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Abstracts

Abstracts of the papers accepted for oral and poster presentation

1 Human α- and β-T-Cell Receptor Gene Rearrangements of Bone-Marrow-Derived T-Cells Propagated with Interleukin 2 in Patients with T-Cell Malignancies in Clinical Remission

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Clinical diagnosis and monitoring of B-cell lymphoproliferative disorders have been aided considerably by the availability of clonal markers such as immunoglobulin gene rearrangements. Such tools habe now become available for the analysis of T-cell malignancies by examining T-cell receptor gene rearrangements. We examined human α- and β-T-cell receptor gene rearrangements in bone-marrow-derived T-cells of patients with T-cell malignancies in clinical remission. Bone-marrow-derived T-cells were cultured in the presence of PHA and highly purified or recombinant interleukin 2. The expanded T-cell population was analyzed for T-cell surface antigens using T3, T11, T4 and T8 antibodies. The cells propagated in this system were T-cells with a purity greater than 99.5%. Restriction endonuclease digestion of DNA from propagated T-cell suspensions was performed, and the fragments were examined by Southern blot analysis. Hybridization probes directed at the constant region were prepared from the Hpa II fragment of PY 14 cDNA and Bgl II fragment of JUR-B_2 cDNA for α and β, respectively. We observed limited patterns of restriction length polymorphism for α-gene rearrangements. However, in some patients gene rearrangements were observed with the β-cDNA probe. This observation suggests that residual disease in patients with T-cell malignancies in clinical remission can be detected by expanding bone-marrow-derived T-cell populations and examining them for T-cell receptor α- and β-gene rearrangements.

2 Monoclonal Rearrangement of T-Cell B-Chain Genes in Acute Undifferentiated Leukemia

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In the course of our study of T-cell receptor (TCR) β-chain gene rearrangements in blast populations of various leukemias, we also analyzed five cases of acute undifferentiated leukemias

(AUL, according to the FAB classification). In Southern blot hybridizations, using the C-region of the TCR β-chain gene as a probe, surprisingly four of the five AUL specimens displayed rearrangements in either part of the constant region gene segment (Cn 1 and Cn 2) on one or both chromosomes. Surface-marker phenotyping of a single AUL showed a distinct reactivity pattern similar to a mature T-cell phenotype. These results led us to investigate whether those leukemic isolates could be propagated in vitro using either PHA or IL 2/IL 3, together with irradiated allogeneic stimulator cells as induction signals for T-cell responses. Two cases in which the entire population underwent proliferation and differentiation in vitro were again subjected to Southern blot hybridization with the β-chain gene probe. In the first case, a growth response could be elicited with PHA alone and was associated with the expression of T-cell-specific antigens. Southern hybridization revealed a polyclonal rearrangement pattern in contrast to the germ-line signals seen in the unstimulated leukemia. In the second instance, the proliferative response did not result in the expression of T-cell-specific antigens, but in the acquisition of monocytic markers and functional characteristics. These latter cells, however, did not retain the monoclonally rearranged band of the unstimulated counterpart seen in Southern blots prior to in vitro differentiation but instead showed a clear germ-line β-chain configuration. Together, these results may suggest the inherent capacity of immature bone marrow cells to activate gene loci that were thought to be reorganized only during T-cell maturation and differentiation in the thymus. These results allow a novel subclassifation of acute undifferentiated leukemia with possible clinical implications.

3 RNA Modification in Human Lymphomas and Leukemias

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Alterations in nucleoside modification particularly concern transfer RNA molecules, and are involved in the regulation of gene expression at the levels of translation and transcription. Transfer RNAs specific for aspartate, asparagine, histidine and tyrosine are characterized by a specific modification of the base in the wobble position of the anticodon. The replacement of guanosine (G) by the 7-deazaguanosine derivative queuosine (Q) in this position was found to be associated with cellular maturation, and was impaired in malignant cells [1]. The number of tRNA molecules having G instead of Q, i.e. (Q^-)tRNAs, can be determined by replacing the unmodified guanosine residue by H^3 guanine, a reaction catalyzed by a specific tRNA transglycosylase from Escherichia coli. After guanylation, tRNAs were separated by polyacrylamide gel electrophoresis in 4 M urea, and (Q^-)tRNA species were specifically detected by fluorography. Using this technique, we found that the amount of (Q^-)tRNA is correlated to the grade of malignancy in human lymphomas [2]. In leukemic cells a wide variation was found in the number of different (Q^-)tRNA molecules identified, ranging from only one band in some leukemias to as many as eight bands in other leukemias. These findings emphasize the complex role of these regulator molecules in the control of cellular transformation and differentiation in human hemopoietic malignancies.

References
1. Kersten W. (1983) Recent Results Cancer Res 84: 255–263
2. Emmerich B., et al. (1985) Cancer Res 45: 4308–4314

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4 Evaluation of Oncogene-Specific mRNA Expression in Hemopoietic Cells by in situ Hybridization

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Northern or dot blotting of total cellular mRNA and solution hybridization using cloned gene probes allow detailed studies of the processes of gene activation and transcription in hemopoietic cells. This technique of population analysis may not be suitable for detecting mRNA in small samples, because sufficient quantities of mRNA cannot be obtained. Such examples include the analysis of small patient probes, of isolated subpopulations of hemopoietic cells or the analysis of cells from in vitro colonies. Moreover, blotting techniques are limited if the mRNA to be hybridized is expressed only in a small subpopulation (e.g., megakaryocytes in bone marrow); extraction of nucleic acids from the whole cell population would miss those particular cells because the specific mRNA would be diluted by the RNA of the other cells that do not express the sequences of interest. In these situations, in situ hybridization allows the localization of specific mRNA sequences at the cellular level. The cells were fixed with ethanol/acetic acid and treated with HCl and proteinase K. In situ hybridization was performed with a DNA probe, labeled by nick-translation to $2-3\times10^8$ dpm/μg with ^{35}S-dGTP. The hybridization (48 h) was followed by autoradiography. The method is explained in detail, presenting examples such as hybridization of c-myc-specific mRNA in phytohemagglutinin-stimulated T_4-lymphocytes.

5 B-Cell Growth Factor Stimulates the Proliferation of Calla-Positive B-Cell Precursor ALL

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Low-molecular-weight B-cell growth factor (BCGF) is a T-cell-derived lymphokine that stimulates the proliferation of preactivated, surface Ig-positive, normal B-cells in vitro. BCGF has also been shown to induce proliferation in neoplastic cells from patients with hairy-cell leukemia and non-Hodgkin's lymphomas. We have analyzed the influence of BCGF on the growth of B-cell precursor acute lymphocytic leukemia (ALL). Bone marrow aspirates from 21 patients with newly diagnosed leukemia ($n = 18$) or in relapse ($n = 3$) were incubated with BCGF for 4 days. Fourteen of 21 aspirates showed a significant increase in ^{3}H-TdR incorporation compared to controls without BCGF. We selected three responders to show that the responses observed were due to proliferation of the leukemic cells and not to contaminating normal B-cells. The CALLA-positive cells from the aspirates of these patients were isolated by cell sorting and incubated with BCGF for 1–6 days. In all three patients a significant increase in the incorporation of ^{3}H-TdR was seen after 3 days, with increases of 3- to 30-fold over the controls. The ^{3}H-TdR incorporation gradually increased until day 6 in two of the patients (nos 1 and 3). The cell number doubled from day 2 to day 6 in two of the patients (nos 1 and 2). In the third patient the cell number remained stable, whereas the cells in the controls died. The CALLA-positive cells exhibited a dose-dependent response to increasing concentrations of BCGF, and additional experiments showed that the BCGF response was not dependent on the presence of fetal bovine serum in the cultures. These data are the first observations showing the influence of a defined growth factor on the growth of B-cell precursor ALL and provide an initial step towards understanding the variables influencing proliferation in this subgroup of acute leukemias.

6 Recombinant GM-CSF Induces Secretion of Autoinhibitory Factors by Leukemic Human Monoblast Line U937

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There is increasing evidence to suggest that colony-stimulating factors (CSF) may stimulate the growth of clonogenic cells from some leukemic myelomonoblast populations in an autocrine pathway (J. Griffin and F. Herrmann, Blood, in press). However, we now demonstrate that recombinant GM-CSF may also display inhibitory effects on differentiation, proliferation and clonal growth of leukemic monoblasts, as represented by the cell line U937, by inducing them to release inhibitory molecules. Exposure of U937 to rGM-CSF (5% vol/vol final) resulted in the release of soluble factors that inhibited DNA synthesis (^{3}H-thymidine incorporation), clonal growth in semisolid media and g-interferon-induced differentiation of U937 cells into more mature monocytic cells. Physical characterization of these factors leads to the identification of a dialyzable and a non-dialyzable factor that acts synergistically in mediating inhibitory properties. The presence of indomethacin 10^{-6} M) during conditioning of medium by CSF-induced U937 cells abrogated the dialyzable inhibitory component. Radioimmunologic assays defined this factor as prostaglandin E2. The non-dialyzable component was different from known cytostatic monokines such as interleukin-1 (IL 1) and tumor necrosis factor-a (TNF-a) in lacking specific biological activities on IL 1 sensitive C3H-HeJ thymocytes and TNF-a sensitive L929 cells, but could be defined as a macromolecular product by gel filtration. These data suggest that GM-CSF may be critically involved in the regulation of leukemic myeloid growth.

7 Molecular Cloning and Restriction Enzyme MAP of a 2.4 kb DNA Hybridizing to the Constant Region of the β-Chain of the T-Cell Receptor from a Patient with Chronic Lymphocytic Leukemia of T-Cell Phenotype

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Antigen recognition by immunocompetent T-cells has recently been demonstrated to be mediated by a heterodimer T-cell receptor (TcR) composed of α- and β-chains. Previous analysis of cDNA from the genes encoding for the α- and β-chains has demonstrated that the TcR chains are transcribed from DNA sequences resulting from the somatic rearrangement of germ-line variable (V), diversity (D), junctional (J), and constant (C) DNA segments. We examined patients with T-cell malignancies by studying patterns of the T-cell receptor α- and β-gene rearrangements in bone marrow and peripheral blood cells, including a patient with chronic lymphocytic leukemia of T-cell phenotype. Southern blot analysis of restriction endonuclease-digested DNA, obtained from peripheral blood cells, revealed a 2.4-Kb-long band by ECO RI digestion, hybridizing to the Bgl fragment of JUR-B2 cDNA (β-constant region). The 2.4-Kb DNA was cloned in the ECO RI site of pBR 322. Colonies that contained DNA clones to the 2.4 Kb fragment were selected by filter hybridization to the nick-translated JUR-B2 probe. The clones were mapped with XbaI, PvUII, Bgl II and Eco RI, using standard techniques. The restriction map of the cloned 2.4 Kb DNA suggests a repetitive constant segment.

8 C-fos and c-fms Expression in Normal Human Blood Cells

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Based on in vivo analyses of permanent cell lines it could be shown that the cellular oncogenes, c-fos and c-fms, are involved in the maturation and differentiation of hematopoietic cells.

Separated, normal, human blood-cell populations and peritoneal and alveolar macrophages were probed for c-fos and c-fms expression by Northern blot analysis. Whereas c-fos was selectively expressed in granulocytes, c-fms transcription was restricted to unstimulated monocytes and monocytes activated in vitro by inflammatory stimuli. These results mean that it may be possible to apply these oncogenes as differentiation markers to myelomonocytic leukemias. Furthermorer, it is hoped that further analyses will show to what extent these oncogenes might be involved in neoplastic transformation.

9 Allelic Polymorphism of Ha-ras Oncogene in Chronic Leukemia

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White-blood-cell DNA from healthy donors shows a polymorphism in restriction fragments of the Ha-ras oncogene locus. Common and rare alleles are found when DNA is cleaved with certain restriction enzymes. Because genetic alterations and polymorphisms seem to be important in the development of hemoblastosis, we investigated the allelic polymorphism of the Ha-ras oncogene in chronic leukemia. Fifteen patients with chronic lymphatic (CLL) and six patients with chronic myelogenous (CML) leukemia were studied. Simultaneously, we determined the Ha-ras alleles in 26 healthy blood donors. In four of the CLL patients Ha-ras alleles were found that could not be detected in the healthy controls. In four of the CML patients a combination of alleles was found that could be detected only once in the controls and once in the CLL samples. In none of the samples tested could an amplification of the Ha-ras gene be observed. Our data indicate a possible involvement of rare alleles of the Ha-ras oncogene in CLL and CML. The different pattern of Ha-ras alleles in CLL and CML emphasizes the influence of gene polymorphism on hematological malignancies.

10 Quantitation of the mRNA Coding for the Heavy Chain of the Immunoglobulin M and the T-Cell Receptor in Acute Unclassified Leukemias with Fluorochrome-Labeled Gene Probes by in situ Hybridization

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Fifteen leukemias that were not classifiable by conventional T- or B-cell markers and did not express the cALL antigen were investigated by in situ hybridization for expression of the μ-gene and the T-cell receptor gene. For this purpose gene probes were labeled with fluorochrome and hybridized to the mRNA in cytocentrifuged cell preparations. The leukemias were not homogeneous as far as expression of the μ-gene was concerned. There were populations that had very high values of expression, some with intermediate expression, and some in which we could not demonstrate any μ-mRNA. We could find no correlation with early B- and T-cell markers (HD 37, 39, and 3 A 1 and WT 1). Surprisingly, a leukemia with blasts of early, incomplete T-cell phenotype (3 A 1 and HLA DR-positive and HD 37, 39, BA-1 and B 1-negative My 7, 9-negative) even showed the highest expression of μ-mRNA. The expression of the T-cell receptor gene was determined in four leukemias in relation to the strong expression of this gene in the T-cell line Jurkat. One leukemia, which expressed the μ-gene, even showed positive values for the mRNA of the T-cell receptor as high as the Jurkat cells. Three other leukemias that were negative for the μ-mRNA were also negative for the T-cell receptor mRNA. Investigations of the rearrangement of the two genes in these leukemias are in progress.

11 Interleukin-2 Studies in Experimental Murine T-Cell Leukemogenesis

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T-cell leukemias were induced by MNU (methylnitrosourea) and typed using various markers. They were heterogeneous with respect to the expression of the IL-2 receptor tested with the monoclonal AB AMT 13. During the latency period a transient increase in the number of AMT-13-positive thymocytes was observed, as during regeneration after treatment with hydrocortisone or radiation. IL-2-receptor-positive and negative leukemias did not differ in their in vitro growth, with or without the addition of IL-2-containing supernatants. The establishment of cell lines from leukemic cell suspensions was very rare and independent of the addition of IL-2. The production of IL-2 was studied in MNU-induced leukemias after stimulation by ConA and PMA. EL-4 cells, IL-2 receptor negative, were used as positive controls, in addition to a newly established cell line derived from an MNU-induced leukemia.

12 Immunological Characterization and Growth-Factor Requirements of Clonogenic Blasts in Acute Myeloblastic Leukemia

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Despite their primitive morphological appearance, the majority of leukemic blasts in acute myeloblastic leukemia (AML) have a limited capacity to differentiate. They are end-stage, non-proliferating cells, and only a small fraction (0.05% – 1%) is capable of a sufficient number of divisions to form a clonal-derived colony in semisolid media. These clonogenic cells (L-CFC) are thought to function as stem cells in vivo to maintain the leukemic clone. To characterize these clonogenic blasts further, we have defined their surface-antigen phenotype, using a panel of monoclonal antibodies recognizing normal hematopoietic progenitor cells and complement-mediated cytotoxicity, and have compared the phenotype to that of the total leukemic population and to the phenotype of normal myeloid progenitors. The results demonstrate that L-CFC in AML are distinct subpopulations of leukemic cells that can be distinguished from the majority of more mature leukemic cells by their surface-antigen phenotype. Distinct stages of L-CFC were defined by their progressive acquisition of surface antigens, in a sequence similar to that detected on normal counterpart progenitors, suggesting diversity of L-CFC in individual leukemias. Like normal progenitor cells, colony growth of L-CFC requires the addition of growth-supporting media, conditioned by activated T-cells or tumor lines such as GCT, MO, 5637. We compared the effects of these media to the effects of gene-cloned GM-CSF-α on L-CFC formation, coming to the conclusion that GM-CSF-α is the major growth factor for in vitro growth of L-CFC.

13 Selection and Characterization of Leukemic Progenitors in "Pluripoetin"-Stimulated Long-Term Cultures

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Due to a lack of suitable stimulators myeloid leukemic cells have been difficult to grow in long-term cultures. We used supernatant from the 5637 cell line which stimulates normal mixed myeloid/-erythroid progenitors in vitro to induce the growth of cells from newly diagnosed acute

myoblastic leukemia (AML) patients in long-term suspension cultures. In 2 of 4 cases cell numbers rose after 6–8 weeks of culture. Morphological analysis and subcloning in agar showed that the cells grew colonies of myeloblasts in a dose-dependent manner. The cells were positive for myeloid antigens (APAAP method): M 522, 1120, My 9, My 10 monoclonal antibodies with intensive staining of early markers. Cytogenetically, most clones were normal. Three of 20 metaphases, however, showed hyperdiploidy with 47 chromosomes and a trisomy 12 (G-banding and methotrexate synchronization). Cocultures with normal bone marrow cells showed strong inhibition by one of the cell lines but no inhibition by the other. These data provide further evidence about the heterogeneity of AML clones and show that these can be enriched by appropriate stimulatory conditions.

14 Phenotypical and Functional Maturation Patterns of Myeloid Leukemia Cells via Human Interleukin 3 (IL 3) Analogue(s) In Vitro

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Phenotypically and cytochemically homogeneous blast cells from myeloid leukemias (M 1, M 2, M 3, M 4) were cultured in partially purified (ppIL 2) and highly purified interleukin 2 (LTHP) for 4, 8, and 12 days in vitro. LTHP cultures led to rapid death of blast cells. In contrast, ppIL 2 induced maturation of most acute myeloid leukemias (ML) following either a monocytic or granulocytic differentiation pattern within this culture period. Surface-marker expression of granulocytic and monocytic clusters of differentiation (CDw 11, 14, 15, 16, 17, 18) and HLA-class II antigen and IL-2 receptor expression were compared with regard to their capacity to present antigen, to stimulate mixed lymphocyte cultures (MLC) and to phagocytose after activation with zymosan and PMA. Ontogenetically early AML (M 1) gained monocytic (CDw 14) more frequently than granulocytic antigens, increased their HLA-class II antigen densitiy, became positive for non-specific esterase, and developed a good ability to stimulate in MLC and to phagocytose. A few AML cases apparently lacked the capacity to respond to the human IL 3 analogue(s), which present in ppIL 2 but not in LTHP, and these leukemic blasts did not further respond to the culture applied in vitro. Comparison of surface-marker expression patterns and function offer the possibility of studying the functional relevance of CD clusters in normal maturation schemes of granulocytes and monocytes and working out selective defects in leukemic blast cells. The results are of possible value for a more selected staging of myeloid leukemia, as well as therapeutic protocols via their differential susceptibility to hematopoetic growth factors.

15 Self-Renewal of CFU-L as Prognostic Factor in Patients with AML

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Unlike acute lymphocytic leukemia (ALL), prognostic factors predicting remission induction and duration in patients with acute myoblastic leukemia (AML) have yet to be identified. Culture techniques for the growth of clonogenic leukemia cells (CFU-L) have recently been described. In the present study we examine the relationship between the CFU-L characteristics and the response to remission induction therapy (TAD 9) in 23 consecutive patients with de novo AML. In 19 (83%) of 23 patients blast-cell colonies could be grown from T-depleted blood cells. Plating efficiency varied between 1.5×10^{-4} and 4×10^{-3} and was significantly correlated with peripheral blast cell concentration ($r = 0.635$; $p < 0.01$). Ara-C suicide levels of CFU-L were not different in patients with sensitive ($71 \pm 21\%$) and resistant ($65 \pm 10\%$) disease. In contrast, self renewal of CFU-L was found to be significantly correlated with therapy outcome ($p < 0.001$). Six of nine patients with low renewal capacity ($5 \pm 3/2\times10^4$) achieved complete remission, while all patients with high self-renewal ($103 \pm 31/2\times10^4$ were resistant to chemotherapy. The results

presented here indicate that studies of CFU-L self-renewal are prognostic significance and may be used to identify AML patients who have not been sufficiently treated with the TAD 9 protocol.

16 Acute Undifferentiated Leukemia: Implications for Cellular Origin and Clonality Suggested by Analysis of Cell-Surface Markers and Immunoglobulin Gene Rearrangement

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We investigated the DNA configuration of immunoglobulin (Ig) genes to reveal the clonality, cellular lineage and stage of differentiation of acute undifferentiated leukemias (AUL). This approach is based on the following properties of Ig recombinations: (a) they represent an early event in B-cell differentiation and thus provide a marker for B-cell commitment, (b) a particular Ig gene rearrangement is specific for a given B-cell clone, and (c) Ig gene rearrangement follows a hierarchical pattern during B-cell development (heavy-chain gene recombination precedes κ- and λ- light-chain rearrangement) allowing a classification of B-cell neoplasms according to their stage of differentiation. Our results suggest that in patients with morphological, cytochemical and phenotypically defined AUL, (1) most blast cells can be allocated to the early B-cell lineage and (2) in some cases more than one abnormal cell clone exists. In addition, our analyses of clonal development in phenotypic conversion of AUL support the view that (1) AUL cells originate from a pluripotent or bipotential stem cell with myeloid differentiation capabilities and (2) confirm the value of Ig gene analysis as marker for cellular clonality.

