXIN STRONGLY REPRESSES SYNTHESIS OF ALPHA-2-MACROGLOBULIN AND STIMULATES THE SYNTHESIS OF ALPHA-1-PROTEINASE INHIBITOR IN MONOCYTES AND MONOCYTE-DERIVED MACROPHAGES

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dedicated to Professor Dr. G.W. Löhr on the onset of his 65. birthday

Physiological functions of proteolytic enzymes range from generalized protein digestion to specific regulation of processes such as the release of active peptides from precursor proteins. Proteolytic release of peptides, which in turn act proteolytically on other enzymes, represents the mechanism by which the cascades of blood coagulation and fibrinolysis as well as the kallikrein-kinin-system and complement are activated. Proteolysis therefore requires control which is provided by a number of proteinase inhibitors. Alpha-2-Macroglobulin (a2M) is a tetrameric protein and is regarded as the most potent proteinase inhibitor in man . Its broad specificity is directed against proteases of all four major classes and includes inhibition of kallikrein,plasmin and thrombin. This indicates its function helping to keep proteolytic activation of complement, kallikrein-kinine and the coagulation cascade under control.
Applying a culture system which enables human blood monocytes to differentiate into macrophages in-vitro, synthesis of a2M and a1PI was studied. The monocyte-macrophage maturation is accompanied by a strong increase of specific a2M synthesis and a concomitant decrease of a1PI. a2M therefore can be designated as a marker protein of the terminal monocyte/macrophage maturation. Submicrogramm quantities of endotoxin (S.typhi) strongly repress a2M synthesis in monocytes and macrophages. The induction of a2M synthesis during monocyte in-vitro maturation is abolished in the presence of LPS indicating that LPS may inhibit the maturation itself. Synthesis of a1PI is stimulated by LPS.

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EFFECTS OF BONE MARROW STORAGE AT 4° C UPON THE SUBSEQUENT GENERATION OF GM-CFU AND F-CFU PROGENITOR CELLS. A QUANTITATIVE AND QUALITATIVE ANALYSIS.

E.S. Gussetis, U. Ebener, J. Cinatl and B. Kornhuber

Autologous bone marrow transplantation is performed with marrow cryopreserved in liquid nitrogen. Freezing and thawing of marrow cells causes red blood cell lysis, cell clumping and a measurable loss of hemopoetic precursor cells such GM-CFU, Meg-CFU and BFU-E. We tested if storage of bone marrow at 4° C may obviate the need of cryopreservation.Whole bone marrow from 12 patients with solid tumours and leukemias in remission was diluted 1:2 with Iscove's medium and stored at 4° C.Aliquots were taken at different times, assayed for GM-CFU and F-CFU and compared to the number prior to storage. To grow GM-CFU colonies 10^5 fresh marrow cells were cultured in a mixture of 0.1 % fibrinogen in Iscove's medium containing 20 % FCS and 10 % plur-CSF (conditioned medium of the human bladder carcinoma cell line 5637). Colonies consisting of 40 cell or more were scored at day 10. GM-CFU recovery in unfractionated marrow was 90 % at day 3, 65 % at day 7 and 20 % at day 15.The recovery of F-CFU was similar (90 % at day 3 and 70% at day 7). The G-CFU/M-CFU ratio remained constant for 5 days. At day 15 the ratio shifted to the M-CFU(90 %). In 3 patients the storage for 15 days resulted in the recovery of Bas-CFU only. Our results suggest that marrow cells preserved at 4° C for up to 5 days may be a reasonable approach for autologous bone marrow transplantation.