17 Significance of Cytochemical Markers for the Identification of T-Lineage Acute Lymphoblastic Leukemia

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The discriminative power of cytochemical markers, regarded to be of value in defining T-lineage acute leukemia, was tested in 334 patients with cytochemically defined acute lymphoblastic leukemia or acute undifferentiated leukemia in the German multicenter trial [1]. Immunologically proven acute leukemia of the T-lymphoblastic type was present in 95 patients (28.4%) while 239 patients revealed other immunological markers (C/pre-B, Null, B). Cytochemically T-lymphoblasts were characterized by the positivity of acid phosphatase (AcP) in 61/95 patients (64.2%), dipeptidylaminopeptidase IV (DAP IV) in 12/68 patients (17.7%), and acid naphthyl acetate esterase (ANAE) in 15/92 patients (16.3%). Cases of non-T immunological type revealed the following cytochemical reactivity: AcP in 14.4%, ANAE in 8.3%, and DAP IV in 0.6% (1 case out of 167; this case was of C/pre-B-type). The difference between T- and non-T type was highly significant for AcP ($p < 0.0005$) and DAP IV ($p < 0.0005$) while that of ANAE was less significant ($p < 0.05$). In conclusion DAP IV showed high specificity for acute lymphoblastic leukemia of T-lineage but was of a lower sensitivity. In contrast, AcP had considerable sensitivity in blasts of T-cell origin but a lower specificity than DAP IV. The value of ANAE as a marker of immature leukemic T-cells is only marginal.

Reference
1. Hoelzer et al. (1984) Blood 64: 38

18 A Comparative Study on the Clinical Value of Subtyping AML Using Immunological and FAB Criteria

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Thirty of 110 cases of acute myelogenous leukemia (AML) diagnosed in 1984–1986 at the St. Georg General Hospital in Hamburg were studied. In addition to morphological and cytochemical findings according to FAB classification, immunological phenotyping of blasts was performed, using a standardized panel of monoclonal antibodies (Mcabs: My 7, Vim 2, VimD 5, 3 C 4, 3 A 1, KiM 1, KiM 2, KiM 5, KiM 6, KiM 7, KiM 8). Characterization of the Mcabs and the immunological subgroups have been presented in an earlier paper by Dr. Bödewadt (Kiel). The results of laboratory investigations and the clinical findings were correlated with FAB subgroups (M 1–M 6) and immunological phenotypes (groups I–IV). [The laboratory and clinical parameters were: myeloperoxidase, esterase (ANAE) activity, including NaF suppression, number of blasts and monocytes in the peripheral blood, cellular volume of blasts, enlargement of lymph nodes and spleen, and response to chemotherapy (TAD-9 regimen)]. Comparison of cytochemical and immunological characterization showed a discrepancy in approximately 50% of cases. Concordance was found in 7 of 8 cases with FAB-M 4, 3 of 4 cases with FAB-M 5, but only 2 of 14 cases with FAB-M 1 + M 2. No significant differences were apparent in the distribution of laboratory values, clinical symptoms and response to chemotherapy. Immunological phenotyping and morphological classification seem to represent two different, independent systems. Further studies on survival time under standardized conditions are necessary to evaluate the prognostic value of immunological phenotyping.

19 Immunological Analysis of Acute Non-lymphoblastic Leukemias

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Monoclonal antibodies specific for myelomonocytic, monocytic cells, or myeloid cells were applied to acute nonlymphoblastic leukemias using immunocytochemical methods. Based on the reactivity patterns, six groups of acute nonlymphoblastic leukemias could be distinguished mirroring different maturation stages of the bimodal differentiation pathway of myelomonocytic cells. Such a continuous differentiation pathway could not be defined on the basis of enzymecytochemical criteria. In addition, enzymecytochemical classification according to FAB criteria did not correspond with the immunological phenotypes of the analyzed nonlymphoblastic leukemias.

20 Immunologic Phenotype of Leukemic Cells

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In addition to the morphological and cytochemical features recognizable in leukemic cells, the application of monoclonal antibodies has provided important insights into the hemopoetic cell differentiation and the cellular origin of leukemias. Peripheral blood from 58 patients with clinical features of leukemia was analyzed by a three-stage diagnostic approach, i.e., flow cytochemistry (H 6000 analyzer), morphology/cytochemistry and immunologic phenotyping. The

lineage specificity of leukemic cells was distinguishable by the H-6000 printout into two main categories, e.g., lymphatic in 42 patients and myelomonocytic in 16 patients. Combined morphological/cytochemical and immunologic cell typing yielded a further differentiation of the leukemic cell phenotype. In 31 patients a B-cell neoplasia (CLL), in 4 patients a pre-B-cell neoplasia (ALL) and in 7 patients a T-cell neoplasia (CLL) were found. By combination of cell-surface markers, enzyme markers and morphology, leukemic blasts were related to myeloid cell series in 15 patients (AML) and to monocytic cell series in one patient (M 5). The clinical usefulness of the three techniques to differentiate leukemic cells is discussed.

21 Immunoenzymatic Characterization of Leukemic Blasts in Routine Bone Marrow Smears – a Comparison with Conventional Immunofluorescence Techniques

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Bone-marrow smears from 25 children with acute leukemia [including cases of acute myoblastic leukemia (AML), acute lymphocytic leukemia (ALL), acute undifferentiated leukemia (AUL) and transforming MDS] were examined for immunological cell-surface markers on leukemic blasts, using a panel of monoclonal antibodies and an immunoalkaline-phosphatase technique. Immunological findings showed no significant deviation from the immunological characterization obtained by routine immunofluorescence on bone marrow aspirates. Hence, the immunocytochemical investigation of bone-marrow smears is a suitable alternative technique for immunological diagnosis in acute leukemias, with special qualitative and practical advantages for multicenter therapy trials. In addition to bone marrow analyses, immunological diagnosis of leukemic CNS involvement or CNS relapse using cerebrospinal fluid samples can be improved by using an immunoenzymatic technique.

22 Karyotype Abnormalities in Childhood Acute Leukemias: A Comparative Analysis by DNA Flow Cytometry and Cytogenetics

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Concomitant measurements of the cellular DNA content by flow cytometry and cytogenetics were carried out in 163 children with acute leukemias to assess the comparability of both techniques for the detection of karyotype abnormalities. DNA aneuploidies were identified in 58 of the 163 cases (36%). Cytogenetic evaluations were successful in 150 patients while no evaluable metaphases could be obtained in 13 children. Seven of these patients revealed DNA aneuploidies. Of 95 patients with normal or pseudodiploid karyotypes, 19 expressed aneuploid DNA stem lines. No DNA aneuploidy was found in 23 of 30 patients with a loss or a gain of 1–3 chromosomes. Of 25 patients with chromosome abnormalities of more than three chromosomes, however, all revealed DNA aneuploidies. These data indicate that additional information can be obtained by the application of cytogenetic evaluation and flow cytometry DNA measurements and that both techniques should be considered complementary methods.

23 Blood-Group H-antigen as a Marker for Myeloid Blast Crises and Secondary Leukemias

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In previous studies it has been shown that the MOAb CLB-ery 3 directed against blood group H-antigen reacts with erythropoietic and thrombopoietic cells. Granulocytic cells and monocytes at any stage of maturation lack this structure. In the present study blast populations of 389 leukemic patients were tested for reactivity with MOAb CLB-ery 3. In contrast to de novo acute leukemia in which CLB-ery 3-positive blasts are rarely found (AML FAB 1–5 12/157, O-ALL 0/2, C-ALL 3/112, B-ALL 5/9, T-ALL 0/12), CLB-ery 3 often reacts with blast cells in secondary leukemias (22/36) and in myeloid blast crises of chronic myeloid leukemia (CML-BC-"M" 30/32, CML-BC-"L" 0/8). Strikingly, we also observed that all blasts which express glycophorin A and/or platelet complex II b/III a usually display this structure. Our findings may indicate that CLB-ery 3-positive cells in secondary leukemia and CML-BC-"M" represent particularly immature hemopoietic precursor cells.

24 Significant Association of Acute Lymphoblastic Leukemia (ALL) with the HLA-C Locus Antigen Cw 7*

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Susceptibility to leukemia induced by Grossvirus in mice has been shown by Lilly and Pincus in 1964 to be linked to H-2^k antigens of the murine major histocompatibility complex (MHC). Since then several studies have suggested different associations of acute leukemias with HLA-A, B or HLA-DR antigens of the MHC in man. Only recently has a significant increase of HLA-Cw 3 in North American and European patients with acute lymphoblastic leukemia (ALL), compared with normal control groups been described. In this study, frequencies of all defined HLA-A, B, C and HLA-DR antigens including the newly characterized HLA specificities of the 8^{th} and 9^{th} International Histocompatibility Workshop, were evaluated in 112 patients who presented with ALL at the University Clinics in Tübingen and Essen between October 1981 and March 1986, and were compared with a random group of 125 healthy individuals. In all patients diagnosis was established by standard bone marrow cytology and cytochemistry. Immunological subclassification revealed 69 patients suffering from c-ALL, 19 from T-ALL, 10 from B-ALL and 2 from Null-ALL; 12 patients were not further analyzed. HLA typing was performed on peripheral blood lymphocytes in the standard microcytotoxicity assay with (locally and internationally) well-characterized HLA-alloantisera defining all known HLA-class I and II specificities. HLA genotypes of patients and their family members were established. Antigen frequencies were statistically evaluated by χ^2 analysis with Bonferroni correction for multiple testing. Comparison of HLA-A, B, C and DR antigen distribution revealed a significantly raised frequency of HLA-cw 7 in the patients (50%) compared with the controls (32%), with a p value of < 0.05 corrected for eight tests. The increase of HLA-Cw 7 was no higher by selective analysis of c-, B-, T- and Null-ALL patients. For all other HLA-A, B, C and -DR specificities, particularly for HLA-Cw 3 and Cw 7-linked HLA-A or -B alleles, similar distributions were observed in ALL patients and controls. No significantly increased frequency of a Cw 7-associated haplotype could be detected. For Cw 7-positive individuals a relative risk of developing ALL was calculated to be 2.125. The difference from the linkage of ALL to HLA-Cw 3 described earlier could be due to previous difficulties in typing HLA-C alleles, including Cw 7. Our results

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further support that in the proposition ALL an HLA-C locus antigen, in particular HLA-Cw 7, could be a marker of a susceptibility gene or be involved in immunoresponsiveness to a putative causative viral infection.

25 Trisomy 12 in Acute Non-Lymphocytic Leukemia

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There are now an increasing number of reports of trisomy 12 in chronic lymphocytic leukemia and Waldenström's macroglobulinemia, and prognostic relevance of this cytogenetic marker has even been demonstrated (N Engl J Med 310: 288, 1984). We report on a 68-year-old patient who presented with anemia, thrombocytopenia and leukopenia. There was no lymphadenopathy or splenomegaly. The bone marrow was packed with blast cells and a diagnosis of acute leukemia was made. Since cytogenetic analysis revealed a trisomy of chromosome 12, an intense cell type analysis was undertaken, including enzyme studies, electron microscopy, monoclonal antibodies and Ig gene analysis. Taken together, all analyses indicated nonlymphocytic differentiation of the leukemia cells, acute myelogenous leukemia with trisomy 12. This patient did not respond to TAD chemotherapy and died 4 weeks after diagnosis. This case illustrates that unique chromosomal markers such as trisomy 12 in CLL per se are not specific for cell lineage or leukemia.

26 Cryptic Antigens of Erythrocytes are Exposed in Carcinoma Cell Lines*

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Human erythrocytes carry cryptic surface antigens that can be exposed upon neuraminidase treatment (Thomsen-Friedenreich antigens). The same determinants have also been found in an exposed form on tumor cells. Moreover, it has recently been suggested that these antigens play an important role in organotropic metastasis. We investigated four anti-T reagents with regard to their specificity and their capacity to bind to the surface of carcinoma cell lines. Two of them – peanut agglutinin and a monoclonal anti-T antibody – recognized epitopes on the cell membrane. PNA showed high specificity for gal-β-1-3-galNAc. The monoclonal antibody, on the other hand, was found to be specific for phenyl-β-gal. The epitope recognized by PNA was frequently distributed in the different carcinoma cell lines and could always be increased by treatment with neuraminidase. Binding sites for the monoclonal antibody were found in significant only on amounts two of ten cell lines. The number of binding sites was unchanged after treatment with neuraminidase. Thus, two independent cryptic erythrocyte antigens may be exposed on the surface of carcinoma cells. Isolation and characterization of these molecules are in progress.

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27 Ki-B3, a Monoclonal Antibody Recognizing Normal and Malignant B-Cells in Formalin-Fixed and Routinely Prepared Biopsy Material

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Production and specificity of the monoclonal antibody Ki-B3, which recognizes a formalin-resistant antigen on human B-cells is described. Investigations of the reactivity to Ki-B3 of B-cells and their precursors reveals a positive reaction at all stages of B-cell ontogeny, including

plasma cells. In specimens from peripheral blood ($n = 5$), tonsils ($n = 15$), thymus ($n = 3$) and bone marrow smears ($n = 5$), cross-reactivity with T-cells or other haemopoetic cells was assessed. Of all of these, only single monocytes and macrophages in lymphatic tissues and bone marrow were stained by Ki-B3. In a comprehensive panel of samples from all human organs and mesenchymal tissues, as well as their malignant variants, reactivity was found to be completely absent. Ki-B3 was positive in 80% of various low-grade ($n = 90$) and 83% of high-grade ($n = 87$) malignant B-cell lymphomas. Forty cases of peripheral T-cell lymphomas were consistently negative for Ki-B3, while 27% of T-lymphoblastic lymphomas/leukemias and one-third of acute myeloid leukemias were positive. The value of this MOAB for examination of routinely formalin-fixed and paraffin-embedded material for diagnosis in malignant lymphomas is discussed.

28 Cell-Cycle Dependent Distribution of the Proliferation-Associated Ki-67-Antigen in Human Embryonic Lung Cells

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The monoclonal antibody Ki-67 has turned out to be a suitable prognostic marker of proliferating cells, not only in malignant lymphomas but also in solid tumors. The antigen and its localization in the cell-cycle are still unknown. Fetal epithelial lung cells (L-132) showed five different patterns of antigen distribution after morphologic evaluation using indirect immunoperoxidase staining. In three of them, the antigen was found only in the nucleus. In the large majority of cells the antigen appeared as a ring around the nucleolus. In some of the nuclei, Ki-67 was diffusely dispersed and attached to the nucleoli. Cells containing only a finely granular Ki-67 karyoplasm were rare. In mitotic cells the antigen was seen on the chromosomes and in the cytoplasm. The antigen was later found to be localized in vacuoles adjacent to the nucleus. The chronological sequence within the cell-cycle was examined in synchronized L-132 cells. Since we assume a strict correlation of the Ki-67-antigegn with cell-cycle phase and an association of the antigen with the nucleic RNA, the results may be of importance to the morphological assessment, of changes in cell-cycle and cycle disturbances in different types of malignancies.

29 Clinical Value of Intermediate Filaments and Tumor-Associated Antigens for the Cytological Diagnosis of Solid Tumors

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In order to establish an antibody panel for the identification of solid tumors, cytology specimens from 100 patients were analyzed with monoclonal antibodies directed against intermediate filaments and tumor-associated antigens (APAAP-technique). Specimens were obtained from 48 fine-needle aspirates of primary tumors or metastases and 52 smears of serous effusions from patients with proven malignancy and a control group of patients with nonmalignant effusions. Except for one case of small cell carcinoma of the lung, major tissue types could be reliably identified with antibodies directed against the five classes of intermediate filaments. Antibodies directed against cytokeratin subclasses discriminated between tumors originating from simple versus stratified epithelia. Otherwise, there was a heterogeneous expression of cytokeratin subtypes without a strict correlation to the cytokeratin pattern known for the respective normal tissues. Tumor-associated antigens such as prostatic acid phosphatase, or monoclonal antibody-defined antigens such as Ca 19-9, SCCL and M 224 provided important additional information regarding the site of the putative primary tumor. Immunocytological analysis of specimens from serous effusions was most valuable for the detection of minimal disease. An antibody panel di-

rected against CEA, Ca 1/2, HMFG 2 and – as a control – Ca 12-5 identified even single malignant cells without misleading nonspecific reactions in patients with nonmalignant effusions.

30 Aspiration Cytology of Lymph Nodes in Inflammatory and Malignant Disorders The Value of Immunocytochemistry for Diagnosis

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Enlarged lymph nodes can be investigated by puncture and aspiration cytology within a few hours without causing great inconvenience to the patient. An assessment of the tissue-structure is, however, impossible. This disadvantage may be partially overcome by the introduction of immunocytochemical methods. Aspirated cells were washed and cytocentrifuged. An indirect immunoperoxidase method was applied using monoclonal antibodies against the antigens OKT 3 (CD 3), BA 1 (CD 24), J 5 (CD 10), Leu 9 (CD 7), OKT 9 (receptor for transferrin), λ- and κ-chains, and common leukocyte antigen T 200. Sufficient material was obtained in 90 of 96 lymph node punctures. In 17 cases the diagnosis of lymph node enlargement from inflammatory (infectious or immunologic) causes was made. In these 17 patients the cytological diagnosis was confirmed by immunocytochemistry and by the further course of the disease. In 73 cases lymph node enlargement was caused by malignant disorders: in 25 cases the unequivocal diagnosis of metastasis from solid tumors was made. Confirmation by immunocytochemical methods was necessary in only 4 of those 25 patients. In 48 enlarged lymph nodes the diagnosis of malignant lymphoma was made. Eight of those patients suffered from Hodgkin's disease; in 6 of those 8 patients diagnosis was made possible by demonstration of Hodgkin and Reed-Sternberg cells. In the remaining 40 patients, malignant non-Hodgkin lymphoma was recognized, which of 29 were of low and 11 of high malignancy. Immunocytochemistry was especially necessary in lymphoma of low malignancy to prove monoclonality of the cells in question. In 8 of 11 lymphomas of high malignancy the diagnosis could be established on morphological grounds alone; in 3 cases it was made possible by the application of immunocytochemistry.

31 Value of Fine-Needle Aspiration Cytology in the Primary Diagnostic Evaluation of Haematological and Oncological Patients

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Since 1981 we have investigated 2,737 peripheral, abdominal, and thoracic tumours using fine-needle aspiration cytology (FNAC) at Ulm University Hospital. We have recently completed a retrospective study in which we obtained the "final objective diagnosis" based on histology or other definitive diagnostic criteria from an analysis of patient records. This study now comprises 2,047 definitive diagnoses. In 960 cases FNAC was the initial diagnostic procedure in the evaluation of a process of unknown etiology. We report on these 960 cases. The cytological diagnosis was based on microscopical evaluation of the FNA smears and basic clinical data without consideration of laboratory data. The following objective diagnoses were obtained: lymphatic hyperplasia 20%, low-grade non-Hodgkin lymphoma (NHL) 21%, high-grade NHL 11%, Hodgkin's disease 8%, carcinomas and sarcomas 16%, extramedullary manifestation of myeloid leukaemias 0.7%, diverse benign lesions 18%. In 5% of cases no cytological diagnosis could be established because of the inadequate quality of the smears. Malignancy was diagnosed with a sensitivity of 96.8%. The specificity was 98%. The correct tumour type was identified in 95.8% of cases. The results establish FNAC as a useful diagnostic procedure in the primary diagnosis of suspected malignant lesions.

32 Immunocytology of Serous Effusions: Reactivity of "Carcinoma-Specific" Monoclonal Antibody KC-4

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Immunocytology is becoming increasingly valuable in the diagnosis of serous effusions. The introduction of "carcinoma-specific" monoclonal antibodies provides an especially useful tool in immunocytology. We tested the recently introduced "carcinoma-specific" monoclonal antibody KC-4 using the Coulter KC-4 kit. Since thoracoscopy and pleural biopsies were performed in all cases, we could compare the results of conventionally prepared cytology, histology of pleural biopsy, and immunocytology. In four of five cases of uniformly positive cytology and histology positive for KC-4, as well as 3/5 cases with positive histology and cytology negative for KC-4, positive cells were revealed. However, in patients with histologically and cytologically verified inflammation of the pleura, KC-4 stained cells in 4 of 12 cases. In one case nearly all "activated" mesothelial cells were stained. Control incubations were always negative. We conclude that KC-4 monoclonal antibody is not "carcinoma-specific."

33 The Use of Glucoseoxidase and Alkaline Phosphatase for Immunocytological Double Staining of Routine Hematological Smears

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We tested different combinations of immunoenzyme techniques to allow simultaneous detection of two monoclonal antibodies on routine hematological smears. The combination of glucoseoxidase and alkaline phosphatase was chosen because of the lack (glucoseoxidase) or potential suppression (alkaline phosphatase) of endogenous enzyme activity. Optimal differential staining and minimal cross-reactivity were observed if an avidin/biotin system (avidin/alkaline phosphatase) was combined with an immunosandwich system (glucoseoxidase/monoclonal anti-glucoseoxidase complex). The use of different disclosing reagents and counterstains is discussed.

34 T-Cell-Controlled Combined Immunosuppressive Therapy of Aplastic Anemia

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Antigen modulation, variable activities in anti-thymocyte globulin (ATG) preparations/charges, and neutralizing antibodies may cause the failure of ATG therapy. Therefore, three patients with aplastic anemia were treated as follows: after the exclusion of neutralizing antibodies (IgM, IgG), rabbit ATG (Fresenius) was given with T-cell count monitoring by multimarker analysis (T_1, T_3, T_4, T_8), until all peripheral T-lymphocytes had been eliminated. Multiple monoclonal markers were used to avoid spurious low T-cell counts due to antigen modulation. To inhibit autoimmunologically induced reproliferation of T-cells, ciclosporin A and prednisolone were given concomitantly. All three patients had a remission (two partial, one complete). They have now been receiving therapy for 14, 9, and 2 months and are currently being treated with CiA maintenance therapy alone. This approach allows measurement of the ATG effect and provides a basis for determining optimal individualized ATG doses independent of differences between the preparations. The total (T-cell depletion) doses required for these patients were 30, 33, and

44 mg per kg body weight, respectively. Furthermore, in cases in which antigen modulation (the isolated disappearance of single antigens) occurs, this approach allows an early switch to different preparations. This concept is currently being tested with a larger group of patients.