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THE IMMUNOLOGICAL CHARACTERISATION OF HUMAN FETAL AND ADULT CFU-F DERIVED BONE MARROW FIBROBLASTS

P.Valent, T.Radaszkiewicz, K.Geissler, W.Hinterberger, K.Lechner and P.Bettelheim

Human fetal and adult bone marrow fibroblasts were obtained from CFU-F cultures, before and after a complete stroma layer was evident. In order to investigate the immunological marker profile of this cell population cells were examined both after trypsinisation and under short term replating conditions. Both fetal and adult bone marrow fibroblasts were found to express an identical phenotype. They are vimentin positive, which provides evidence for their mesenchymal nature. Furthermore, no reactivity with the pan leucocyte marker T200 (CD 45) could be observed. Nevertheless bone marrow fibroblasts are recognized by a number of moabs which are usually used as hemopoietic surface markers: KIM7, MY 7, anti-CALLA, anti-platelet IIb/IIIa and OKT 9. MoAbs directed against the endothelial cell associated F.VIII antigen do not bind to CFU-F derived fibroblasts. We conclude that bone marrow fibroblasts represent a distinct cell lineage which expresses a unique phenotype.

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ENRICHMENT OF CLONOGENIC BONE MARROW CELLS BY DUAL FLUORESCENCE-FACS-SORTING

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Clonogenic cells from normal human bone marrow were enriched by FACS-sorting according to light scatter and immunofluorescence criteria. Cells were labeled with anti-HPCA (Mylo) which was developed by fluorescein-isothiocyanate (FITC) conjugated goat-anti-mouse IgG and with My9 conjugated with phycoerythrin (PE). Blast cells were preselected by dual scatter gating. Sort windows were set around the antibody-positive populations. The following populations were tested on their capacity to form colonies: $Mylo^+$-, $My9^+$-, $Mylo^+My9^-$-, $Mylo^+My9^+$-, and $Mylo^-My9^+$-cells. The sorted cells were cultured together with growth factors which stimulate colony growth of the myeloic and granulocytic lineage (CFU-GM), the early erythroid lineage (BFU-E), and of the mixed type lineage (CFU-GEMM). Our data indicate that clonogenic $My9^+$ cells formed mainly colonies of the more mature (7 day culture) CFU-GM type and could be enriched upto 10-fold. Contrarily, clonogenic $Mylo^+$ cells formed colonies of all types and could be enriched upto 80-fold.

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COLONY FORMATION OF SOLID HUMAN TUMORS TAKEN DIRECTLY FROM PATIENTS AND AFTER GROWTH IN NUDE MICE

C.Scholz, H.H.Fiebig, B.Winterhalter, K.Meinhardt, J. Schildge, and G.W.Löhr

We review to date colony formation using solid human tumor material directly from the patient and after one serial passage in nude mice. Employing a slightly modified double-layer-soft-agar assay described by Hamburger and Salmon, approximately 300.000 cells were plated per dish. Only those plates containing a minimum of 50 colonies of 60 µ or 30 colonies of 80 µ were scored as growth.
Twenty-three various solid human tumors have been directly tested and 8/23 (35%) showed growth. The use of tumor material following one passage in nude mice presents the advantage of having ample tumor material and assay reproducibility. It has been shown that about 50% of human tumors can be successfully grown subcutaneously in athymic nude mice (medwelt 35:52-58 and 81-86, 1984). Of these rapidly growing tumors 45/63 (71%) effected a colony formation sufficient for drug testing. Bronchogenic carcinomas 20/23 (87%) and colon tumors 15/16 (94%) gave especially favorable results. A diverse third group of tumors including renal, gastric, testicle, mammary as well as sarcomas and pleuromesotheliomas showed a growth rate of 12/23 (52%). Comparison of chemosensitivity of direct testings and further passages has been studied only for a few tumors and has correlated well.
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INTERACTION OF RECOMBINANT GRANULOCYTE COLONY STIMULATING FACTOR (rhG-CSF) WITH HUMAN MEGAKARYOCYTIC PROGENITOR CELLS (CFU-M)

L.Kanz[a], E.Platzer[b] and G.W.Löhr[a]