35 The Effect of Androgens on the Response to ATG in Patients with Aplastic Anaemia

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Antithymocyte globulin (ATG) is an effective therapeutic agent for patients with aplastic anaemia (AA). It has been suggested that androgens enhance the response to ATG. Champlin et al. (Blood 66: 185, 1985) published a study which contradicts that suggestion. We report a prospective randomized study in which ATG (ATGAM, lot. no. 17924, Upjohn, USA) was used together with the androgen methenolone. The study was designed in order to determine whether ATG plus methenolone is superior to ATG alone. Only patients with moderate or severe AA were studied. To date 29 patients (18 men and 11 women, aged 4–70 years) have been included in the study. ATG was given according to a low-dosage protocol (20 mg/kg×5 days). Fourteen patients received ATG only, and 15 were treated with ATG plus androgen (methenolone, 3 mg/kg per day). Eleven of 15 patients (73%) responded to ATG plus androgen, whereas 4 of 14 (29%) responded in the group treated with ATG alone. The quality of response – 8 complete responses (53%) compared with 2 (14%) – was also superior in patients treated with ATG plus androgen. The differences in response and survival rates are statistically significant ($p < 0.05$). We conclude that the addition of methenolone may improve the response to ATG in patients with aplastic anaemia.

36 The Structure, Function, and Monoclonal Antibodies of Class II Transplantation Antigens

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Transplantation antigens of class II of the human homozygous B-cell lymphoblast line H 2 LCL were isolated by biochemical preparative procedures [G. Egert et al. (1984) Hoppe Seyler's Z Physiol Chem 365: 1291–1308]. The amino acid sequence was determined using micromethods. It consists of two noncovalently associated subunits: an α-chain of molecular weight 34 kilodaltons (229 amino acid residues) and a β-chain of 29 kilodaltons (237 amino acid residues). The membrane glycoproteins consist of three parts: first, the long N-terminal part, encompassing 191 amino acid residues of the α-, and 198 β-chain; residues of the second, hydrophobic regions comprising 23 residues in both the heavy (α) and light (β) chains which penetrate the cell membrane; and third, a basic hydrophilic short C-terminus of 15 and 16 amino acids located in the cytoplasm. The higher molecular weight of the α chain originates from two N-glycoside-linked side chains, as opposed to the β-chain, which has only one carbohydrate moiety attached to the extracellular part. The almost complete determination of the amino acid sequence of the DC 1 α-chain, and the characterization of at least seven class II β-chains provide an insight into the isotypic complexity of human class II antigens. In addition, four monoclonal antibodies (moAb) were prepared and characterized. MoAb 35.12 and moAb 43.7 specific for peptide antigen p 34 were shown to be different after two-dimensional separation of p 34. After immunoelectrophoresis (IEF) of p 34, moAb 43.7 was shown to react with all α-chains, whereas moAb 35.12 reacted only with a single α-chain. MoAb BE 11.10 and BE 44.23 were shown to be specific

for p 29; moAb BE 11.10 reacted with a single band of p 29, moAb 44.23 reacted with two bands of p 29 after IEF. MoAb 44.23 also reacts with a well-defined peptide of the DR 2 β chain, in position 22–36. Comparison of sequences of other chains showed distinct differences in their isotypes specifically in this region.

37 Non-Responsiveness to Hepatitis Vaccination and HLA Gene Distribution in Healthy Persons

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It has been noticed in hepatitis (HBV) vaccine trials that 5% of healthy subjects do not respond to vaccination. Recently, the murine humoral response hepatitis B surface antigen (HB_SAG) was shown to be regulated by H 2-linked immune response (Ir) genes. We have studied HLA class 1 and class 2 antigens – the human equivalent of the murine H 2 system – in healthy adults who were immunized with the German hepatitis vaccine (Thomssen). A standard schedule of three doses at 0, 30, and 180 days was administered to 217 persons; they were then observed according to a number of indices, including periodic titration of anti-HB_SAG antibody. Six individuals who failed to produce anti-HB_SAG and 19 of 26 who produced weak and transient anti-HB_SAG were typed for their HLA-A, -B, -C, and -DR antigens. The results revealed a significant increase of -DR 5 (41.7% compared with 19.5% in the normal Caucasian population, Chi-square 6.1, $P < 0.02$) and a decrease in HLA-DR 2 (8.3%). Our data suggest that failure of antibody production to HB_SAG is associated with genes in the HLA-DR region and, hence, that major histocompatibility complex (MHC)-linked Ir genes may control this very important antibody response. Further studies of the MHC in relation to the defective generation of specific immune responses could offer a possible explanation for aspects of the genetic basis of immune dysfunctions, such as the differences in the clinical evolution of natural HBV infection, and the development of a chronic carrier state.

38 Oxygen-Radical Mediated Changes in Membrane Fatty Acid Composition in Red Blood Cell Aging

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The removal of old red blood cells is generally believed to be a consequence of declining deformability (indicated by spherocyte formation, increase in mean cell haemoglobin concentration (MCHC), decrease in mean cell volume (MCV), and decrease in osmotic resistance) due to changes of the physicochemical properties of the red blood cell membrane. The reduced antioxidative enzymatic activity in old red blood cells [involving glucose-6 phosphate dehydrogenase (G-6PDH), glutathione reductase (GR) and glutathione (GSH)-peroxidase] implies that increased susceptibility to lipid peroxidation might result in alterations of the cellular membrane. Incubating isolated erythrocytes in phosphate-buffered saline (PBS) supplied with sufficient glucose (20 mM) for several days resulted in methaemoglobin formation, decrease in glycolytic and antioxidative enzyme activities, release of volatile hydrocarbon gases (ethane, ethylene, propane, butane, isobutane and pentane) and loss of polyunsaturated fatty acids – arachidonic acid, (C 20:4), linoleic acid (18:2) and docosahexaenoic acid (22:6) – from the erythrocyte membrane, indicating possible involvement of peroxidative reactions in cellular aging processes.

39 Correlation of Cytostatic Drug Efficacy Between the Colony Assay and the Nude Mouse System

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The colony assay (CA, a modification of the assay described by Hamburger and Salmon) can be used for in vitro screening of new cytostatic agents, in vitro phase II studies, and for pretherapeutic sensitivity testing. We perform the assay with human tumors established in nude mice. Due to the high correlation between the nude mouse system and clinical response rates (correct prediction of tumor resistance in 96% and of response in 92%: Fiebig et al. 1984), the in vivo findings may provide a reference system for the verification of CA results. In order to evaluate the predictive value of the in vitro procedure, five clinically used agents were tested in several dosages in 52 different tumors. Tests were performed in the CA and nude mouse systems. Tumor response in the athymic mouse was defined as regression to less than 75% of the individual initial tumor size, sensitivity in vitro as reduction of colony formation to less than 30% of the control group. The following criteria were established for evaluation of the in vitro results:
- Mean number of colonies in the control group at least 100 (colony diameter 60 μm)
- Initial plate counts (negative control group days 1 and 3) and positive control group (incubated with high-dose 5-flurouracil) smaller than 20% of the control group
- Coefficient of variation in the control group less than 40%

It was possible to make 152 comparisons of in vitro and in vivo results:

Tumor Response CA	Nude Mouse	Prediction of CA	Results Number	Percentage
Sensitive	Sensitive	True positive	29	77%
Resistant	Resistant	True negative	88	
Sensitive	Resistant	False positive	29	19%
Resistant	Sensitive	False negative	6	4%

With the establishment of exact criteria for evaluation, the testing of cytostatic agents in the CA and athymic tumor-bearing nude mouse shows marked correlation between the in vitro and the in vivo systems.

40 A Method for the Production of DNA Polymers Containing 1-β-D Arabinofuranosyl Cytosine (Ara-C) to Study the Interaction of Ara-C with Eukaryotic DNA Methylases

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The aim was to establish a new method for the substitution of cytosines in DNA polymers by Ara-C in order to produce a suitable substrate for evaluating the interaction of DNA-incorporated Ara-C with eukaryotic DNA methylases. It is known that Ara-C applied in vivo is incorporated into DNA and modulates the activity of some nuclear enzymes. One of the most important changes, which may be the reason for the arrest of proliferation and the induction of differentiation of Ara-C treated cells, may be modulation of DNA methylation by incorporated Ara-C molecules, since DNA methylation plays an essential role in the activation or repression of many eukaryotic genes. In former studies we demonstrated that Ara-C induces significant hypermethylation of DNA, accompanied by phenotypic changes and a loss of cellular proliferative activity. We have now established a method which enables us to substitute Ara-C for various amounts of cytosines of any DNA. This method is based on a modified nick-translation reaction

mic cell lines (HL-60 and K 562) and purified them on immobilized, highly specific monoclonal anti-DNA-methylase antibodies. We are now investigating the effects of DNA polymers containing Ara-C on the enzyme kinetics of purified eukaryotic DNA methylases, and the modulation of their de novo and maintenance activity. The results may form a basis for understanding the induction of differentiation by "low dose" Ara-C.

41 Membrane-Sterol-Modulated Exposure of Membrane Antigens by Vertical Phase Separation as the Basis for a New Approach to Immunotherapy of Neoplastic Diseases

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Cell differentiation and proliferation entail a series of events involving the cell membrane, which lead to the modulation of proteins at the cell surface. In the case of malignant differentiation this may enable the tumour cell to escape immune surveillance. Vertical phase separation of membrane proteins appears to play an important role in the modulation of membrane proteins. Our experiments, using a variety of haematological cells derived from animals and humans, strongly suggest that it is the membrane lipid fluidity which modulates the expression of membrane proteins via vertical phase separation. When the membrane fluidity is elevated, the surface expression of some membrane proteins increases, whereas their expression decreases when the membrane becomes more rigid. These proteins e.g. H-2 antigens, hormone receptors, etc. were termed "syndromic". The membrane proteins which displayed the opposite behaviour with respect to lipid fluidity were referred to as "antidromic" proteins e.g. human blood group antigens, Thy 1.2 and neuroreceptors. The possibility that the tumour cell plasma membrane contains cryptic antidromic antigens, which may become exposed by manipulating the membrane lipid fluidity, led to a new experimental approach in the treatment of solid tumours and leukaemias. Our results are discussed in the light of reports that autologous tumour cells that have been pretreated to decrease their membrane lipid fluidity have an increased capability to elicit specific immune responses when compared with normal control cells subjected to the same treatment.

42 Immune Status and T-Lymphocyte Subsets in Patients with Aplastic Anaemia

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There is increasing evidence that immunosuppressive treatment may induce substantial haematological improvement in patients with moderate and severe aplastic anaemia (mAA, sAA). However, there is currently no in vivo or in vitro test available to predict the response to immunosuppression prior to treatment. We report a prospective study on the immune status of patients with AA. Fifteen patients (11 sAA, 4 mAA) were included. Prior to treatment with antithymocyte globulin (ATG) peripheral blood counts, bone marrow histology, immunoglobulins and lymphocyte subsets were evaluated. T-lymphocyte subsets were also determined during and after treatment with ATG (ATGAM, Upjohn). The numbers of circulating lymphocytes and monocytes as well as serum immunoglobulin concentrations were partly reduced. There was, however, no significant difference between responders and non-responders to ATG. T-4 (0.42 ± 0.25), T-8 (0.38 ± 0.15), and Leu-7 ($0.13 \pm 0.1 \times 10^9$/l) concentrations were lower in non-responders than in responders (0.79 ± 0.27; 0.51 ± 0.12; $0.18 \pm 0.15 \times 10^9$/l), but the T 4 : T 8 ratio was in the normal range in both groups. During ATG administration T-lymphocyte subsets showed a marked reduction, which returned to normal within 2–6 weeks. We conclude that ATGAM has a specific and reversible suppressive effect on T-lymphocytes. Whether the differences

in T-cell subsets between ATG responders and non-responders prior to treatment may be considered as a prospective indicator of those patients likely to respond to ATG requires further confirmation.

43 Successful Treatment of Aplastic Anaemia with Cyclosporin A after Repeated Relapses

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In a prospective, randomized study on the effect of antithymocyte globulin (ATG) in 30 patients with aplastic anaemia (AA), we observed a twofold relapse in one female patient. The diagnosis of severe AA was made in June 1984 by peripheral blood count and bone marrow histology. Treatment with ATG (ATGAM, Upjohn, USA) was started in July 1984 with 20 mg/kg per day on 5 consecutive days and the addition of methenolone acetate at 3 mg/kg per day for 6 months. Complete remission (CR) was obtained 3.5 months later. In January 1985, the first relapse was observed and the patient was again treated with ATG (ATGAM, Upjohn, USA) in addition to 1000 mg/day of methylprednisolone (MP); androgen treatment was continued. Again CR was obtained (June 1985). In January 1986, a second relapse occurred, followed by treatment with cyclosporin A (CSA): initial dosage, 400 mg/day. Three months later a partial remission could be observed. We conclude from this case that relapse of AA after treatment with ATG may be successfully treated by repeated use of the same ATG in combination with high dosage MP. Finally, the case report shows that immunosuppression with CSA can also be effective in AA.

44 Severe Haemolytic Anaemia Caused by Ionescu-Shiley Cardiac Valve Bioprostheses

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There are very few reports on haemolysis following heart valve replacement with biological prostheses, especially of the bovine pericardial xenograft type; haemolysis is then either very mild or, in a few cases, moderate and associated with valve dysfunction. Here I report on two patients who had undergone aortic (patient 1), or aortic and mitral (patient 2) valve replacement with biological Ionescu-Shiley prostheses (patient 1: size, 23 mm; patient 2: size, 27 and 31 mm). Massive haemolysis developed 3–4 weeks after valve insertion. Both direct and indirect Coombs' tests were negative. Pertinent data are shown in the table

Patient (sex, age)	Haemoglobin (g%)	Reticulocytes (‰)	Lactate dehydrogenase (U/l)	Free haemoglobin (i.p. mg/l)	Haptoglobin (G%)	Haemosiderin i.U.	Schistocytes (IU)	Bilirubin μmol/l)
1 (M, 60)	7.9	38	1851	1565	under 0.1	++++	++	29
2 (M, 61)	7.4	45	2592	380	under 0.1	+++	++	51

Patient 2 had a paravalvular leak; however, in patient 1 a valve dysfunction or leak could be ruled out repeatedly by several investigations, including transoesophageal echocardiography. The true nature of the haemolysis, i.e. intravascular and physical activity dependent, could be confirmed by serial measurements of several haemolytic parameters, detection of schistocytes, and aggravation of the haemolytic activity by vigorous exercise. The cases demonstrate that the Ionescu-Shiley xenografts can cause not only mild but also overt haemolysis – even in the absence of valve dysfunction; moreover, screening for haemolytic activity could be useful for early diagnosis of valve dysfunction.

45 Porcine Cardiac Valve Prostheses (Hancock) and Haemolysis

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Until very recently it has not been anticipated that heart valve bioprostheses will cause haemolysis. Since 1978, however, several cases of haemolysis following valve replacement with a biological xenograft have been described, but to date there are few data on this important subject, and there is some controversy. This is probably due to differences with regard to type, size, and position of the valve, and particularly the accuracy of the haematological investigation and the time after the valve insertion at which the observation was made. In the present study on haemolysis caused by bioprostheses, we measured several parameters of haemolytic activity among 85 patients with aortic (35), mitral (37) or aortic/mitral (13) replacement with Hancock bioprostheses. The haematologic studies were performed at a mean interval of 81 months (range 65 to 101 months) after valve replacement. Clinically significant haemolysis occurred in only one patient (haemoglobin values: mean 14.5 g%, range 11.2–17.0 g%). However, 27/80 patients showed decreased haptoglobin, 11/81 elevated lactate dehydrogenase, and 53/59 slightly increased osmotic fragility. Several other biochemical and haematological parameters, including schistocytes and urinary haemosiderin were documented. Our study showed that mild haemolysis often occurs, but that severe haemolysis is rare. The lack of overt haemolysis is probably due to the selection of patients with regard to the long period after valve insertion: it is quite possible that at this time the valve structures had become completely covered with endothelial tissue.

46 Red-Cell-Volume Distribution Width (RDW): a New Index for Differentiating Between Iron Deficiency and Heterozygous Thalassaemia. A Comparative Evaluation with Some Other Discrimination Functions

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Since the introduction of electronic particle-counting systems, several discriminant function formulae have been proposed for the differentiation of iron-deficiency anaemia from heterozygous thalassaemia [MCV-(5×Hb)-RBC-8.4; MCH to RBC ratio; MCV to RBC ratio; $(MCV)^2 \times MCH$][1]. In this study I attempted to estimate the value of the new erythrocyte index RDW in distinguishing the microcytosis due to iron deficiency from the microcytosis due to heterozygous thalassaemic conditions. All CBC measurements were performed by the Coulter counter model S Plus IV. The results are given in the table.

Diagnosis	Patients *n*	Mean haemoglobin values (g%)	Mean MCV values (fl)	RDW values (%)	
				Mean	Range
Iron deficiency anaemia	25	10.9	74.4	17.4	15.0–24.0
Heterozygous β-thalassaemia	21	12.4	66.5	15.3	12.1–16.0
Normal blood donors[2]	110	14.6	89.6	13.1	12.0–14.4

[1] MCV, mean corpuscular volume; Hb, haemoglobin; RBC, ned blood cell count; MCH, mean corpuscular haemoglobin; CBC, complete blood count

[2] Before first blood donation

RDW, which has the advantage of being automated and routinely available as an addendum to the haematological report, has proved useful in differentiating between the conditions mentioned. The diagnostic accuracy of this new index is also compared with the previously advocated discriminant function formulae, which are tedious to perform.

47 Microcytosis: Incidence and Causes

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Although microcytosis (MCV < 80 fl)[1] is a common finding, it has gained surprisingly little attention; moreover, despite the recent development of highly precise electronic particle-counting systems, microcytosis is often overlooked or misinterpreted. We attempted therefore to define the incidence and causes of microcytosis among in- and outpatients (group A: 200 consecutive CBCS; group B: 110 prospectively investigated cases) of a teaching general hospital with 1200 beds. All CBC measurements were performed by Coulter counter model S Plus IV. The incidence of microcytosis was 3.1% (11 445 consecutive CBC). The causes of microcytosis were (percentages in parentheses for group B): iron-deficiency anaemia, 22.5 (30.9%); anaemia of chronic diseases, 20.5 (37.3%); phlebotomy for polycythaemia vera 13.0% (1.8% = 2 cases of secondary erythrocytosis); combined anaemia of chronic disorders/iron deficiency, 12.0% (18.2%); heterozygous β-thalassaemia, 1.5% (9.1%); normal childhood microcytosis, 11.5% (children were not included); undefined – mainly because of lack of sufficient data – 19% (2.7%). In 3 adults (group B), the cause of the microcytosis could not be found despite exhaustive investigations, including estimation of the ratio of β/a-globin chains. Although a patient with microcytosis of unknown origin (constitutional) has been described, we suggest that the microcytosis in 2 cases was due to a silent heterozygous thalassaemic condition (DNA analysis could not be performed) and in a third (uraemic) patient to aluminium intoxication. The patient characteristics, the Hb values and several profiles of the erythrocyte indices, including the new index RDW, and their diagnostic significance are discussed.

[1] MCV, mean corpuscular volume; CBC, complete blood count; Hb, haemoglobin; RDW, red-cell-volume distribution width

48 In Vitro Function of Platelets Stored for Up to 5 Days

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The supportive therapy of various hematological diseases depends in particular upon the replacement of thrombocytes. Transfusion therapy could be facilitated if stored platelet concentrates were available. More than 120 preparations of platelets (PRP, PC) were stored at room temperature for up to 5 days using first-generation (PL 146, F 76) and second-generation containers (PL 1240, F 702, CLX-TM). We investigated a broad spectrum of in vitro function parameters: platelet counts, pH, adenosine diphosphate (ADP)/adenosine triphosphate (ATP) concentration of platelets, platelet adhesion and aggregation (stimulated by collagen, ADP, and ristocetin), plasma concentration of serotonin and β-thromboglobulin, and C 14-serotonin uptake. After storing the platelet preparations for 5 days with vertical-elliptical agitation, the pH never became critical (< 6.4). In contrast, horizontal agitation caused a more marked decrease in pH and of in vitro function (even C 14 serotonin, $p < 0.01$). After vertical-elliptical storage of PRP in first-generation containers, a significant decrease in ATP/ADP concentration was observed ($p < 0.05$ and 0.01), ADP-induced aggregation ($p < 0.01$ and 0.05) and adhesion ($p < 0.001$). PC in second-generation containers showed even more significant changes, especially higher β-TG values ($p < 0.001$). In addition, we report on the storage of platelet concentrates with a new monitoring system (PMS, Bellhouse).

49 Immunological Aspects of Transfusion Reactions

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Fifty-four cases (52 patients) suspected of having transfusion reactions to packed red blood cells were surveyed for hemolysis (using transfused red cell concentrate and post-transfusion blood sample from the recipient), infection (transfused red blood cell concentrate), red cell incompatibility, IgA deficiency, platelet, and/or leukocyte alloantibodies (screening with 10 different donors, in 22 cases cross-matching the recipient's serum with the cells of the appropriate donor). Nine patients were excluded from the study because of their case histories. Six patients had hemolytic transfusion reactions: one case with anti-c (not in the hospital), one case with anti-S (anamnestic reaction), in four cases transfusion of hemolyzed blood. One patient with IgA deficiency was revealed. Of 38 patients, 33 (86.6%) with platelet and/or leukocyte antibodies were found in the screening. Cross-match was positive in 16 of 20 cases (80%). All patients with a positive cross-match were also positive in the screening. Detectability of the antibodies in the different test methods did not correlate with the clinical symptoms of the reported transfusion reactions. In 31 cases (81.6%) the TFT (platelet fluorescence antiglobulin test) was positive, whereas the LCT (lymphocytotoxic test) was positive in only 50%. In the two cases that had negative TFT findings, leukocyte antibodies could be detected with the GAT (granulocyte agglutination test). The GAT was positive in 27 cases (71%). Our results confirm that platelet and/or leukocyte alloantibodies are frequently responsible for nonhemolytic transfusion reactions.

50 Proliferation Kinetics of Erythroid Progenitors in Relation to Compensation of Hemolytic Anemia in a Lactate Dehydrogenase Mutant Mouse

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A few years ago, a mouse mutant (+*/+*) was discovered with an LDH-A deficiency (muscle type). This mutant possesses a severe hemolytic anemia, which is expressed by an extreme reticulocytosis of 95%. We have shown that the hemolytic disease is largely compensated for at the level of more mature erythroid progenitors (CFU-E) (Blut 52: 179–183, 1986). In mutants, 90% of the total body CFU-E is found in the spleen. Our purpose was to study the proliferative behavior of different hemopoietic cells and their role in the compensation mechanism of hemolytic anemia. Cell cycle status, determined by the ^{3}H-TdR suicide method, showed no significant genotypic difference for hemopoietic stem cells (CFU-S) or for early and late erythroid progenitors (BFU-E and CFU-E, respectively). Quantitative ^{14}C-autoradiography and ^{3}H-TdR labeling were carried out to study the proliferation kinetics of the three different (pro-, basophilic, polychromatic) erythroblast compartments in the spleen. In mutants, the erythroblasts showed a shortened DNA synthesis time and an increased labeling index, indicating a reduction in the cell cycle time. It could be shown that approximately four additional cell divisions must have occurred between the BFU-E and CFU-E compartments in the mutants. On the other hand, no extra division was necessary from CFU-E to reticulocytes in the mutants. Hemolytic anemia is primarily compensated for by an enlargement of the CFU-E pool and, to a minor degree, through a considerable shortening of the erythroblast maturation time.

51 Correlation Between Metabolic Rate and Lifespan in Neonatal and Adult Red Blood Cells

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One of the most striking differences between the red blood cells of adults and those of newborns lies in the shorter lifespan of the neonatal red cells. The cause of the reduced lifespan of neonatal red cells is not yet known. Our experiments were carried out to examine whether certain unique metabolic features of the neonatal erythrocyte are responsible for its shortened survival time. Adenosine triphosphate (ATP) content, lysolecithin sensitivity, filterability, lipid peroxidation and superoxide dismutase (SOD), glutamic oxaloacetic transaminase (GOT), G-6-PD, and lactate dehydrogenase (LDH) activity of red blood cells were measured. The measurements were carried out on fresh red blood cells (RBC) and on 24-h incubasted RBC of calves 0–6 weeks old and cattle 1–5 years of age. The incubation of erythrocytes at 37°C for 24 h in the absence of glucose was considered to be a model system for studying the in vitro aging of red blood cells. Our results seem to support the hypothesis that there is an inverse relationship between metabolic rate and lifespan – not only at the organism level but at the cellular level, too.

52 Atypical Congenital Dyserythropoietic Anemia

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We observed a 26-year-old male patient who had suffered since infancy from thrombocytopenia with hemorrhagic diathesis. At age 12 (1972), congenital dyserythropoietic anemia (CDA) was diagnosed which, however, could not be grouped properly with one of the three known variants of CDA. In spite of a spleenectomy (1973), anemia and thrombocytopenia persisted. In 1985, an extremely enlarged accessory spleen was removed that histologically disclosed extensive infiltrates of megakaryocytes, erythroid, and myeloid precursors. A bone marrow biopsy showed pronounced hyperplasia of the three cell series, so that the condition could not be distinguished from a myeloproliferative syndrome. Only two cases of CDA variants with atypical findings involving megakaryocytes and granulopoietic cells have been reported so far. On the basis of such variants it is postulated that the genetic lesion underlying CDA affects very early precursors (for instance, stem cells), so that not only erythropoietic cells but also other bone marrow elements are afflicted with the disease.

53 Distribution of Mononuclear Cells in Bronchoalveolar Lavage (BAL) and in Peripheral Blood in Patients Suffering from Acquired Immune Deficiency Syndrome (AIDS)

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BAL has been proposed as a diagnostic tool for assessement of the activity of interstitial lung processes by characterization of the cell material obtained. Therefore, BAL was carried out in 14 patients with AIDS and radiological signs of interstitial pneumonia. By bronchofibroscopy, 100 ml 0.9% NaCl solution was instilled into subsegments of the lingula or the middle lobe and aspirated immediately thereafter. Taking the whole cell count into consideration, the percentage of macrophages and lymphocytes were estimated by light microscopy and the lymphocytes dif-

ferentiated into subtypes by immunofluorescence. On the same day, peripheral blood estimations were done. The present pilot study indicates that all patients investigated showed identical distributions of lymphocyte subtypes in blood and BAL fluid. In all cases the helper-supressor cell ratio was below the normal range, with a median value of 0.45 (range 0.1–0.9, blood) and of 0.30 (range 0.2–0.8, BAL). The percentage of lymphocytes was therefore higher and the macrophage counts lower, the total T-cell count was reduced in contrast to shifted counts of the activated forms that could be identified by the expression of interleukin-2 receptors.. Further studies are necessary to investigate the predictive value of BAL regarding the risk of developing pneumonia.

54 An Illness like Infectious Mononucleosis as a Clinical Manifestation of an Acute Infection with the AIDS Virus

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Two homosexual patients, aged 29 and 40, presented with an acute infectious illness with fever of sudden onset, a sore throat, and severe myalgia. Both had tonsilectomies. Bilateral submandibular, cervical and axillary lymphadenopathy, and slight enlargement of the spleen were noted in both patients. There was no inguinal lymphadenopathy. Repeated serological analyses for Epstein-Barr virus (EBV), cytomegalovirus (CMV), and toxoplasmosis were negative. Seroconversion to anti-LAV/HTLV-III positivity, as confirmed by Western blot analysis, was closely related in time to the acute illness in one case. In the other patient, who presented later during his disease, we did not have the opportunity to determine the time of seroconversion. The demonstration of IgM antibodies of high titer, together with IgG antibodies by ELISA, immunofluorescence and Western blot, also indicated an acute infection from the AIDS virus in this case. Correspondingly, an increase in circulating T8+ cells was recorded, resulting in an inversion of T4:T8 ratio. This T8 response is known to occur in acute virus infections such as EBV, CMV, and hepatitis-B virus (HBV). Concerning the infection route, we received information that fellatio had been practiced. This first report on IgM antibodies against the AIDS virus and a preceding one on acute AIDS infection (Lancet I: 537, 1985) suggest that the AIDS virus infection should be considered in any person at high risk with acute febrile mononucleosis-like illness.

55 Alterations in the Hematopoietic Stem-Cell Compartment in Patients with AIDS

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In 50% of patients suffering from acquired immune deficiency syndrome (AIDS) in LAV/HTLV-III virus infection, the clinical course is complicated by anemia, thrombopenia, neutropenia, or combined cytopenias of unknown cause. To find out whether a change in the hematopoietic stem-cell pool is the underlying cause of the observed cytopenias, we determined the incidence of the peripheral blood stem cells. Mononuclear cells from the peripheral blood of seven patients with AIDS were cultured in a methylcellulose assay. There was a reduction in the colony growth of pluripotent and committed stem cells. The colony number of the pluripotent CFU-GEMM was decreased to 1.8 ± 1.1 ($\bar{x} \pm$ SEM; normal: 4.4 ± 1.8) per milliliter blood, the megakaryocytic precursors CFU-Mk to 6.9 ± 2.5 (17.3 ± 7.2), the erythroid BFU-E to 116.5 ± 25.6 (401.0 ± 71.6), and the granulocytic-monocytic CFU-GM to 79.7 ± 13.0 (114.3 ± 20.0). The decrease in the peripheral blood stem cells might indicate that stem-cell loss is the cause for cytopenias in the blood of patients with AIDS. It is not clear if this is a direct effect of LAV/HTLV-III infection or a disorder in hematopoietic regulation.

56 A Comparative Study of Chemiluminescence in Granulocytes*

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There is great variation in chemiluminescence (CL) in granulocytes, which makes it difficult to compare absolute values by various authors. Hence, we studied CL in whole blood as well as in isolated granulocytes of healthy volunteers in the presence of buffer (KRPG) and serum. The cells were stimulated with phorbol myristate acetate (PMA), zymosan and the stimulus "ZAP," which can be purchased. In whole blood, only ZAP induced measurable CL. On stimulation with ZAP, with 68×10^3 counts/min $\times 10^3$ granulocytes, isolated granulocytes resuspended in KRPG produced the greatest CL. However, following stimulation with zymosan or PMA, only one-third of this CL value was observed. CL was not enhanced by increasing the zymosan or PMA concentration, but on stimulation with ZAP, zymosan and PMA in the presence of serum, we determined only 50%, 16%, and 20%, respectively, of the CL value measured in the presence of KRPG. Furthermore, the PMA concentration had to be increased sevenfold to achieve maximal stimulation. The absolute CL is not only dependent on the used stimulants but also on the composition of the cell suspension medium. In addition, good knowledge of the measuring instrument is of great importance to compare absolute CL values by various investigators.

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57 Characterization of the Main Component of the Epstein-Barr Virus (EBV)-Associated Early Antigen (EA) Complex*

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By means of two-dimensional immunoblot analysis, it was possible to map the main component of the EBV-associated EA complex (a series of polypeptides) to pH 4–8 and 50–58 kDa. These polypeptides react with human antisera, rabbit antibodies raised against a purified p52, and monoclonal antibodies. Therefore, post-translational modifications of a single or a few initially synthesized polypeptides may be responsible for this migration pattern. One reason for the varying molecular weights may be a different degree of glycosylation; this could be ruled out by incubation of chemically induced Raji cells with tunicamycin. The function of EA is still not clear. However, we demonstrated that a purified p52 fraction has an inhibitory effect on the cell-free translation of mRNA; the physiological relevance of this must be investigated further.

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58 Change of Noninvasive Parameters of Global Left Ventricular Function in Anemic Patients

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Systolic time intervals (STI) and echocardiographically determined fractional shortening (fs) are recommended for noninvasive monitoring of global left ventricular function in patients treated with anthracyclines. Anemia is a common clinical condition in such patients; however, little information is available regarding the influence of this symptom on STI and fs. We determined

these parameters prospectively in 23 patients with subacute and chronic anemia before and following erythrocyte transfusion: fs could be assessed in 16 and STI in 20 patients. Results (median; $*P \leq 0.05$); $**P \leq 0.01$):

	Hemoglobin (g/dl)	Frequency (bpm)	fs (%)	PEPi (ms)	PEP/LVET
Before transfusion	7.9	91	39.7	113.1	0.302
Following transfusion	9.8	76.5	35.1	119.4	0.330
Difference	2.2	−7	−4	9.1	0.024
P	**	**	**	**	*

Patients treated with anthracyclines tended to have smaller differences in fs (diff-fs = 3.2%; $n = 7$) than others (diff-fs = 7.1%; $n = 9$; $P \leq 0.10$). These results indicate that anemia changes fs and STI significantly and must be considered in patients on anthracycline therapy who should be monitored for left ventricular function.

59 In Situ Staining of Blood Progenitor Cells Cultured in Fibrin Layer for Hematological Diagnosis

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Morphological differentiation of blood progenitor cells cultivated in agar or methylcellulose is possible only in cytocentrifuge preparations when Pappenheim staining is used. However, structure and interrelation of the different colonies cannot be judged with this method. Using these media, staining in situ with POX, PAS, α-naphthyl-acetatesterase, etc. means a lot of work and expense. These reasons led to the development of a technique to stain cultivated blood progenitor cells in situ on fibrin-layer medium. The cultures were performed with 5×10^4 mononuclear cells (MNC) in slide flasks containing medium (IMDM with 20% – 30% FKS: 10% HPCM; erythropoetin, 1 U/ml medium; 0.1% fibrinogen). After cultivation, the slide is detached from the flask and the fibrin layer dried with filter paper. Afterwards, the preparation can be stained with May-Grünwald-Giemsa staining. Before staining with peroxydase, alkaline and acid phosphatase, α-naphthyl-acetatesterase, naphthol-ASD-chloracetatesterase and glykogen (PAS), the preparation has to be fixed. In these preparations, cells and colonies can be stained as easily as bone marrow or blood cells in smears.

60 The Effect of Red Cell Lipid Composition on Lipid Peroxidation

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Red blood cells from 31 healthy donors were examined for cholesterol content, fatty acid composition, and susceptibility to lipid peroxidation induced by hydrogen peroxide or phenylhydrazine. Lipid peroxidation was monitored by the release of pentane and ethane. In addition, plasma fatty acids were measured to find out whether plasma and red cell fatty acids are correlated. In experiments with hydrogen peroxide, a significant positive correlation was found between the proportion of arachidonic acid (C 20:4, *n*-6; $r = +0.57$, $p < 0.01$) and docosahexaenoic acid (C 22:6, *n*-3; $r = +0.71$, $p < 0.01$), and the release of pentane and ethane, respectively, whereas a significant negative correlation was found between membrane cholesterol content and pentane release ($r = -0.44$, $p < 0.05$). In experiments with phenylhydrazine, red cell membrane lipid composition did not influence the susceptibility of red cells to lipid peroxidation. A close corre-

lation was found between plasma and red cell fatty acids (palmitic acid, $r = +0.46$, $p < 0.01$; linoleic acid, $r = +0.41$, $p < 0.05$; aracidonic acid, $r = +0.59$, $p < 0.01$; docosahexaenoic acid, $r = +0.67$, $p < 0.01$). The results demonstrated that the degree of peroxide-induced autoxidation of erythrocyte lipids depends on the content of polyunsaturated fatty acids in the membrane which, on the other hand, is determined by plasma fatty acids. It is suggested that dietary variations may influence the susceptibility of red cells to lipid peroxidation.

61 Oral Vitamin B_{12} Replacement in Hereditary Transcobalamin II Deficiency

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A report is presented on siblings with hereditary transcobalamin II deficiency. Regular intramuscular application of cyanocobalamin resulted in complete clinical and hematological reconstitution. Thrombocytopenia was the most sensitive laboratory parameter regarding insufficient substitution; clinical signs were tongue ulcers and loss of appetite. A theoretical understanding of this metabolic disorder has led us to consider the possibility of oral vitamin replacement. Different oral replacement regimens using cyanocobalamin have proved unsuccessful. For the past 18 months, normalization of clinical and hematological parameters has been obtained by administering the extremely high oral dose of 1 mg hydroxycobalamin twice daily. The physiology of hydroxycobalamin metabolism is discussed.

62 Reduced Platelet Thromboxane Formation in Acute Thrombotic Thrombocytopenic Purpura: Evidence for a Transient Cyclooxygenase Defect

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We have recently shown that circulating degranulated platelets occur in acute thrombotic thrombocytopenic purpura (TTP). These platelets are hemostatically defective due to partial loss of granular constituents and/or metabolic abnormalities. To further evaluate biochemical alterations of "exhausted" platelets, we studied their thromboxane A_2 (TXA_2) formation in two patients with primary TTP. TXB_2 production was measured radioimmunologically after incubation of platelet-rich plasma (PRP) with thrombin (10 IU/ml) or arachidonic acid (AA, 450 μM) for 5 min. During the acute phase of TTP the thrombin-induced platelet TXB_2 formation was significantly reduced ($n = 6$, 0.32 ± 0.08 nmol/10^9 platelets) as compared with controls ($n = 10$, 4.7 ± 1.9 nmol/10^9 platelets, $P < 0.001$): Incubation of PRP with exogenous AA failed to restore normal TXB_2 production in platelets from patients with acute TTP. Following remission of TTP achieved by repeated plasmapheresis and substitution of fresh frozen plasma, the platelets recovered normal thrombin-inducible thromboxane synthesis capability in vitro. We conclude that an abnormality of platelet arachidonic acid metabolism exists in acute TTP, leading to reduced TXA_2 production. This dysfunction may reflect the previous activation of platelets in vivo. The reduced platelet thromboxane synthesis is consistent with a transient cyclooxygenase defect which disappears following remission of TTP.

63 Platelet Prostacyclin Binding in Smokers

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The binding capacity and dissociation constant of platelet prostacyclin receptors in 23 male smokers and 14 nonsmokers were determined by direct binding studies in order to investigate

whether platelet prostacyclin binding is altered in smokers. Additionally, the inhibitory effect of PGI_2 (IC_{50}) on ADP-induced platelet aggregation was measured. As confirmed by discriminant analysis, 69% of the smokers had significantly different B_{max} and K_D as well as antiaggregatory potency (IC_{50}) of PGI_2. Binding capacity was increased in 12 smokers (B_{max} +70%) with a concomitant decrease in affinity (K_D +104%). In these volunteers, the postreceptor responses (PGI_2-induced cyclic AMP accumulation in platelet-rich plasma as well as IC_{50}) did not differ from controls. In contrast, 4 smokers with considerably reduced PGI_2 binding capacity (B_{max} −64%) exhibited a lower antiaggregatory effect (IC_{50} +76%) although the affinity was slightly increased (K_D −59%). Nicotine, L-epinephrine, and prostaglandin E_2 did not significantly compete with the binding of 9-^{3}H-PGI_2-sodium salt. The antiaggregatory effect of PGI_2 an ADP-induced platelet aggregation, however, was inhibited by L-epinephrine (K_i 26 nmol/l) and prostaglandin E_2 (K_i 230 mnol/l) by 50%. Our data suggest that with respect to platelet PGI_2 binding and in vitro responsiveness to PGI_2, smokers are a heterogeneous population. Although increased binding capacity was observed in 50% of the smokers investigated, our data provide no evidence that a biologically relevant up regulation – e.g. with concomitantly enhanced postreceptor reaction – occurs in smokers.

64 Heterologous Desensitization of Platelet Adenylate Cyclase by the Stable PGI_2-Analogue Iloprost (ZK 36 374). Evidence for Multireceptor Down-Regulation

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When treatment with epoprostenol (PGI_2-Na) and iloprost (IP) is stopped, impaired sensitivity of platelets to these eicosanoids and hyperaggregability have been observed. Thus we investigated whether the pretreatment of human platelets with IP causes alteration of specific platelet IP binding. Specifically bound radioactivity (N) and affinity (K) of IP receptors have been determined in intact washed platelets by direct binding studies with 5×10^{-9}M ^{3}H-IP (Schering AG, Berlin) and increasing concentrations of unlabeled ligand up to 2×10^{-5}M. Platelet adenylate cyclase activity (AC/EC 4.6.1.1) was measured in platelet membranes according to the method of Salomon. The incubation of platelets (12 h, 20°C) with 10^{-7}M IP resulted in a loss of specific binding sites (N 716 ± 42 vs 364 ± 76 fmol/10^9 platelets) without significant changes of affinity (K 1.4 ± 0.1 vs 1.9 ± 0.9$\times10^{-8}$M) This down-regulation has been demonstrated to be time- and dose-dependent (T/2 = 1 h, $IC_{50} = 3.5\times10^{-8}$M). After complete removal of the agonist, time-dependent resensitization occurred (M_{t0h} = 29, M_{t8h} = 48, M_{t24h} = 60% control). After treatment with IP a loss of specific ^{3}H-PGD_2 binding was also observed (−40%). The V_{max} of IP-stimulated AC amounted to 61% of control incubations (269 ± 20 pmol$\times$min$^{-1}\times$ mg^{-1} protein), whereas Menten-constants showed only minor changes (1.0 ± 0.2 vs 1.5 ± 0.2$\times10^{-8}$M). The pretreatment of platelets with IP also caused a decrease of basal AC activity (−65%) and activity of this enzyme was stimulated by 10^{-2}M NaF (−31%), 10^{-6}M PGD_2 (−48%), and 10^{-4}M forskolin (−42%). Because the pretreatment of platelets with 5×10^{-3}M 8-bromo-cyclic AMP and 3×10^{-3}M 3-isobutyl-1-methylxanthine (12 h, 20°C) did not result in an impaired activity of AC, to our present knowledge IP-induced desensitization appears to be unlikely to occur via cyclic-nucleotide-dependent protein phosphorylation.

65 Coagulation Parameter and Platelet Function in Diabetic Children

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In 52 children with type I diabetes mellitus factor VII C, factor VIII C, von Willebrand factor, fibrinogen, plasminogen, antithrombin III, platelet count, spontaneous platelet aggregation

(PAT III), ADP-induced platelet aggregation, collagen-induced platelet aggregation, adrenalin-induced platelet aggregation, platelet adhesion, platelet volume and β-thromboglobulin were assayed. In our patients no signs of vascular diseases could be found based on negative fundoscopy, normal serum creatinine and the absence of proteinuria. Compared to an age-matched control group spontaneous platelet aggregation, β-thromboglobulin, factor VIII C and von Willebrand factor were significantly increased in diabetic children. These changes were not related to the mean metabolic equilibrium (HBA 1 C). A positive correlation could be observed between increased spontaneous platelet aggregation, von Willebrand factor and factor VIII C to the duration of diabetes mellitus. Spontaneous platelet aggregation seems to be a useful parameter for assessing the onset of atherosclerotic diseases in diabetic children. High values of von Willebrand factor may indicate reversible and/or irreversible damage of vascular endothelium.

66 Follow-up of 105 Patients with Idiopathic Thrombocytopenic Purpura from 1974 to 1984 with Respect to DNA content of Megakaryocytes

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A total of 105 patients (100%) with idiopathic thrombocytopenic purpura (ITP) have been followed up during the last 10 years. The disease was considered to have remitted if there was no relapse for 1 year or more after the end of therapy. Spontaneous remission was observed in 13 patients (12.4%). Seventy-eight patients (74.3%) were treated with steroids; 26 (24.8%) of them – 17 with an initial dose of more than 100 mg prednisolone/day – were cured after one treatment cycle. In 9 patients (8.6%) a second cycle was necessary in order to achieve remission after there had been an unsatisfactory response to initial steroid treatment. In 22 (21%) patients, splenectomy had to be performed after steroid treatment had failed. The operation resulted in cure in 18 (17.2%) of these patients; 2 patients (1.9%) died of bleeding complications. The DNA content of bone-marrow megakaryocytes was measured by Feulgen cytometry in bone-marrow smears before therapy. Nuclear ploidy correlated inversely with the stages of maturity of the megakaryocytes and the peripheral platelet count. A prediction about the course of the disease could not be made on the basis of megakaryocyte DNA distribution patterns. Our study has shown (1) that the rate of spontaneous remission of ITP is higher and the rate of lethal bleeding complications is lower than was previously believed; (2) a second cycle of therapy with prednisolone must be considered and weighed against the risks of splenectomy; (3) megakaryocyte ploidy levels compared with megakaryocyte maturity and peripheral platelet counts correspond to the experimental results in thrombocytopenic animals.