Human rhG-CSF - formerly called pluripotent CSF - has been shown to stimulate colony formation by granolocyte-monocyte progenitor cells, as well as to enhance erythroid and mixed colony formation.
After stimulation of mononuclear bone marrow cells with rhG-CSF, we observed a pronounced increase in colony number and colony size of CFU-C, compared to stimulation with PHA-LCM (phytohemagglutinin-stimulated leukocyte conditioned medium) or a combination of G-CSF and PHA-LCM; only limited colony formation by megakaryocytic progenitor cells and by BFU-E and CFU-GEMM could be induced. Following depletion of T- and B-lymphocytes and monocytes by indirect immunopanning, G-CSF again enhanced the generation of large CFU-C colonies, whereas growth of CFU-M, BFU-E and CFU-GEMM was no longer observed. The addition of G-CSF to PHA-stimulated cultures, however, consistently resulted in a markedly increased size of megakaryocytic colonies, and - though less prominent - of BFU-E and CFU-GEMM colonies. The stimulatory effects of G-CSF were blocked by anti-G-CSF-antibodies.
We conclude that rhG-CSF - while being highly active on CFU-C - neither supports the formation of megakaryocytic colonies nor of erythroid and mixed colonies when added to cultures depleted of accessory cells. Stimulatory activities for CFU-M, BFU-E and CFU-GEMM, however, can be induced in accessory cells. The enhanced colony size particularly observed for megakaryocytic colonies after addition of G-CSF to cultures depleted of accessory cells in combination with stimulatory activities within PHA-LCM, suggests a role for G-CSF in early events of human megakaryocytopoiesis.

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GROWTH OF BFU-E FROM CANINE BONE MARROW AND PERIPHERAL BLOOD STIMULATED BY FETAL CALF SERUM AND/OR SERUM FROM TOTAL BODY IRRADIATED DOGS.

L. Kreja, K. Baltschukat and W. Nothdurft

A reproducible BFU-E culture system was established in order to increase the repertoire of stem cell assays in the dog as they are preformed in our group.

The BFU-E from canine bone marrow and peripheral blood could be grown in cultures containing methylcellulose in the presence of appropriate batches of FCS, transferrin and erythropoietin (Epo). However, best colony formation (size and number of bursts) was obtained after the addition of serum from total body irradiated dogs (TBI-S) to the culture medium. The BFU-E concentration in the bone marrow from the humerus, the iliac crest and the sternum in several normal beagles ranged from 34 to 116/10^5 bone marrow cells after stimulation with FCS and TBI-S and from 27 to 89/10^5 cells, if stimulated with FCS alone. In the presence of TBI-S good BFU-E growth was also obtained in FCS-free medium, containing bovine serum albumin and cholesterol as serum substitutes. The TBI-S reduced the Epo requirement of BFU-E markedly. At high concentrations of TBI-S burst formation occured even in the absence of added Epo.

The TBI-S used by us as a source of colony stimulating activity (CSA) in the canine GM-CFC assay seems to be a multifactoral mixture containing CSA, BPA-burst promoting activity (Interleukin-3) and erythropoietin.

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HEMOPOIETIC CELL REGENERATION AFTER THIAMPHENICOL

H.Goris*,M.Loeffler*,B.Bungart*,S.Schmitz*,W.Nijhof+

Previous reports (1) suggested that the antibiotic Thiamphenical (TAP) has drastic effects on murine erythropoiesis affecting the dividing cell stages and the marrow to spleen ratio. Stem cells seemed relatively refractory to TAP. These features make TAP an interesting drug to investigate the regulatory hemopoietic controls. C57bl mice received TAP continuously for 3 days by subcutaneously implanted dialysis bags (d-3 to d0). After treatment (d0) we found the following values in the marrow and spleen respectively (in % of control): CFU-S 60% both, BFU-E 160% and 130%, CFU-E both below 5%, erythroblasts 10% and 30%, CFU-GM 220% both, myeloblasts 80% and 30% all parameters showing a parallel behaviour of spleen and marrow. After removal of the bags stem and progenitor cells started a recovery with a drastic increase in the spleen peaking at day 4 (CFU-S and BFU-E about 30-fold, CFU-GM and CFU-E about 100-fold increase). Control spleens contained about half of a femur's progenitor cell counts. In contrast, marrow progenitor counts dropped to a nadir at day 4 with CFU-S being 20%, BFU-E and CFU-GM 50% and CFU-E 30% of control. Thereafter all cell stages recovered with a slight overshoot at day 9-11. Recovery of erythroblasts (splenic peak value 30-fold, marrow nadir 10 %) followed CFU-E patterns generating a small peak of blood reticulocytes at day 6 (180%) which compensated a mild anemia. Myeloblasts in contrast showed a peaking behaviour in the spleen (2-6 fold) and in the marrow (2 fold). We conclude that most rapidly dividing hemopoietic cells are susceptible to TAP,but CFU-S,BFU-E and CFU-GM are less affected.It is unclear on the basis of these data to which extent local microenvironmental regulation or an exchange of cells between the marrow and spleen contribute to the observed marrow to spleen antagonism.(Supported by the DFG Lo 342/1-1, FRG); (1) Nijhof et al Exp. Hematol 10(1982)36; *:Med Uni-Klinik,LFI-EDV, D 5 Koeln 41, FRG; +:Lab Physiol Chem, Bloemsingel 10, 9712 KZ Groningen,Netherlands