67 Analysis of Lymphocyte Subsets in Patients with Chronic Autoimmune Thrombocytopenic Purpura

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Idiopathic thrombocytopenic purpura (ITP) is a syndrome characterized by persistent thrombocytopenia caused by circulating antibodies that result in platelet destruction. The pathogenesis of these antibodies is not fully understood. Therefore, we studied the lymphocyte subpopulations of 17 patients with ITP prior to therapy. To isolate the mononuclear cells, density gradient centrifugation was performed. The cells were incubated with FITC-conjugated monoclonal antibodies. The percentage of positive cells was counted using a fluorescence microscope. The results, when compared with those in healthy volunteers matched for sex and age, showed no

significant differences in the subsets of lymphocytes: median helper/suppressor cell ratio 1.9 (range 1.6–4.9); pan T-lymphocytes 65% (50%–79%); pan B-lymphocytes 31% (14%–36%); NK cells 8% (3%–26%); helper T-cells 43% (28%–66%); suppressor-cytotoxic T-lymphocytes 20% (10%–34%). No activated T-cells, as detected by the expression of the IL-2 membrane receptor, were identified. In contrast to most other autoimmune diseases, ITP does not show a typically increased helper/suppressor ratio of lymphocyte subsets, nor are the B-lymphocytes significantly increased. Additional immunological studies are necessary to identify the disorder underlying ITP.

68 Danazol in the Management of Adult Idiopathic Thrombocytopenic Purpura

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Danazol, a 17 α-alcyl derivate of ethinyl testosterone, is an anabolic steroid with mild androgenic side-effects. According to Ahn et al. (N Engl J Med 1983, 308: 1396) this drug has been used successfully in the treatment of 22 patients with ITP who did not respond to corticosteroid therapy and in whom splenectomy did not lead to the desired results. Of the 22 patients, 15 responded to danazol. We tried to confirm these positive results in 14 patients who did not respond to corticosteroids. However, only 3 of the 14 patients underwent splenectomy. Danazol was given orally in a dosage of 200 mg, 3 times a day, for a minimum of 4 weeks, if tolerated. Of the 14 patients, 4 showed a response: in 1 patient the platelet count normalized for 10 months, whereas in 3 patients the platelet count rose to 50–100 000/μl. More severe side-effects were seen in 3 patients: 2 developed a generalized, itching drug rash and one showed symptoms and laboratory signs of cholestasis. In all 3 patients, the drug had to be discontinued within 5–14 days. Four patients objected to taking danazol as a result of gaining weight due to water retention. We conclude that danazol is certainly not as effective as it was claired to be by Ahn et al. It may be useful as a third-line therapeutic agent in some patients who do not respond to conventional therapy. Side-effects seem to occur relatively frequently.

69 Alterations of the Fibrinolytic System in Patients with History of Deep Venous Thrombosis

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Changes in the fibrinolytic system opf 50 consecutive patients of both sexes (mean age 39.7 ± 16.5 years) with a history of deep vein thrombosis (DVT) and/or pulmonary embolism were studied. Twenty-nine healthy individuals (mean age 31.3 ± 7.8) served as control subjects. Tissue plasminogen activator (t-PA) activity and antigen before and after venous occlusion (VO), as well as PA inhibitor levels before VO and euglobulin clot lysis time (ECLT) after VO were determined in the plasma of all subjects. The ECLT of the patients was significantly longer than that of the controls (75.8/30.7 min). No other significant differences were observed between these groups. When defining the normal range as the mean value of the normal controls ± 1 SD, the patients could be divided into one group ($n = 17$) with normal ECLT and t-PA activity after VO and another group ($n = 33$) with prolonged ECLT, who largely ($n = 26$) presented diminished t-PA activity levels after VO. While release of t-PA antigen was impaired in 13 of these 26 patients following VO, 8 patients were shown to have increased levels of t-PA inhibitor. ECLT and t-PA activity after VO were negatively correlated in controls ($r = -0.41$) and in patients ($r = -0.72$). In conclusion, alterations of the different components of the fibrinolytic system appear to be a common disorder in patients with a history of DVT. Obviously, the pathogenetic importance of these findings needs to be elucidated further.

70 Effects of Intravenous Recombinant Tissue-Type Plasminogen Activator on Coagulation and Fibrinolysis System in Healthy Volunteers

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Streptokinase and urokinase, the thrombolytic substances now available, induce the activation of systemic fibrinolysis resulting in a serious bleeding tendency. Since the transfer of animal DNA to Escherichia coli or eukarytic cell lines became possible, recombinant plasminogen activator (rt-PA) has been available. In animal studies, rt-PA has been shown to induce specific thrombolysis without systemic fibrinolytic activation. In the present study we investigated the effects of rt-PA on some parameters of coagulation and fibrinolysis in healthy volunteers. Eighteen volunteers (group I) received 0.25 mg/kg body wt. i.v. over 10 min; 6 males (group II) were administered 0.25 mg/kg body wt. i.v. over 60 min.; 6 persons (group III) received 0.50 mg/kg body wt. i.v. over 60 min and a last group of 6 volunteers (group IV) received a placebo infusion. Prothrombin time, partial thrombin time, factors II, V, VII, VIII, IX, X, XI and XII, plasminogen, α_2-antiplasmin, α_2-macroglobulin and fibrinogen levels were measured in thawed plasma samples of citrate or citrate/aprotinin blood; fibrinogen-fibrin degradation products were determined in serum collected on aprotinin at the beginning of infusion, and 15, 30, 45, 55, 120, 180 min and 8, 12 and 24 h after infusion. Thirty min after the beginning of the infusion, fibrinogen was 96% ± 18%, 85% ± 29% and 91% ± 20%; plasminogen was 85% ± 6%, 74% ± 4% and 52% ± 13%; and α_2-antiplasmin was 69% ± 9%, 44% ± 17% and 23% ± 14% of the pre-infusion values in groups I, II and III respectively. After 24 h most of the volunteers had reached their initial values. No significant changes could be detected in the placebo group.

71 Monoclonal Antibodies to Idiotypes of Human Antibodies to Factor VIII

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The generation of antibodies to factor VIII (anti-VIII : C) is a serious complication in patients with severe classic hemophilia and can be the cause of acquired hemophilia. To understand better immunological phenomena and eventually design a therapy for these patients, we raised a monoclonal antibody (MOAB) to the idiotypes of anti-VIII : C from one patient with inherited hemophilia. The antiidiotype specificity of the MOAB was proven by specific inhibition of anti-VIII : C in solution. The MOAB only partially decreased the patients' anti-VIII : C activity and did not alter the anti-VIII : C activity of two other patients; thus the heterogeneity of anti-VIII : C was confirmed. In a solid-phase study using the MOAB, we were able to partially deplete the plasma anti-VIII : C activity. Monoclonal antiidiotype antibodies to anti-VIII : C of well-defined specificity can be produced in unlimited quantities. They may be useful in the treatment of bleeding complications in patients with hemophilia and anti-VIII : C.

72 The Biochemistry of Tumor Cell Thrombosis: Thrombin Acts as Growth Hormone

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In tumor cell thrombosis, thrombin not only acts as a clotting enzyme but also as a tissue hormone stimulating the proliferation of malignant cells as indicated by an increase in cell count and thymidine uptake in cell cultures. Spontaneous decay of tumor cells and that induced by

cytostatic treatment leads to the release of tumor cell thromboplastins, which induce coagulation locally and liberate thrombin. The local liberation of thrombin in tumor cell thrombosis again stimulates tumor cell proliferation and can be considered a biochemical substrate of tumor spread and growth in tumor cell thrombosis.

73 Hypercoagulability in Cytostatic Tumor Therapy

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Activation of the clotting system is believed to be implicated in some thrombotic complications of cytostatic therapy. Clot analyses were performed in 10 patients with acute leukemia, 16 patients with small cell and 11 patients with non-small-cell carcinomas of the lung, 8 patients with malignant lymphomas and 8 patients with plasmocytomas before and 4 and 24 h after chemotherapy. Following a thrombin-induced activation of the clotting system, we observed as early as 4 h after the initiation of therapy significant elevations of fibrinopeptide A (FPA) and delta FPA levels (FPA generation rate in vitro): Small cell carcinomas of the lung: delta FPA from $\tilde{x} = 0.59$ ng/ml/10 min to $\tilde{x} = 3.0$ ng/ml/10 min, $P < 0.05$; malignant lymphomas: delta FPA from $\tilde{x} = 0.71$ ng/ml/10 min to $\tilde{x} = 1.75$ ng/ml/10 min, $P < 0.01$. We subsequently observed a significant rise in factor VIII activity in small cell carcinomas of the lung from $\tilde{x} = 155.5\%$ to $\tilde{x} = 205\%$ ($P < 0.01$) and in malignant lymphomas from $\tilde{x} = 176\%$ to $\tilde{x} = 260\%$ ($P < 0.05$). The plasma fibrinogen level decreased significantly. Protein C increased in patients who received cortisone. These results support the thesis of activation of the exogenous clotting system by release of tumor cell thromboplastins in therapy-induced cell destruction.

74 The Antiaggregatory Effectiveness of Prostacyclin and Its Binding to Specific Platelet Receptors. A Comparative Study

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The antiaggregatory effect of PGI_2 on primary platelet aggregation induced by 0.6 μM ADP (IC_{50}) was measured in 85 volunteers and compared with the specific binding of 9-^{3}H-PGI_2-sodium salt, i.e. B_{max} and K_D, which were calculated by computerized analysis of binding isotherms obtained at equilibrium. The relationship between IC_{50} and B_{max} was fitted to the equation $IC_{50} = 0.3066 \cdot B_{max} \cdot \exp{-0.2430}$ ($F = 33/P = 0.000$). Nevertheless, the low correlation coefficient ($r = 0.5330$) suggests that in addition to the binding of PGI_2 to its receptor other variables might also influence the biological effectiveness. As shown by multiple regression analysis, free fatty acids ($P = 0.0005$) and albumin ($P = 0.0018$) were proven to correlate with the IC_{50}, whereas cholesterol, triglycerides, and apolipoproteins (A, B) had no significant effect. In vitro, PGE_2 and L-epinephrine have also been shown to impair the antiaggregatory effects of PGI_2 ($EC_{50} = 230$ nM and 26 nM respectively), although neither compound competed with the binding of labeled PGI_2 in concentrations up to 10 μM. Furthermore, based on the observation that the ratio between the equilibrium dissociation constant ($K_D = 54$ nM) and the Menten constant of PGI_2-induced adenylate cyclased activation ($K_M = 28$ nM) is close to 2, the existence of spare receptors seems likely and is further supported by the observation that an increased B_{max} did not necessarily cause an enhanced postreceptor response. Finally, the analysis of dose response curves of antiaggregatory effects of PGI_2 and its binding to specific binding sites ($IC_{50} = 0.9$ nM vs $K_D = 54$ nM and Hill coefficients 1.9 vs 0.9) suggests a considerable positive cooperative signal amplification beyond the receptor (Chock, Stadtman PNAS 74: 2766, 1977). In conclusion, since the biological effectiveness of PGI_2 has been shown to depend on multiple variables, determinations of IC_{50}-PGI_2, although feasible, cannot replace the more expensive and time consuming radioligand assay in obtaining reliable data on platelet PGI_2 receptor density.

75 Effects of Heparin on Prostacyclin Binding and Platelet Adenylate Cyclase Activity Stimulated by Iloprost and Forskolin

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Heparin is known to impair the antiaggregatory effectiveness of prostacyclin (PGI_2) for adenosine-diphosphate-induced aggregation in citrated platelet-rich plasma. Thus, porcine mucosal heparin (PMH) from different commerical sources was investigated for its ability to compete with specific platelet prostacyclin binding. In concentrations up to 250 IU/ml PMH itself did not interfere with the binding of 3×10^{-9}M 9-^{3}H-PGI_2-sodium salt to intact washed platelets. The displacement of specifically bound PGI_2 observed by PMH (K_i 60 IU/ml) containing 4-chloro-m-cresol (4-CC) was caused by the preservative (K_i 4-CC 3.0×10^{-4}M). Although 60 IU/ml PMH (free from 4-CC) has been shown to inhibit basal 3×10^{-5}M forskolin- and 10^{-8}M iloprost-stimulated adenylate cyclase in platelet membranes (−63%, −62%, −83% respectively), no effect of PMH on ^{3}H-cyclic-AMP formation has been observed when intact platelets were studied by a ^{3}H-adenine prelabeling technique. There is also no evidence that the preincubation of PGI_2 (5×10^{-7}M) with 1000 IU/ml PMH might neutralize the effectiveness of this eicosanoid. In contrast, PGI_2 preincubated with PMH (10 min, 37°C) caused a more pronounced increase of platelet ^{3}H-cyclic AMP (+65%) compared with PGI_2 incubated in 5×10^{-2}M Tris-HCl, pH 7.4. Thus our data provide no evidence that PMH interferes with (i) specific platelet PGI_2 binding, (ii) PGI_2-stimulated cyclic AMP synthesis or (iii) that it neutralizes PGI_2 by the formation of a biologically less active PGI_2-PMH complex.

76 Serial Determinations of Fibronectin and Coagulation Parameters in Patients with Acute Myelogenous Leukemia

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Serial determinations of coagulation parameters and fibronectin were performed in 19 patients with acute hematologic neoplasias (3 acute myelogenous leukemia M 1, 8 M 2, 1 M 3, 4 M 4, 2 M 5, 1 myelosarcoma) and a peripheral leukocyte count of $> 2000/\mu l$ before therapy. All patients were treated with intensive polychemotherapy (TAD, DAV, Hd-AraC/VP 16). Prothrombin time was prolonged initially. The activity increased after reduction of tumor burden. Fibrinogen increased substantially under therapy. The course of thrombin time and aPTT were uncharacteristic. There was a moderate increasing tendency in F II, marked in F V up to day 11 after the maximal drop in leukocyte count. F XIII a levels rose markedly after initially diminished values. Maximal reduction of leukocyte count was followed by an extreme increase in the level of F VIII : AG with F VIII : C remaining unchanged. FDP levels were found to be inconstant. The course of AT III was uncharacteristic; plasminogen levels rose moderately. Plasma fibronectin had an inconclusive course in general, whereas marked drops were seen in individual patients.

77 Management of Disseminated Intravascular Coagulation in Children Using Antithrombin III Concentrate

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Prothrombin time, partial thromboplastin time, thrombin time, antithrombin (AT) III, fibrinogen and platelet count were determined in 52 children with acquired AT III deficiency immediately before and during substitution therapy with AT III concentrate. Consumption coagulopathy and AT III deficiency were caused by septicaemia, shock situations, necrotizing enterocolitis, haemolytic-uraemic syndrome, rh incompatibility, idiopathic respiratory distress syndrome, acute hepatic failure, hereditary fructose intolerance and immune complex vasculitis. AT III concentrate was substituted during an average period of 6–8 days. Only when bleeding occurred fresh frozen plasma was also used, in two children supplemented by PPSB and factor XIII concentrate. Low-dose heparin was administered in premature infants to prevent catheter occlusion. Consumption coagulopathy could be stopped in nearly all children with sufficient amounts of AT III concentrates. The coagulation parameters determined showed an improvement 24–48 h after normalization of plasma AT III levels. The platelet count was low at the beginning and started to increase by the end of the observation period (mean 9–11 days). Consumption coagulopathy in children was treated successfully by substitution of AT III concentrate without any supporting heparin therapy.

78 In Vivo Plasma Level Studies After Infusion of Recombinant Tissue-Type Plasminogen Activator in Healthy Volunteers

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Different molecular forms of plasminogen activators are synthesized and secreted by vascular endothelium and other normal and transformed mammalian cells in culture. They can be distinguished on the basis of their specific properties and immunological relationship with either urokinase or tissue-type plasminogen activators (t-PA). Recent success in the cloning of the t-PA gene in animal cells capable of synthesizing the thrombolytic agent has opened up the possibility of obtaining recombinant t-PA for clinical investigations. In this study plasma levels were determined in 36 healthy volunteers. Eighteen volunteers received 0.25 mg/kg body wt. rt-PA i.v. for 10 min; 6 volunteers were administered the same dosage for 60 min; a third group of 6 males received 0.50 mg/kg body wt. i.v. for 60 min; and a last group received a placebo infusion. Blood samples were taken at 21 different times after the beginning of the infusion and rt-PA antigen levels were measured in plasma using monoclonal antibodies to t-PA. During the infusion there was a rapid increase in plasma rt-PA levels with a maximum of 1200 ± 80 ng/ml (SEM), 450 ± 60 ng/ml and 880 ± 150 ng/ml in groups I, II and III respectively. No significant differences in rt-PA plasma levels could be detected at the different times in the placebo group. The disappearance of rt-PA from plasma after infusion could be described by the sum of three exponentials with α, β and γ half-lives of about 2, 10 and 60 min respectively; the γ-phase seemed to be biological insignificant.

79 Chronic Thrombotic-Thrombocytopenic Purpura: Report of a Case and Review of the Literature

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Thrombotic-thrombocytopenic purpura (TTP) is an uncommon disorder, which usually occurs in young adults. Since its initial report in 1924, over 500 cases have been reported. TTP is characterized by a pentad of findings: fever, neurological abnormalities, renal dysfunction, microangiopathic hemolytic anemia and thrombocytopenia. The histological changes (hyaline thrombi occluding capillaries and arteries) occur in the microcirculation of multiple organs. TTP is usually an acute and fulminant disorder, which leads to death in the majority of patients within 3 months. Chronic TTP is a rare variant of this disorder. The best reported survival for chronic TTP is 18 years. The literature was reviewed and about 15 cases of the chronic variant of TTP were found. Our present patient is a 45-year-old female, whose history of the disorder started in 1974 with the onset of hemolytic anemia and thrombocytopenia. She first visited our clinic in December 1985; the findings were: neurological abnormalities, renal dysfunction, hemolytic anemia with red cell fragmentation and thrombocytopenia. She did not respond to corticoids or antiplatelet agents. Following therapy with fresh frozen plasma the blood counts returned to normal levels, but only for 2 weeks.

80 Protein C Concentrate and Coumarin Necrosis: A Case Report

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A 74-year-old male patient with recurrent thromboembolic episodes developed skin necrosis 3 days after the initiation of phenprocoumon therapy. Heparin treatment was continued, which resulted in severe thrombocytopenia. The patient was found to have decreased protein C (PC) activity, whereas the antigen amounted to 73%. Therefore, oral anticoagulation was restarted and additional substitution with a human PC concentrate was planned. Twelve hours after the first dose of coumarin (prior to the first PC application) there was acute occlusion of the right femoral artery. Two hours after i.v. administration of 2000 units of PC, the popliteal pulse was palpable again and it was not necessary to perform an operation. Oral anticoagulation was continued and 13 000 units PC was given for 5 days in divided doses ranging from 500 to 2000 units. The coumarin-induced decrease of PC in plasma was partially prevented and no new necrosis developed. PC recovery ranged from 20% to 35% (antigen) and 12% to 56% (activity). The PC concentrate was well tolerated. The patient completely recovered from the symptoms of acute ischemia and the skin necrosis cleared. This case report supports the concept that a reduced level of PC activity is pathogenetically involved in coumarin necrosis. Furthermore, PC may have a clinically important profibrinolytic activity.

81 Successful Treatment of Amegakaryocytic Thrombocytopenic Purpura with Cyclosporin A

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We report the case of a 19-year-old man who developed amegakaryocytic thrombocytopenic purpura, probably as a result of treatment with thiazide diuretics. The platelet count was 4000/μl,

haemoglobin 14.2 g/dl and white blood cells 2600/μl with 58% lymphocytes and 35% granulocytes. Bone marrow biopsy showed a marked reduction of megakaryocytes. The karyotype of the bone marrow cells was normal. After unsuccessful treatment with antilymphocyte globulin (ALG; Pressimmun; 1750 mg for 5 days) and corticosteroids, cyclosporin A (CSA; 4 mg/kg per day) was given daily to keep the plasma levels in a therapeutic range. After 3 months the platelet count began to rise above 40000/μl and is now about 60000/μl. Investigation of bone marrow by monoclonal antibody (C 17) showed an increase of megakaryocytes from $<$ 10% before CSA treatment to 30% of the normal value. The incidence of haematopoietic progenitor cells CFU-GEMM, CFU-Mk, BFU-E and CFU-GM from the bone marrow was markedly reduced at the time of diagnosis. In vitro treatment of the marrow cells with CSA prior to culture resulted in increased colony growth, while corticosteroids, two different preparations of ATG and T-cell depletion were ineffective.

82 Platelet Size and α-Granule Secretion in Thrombotic-Thrombocytopenic Purpura and Hemolytic Uremic Syndrome

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Platelet parameters were evaluated in a 35-year-old man with thrombotic-thrombocytopenic purpura (TTP) and a 17-year-old girl with hemolytic-uremic syndrome (HUS) during the acute phase of the disease until the platelet count normalized. The volume distribution in whole blood and platelet-rich plasma was determined using the impedance method. The plasma levels of β-thromboglobulin (βTG) and platelet factor 4 (PF4) were estimated and fibrinopeptide A (FPA) plasma levels reflected the amount of thrombin generation. The acute phase of the disease in both patients was characterized by a platelet population consisting of very small platelets only. With rising platelet count, these were gradually replaced by a normal platelet population. During the acute phase, the volume distribution of the TTP patient showed a peak suggestive of the presence of microaggregates. A bimodal platelet distribution was observed in the HUS patient during normalization of the platelet count. Platelet α-granule secretion was elevated in both patients (maximum βTG: 79 and 137 ng/ml) and slowly returned to normal. FPA plasma levels were high in the patient with HUS. In these two patients with TTP and HUS respectively, there was an abnormal platelet population in the acute phase of the disease, characterized by very small platelets and increased α-granule secretion.