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DNA METHYLATION - A PATHWAY OF GENETIC REGULATION OF HUMAN LEUKEMIC CELL DIFFERENTIATION?

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We studied genetic regulation of in vitro differentiation of human leukemic cells by using the promyelocytic cell line HL-60. These cells contain an amplificated c-myc oncogene which is probably responsable for their transformed status. With different compounds HL-60 cells can be induced to differentiate into mature granulocytes or macrophages with concomitant decrease of c-myc expression.
It has been suggested that cytosine methylation is a possible regulatory pathway of gene transcription. Here we present evidence that inhibition of methyltransferase in HL-60 cells does not prevent differentiation induced by dimethylsulfoxide. Restriction enzyme analysis of the c-myc gene indicates that the gene including the 5'-promoter region is highly methylated in both differentiated and undifferentiated HL-60 cells. The methylation pattern of the gene is not altered after dimethylsulfoxide-induced differentiation of the cells. In addition, there is only one un-methylated CCGG-site which is located in the second exon of the c-myc gene.

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THE 5q- ANOMALY IN 23 PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND ACUTE MYELOID LEUKEMIAS*

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From January 1981 to December 1986 we have found a deletion of the long arm of chromosome 5 (5q-) in 23 consecutive patients (15 female and 8 male; median age, 65 years) referred for cytogenetic investigations with the following blood disorders: refractory anemia (3), sideroblastic refractory anemia (4), refractory anemia with excess of blasts (7), and acute myeloid leukemia (9). The common region deleted in all patients was between bands q21 and q33 with 3 variable proximal breakpoints: q12, q14 and q21. The 5q- anomaly was the only chromosomal change in 8 patients, whereas 15 patients had additional numerical and/or structural aberrations. The clinical and morphological characteristics of the patients with a 5q- chromosome as the sole anomaly will be presented and compared with those patients who had additional aberrations.

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INDUCTION OF CHROMOSOMAL REARRANGEMENTS IN LYMPHOBLASTOID CELL LINES FROM PATIENTS (PTS) WITH HODGKIN'S DISEASE BY IN VITRO TREATMENT WITH CYTOSTATIC DRUGS

H.H. Kirchner, C. Fonatsch[1], J. Rademacher[1], B. Brüggenjürgen

Secondary neoplasias especially non-lymphocytic leukemia are more often found in pts with Hodgkin's disease (HD) after chemotherapy than in pts with comparable intensive therapies because of non-Hodkgin's lymphoma or solid tumors. The following experiments were performed to prove the hypothesis that there may be a predisposition for chromosomal changes due to environmental noxae in HD-pts. Epstein-Barr virus transformed B-lymphoblastoid cell lines (LCL) from pts with HD before treatment as well as from healthy controls were used either for long term or for short term experiments. In long term investigations the cell lines were treated 5 times for 2 h each over a period of 3-6 mos. For short term experiments the cell culture was exposed only once for the last 24 h before chromosome preparations were performed. In both series a panel of cytostatics used in standard chemotherapy of HD were tested. After long term in vitro treatment with activated cyclophosphamide in a LCL from a HD-pt., a clonal aberration, namely a translocation between the long arm of chromosome 1 and the short arm of chromosome 17 was found. After treatment with bleomycin the same LCL showed other clonal rearrangements: a translocation t(1;11)(q21;q23/25) and a translocation t(3;5)(q27/29;q31). Because the same chromosomal regions are often involved in marker formation of secondary malignancies in HD, a possible predisposition for chromosomal breakage in HD pts will be discussed.