83 Autologous Bone Marrow Transplantation in Acute Leukemia Using Ex Vivo Marrow Treatment with Mafosfamide

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In addition to the well-known advantages of autologous bone marrow transplantation (ABMT) over allogeneic bone marrow transplantation, one of the major problems with ABMT in acute leukemia is the potential presence of clonogenic tumor cells in the remission marrow. The use of ex vivo treatment of the stem cell graft with cyclophosphamide (CY) derivatives, such as mafosfamide, makes it possible to "purge" such tumor cells without destroying most of the early pluripotent stem cells. Eighteen patients with acute myelogenous leukemia (AML) and six with acute lymphoblastic leukemia (ALL) have been transplanted in complete remission (AML: ten patients in CR 1 and eight patients in CR 2 or 3; ALL: 5 patients in CR 1 and 3 in CR 2 or 3). The preparative regimen consisted of total-body irradiation (1320–1440 cGy), superfractionated over 4 days and CY (200 mg/kg given over 4 days). Actuarial analysis of relapse-free

survival (RFS) for patients with AML reveals a plateau of 60%. No case of death resulting from transplantation has been observed. The longest follow-up period is 33.5 months in a patient with AML transplanted in CR 2; the mean period of follow-up is 9 months. Although the duration of follow-up is still too short to draw definite conclusions about the therapeutic benefit of ABMT, there is certainly no doubt that the supralethal preparative regimen can safely be used even in heavily pretreated patients up to the age of 50 years.

84 Autologous Bone Marrow Transplantation in High-Risk Acute Lymphoblastic Leukaemia: Depletion of Leukaemic Cells from the Graft Using Monoclonal Antibodies and Human Complement

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One of the major problems of autologous bone marrow transplantation (ABMT) in leukaemia is the risk involved in retransfusion of clonogeneic leukaemic cells in the graft. Selective depletion of leukaemic cells from the graft using monoclonal antibodies (MOABs) and complement could solve this problem. In 13 of 15 patients with C-ALL a cocktail of three MOABs (VIB Pool) combined with human complement and in 4 of 7 patients with T-ALL the MOABs Campath 1 + 2 combined with human complement were lytic for more than 95% of the leukaemic cells. Stem cell toxicity of all MOABs used was excluded by in vitro culture assays. Two patients received ABMT after the lytic efficacy of MOABs had been demonstrated. The marrow of the first patient with C-ALL was harvested in incipient second relapse and contained 30% leukaemic cells. A purging efficacy of more than 98% was achieved in the graft by incubation with VIB Pool and human complement. The graft of the second patient in CR 1 of a primary high risk T-ALL was incubated with Campath 1 + 2 and autologous complement. Both patients showed complete haemopoietic reconstitution and a CR without further therapy 9 and 6 months after ABMT respectively. The results indicate that (1) depletion of leukaemic cells from the graft is possible using MOABs + complement; (2) haemopoietic reconstitution is not impaired by this treatment; (3) the lytic efficacy of MOABs cannot be predicted by the phenotype of ALL; (4) the lytic efficacy of MOABs should be tested in vitro in each patient before ABMT.

85 Immature B Cells During Reconstitution After Autologous Bone Marrow Transplantation: Correlation with Leukemic Phenotypes

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B-cell reconstitution in bone marrow and peripheral blood was evaluated in 12 patients following autologous bone marrow transplantation (ABMT). All patients had acute myeloblastic leukemia and were in complete remission at the time of transplantation. Conditioning therapy consisted of supralethal fractionated total-body irradiation and high-dose cyclophosphamide. Bone marrow was treated by the active cyclophosphamide derivative 4-HC to purge the marrow from residual leukemic cells. More than 95% of committed myeloid progenitor cells are killed by the 4-HC treatment at the concentration used. Reconstitution of the lymphoid compartment was studied using a panel of lineage-specific monoclonal antibodies (MOABs). Our MOAB HD 37 recognizes the earliest cell surface antigen in B-cell differentiation (B 4, CD 19). Our MOAB

HD 39 is directed against a B-cell-specific antigen (B 3, CD 22) which is present in the cytoplasm of all B cells but is expressed on the cell surface only in late stages of maturation. Anti-B 1 detects a pan-B antigen on the cell surface (B 1, CD 20). Reconstitution of B cells seems to proceed much more slowly than reconstitution of T cells. B cells does not exceed 1% of bone marrow mononuclear cells until 30 days post-ABMT. The first B cells to appear express the HD 37 (B 4) antigen on the cell surface and the HD 39 antigen in the cytoplasm, but are B 1 negative. B 1-positive cells do not exceed 1% until more than 70 days post-ABMT. Thus the immature B cells after ABMT (4-HC) have a $B4^+$ $cB3^+$ $B1^-$ phenotype comparable with the phenotype found in non-T ALL.

86 A New Immunotoxin HD 37-Ricin A Conjugate for the Purging of Bone Marrow for Autologous Transplantation in Patients with B-Cell Malignancies

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A ricin A conjugate of the monoclonal antibody HD 37 (IgG_1) recognizing the B 4 antigen, the broadest B-cell-lineage-specific marker, was studied for purging bone marrow of leukemic B cells in autologous bone marrow transplantation. The cytotoxic effect was assessed in a quantitative and sensitive clonogenic assay using various lymphoma B-cell lines expressing different surface B 4 antigen densities. Treatment with the conjugate (IT) at 10^{-8}M, including ammonium chloride (NH_4Cl) at 10^{-2}M, proved to be effective in eliminating more than 99.9% of the clonogenic tumor cells after a minimal incubation period of 8 h at 37°C. Without NH_4Cl a kill rate of only 75% could be achieved under identical conditions. Flow cytofluorimetry of the surviving clones demonstrated that only the cells weakly expressing the B 4 antigen were resistant. On lymphoma cell lines negative for B 4, a slight decrease in the plating efficiency after IT and NH_4Cl incubation could be observed, reflecting a nonspecific toxic effect. A similar reduction was demonstrated for the normal hematopoietic precursors, as assessed in a mixed colony assay. Despite this suppressive effect on normal in vitro hematopoiesis, the application of the HD 37-ricin A immunotoxin, including NH_4Cl, is a safe and highly effective method for the elimination of B 4-positive leukemic cells from autologous bone marrow in patients with B-cell malignancies.

87 Immunohistology of Liver Biopsy Specimens Following Bone Marrow Transplantation*

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Graft-versus-host disease (GvHD) frequently also affects the liver after allogeneic bone marrow transplantation (BMT). Liver specimens (5 biopsies, 10 autopsies) obtained from 15 patients with acute leukaemia at days 43–512 after grafting of bone marrow from an HLA-identical sibling were analysed immunohistologically using monoclonal antibodies against differentiation and HLA antigens. These specimens were compared with two normal liver autopsy specimens and one hepatic specimen from a neuroblastoma patient following autologous BMT to evaluate specific GvHD-related organ lesions. Histological evidence of liver GvHD was found in 5 patients; 7 patients revealed infectious liver diseases and 3 had nonspecific cholestasis. Due to elevated bilirubin levels GvHD was diagnosed clinically in 9 patients. In all of the patients with histological GvHD, as well as in 5 patients with infectious hepatitis, an increase in the expression

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of HLA class-I antigens was observed in liver cells. Moreover, vascular endothelial HLA-DR/DP antigens were found on Kupffer cells and macrophages, as well as on the majority of lymphocytes of the periportal area. Furthermore, in all of the patients with GvHD as well as the patients that had undergone autologous BMT and 5 patients with infectious diseases, bile duct epithelia revealed focal or generalized expression of HLA-DR/DP but not of -DQ antigens whereas they were negative for all HLA class-II antigens in the 2 normal biopsy specimens. HLA-DQ antigens were also present on rare Kupffer cells and macrophages in all specimens. A slight increase of $T11^+$, $T8^+$, but not $T4^+$ T-cells in periportal areas and between liver cells was observed in all 5 patients with histological GvHD as well as in 4 patients with infectious hepatitis. Between liver cells $T4^+$, $TM3^+$, and Kupffer cells could not be differentiated from infiltrating macrophages due to the lack of specific surface antigens. In 10 of 15 patients who had undergone BMT, partial or complete destruction of sinus endothelia was indicated by lack of response to antibody TÜ 70. These findings indicated immunologically mediated liver injuries after BMT, but did not allow differentiation between GvHD-mediated and infectious lesions.

88 Transplantation of Bone Marrow with a Defined Number of Residual T-Cells

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An attempt was made to define the number of T-lymphocytes in bone marrow grafts preventing graft-versus-host-disease (GvHD) in HLA-identical bone marrow transplantation without further GvHD prophylaxis. The number of T-cells in bone marrow transplants was reduced to less than 1×10^5/kg body weight by the E-rosetting technique. T-lymphocytes were separated from peripheral blood by Ficoll-Hypaque centrifugation and were added to the transplants at a final concentration of $0.5\times10^6-1\times10^6$ cells/kg body weight. All of the 8 patients in the study were promptly engrafted. Two patients had no signs of acute GvHD; five had only diseases of the skin of grade II or less. In one patient GvHD was classified as grade IV. Two patients developed chronic GvHD of the skin: in one of them it was limited (grade I, 1), in the other generalized (grade II), but there was no involvement of other organs. It is concluded from this study that the reduction of T-cells in a bone marrow graft to $0.5-1\times10^6$ cells/kg body weight is not effective in preventing GvHD.

89 T-Cell Clearance by Antibodies with High Affinity for C1q: An Approach to Prophylactic Suppression of Graft-Versus-Host and Host-Versus-Graft Reaction

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Different subclasses of monoclonal antibodies of the same pan-T-cell specificity were tested and it was observed that certain isotypes proved useful for the prevention of graft-versus-host disease (GvHD) if applied in vitro in the absence of heterologous complement. This immunosuppressive potency correlates with the uptake of the C1q complement subunit on antibody-coated T-cells. Mouse and rat monoclonal antibodies of the IgG2a and IgG2b subclasses respectively – both known to exhibit a high intrinsic affinity for the C1q subunit – form stable antibody-C1q complexes on the T-cell surface, whereas the other isotypes to Thy-1 fail to generate these complexes. Obviously, isotypes with a high affinity for C1q caused profound immunosuppression by effectively using the hosts' own effector mechanisms to achieve more than transitory cell elimination. The interpretation of this observation led to the pretreatment of prospective marrow recipients with a single injection of these antibodies before transfusion of fully H-2/I-A incompatible donor marrow (bone together with spleen cells). Like the in vitro treatment, this approach is effective in abolishing acute and chronic mortality of GvHD. In addition, this treat-

ment also suppressed the host-versus graft (HVG) reaction, so that the radiation dose necessary for engraftment of donor cells could be reduced to 6 Gy (600 rad). Our transplantation experiments in the mouse model have shown that: (1) measurement of the uptake of the C1q complement subunit is of value in the prediction of the effectiveness of immunosuppression in vivo; (2) with regard to this in vitro experiment, selected anti-Thy-1 antibodies not only prevented GvHD when a single injection was administered in vivo before marrow transplantation, but also suppressed the HVG reaction, so that the radiation dose necessary for the engraftment of donor cells differing in H-2 and I-A (both haplotypes) major histocompatibility antigens could be reduced to 6 Gy.

90 Ex Vivo Treatment of Bone Marrow Donor Cells with Immunotoxin IT_{101} to Prevent Acute Graft-Versus-Host Disease in Allogeneic Bone Marrow Transplantation

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Acute graft-versus-host disease (GVHD) remains a principal cause of morbidity and mortality in allogeneic bone marrow transplantation. GVHD can affect 30%–70% of the recipients of transplants from fully matched siblings and may be an indirect cause of death in 20%–40% of affected individuals. Certain host characteristics have been associated with an increased risk of GVHD in multiple studies, such as increased host age, clinical status, and nature of the disease. We report our clinical experience of the ex vivo treatment of allogeneic donor marrow using a pan-T monoclonal antibody (T_{101}) coupled to ricin-A chain. Ten patients received allografts from HLA-identical and MLR non-reactive sibling donors. All patients had malignant hematological disease with poor prognosis (acute leukemia in second or third remission, non-Hodgkin's lymphoma stage IV, Hodgkin's disease stage IV, Burkitt's lymphoma stage IV). Most of the patients were at high risk for acute GVHD (older than 30 years and/or subsequent remission). The patients at high risk received marrow treated ex vivo with IT_{101}. The IT_{101} treatment removed 97.5% ± 2.1% of the T-lymphocytes. Engraftment was achieved in these patients within 18 days to reach 500 granulocytes/μl. No severe, acute or chronic GVHD was observed in the group of patients treated with IT_{101}.

91 Graft Rejection After Transplantation of T-Cell-Depleted Bone Marrow

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Forty-two patients received HLA-identical bone marrow transplants after T-cell depletion with Campath 1 and complement as graft-versus-host disease (GVHD) prophylaxis. In 8 patients the graft was rejected (m = day + 46). One primary graft failure was observed. Graft rejection was associated with cytopenia, bone marrow hypoplasia and fever. In six patients rejection was confirmed by the detection of activated recipient lymphocytes. Three patients recovered after the administration of cyclosporin A and prednisolone alone or in combination with in vivo application of the monoclonal antibodies Campath 1 + 2. Retransplantation without T-cell depletion was performed in five patients who had rejected the graft and in the patient with primary graft failure. Five patients were engrafted; two of them are currently still alive. Four patients died following infectious diseases, acute GVHD or VOD. There was no correlation between the occurrence of graft rejection and conditioning regimen, underlying disease, age, sex and ABO compa-

tibility of donor and recipient, transplanted cell dose or efficacy of T-cell depletion. The absence of risk factors for rejection indicates the need for prophylaxis in all patients receiving a T-cell-depleted graft. Prophylaxis requires additional immunosuppression, which could be achieved by in vivo application of anti-T-cell monoclonal antibodies.

92 Haemopoetic Reconstitution After Allogeneic Bone Marrow Transplantation

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Haemopoietic reconstitution after allogeneic bone marrow transplantation was studied in 24 patients receiving methotrexate (MTX) for graft-versus-host disease (GVHD) prophylaxis and in 35 patients receiving a T-cell-depleted bone marrow transplant. In the MTX group the number of transplanted nucleated cells, the number of transplanted granulopoietic progenitors (CFU-GM) and the number of transplanted erythroid progenitors (CFU-E) correlated significantly with the time required until granulocyte and reticulocyte recovery was achieved. In contrast, in the Campath group only the number of nucleated cells and the number of transplanted erythroid progenitors (BFU-E and CFU-E) correlated significantly with the time to reticulocyte recovery. There were wide variations in granulocyte recovery in the Campath group. Retrospective analysis revealed that delayed granulocyte recovery is associated with late rejections, viral infections and early relapses. In both groups analysis of the reconstitution of progenitors (CFU-GM) showed decreased incidences in the early phase (up to 3 months) after bone marrow transplantation, followed by a normalization of progenitor incidences in long-term survivors in the Campath group only.

93 "Minor Transplantation Antigens" Defined by Graft-versus-Host (GVH) Reactive T-Cell Lines Derived from Peripheral Blood Before Clinical Manifestation of GVHD

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Using purified interleukin 2 (IL 2) in vivo activated T cells could be propagated from peripheral blood mononuclear cells (PBMCs) derived 1–2 days prior to the clinical manifestation of acute (GVHD) in patients after allogeneic HLA-compatible bone marrow transplantation (BMT). These uncloned T-cell lines were tested for their proliferative (PLT) and cytotoxic response to PBMCs from the BMT donor and recipient isolated before and BMT as well as to PBMC from available family members and normal allogeneic donors, including other BMT donor-recipient pairs. The dominant T-cell subpopulation in these lines was CD 4-positive and mediated PLT rather than allospecific cytotoxic function in vitro. Interestingly, the PLT specificity by 6 out of 8 T-cell lines did not segregate with certain HLA haplotypes since neither parent gave high restimulation values. In contrast, certain siblings typed positive for the antigen(s) which had apparently caused sensitization in vivo. Thus most GVHD-specific T-cell lines recognized novel antigenic determinants possibly resulting from transcomplementation and which can be expressed on PBMC of the progeny – not on parental cells. Responses to such "hybrid antigens" were restricted by HLA class-II antigens, since the in vitro response could be blocked by specific monoclonal antibodies. Such T-cell lines derived from in vivo activated T-cell populations can be utilized for selecting "minor-transplantation antigen"-compatible BMT donors, if more than one HLA-identical sibling is available.

94 Relevance of Cytogenetic Analysis in Leukemic Relapse Following Allogeneic Bone Marrow Transplantation

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Following allogeneic bone marrow transplantation (BMT), 46 patients were analyzed cytogenetically. In 5 patients with acute leukemia karyotype analysis was carried out during relapse after BMT. Four showed only metaphases of the donor, but 1 patient had metaphases of both donor and recipient karyotype in the bone marrow. Concomitant analysis of Y-chromatin and PGM-1 isoenzyme type revealed that the leukemic blasts at time of relapse were the recipient type in all cases. Thus we repeated cytogenetic analysis with PHA stimulation or MTX synchronization and found metaphases of the recipient karyotype in 2 patients. In 2 of 15 patients with chronic myelocytic leukemia, Ph[1]-positive metaphases were detected after BMT without clinical evidence of relapse. Our results suggest that cytogenetic analysis of direct bone-marrow preparations after BMT alone is not sufficient when differentiating donor and recipient type in acute leukemic relapse. On the other hand, it is capable of detecting "early" abnormalities in CML patients after BMT.

95 Polymorphic Enzymes for Investigation of Chimerism and Leukemic Relapse After Allogeneic Bone Marrow Transplantation

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Proof of chimerism and the origin of leukemic relapse can be obtained independent of the proliferative state of cells, if the donor and recipient differ with regard to polymorphic enzymes (phosphoglucomutase 1, phosphoglucomutase 3, acid phosphatase, glutamate pyruvate transaminase, esterase D, glyoxalase I, adenosine deaminase and adenylate kinase). The electrophoretic pattern of these isoenzymes provided valuable information in 36 of 51 patients; most frequently helpful was the pattern of phosphoglucomutase 1 (22/51). Twenty-four patients were studied repeatedly; five of these patients had a mixture of donor- and host-type cells. The recurrence of host-type cells was associated with leukemic relapse in four patients and was restricted to the cell line of the leukemic clone. In one patient with C-ALL, mixed chimerism was repeatedly found in all cell lines with the exception of T-lymphocytes, which were exclusively of donor type. In this patient mixed chimerism has persisted for more than 1 year without evidence of leukemic relapse. Our results indicate that the recurrence of leukemia is not necessarily associated with mixed chimerism and also that mixed chimerism does not imply that a relapse is impending.

96 Identification of the Origin of Blood Cell Populations Using DNA Sequence Polymorphisms After Allogeneic Bone Marrow Transplantation

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In order to document and characterize engraftment after bone marrow transplantation (BMT), donor and recipient cells must be distinguished from each other. In six patients in whom trans-

plantation was successful, DNA sequence polymorphism analysis on PHA blasts was employed for this purpose with HLA-identical transplant combinations. A selected panel of five cloned DNA probes (pDP 34, PAW 101, pAT 3, pGK, pIVS 2) and their associated sequence polymorphisms made it possible to distinguish unequivocally between subpopulations of patient and donor cells. In addition, two mini-stellite core probes (15.11.4 and 4.3 from A. J. Jeffreys) were employed as individual genetic fingerprints in these BMT cell-DNA samples for examination by DNA restriction enzyme digestion and Southern blot hybridization. Using this method, it was possible to detect mixed lympho-hematopoetic chimerism in two patients. The same approach, employing this molecular immunological method, is being utilized now on over 23 PHA blast cell populations from BMT patients. It is also expected that these techniques will enable earlier detection of a leukemic relapse or the occurrence of a secondary lymphoma after allogeneic BMT.

97 Ten Years' Experience of Clinical Bone Marrow Transplantation

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Since the inception of our program in May 1976, 360 patients with hematologic disorders have undergone bone marrow transplantation (BMT) at the City of Hope National Medical Center. The program has grown continuously, with 11 patients receiving transplants during the 1st year and 67 patients being treated during the 10th year. The majority of patients ($n = 335$) had hematologic malignancies; 244 had acute leukemia (AL), 55 had chronic granulocytic leukemia (CGL); and 36 had other hematologic malignancies, such as advanced Hodgkin's disease, non-Hodgkin's lymphoma, and myelodysplastic or myeloproliferative disorders. In addition, 25 patients with severe aplastic anemia (SAA) were accepted for BMT. After myeloablative/immunosuppressive radiochemotherapy, 351 patients received bone marrow from histocompatible sibling donors, 8 patients marrow from syngeneic donors and 1 patient from a phenotypically matched paternal donor. Graft-versus-host disease (GVHD) prophylaxis/therapy consisted of methotrexate/prednisone or cyclosporin A/prednisone. At the time of analysis (end of April 1986), 168 patients were alive and in complete continued remission (CCR), of whom 121 patients had been followed for more than 1 year; maximum follow-up has reached the 10th year. The following disease-free survival rates have been achieved: 51% – 67% of patients with AL in first remission or CGL in chronic phase; 19% – 44% for patients who had relapsed at least once with AL or had accelerated/blastic CGL; 50% for patients with the various other hematologic malignancies:'; and 73% for patients transplanted for SAA. The leading causes of failure have been recurrent malignancies and interstitial pneumonia following GVHD. The data of this ten year study will be presented in detail.