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DELETION 11q- AND ISOCHROMOSOME 17q IN A PATIENT WITH OSTEOMYELOFIBROSIS

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Chronic myeloproliferative disorders which are not Ph-positive represent a heterogenous group. A terminal deletion of chromosome 11 (del 11q14) was observed in patients who presented with an acquired idiopathic sideroblastic anemia (AISA). These patients showed no further clonal evolution nor a leukemic transformation.

We report on a 37 year old patient who developed leukocytosis in 1978 and was diagnosed as osteomyelofibrosis (OMF) with a stable clinical course for 5 years. The first chromosome analysis showed a normal male karyotype (46XY). In 1983 the disease accelerated leading to a marked leukocytosis and hepatosplenomegaly. At that time cytogenetic analysis revealed an abnormal clone with a terminal deletion of the long arm of chromosome 11 (q14) in about 50% of metaphases. Two years later a clonal evolution was observed characterized by an additional isochromosome 17q, [i(17q)] with increasing relative frequency in subsequent analyses and atypical eosinophils and basophils appeared in the bone marrow. In August 1986, 8 years after diagnosis the disease transformed into a terminal acute non lymphocytic leukemia (ANLL). At that time the proportion of metaphases with an additional i(17q) to the del 11q14 had increased to 90%.

Clonal evolution with appearence of an i(17q) is frequently observed in the terminal phase of Ph positive chronic myeloid leukemia (CML). In order to exclude a cytogenetically not detectable "Ph-positive" CML southern blot analysis was performed which showed no bcr rearrangement.

In conclusion, this case illustrates that a deletion 11q14 cannot only be seen in AISA but also in myeloproliferative disorders. Furthermore, our observation gives evidence that similar to Ph-positive CML, clonal evolution with an i(17q) may also be associated with transformation to a terminal acute phase in Ph-negative myeloproliferative disorders.

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CORRELATION OF VIRUS-INDUCED BREAKS AND INTEGRATION SITES WITH FRAGILE SITES, CANCER BREAKPOINTS AND ONCOGENE LOCATIONS ON HUMAN CHROMOSOMES

O.A. Haas

The strong connection between the chromosomal locations of 89 accepted fragile sites, 83 cancer breakpoints and 32 oncogenes prompted me to investigate, whether virus-induced chromosome breaks and viral integration sites also cluster within these highly interesting regions. Extensive literature search revealed the locations of 73 viral sites on human chromosomes; 63 of which have been exactly mapped. They consist of 39 breaks (Br) and 24 integrations sites (Int) of the following viruses: Herpes simplex I & II (26 Br), Epstein-Barr (1 Br/16 Int), Hepatitis B (4 Br/19 Int), Human Papilloma (7 Int), Adeno (5 Br/1 Int), Rous sarcoma (1 Br) and SV40 (1 Int).

				STRINGENT		NONSTRINGENT	
CORRELATION WITH		TOT	EXP	OBS	p	OBS	p
89 FRAGILE SITES	INT	24	5.2	10	n.s.	20	<.001
	BR	39	8.3	23	<.001	31	<.001
	INT+BR	63	12.9	33	<.001	51	<.001
83 CANCER BREAKPOINTS	INT	24	4.9	8	n.s.	17	<.005
	BR	39	7.8	21	<.001	24	<.001
	INT+BR	63	12.2	29	<.001	41	<.001
32 ONCOGENES	INT	24	2.0	3	n.s.	17	<.001
	BR	39	3.2	9	<.005	24	<.001
	INT+BR	63	5.0	10	<.005	41	<.001

Both virus-induced breaks and viral integration sites are significantly correlated with fragile sites, cancer breakpoints and oncogene locations.

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CHROMOSOMAL INTEGRATION OF HUMAN PAPILLOMAVIRUS (HPV 18) IN THE HELA CELL LINE

P.F.Ambros [1] and H.I.Karlic [2]

Considering the importance of specific viral integration sites in cancerogenesis, we designed a study for detecting the chromosomal location of DNA sequences homologous to HPV 16 and 18. A nonisotopic high resolution in situ hybridization technique and subsequent staining of the hybridized chromosomes with a modified Chromomycin A3/Distamycin/DAPI banding enabled an exact assignment of the HPV 18 in situ hybridization signal to band 8q24. This site is closely associated with the c-myc-oncogene. Recent data of two cell lines derived from cervical carcinomas, show a mean distance between the virus sequences and c-myc of less than 50 kb (Dürst et al., 1987, PNAS 84, 1070).

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EXPERIENCES WITH THE COMBINED TREATMENT OF PRIMARILY EXTRANODAL LOCALIZED MALIGNANT LYMPHOMAS.

J.Fleischer, M.Herrmann, M.Plat, A.Lesche, H.Wolf, U.Reinhardt

Patients with primarily extranodal localized malignant lymphomas were selected out of 201 patients with Hodgkin's disease and 80 patients with combined treated Non-Hodgkin-Lymphomas (except CLL) of the last 10 years of the area Dresden and were assessed. On the other hand 34 patients with primarily gastrointestinal Non-Hodgkin-Lymphomas (1961 up to 1984) were examined. Gastric, small intestinal and colonic involvement in the relation 4:2:1; one lymphome of low malignant degree on six lymphomas of high malignancy. 14 patients belonged to stage I E, 10 to stage II E, 10 to the stages III E and IV. 8 patients could not be operated. The microscopically in sano performed operation gave the best therapeutic results. The irradiation always has to include tumor and lymph-node area. An irradiation of regional lymph-nodes alone is justifiable with radical operative removal of tumor only. The submitted study showed no better results with chemotherapy being performed additionally to irradiation.
After 30 months the survival rates werde 70 % in stage I E, 75 % in stage II 1 E, 60 % in stage II 2 E, 66 % in stage III E and 33 % in stage IV.

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CHEMILUMINESCENCE INVESTIGATIONS FOR THE ASSESSMENT OF THE REGENERATION CAPACITY OF THE BONE MARROW

U.Reinhardt, J.Fleischer

The native bone marrow shows a spontaneous chemiluminescence whi ch develops in connection with proliferation processes of the cell systems probably. A normality standard range can be settled. Chemiluminescence investigations in 11 healthy persons and 47 patients with aplastic anemia, iron deficiency anemia, acute bacterial infection, acute and chronic myeloid leucemia proved a close correlation between the spontaneous chemiluminescence and the regeneration capacity. Further the behaviour of the chemiluminescence was tested in 5 patients with metastasizing breast cancer during a CMF-therapy and in 25 pretreated patients with breast cancer before the chemotherapy, with the same result. 8 of the 25 patients had a very low spontaneous chemiluminescence; bacterial infections and premature interruptions of the therapy arised in this group nearly exclusively.

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ERYTHROCYTE FERRITIN IN PATIENTS WITH NORMAL AND ABNORMAL IRON METABOLISM

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Serum ferritin (SF) and erythrocyte ferritin (EF) were measured by an immunoradiometric assay. Venous blood samples were taken from patients with normal iron metabolism, with slight iron deficiency, with idiopathic haemochromatosis (IH) before therapy, with IH and slight iron deficiency anaemia due to venesection therapy, further with IH 6 months after therapy and with iron overload (i.o.) due to sideroblastic anaemia.

Results:	n	SF(ug/ml)	EF(ug/cell)	SF/EF
Normal subjects	10	84,3	21,2	4,0
Iron deficiency	7	13,0	18,3	0,7
IH before therapy	7	1766,0	269,0	6,6
IH after therapy	7	16,7	25,2	0,6
6 months after therapy	4	45,0	235,0	0,19
Sideroblastic anaemia	2	450,0	309,0	1,5

There were no differences between EF in normal subjects and with slight iron deficiency. The mean values of SF and EF are significantly increased in IH. On the contrary, patients in this group with a moderate i.o. and normal SF had an increased EF. After all, EF seems to be a better indicator for i.o. than SF, especially in cases of early IH.

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