98 Bone Marrow Transplantation: Results Obtained in Essen

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At the West German Tumor Centre in Essen 131 allogeneic, 9 isologous, and 6 autologous bone marrow transplantations (BMT) had were performed up to April 1986. The main indications were severe aplastic anemia ($n = 19$), acute leukemia ($n = 79$), and chronic myeloid leukemia ($n = 44$). In cases of severe aplastic anemia, cyclophosphamide alone or in combination with total nodal irradiation ($n = 4$) was used for conditioning. For acute or chronic leukemia the pretreatment consisted of cyclophosphamide and total body irradiation; 12 patients received cyclo-

phosphamide and busulfan without irradiation. Methotrexate was given as graft-versus-host disease (GVHD) prophylaxis. In all patients, the gastrointestinal tract was decontaminated and they were strictly isolated. Following allogeneic BMT, 10 of 15 (66.7%) patients with severe aplastic anemia survived; for syngeneic BMT the figure was 4 of 4. The median disease-free survival in both groups was 34 and 16 months, respectively. Out of 24 patients with acute leukemia grafted during relapse, only 2 survived. Grafting during remission provided superior results; out of 55 patients, 30 survived (54.5%). Of 29 patients with acute myelogenous leukemia, 16 (55.2%) survived disease free if the allogeneic transplantation was performed during the first stage of remission. The median disease-free survival in this group was 43 months. In chronic myeloid leukemia, BMT was performed during the first chronic phase, in the phase of acceleration or during the second chronic phase after acute transformation. The survival rates were 15 of 32 (46.9%), 4 of 7 (57.1%) and 0 of 3 respectively. The median disease-free survival was 9 and 16 months respectively. The actuarial risk of developing GVHD in severe aplastic anemia and acute leukemia was 24%. In chronic myeloid leukemia patients the risk of GVHD appeared to be 2.6 times higher. The main cause of death was interstitial pneumonia, from which 36 patients died.

99 Side Effects Caused by Cyclosporin A in Bone Marrow Transplantation

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During the last 4 years, 48 patients (10–50 years old) have been given cyclosporin A for the prevention of graft-versus-host disease (GVHD) following allogeneic bone marrow transplantation. Despite this therapy 25% of the patients developed GVHD stage III or IV, which was treated with steroids. The intended dose of cyclosporin A (5 mg/kg body wt. twice a day) had to be reduced in all patients because of nephrotoxicity (increase in serum creatinine) or, less frequently, because of hepatotoxicity (increase in bilirubin). The aim of reducing the cyclosporin A dose was to achieve serum levels between 100 and 400 ng/ml. Serum creatinine should not exceed 1.5-fold the baseline level. As soon as cotrimoxazole was administered in addition to cyclosporin A, the nephrotoxicity of the drug increased. Between 1 and 2 years after bone marrow transplantation, the immunosuppressive therapy could be reduced and after a while it was stopped. Up to now we have not seen any positive evidence that cyclosporin A treatment causes permanent, severe disease. Further observations will be presented and analysed.

100 Fractionated Total-Body Irradiation and High-Dose VP 16 Before Allogeneic Bone Marrow Transplantation for Poor-Risk Acute Leukemia*

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High-dose VP 16 in combination with total-body irradiation (TBI) is becoming increasingly recognized as an effective conditioning agent before bone marrow transplantation (BMT) (Blume et al.: Blood 66 [Suppl 1] 249 a, 1985). Eleven patients with acute myelogenous leukemia ($n = 7$), acute lymphoblastic leukemia ($n = 2$), biphenotypic leukemia ($n = 1$), and high-grade malignant lymphoma ($n = 1$) received fractionated TBI (6×200 rad) from day -7 to day -5 followed by infusion of VP 16 on day -3. Patients were given 50 mg/kg ($n = 3$), 60 mg/kg ($n = 2$), or 70 mg/kg ($n = 6$) of the drug. Bone marrow from HLA-identical donors was transplanted on

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day 0. The toxic side-effects of the fTBI/VP 16 regimen mainly consisted of severe oral mucositis in all patients. The incidence and severity of other side-effects observed did not differ from our experience with the standard fTBI/cyclophosphamide protocol. At the time of BMT, 5 of 11 patients were refractory to conventional chemotherapy, 3 patients were in ≥ 3rd remission and two children were in early relapse. A woman with acute myelogenous leukemia in 1st remission was given VP 16 because cardiac disease precluded the use of cyclophosphamide. Seven patients are still alive and free of disease 14–436 days (median: 150 days) post-BMT. Three patients died following BMT-related complications (graft-versus-host disease, interstitial pneumonia, infection); so far only one patient has had a relapse and this patient died 122 days after BMT.

101 Successful Bone Marrow Transplantation Across HLA Barriers Using an Improved Conditioning regimen

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In most centers the treatment of leukemia and severe aplastic anemia by bone marrow transplantation has been limited to patients with an HLA-identical sibling as donor. We have studied a conditioning regimen for HLA-mismatched transplantation derived from studies in DLA-haploidentical canine littermates. The regimen consists of hypofractionated total-body irradiation, buffy coat transfusion and cyclophosphamide. The combination of cyclosporin A and a short course of methotrexate was given as prophylaxis of graft-versus-host disease (GVHD). So far three patients with advanced leukemia have been treated according to this regimen and grafted with marrow from HLA-mismatched relatives. Presently, they are alive and well more than 1, 4 and 10 months after grafting respectively. This is in contrast to previous experiences in 8 patients who had been grafted with marrow from donors other than HLA-identical siblings and died within 4 months after grafting. The causes of death were infections prior to engraftment, persistence of leukemia, graft failure and infections following GVHD. This improved conditioning treatment means that more patients may benefit from bone marrow transplantation.

102 Bone Marrow Transplantation in Partial HLA Compatibility

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Although the significance of HLA incompatibility regarding the etiology of graft rejection and graft-versus-host disease (GVHD) is well defined, there have only been a few reports concerning long-term disease-free survival rates in patients who have undergone bone marrow transplantation (BMT) with partially HLA-compatible marrow from a family member. Since 1977, 11 patients (5 females, 6 males; median age, 29 years) have received bone marrow grafts that were partially compatible for HLA (related donors, $n = 10$; unrelated donors, $n = 1$). The indications for BMT were[1]: AML/ALL recurrence ($n = 3$), AML 1st or 2nd remission ($n = 2$), CML chronic phase ($n = 2$), CML accelerated or 2nd chronic phase ($n = 3$), SAA ($n = 1$). GVHD prophylaxis was performed using methotrexate according to the Seattle regimen (2 patients received cyclosporine A concomitantly). Engraftment was documented in all cases. Of the 11 patients, 5 (45.5%) exhibited a median disease-free observation period of 44 months (2–56 months).

[1] AML = acute myeloblastic leukemia; ALL = acute lymphocytic leukemia; CML = chronic myelocytic leukemia; SAA = severe aplastic anemia

GVHD occurred in 4 patients (36%) (acute grade II $n = 2$, acute grade IV $n = 1$, de novo chronic $n = 1$). The principal cause of death was interstitial pneumonia (IP), which had developed in 5 patients (45%) (idiopathic IP $n = 4$, CMV-IP $n = 1$). This was associated with GVHD in only one case. In patients without HLA genotypically identical bone marrow donors, a 10% probability exists that a haploidentical partially HLA-compatible donor will be found within the family (i.e. one or two antigen disparities on one D/DR identical chromosome). Based on the results of studies conducted to date, BMT with partially compatible marrow (especially in prognostically favorable subgroups) appears to lead to comparable disease-free survival rates compared with the transplantation of bone marrow from genotypically identical donors.

103 Bone Marrow Transplantation in the Fifth Decade of Life

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In considering the age distribution of malignant hematologic diseases, the question is raised whether bone marrow transplantation (BMT) in patients over 40 years of age is associated with acceptable risks and should therefore be regarded as the treatment of choice for patients with histocompatible donors. Allogeneic BMT was performed in 13 patients (8 females, 5 males) over the age of 40 for acute leukemia (AML relapse, ALL 4th remission, $n = 1$; AML 1st remission, $n = 3$) and chronic myelocytic leukemia (CML) (chronic phase, $n = 4$; accelerated or 2nd chronic phase, $n = 4$). The median age at the time of transplantation was 43 years (40–48 years). Of 13 patients, 8 (60%) are still alive with a median disease-free observation period (DFS) of 22 months (2–58 months). Of the patients grafted in the 1st remission of AML or in the chronic phase of CML, 70% (5 of 7) are still alive with a median DFS of 30 months (9–58 months). Acute graft-versus-host disease developed in 3 of 13 patients (23%). The principal cause of death was interstitial pneumonia (IP), which occurred in 4 of 13 patients (31%) (idiopathic IP, $n = 3$; CMV-IP, $n = 1$). In conclusion, these preliminary results justify the wider employment of BMT in patients over 40 years of age.

104 Bone Marrow Transplantation in Fanconi's Anemia

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Six children with Fanconi's anemia have been transplanted during the last 2 years at the Children's Hospital in Ulm, using HLA-identical siblings. The conditioning protocol consisted of cyclophosphamide (20 mg/kg) and thoracoabdominal irradiation (5 Gy) (Gluckman et al., Semin Hematol 21: 20–26, 1984). Three patients received unseparated bone marrow and the other three patients T-cell depleted marrow by E-rosetting for graft-versus-host disease prophylaxis. Donor-derived, hemopoietic reconstitution was prompt in all patients receiving an unseparated marrow graft. After T-cell depletion only one engraftment and two graft failures occurred. Retransplantation with unseparated bone marrow from the same donor was successful in one case; the other child died of a fungal infection without signs of engraftment. A long-term persistence of functioning, host-type lymphocytes could be detected throughout the posttransplant course in all children, independent whether or not engraftment could be achieved.

105 Repeated High-Dose Cyclophosphamide Administration in Bone Marrow Transplantation: Exposure to Activated Metabolites

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Blood levels of cyclophosphamide (CP) and activated metabolites were measured in 11 patients undergoing conditioning chemotherapy prior to total-body irradiation (TBI) for bone marrow transplantation for 2–4 days. The level of CP in urine was determined in 5 patients. CP half-lives decreased after pretreatment from an average of 7.1 h on the 1st day to 5.53 on the 2nd day ($P < 0.005$) and 4.33 h on the 4th day ($P < 0.005$). No characteristic changes in urinary excretion could be observed. At the same time, exposure to non-protein-bound activated metabolites increased from 10.5 to 19.5 and 26.0 nmol *h/ml respectively ($P < 0.005$ and $P < 0.04$). Thus, in contrast to in vitro and animal studies no evidence of inhibition of activating enzymes could be found. On the contrary, pretreatment seems to enhance the production of the cytotoxic metabolites. The possibility that these changes may be explained by enzyme induction and by the role of saturated protein binding sites is discussed. Exposure to active metabolites might be altered by dose-splitting or even a change in the duration of the infusion. The data of an ongoing study with cyclophosphamide after TBI are also presented.

106 Microbiological Investigations in Patients Undergoing Bone Marrow Transplantation

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In 20 patients, the effectiveness of gastrointestinal and topical decontamination as well as of isolating the patients in a laminar airflow (LAF) unit, was studied. On a weekly basis, surveillance cultures were made from throat, sputum, mouthrinse, axilla, hand, inguinal, perianal, vulva or prepuce, urine, feces and from the point of entry on the skin after implantation of a catheter. Environmental control samples were taken from the medical ward outside the LAF units and from the LAF unit itself when accommodating a patient. The results of the environmental controls showed that the protection of the patient is of major significance; however, complete protection is not possible. There are two weak points in the isolation measures: the opening of the tent with a free entry way into the tent itself and the water delivery system. By using appropriate decontamination measures, it is possible to greatly reduce the number of bacteria and species of the normal human commensal flora, with the exception of the oropharynx. All individual patients who had flucloxacillin-resistant coagulase-negative staphylococci and/or *Candida albicans* continued to show the presence of these organisms all the time. A meaningful observation was the detection of Clostridium difficile and/or toxin B in eight patients at the beginning of the individual observation period. Three out of 15 episodes of fever were attributable to bacterial infections with gram-positive organisms. All three pathogens had already been isolated in the preceding surveillance cultures. As was demonstrated by the low incidence of infectious complications in these patients, gastrointestinal decontamination and topical decontamination of the skin as well as reverse isolation in LAF units provide effective protective measures for patients undergoing bone marrow transplantation.

107 Fetal Liver Transplantation in Dogs: A Model for Hemolymphopoietic Restoration with a T-Cell-Depleted Stem Cell Graft

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Fetal liver transplantations (FLT) were carried out in 25 beagles under various conditions. Graft recipients were prepared with fractionated total-body irradiation (TBI) of 3×6 Gy or 2×6 Gy and rescued with cryopreserved fetal liver cells from 43- to 46-day-old or from 51- to 52-day-old, DLA-identical siblings or DLA-haploidentical, homozygous half-siblings. In all groups, grafts contained comparable numbers of granulocyte-macrophage progenitor cells. Initial engraftment was achieved in all dogs, with rapid restoration of hemopoiesis in most of them. However, low TBI dose and DLA haplotype disparity between donor and recipient resulted in graft failure in 1 of 3 and 2 of 9 recipients, respectively, within 10–16 days of treatment. Lectin-responsive host-type lymphocytes circulated for more than 5 weeks. Young donor age and, although less marked, reduced TBI dose resulted in protracted recovery of circulating granulocytes, platelets, and both total lymphocytes and T- and B-cells. Delayed cutaneous hypersensitivity reactions were normal 1 year after FLT, but specific antibody responses to sheep red blood cells and to recall antigens remained defective in some cases. In mixed leukocyte cultures, chimeric lymphocytes were tolerant to host antigens. Nonetheless, 10 of 25 recipients manifested clinical and histological abnormalities compatible with low-grade graft-versus-host disease (GVHD). Thus, FLT in dogs could successfully be carried out, even across a DLA haplotype barrier, without severe GVHD. However, low TBI dose, incomplete DLA match, and young age of the fetal donor may compromise the outcome, resulting in graft failure or protracted restoration of hemopoiesis and immune functions.

108 An Experimental Model for Studying the Host-Versus-Graft Reaction in Allogeneic Bone Marrow Transplantation (BMT): Implications for Human T-Depleted BMT

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Rats were irradiated lethally (12.3 Gy) or sublethally (10.5 Gy) and received an allogeneic skin graft as an indicator for host-versus-graft reactions. Irradiated rats receiving only the allogeneic skin graft retained it for a median of 28 days (10.5 Gy) or until death (12.3 Gy). The additional i.v. injection of bone marrow from the irradiated (GVHR-inactive) skin donor induced skin graft rejection within 9 days after lethal irradiation. The maximal effect was seen when the bone marrow of the irradiated skin donor was injected within 24 h after total-body irradiation (TBI), and fell to zero by day 4. In contrast, 4×10^8 nonirradiated (GVHR-active) BM cells led to the skin graft surviving until the death of the recipient. Treatment of the recipient with methotrexate or cyclosporin A almost abolished the effect of injecting irradiated BM, whereas prednisone was ineffective. It can be concluded that: (1) the strong classical immune reactivity that can be induced by the injection of allogeneic BM persists for several days after lethal TBI; and (2) if GVHR-inactive BM is transplanted, additional measures are needed to sustain engraftment. Our data suggest that the interval between TBI and BMT should be extended and that immunosuppression following BMT may be helpful.

109 Restoration of Normal Hemopoiesis Through Bone Marrow Transplantation in a Lactate Dehydrogenase Mouse Mutant with Hemolytic Anemia

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A codominant mutation of the lactate dehydrogenase A (LDH-A, muscle type) locus in the C3H/El mouse causes severe hemolytic anemia with a red blood cell (RBC) count of 50% of wild-type (+/+) mice, reticulocytosis of 95.3% (2.3% in +/+ mice), and splenomegaly. Several bone marrow transplantations were performed to answer the following questions: (1) Is the hemolytic anemia transplantable, i.e., does the mutant hemopoiesis have a normal repopulating capability? (2) Is the hemolytic anemia curable, i.e., does the mutant environment influence normal hemopoiesis? A dose (8 Gy) sufficient to cause complete hematological toxicity was used to irradiate +/+ mice, and the blood cell mass was reconstituted with 3×10^6 mutant bone marrow cells (BMCs). All of the above-mentioned characteristics of the mutant could be transplanted. The mutants turned out to be extremely radiosensitive ($LD_{50/30}$ 4.4 Gy vs. 7.3 Gy for +/+ mice). They died within 5–6 days after irradiation with only 5 Gy due to rapid depletion of short-lived RBCs. On the other hand, 4.4 Gy was not sufficient to establish a permanent chimerism with +/+ BMCs. Therefore, the mutants received a blood transfusion (more than 5.5×10^9 +/+ RBCs/mouse) 3 days before, and 7 and 11 days after lethal irradiation (8 Gy) and hematological reconstitution (20×10^6 BMC/mouse). The chimerae were examined up to week 16. They exhibited RBC and reticulocyte counts as in +/+ mice and a normal LDH activity in peripheral blood. The degree of radiosensitivity was also normalized.

110 The Effect of Ether-Lipid Derivatives on Tritiated Thymidine Uptake and Trypan Blue Dye Exclusion In Vitro of Non-Neoplastic Bone Marrow Cells and Leukemic Cells from Humans

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The effects of the alkyl-lysophospholipid derivatives (ALPs) 1-0-octadecyl-2-0-methyl-rac-glycero-3-phosphocholine (ET-18-OCH), and 1-0-hexadecyl-sn-glycero-3-phospho-trimethyl-ammonio-hexanol, the platelet-activating factor (PAF) analog 1-0-octadecyl-2-acetamide-rac-glycero-3-phosphocholine, the thioether-lysophospholipid derivative (TLP) BM 41.440 and the ether-linked lipoidal amine CP-46,665 on tritiated thymidine uptake and trypan blue dye exclusion were tested in vitro in various freshly explanted cell samples from human non-neoplastic bone marrow (BM) and human leukemias. In both assay systems a dose range of between 1 and 20 μg/ml of the compounds was tested after 24, 48, and 72 h of coincubation with the cells. Trypan blue dye exclusion revealed statistically significant selective cytotoxicity on a single cell level towards leukemic cells for three compounds with the order of selectiveness: ET-18-OCH_3 > BM 41.440> PAF analog. CP-46,665 was the most toxic compound, but did not reveal significant differences between non-neoplastic BM and leukemic cells when added in concentrations greater than 1 μg/ml. The trimethyl-ammonio-hexanol compound showed only minor activity in the majority of tests. The tritiated thymidine uptake showed only selective antiproliferative effects towards leukemic cells of ET-18-OCH_3, and in a few exceptions within the dose-time frame tested of BM 41.440 and CP-46,665. All compounds tested except the trimethyl-ammonio-hexanol compound were also active in this assay (inhibition of uptake > 50%). Based on these results, ET-18-OCH_3, BM 41.440 and the PAF analog are recommended for experimental bone marrow purging.

111 Cell-Cycle-Phase-Specific Activity of Alkyl-Lysophospholipids on Lymphoblastoid Cell Lines of Human Origin

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The value of alkyl-lysophospholipids (ALPs) for purging leukaemic marrow prior to autologous transplantation has been discussed controversial (Vogler et al., Exp Hematol 12: 569, 1984; Andreesen et al., Blut 51: 194, 1985). We studied the effect of the ALP "BM 41 440" on lymphoblastoid cell lines using a supravital dye (Hoechst 33342) and flow cytometry. Lymphoblastoid cell lines (Reh, subclones of Reh, Im 9) were compared with other cell lines of high (HL 60) and low (K 562) sensitivity to ALP. The following incubation conditions were studied: concentration of cells, concentration of ALP, duration and temperature of incubation. For the inhibition of cell growth, an incubation time of at least 24 h was required and this effect was enhanced at high temperatures. The inhibition was most marked for cells in S-phase and this was shown remarkably clearly in histograms obtained by flow cytometry. The fate of cells that survive in G_0-phase is unknown. Clonogenic assays will be necessary for further clarification of this question.

112 Elimination of Leukemic B Cells in Autologous Bone Marrow Transplantation with the Monoclonal Antibodies HD 237 (Anti-B 4) and B 1 with Complement in Patients with B-Cell Malignancies

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A sensitive and quantitative clonogenic assay was used to study different variables, such as number of antibody-complement incubations, effect of excess bone marrow cells on the efficiency of eliminating leukemic B cells from bone marrow with anti-B 1 and HD 237 (anti-B 4) by a complement-mediated lysis. These cell lines are derived from non-Hodgkin lymphoma patients and co-express B 4 and B 1 with different densities. We demonstrated that the combination of anti-B 1 and HD 237 was more effective than any single antibody. This mainly reflects the varying antigen densities for B 1 and B 4 on different cell lines as seen by flow cytofluorometric analysis of the surviving clones. Because of the individually varying antigen density pattern, we strongly recommend the combination of antibodies for bone marrow purging. Additionally, HD 237 is very important for the purging of early B-cell malignancies which are B 4 positive and B 1 negative. With three incubation cycles an elimination of 99.9% clonogenic tumor cells is achieved. In a mixed colony assay the antibody-complement treatment failed to demonstrate any toxicity to either single lineage or multipotential normal hematopoetic progenitors. Subsequently, the biological properites of the surviving cells expressing a low antigen density especially with respect to the origin of the early clonogenic tumor cell are currently being investigated.

113 Enrichment of Stem Cells From Bone Marrow and Peripheral Blood by Counterflow Centrifugation With the Beckman Elutriator System

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It was recently shown by our group that stem cells from the peripheral blood are capable of repopulating the haematopoietic system of patients who have been conditioned for autologous

transplantation. For autologous bone marrow transplantation in advanced Burkitt-type lymphomas we have also used purged stem cell preparations. In the present study, we used counterflow centrifugation to enrich by different in-vitro culture systems (CFU-C and BFU-E) those cell fractions from a suspension of human blood leucocytes that show the potentialities of haematopoietic progenitors. A Beckman J-21 B centrifuge equipped with an elutriator rotor was used with a newly developed separation chamber, in which up to 5×10^7 cells can be separated without clumping. With this system we were able to show that the above-mentioned cell fractions can be separated from lymphocytes and from granulocytes. Since it is possible with our separation chamber to enrich haematopoietic stem cells on a large scale, this principle will perhaps be useful in several clinical applications, such as in T-cell depletion for mismatched allogeneic BMT or in stem cell purification for autologous transplantations.

114 BFU-E Growth During Acute Graft-versus-Host-Reaction (GVHR) and Virus Infections After Allogeneic Bone Marrow Transplants (BMT)

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Six patients had received allogeneic bone marrow transplants in accordance with the Seattle protocol for various basic illnesses. Their hematological and immunological reconstitution was monitored by means of graft-versus-host prophylaxis with cyclosporin A or methotrexate for up to 24 months. If a GVHR occurred, high-dosage prednisolone was also administered. In parallel to the determination of CD 4- and CD 8-positive T-lymphocytes from the peripheral blood in complications such as infections and/or GVHR, and corresponding to the described stimulating effect of CD 4-positive cells on hematopoesis, patient blood samples were obtained at various times and investigated for stimulating substances for erythropoesis (BPA) in the BBU-E system. After BMT, as of day 12–14 an increase in CD 4-positive lymphocytes was observed, which occurred in parallel with an increase in peripheral leukocytes and led to a CD 4-CD 8 relationship of 2.0. A return of the CD 4/CD 8 relationships to a value of around 0.5 occurred in cases of acute GVHR and/or virus infections. Patient sera stimulated the growth of BFU-E exclusively in the regeneration phase in cases of anemia still requiring transfusion, without correlation to the quantity and relationship of CD 4- and CD 8-positive T-lymphocytes. Although an acute GVHR and/or virus infection, mediated by a CD 4-positive inducer population, led to a CD 8-positive effector population, it was not possible to demonstrate produces by peripheral lymphocytes – either stimulating or restrictive – in the growth of BFU-E.

115 Incidence of Graft-Versus-Host Disease After Bone Marrow Transplantation in 42 Patients with Chronic Myeloid Leukemia

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Between April 1982 and April 1986, 42 allogeneic bone marrow transplantations (BMTs) were carried out at the West German Tumor Center for chronic myeloid leukemia (CML); 32 patients were in the chronic phase, 7 patients in the accelerated phase and 3 patients in the second chronic phase following acute lymphatic transformation. The median age of the patients was 32 years (14–48 years). Over the same time period allogeneic BMT was performed in 45 patients with acute leukemia (AL). The two groups were similar as to age, sex, conditioning regimen and prophylaxis of graft-versus-host disease (GVHD) with methotrexate. Retrospective analysis showed that CML patients were at a higher risk of developing GVHD as compared with patients with AL. The cumulative probability of developing GVHD for patients "at risk" was 68% in the CML group versus 28% in the AL group ($P < 0.0002$; log-rank test). The severe forms of acute GVHD (clinical grade III–IV) amounted to 25% in the CML group of patients compared

with only 6% in the AL group. Multivariate analysis (Cox model) showed that the factor "diagnosis of CML" was associated with an increased risk for GVHD. The cause of the higher GVHD incidence remains unclear. In conclusion, we feel that GVHD prophylaxis in patients suffering from CML should be intensified.

116 Kinetics of Restoration of Interferon Production After Bone Marrow Transplantation in Man

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Peripheral blood mononuclear cells (PBMCs) from patients that had undergone bone marrow transplantation (BMT) were studied for their capacity to produce interferon (IFN) in vitro. The basal and interferon-stimulated 2–5 A synthetase activity was also investigated as an indicator of the cells' ability to respond to exogenous IFN. Interferon production stimulated in vitro was not detectable in most patients without graft-versus-host disease (GVHD) until 7 months after grafting. The impairment of IFN production could not be restored by adding IL 2 in vitro. In contrast to patients without GVHD, PBMCs from patients with GVHD produced stable high levels of IFN when stimulated in vitro. The basal activity of 2–5 A synthetase in PBMCs from patients without GVHD was normal, but during the first 3 months after BMT, the PBMCs did not respond to stimulation with IFN-α. The basal activity of 2–5 A synthetase in PBMCs from patients with GVHD was 3 times higher than normal.

117 Immunoreconstitution Following Allogeneic Bone Marrow Transplantation Using Donor Bone Marrow Pretreated with Immunotoxin-101

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Bone marrow transplantation (BMT) can cure patients with leukemia and aplastic anemia. Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after BMT and limits its use. We are attempting to prevent GVHD by depleting the marrow of mature T lymphocytes, using immunotoxin-101 (IT-101) prior to infusion. The effect of T-cell depletion on the speed and efficacy of lymphocyte reconstitution is unknown. We have tested the peripheral lymphocytes of patients receiving untreated bone marrow or bone marrow depleted of T-cells to determine whether there are differences between the two groups of patients when examining the absolute numbers of lymphocytes bearing surface Ig, T 3, T 4, T 8, Leu 7, and Leu 11 antigens, as well as the ability of T-lymphocytes from the patients to support PWM-stimulated Ig synthesis. Absolute B-lymphocyte numbers tend to be lower in the T-cell-depleted patients, as do numbers of T 4-bearing cells. There is no clear difference between the two groups in the ability of T-cells to support immunoglobulin synthesis. Thus, although cell numbers are abnormal longer in the T-cell-depleted patients, functional recovery seems not to be impaired.

118 The Influence of T-Cell Depletion on the Occurrence of Infections After Bone Marrow Transplantation

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In allogeneic bone marrow transplantation (BMT), incubation with Campath 1 (monoclonal anti-lymphocyte antibody) results in the transplantation of a T/B-cell-depleted graft. Up to now

data concerning infections after T-depleted allogeneic BMT are very limited. Thirty-four patients with leukaemia were treated by BMT using Campath 1 for the prevention of graft-versus-host disease (GVHD). Infection prophylaxis in all patients consisted of either total decontamination in strict reverse isolation ($n = 27$) or selective decontamination ($n = 7$). In addition, cytomegalovirus (CMV)-hyperimmunoglobulin and cotrimoxazole were given prophylactically. GVHD occurred in 18% of patients and 53% remained free of fever during the period of severe granulocytopenia ($< 1000/\mu l$). In contrast 68% ($P < 0.0001$) of patients in our historical control group without T-cell depletion ($n = 41$) developed GVHD and only 24% remained free of febrile episodes ($P < 0.02$). The incidence of late infections (> day + 100 after BMT) was also reduced in BMT by using Campath 1. In conclusion, the significant reduction of GVHD in patients with Campath 1 reduced early and late infections after BMT, which were often associated with GVHD in patients without Campath 1.

119 Oral Acyclovir Prophylaxis of Herpes Simplex Virus Infections After Bone Marrow Transplantation: A Clinical and Clinicopharmacological Study

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Viral infections represent one of the major complications following bone marrow transplantation (BMT); mortality and morbidity are both high. Forty-six patients between the ages of 3 and 48 years (median 15 years) received 400 mg (those under the age of 6: 200 mg) acyclovir orally four times daily from day −12 to day 84 after BMT. All patients were isolated in laminar airflow units for at least 23 days with total enteral decontamination. They were concomitantly treated with anti-CMV hyperimmunoglobulin and cotrimoxazole. During acyclovir prophylaxis, seven patients developed herpes simplex virus (HSV) infections; all of them were seropositive before BMT. Acyclovir plasma concentrations were measured using a new HPLC method. No acyclovir was present (detection limit 40 ng/ml) in the plasma of five out of six patients with HSV infections. Three of them were non-compliant; a lack of acyclovir absorption developed in two patients receiving the conditioning regimen. No drug-related side-effects were observed. Laboratory tests did not show liver or renal toxicity. Take and hematologic reconstitution were unchanged. In our study, oral acyclovir reduced the incidence of HSV infections after BMT. HSV infections only occurred in patients with non-compliance or lack of acyclovir absorption.

120 The Occurrence of Cytomegalovirus (CMV) Infections After Allogeneic T-Cell Depleted Bone-Marrow Transplantation (BMT)

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Use of the monoclonal antilymphocyte-antibody Campath 1 in allogeneic BMT eliminates mature T- and B-lymphocytes from the graft and provides effective GVHD prophylaxis. In this study we analyzed whether or not T/B-depleted BMT induces an increase in CMV infections as an expression of delayed immunoreconstitution. Thirty-four BMT patients received a Campath-1 incubated graft. CMV prophylaxis was administered with CMV-IgG hyperimmunoglobulin every 2 weeks up to day 112 after BMT. In all patients CMV-KBR, CMV-ELISA-IgG and CMV-ELISA-IgM were investigated. Nine of 34 patients developed a CMV infection; 6 of these 9 progressed to interstitial pneumonia, which was lethal in 4 patients. Six patients produc-

ed CMV-IgM between the 4th and 40th week (median: 12th week) after BMT. Five of 6 patients with seroconversion (IgM negative → positive) survived, whereas all 3 patients without seroconversion died. These results indicate that an early antigen-specific immune response is possible in BMT with T/B-cell-depleted grafts.

121 Rubella Virus Infection Following Bone Marrow Transplantation

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A 5-year-old boy in the second chronic phase of chronic myelocytic leukemia received a bone marrow transplant (BMT) donated by a HLA-identical sister. Twelve days after BMT, the patient developed fever up to 38°C and a maculopapular exanthem. IgM antibodies against rubella virus could be demonstrated by ELISA and after separation by sucrose density-gradient centrifugation. Rubella virus was isolated from the throat 24 days after BMT. Virological and serological surveillance of the patient showed no further virus excretion, but there was persistent elevation of rubella-specific IgM antibodies; however, only low levels of HAI antibodies could be demonstrated. Virus-infected donor bone marrow was identified as a possible source of the rubella virus infection.

122 Interstitial Pneumonia After Human Bone Marrow Transplantation

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Idiopathic and infective interstitial pneumonia contributes to a substantial fraction of deaths after bone marrow transplantation. At the West German Tumor Center, 37 of 146 patients with bone marrow transplants died with clinical signs of interstitial pneumonia. The respective autopsy findings in these cases are analyzed and compared with pulmonary changes in conventionally treated leukemias and transplantation cases without clinical evidence of interstitial pneumonia. In addition, the results are discussed with regard to the therapeutic modalities preceding the bone marrow transplantation and the different underlying leukemias. Finally, the genesis of interstitital pneumonia after bone marrow transplantation is discussed.

123 Pregnancy Two Years After Allogenic Bone Marrow Transplantation

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Case report. A 22-year-old female was treated by allogenic bone marrow transplantation because of severe aplastic anemia. She received the bone marrow from her HLA-identical brother. Before transplantation she was treated with 50 mg cyclophosphamide/kg body weight for 4 days. Cyclosporin A was given to prevent graft-versus-host disease. Eight weeks after bone marrow transplantation, regular menses started. She became pregnant 1.5 years later. One week before normal term a healthy boy was delivered by cesarean section. Two further female patients were treated in the same way because of severe aplastic anemia. Shortly after the treatment regular menses started in both of them. Female sex hormone levels were also within the normal range.

124 Accidental Cyclosporin A Overdose

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An 11-year-old female patient with severe aplastic anemia received a syngeneic bone marrow transplantation (BMT) without any form of conditioning. A graft rejection was treated with corticosteroids and cyclosporin A. The child accidentally took a peroral dose of 5000 mg (= 140 mg/kg) cyclosporin A, which is 17 times the usual dose. Six hours later the mistake was discovered. In the meantime the child had already vomited, but there was no cyclosporin A in the vomitus. Ten grams of charcoal was administered every 2 h in addition to sorbite for catharsis. No further toxic effects were observed. The first plasma sample was analyzed by radioimmunoassay 8 h after ingestion; the plasma drug concentration was 8200 ng/ml. The pharmacokinetic data were calculated with the TOPFIT computer program: terminal half-life 21.7 h, volume of distribution in steady state 101.8, and clearance 211.3 ml/min. The fact that the pharmacological data in this case of overdose were unchanged indicates normal renal and hepatic function. The second BMT was done after administration of cyclophosphamide (4×50 mg/kg). The patient is now alive 214 days after BMT with normal graft function.

125 Three New Precipitating Mitochondrial Antibodies Detected in Patients After Bone Marrow Transplantation Are Identical with Three Yet Undefined Precipitating Antibodies in Patients with Primary Biliary Cirrhosis

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Mitochondrial antibodies (AMAs) of different specificities have been demonstrated in patients with primary biliary cirrhosis (PBC), connective tissue diseases, drug-induced disorders and syphilis. In view of the similar clinical findings in patients with PBC, collagen diseases and graft-versus-host disease (GVHD), we were interested to find out whether AMAs may also occur in patients after bone marrow transplantation (BMT). A total of 34 patients who had received bone marrow because of leukemia were examined. Tests for AMAs included immunofluorescence, complement fixation, immunodiffusion and ELISA. As antigens we used either mitochondria from rat liver, pig kidney or the ATPase fraction prepared from beef heart mitochondria. The overall incidence of AMAs measured by these different methods was 47%. In most instances AMAs were detected only transiently. Five patients had persisting precipitating antibodies, which reacted with three different antigens present in (a) liver mitochondria, (b) kidney mitochondria and (c) the ATPase fraction. None of these three different AMA specificities recognized the PBC-specific antigen M 2, the cardiolipin antigen M 1 or the precipitating antigen system M-A, M-B, M-C described in PBC. However, the same three different AMA types were also found in sera from patients with PBC, proving that BMT and PBC patients have at least three different mitochondrial antibodies in common. Of the PBC patients, 25% had the ATPase-associated antibody specificity and 31% antibody specificity associated with liver and/or kidney mitochondria.

126 Bone Marrow Transplantation The Psychological Situation of the Recipient Just Before Isolation

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From an ongoing study on coping strategies and psychosocial changes in the context of inpatient and outpatient treatment for bone marrow recipients, the psychological situation shortly before isolation is presented as a preliminary aspect. To date, seven patients (aged 20–48 years; average age 33 years; 1 woman and 6 men) have been given semistructured interviews, projective tests (Rorschach test, TAT, tree test) and psychometric tests (such as the Freiburg Personality Inventory or FPI, WAIS, HAMA). The following tendencies were found: (1) Clinically speaking, the patients presented the picture of an anxious-depressive stress reaction; (2) The FPI and tree test both revealed good basic dynamics oriented towards development, with concurrent high sensitivity and increased efforts towards adjustment, combined with reduced performance ambition; (3) The Rorschach test indicated an increased tendency towards anxiety; (4) From a psychodynamic point of view, the situation of helplessness and extreme dependence on the donor and the medical team often led to the reappearance of early relationship patterns.

127 Strikt Indications for a "Wait and See" Attitude in Stage-I "Nonseminomatous Germ-Cell Tumor," Based on a Retrospective Analysis of Relapse

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A "wait and see" attitude in the therapy of stage-I tumors without retroperitoneal lymphadenectomy (RLA) is increasingly in favor. To date there have been few long-term results; therefore, we conducted a retrospective analysis of relapses in our patients with nonseminomatous germ-cell tumor (NSGCT) after surgery or polychemotherapy. Between 1979 and 1986, 47 patients, stages I–II a, were documented in our hospital. All 47 had complete remission (CR) after surgery; 23 had relapses (48%). For comparison, 160 patients with primary chemotherapy were analyzed. A total of 122 (75%) achieved CR; 15 had relapses (12%). Concerning possible risk factors in stages I–II a, we observed 7 cases without sufficient histological documentation of lymph node status. In contrast, the suspected high risk group of patients with MTU was not overrepresented nor were the majority of the marker-negative patients (10/23). In 16 patients the relapse was detected by laboratory investigations; there were elevated tumor markers in 13; the remaining 7 already had clinical symptoms. CR was induced by chemotherapy in 20 of 23 patients (87%). In conclusion, the fact that 3 patients did not achieve CR shows that it is dangerous not to determine the prognostically relevant lymph node status histologically. Therefore, we recommend RLA as standard therapy; "wait and see" is justified only in controlled trials, in which we suggest excluding patients with MTU and primary negative tumor markers because of the risk of delated detection of relapse.

128 Prognostic Significance of Chemotherapeutic-Induced Initial Tumor Reduction in Rhabdomyosarcoma (RMS). A Report on the CWS-81 Study

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Since most RMS are quite large or unfavorably localized, in order to allow primary resection without mutilation at the time of diagnosis, cytostatic treatment ensuring tumor reduction is preferred as pretreatment instead of resection or adjuvant radiotherapy. To prevent the development of resistance this procedure raises the question of how much tumor reduction is absolutely necessary within a definite time period. An analysis of the CWS-81 study determined the degree of tumor reduction under initial chemotherapy in relationship to the prognosis. This analysis showed that the degree of tumor reduction achieved its highest prognostic value after 7 weeks of chemotherapy, independent of tumor size, localization, and histologic subtype. Patients with clinically complete remission had a DSF rate of 100%, those with a tumor reduction of two-thirds of the original size 67%, and those with a tumor reduction of two-thirds to one-third a DSF rate of 25%. The importance of this tumor-reduction factor per unit of time is emphasized by the fact that those patients with partial remission at week 7 who achieved complete remission by week 16 showed a DSF rate of 64%, in contrast to complete responders at week 7 with a DSF rate of 100%. We conclude from this that a remission achieved earlier is of higher quality. With the determination of more exact tumor kinetics under initial chemotherapy, in the future RMS treatment could become more individualized and improved.

129 Synovial Sarcoma –A Report on Treatment Results of the Soft Tissue Cooperation Study (CWS-81)

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Thirty children and adolescents with synovial sarcoma underwent treatment between 1981 and 1985 following the concept of the multicenter therapy study (CWS-81). The age of the patients was between 1 and 21 years (median age 13); 13 patients were male, 17 female. In 26 patients, the tumor was localized in the extremities, especially the lower extremities (16 of 26). For the whole group, there is a relapse-free survival rate (Kaplan and Meier) of 66%, with a median observation time of 30 months. Patients with synovia sarcoma of the extremities had a DSF rate of 88%. In 8 patients with stage I (complete removal of primary tumor), 6 are still free of tumor. In 9 patients with stage II (microscopic residue) 7 are still in remission. In 8 patients in initial stage III (primary macroscopic residue or inoperable tumors) there are 6 patients in first remission. Two of the 8 patients were nonresponders to initial chemotherapy and tumor progression was the cause of their death. Five patients already had metastasis at the time of diagnosis (stage IV); 3 of them are still in remission. For both stages III and IV, there was a response rate to initial chemotherapy (VACA = vincristine, actinomycin D, cyclophosphamide, Adriamycin) of 77%. Rhabdomyosarcomas had, with the same combination, a response rate of 89%. Chemotherapy was obligatory for all patients, and radiotherapy was obligatory for all patients with stages I–III and optional for patients with stage IV. Primary stage, tumor size, and initial chemotherapy response in stage III and IV patients are the essential prognostic factors that should determine therapeutic measures.

130 Medroxyprogesterone Treatment of Seven Patients with Aggressive Fibromatosis: Preliminary Results

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Aggressive fibromatosis is a common complication in patients with familial polyposis. These benign desmoid tumors can grow very large, thus causing local obstruction; these patients usually die within a few years. In seven cases with aggressive fibromatosis, we started treating them with oral medroxyprogesterone (500 mg daily). In six patients either no change or a regression of the tumor masses occurred. One patient showed a progression 5 months after the onset of treatment. These seven cases have now been observed for 6–30 months. These preliminary results indicate that gestagens might be beneficial to patients with desmoid tumors.

131 Polychemotherapy with 5-Fluorouracil, Adriamycin and Methotrexate in Medium-Dose Range in Advanced Gastric Carcinoma

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Twenty-one patients (aged 32–66 years; Karnofsky index 50–100) suffering from advanced gastric carcinoma were treated with three-drug combination chemotherapy containing 5-fluorouracil (5-FU) at 900 mg/m^2, adriamycin (ADM) at 40 mg/m^2, and methotrexate (MTX) at 300 mg/m^2 with a leucovorin rescue. Nine patients responded with a measurable reduction in tumor size (response rate 43%). So far, the mean duration of remission has been 5.8+ months (2–11+). Four patients showed stable disease for 2.4+ months (1.5–4); 8 did not respond to chemotherapy. Mean survival of 9 patients responding to chemotherapy is 8.4+ months (3+–15). Mean survival of nonresponders is 3.6 months (1.2–7) and of patients with stable disease, 4.2+ months (1.9+–11). Chemotherapy was well tolerated. Minor side effects included alopecia, mild nausea and fatigue. Seven patients suffered from stomatitis. Mild transient leukopenia occurred in 3 patients; no septic complications were seen. Although remission incidence is not as frequent as in high-dose MTX/5-FU + ADM regimen, our preliminary data suggest that combination chemotherapy with 5-FU, ADM and MTX in a medium-dose range is effective in advanced gastric carcinoma.

132 Intrapleural Mitoxantrone for Treatment of Malignant Pleural Effusion in Metastatic Breast Cancer

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The initial results of intrapleural mitoxantrone treatment in eight patient with metastatic breast cancer and recurrent malignant pleural effusion are reported. In six of the eight patients a Matthys catheter was inserted for pleural drainage for 24–48 h prior to drug administration. Then mitoxantrone at a dose of 30 mg, or two times 30 mg, was given via the intrapleural catheter in a solvent volume of 10 or 20 ml, respectively. Mitoxantrone was left intrapleurally once or twice for 48 h and then drawn off. No side effects due to local mitoxantrone application were seen. There was also no nadir of leukocytes when mitoxantrone was given intrapleurally without additional systemic cytostatic therapy, which indicates that there is a small amount of pleural

clearance of mitoxantrone which is suitable for local therapy due to its physicochemical properties (high molecular weight and low lipophilia). Good clinical response to intrapleural mitoxantrone (partly administered with 5-FU, tetracycline-HCl and fibrin glue) was documented. Four of eight patients showed no recurrent effusion for up to 6 months under subsequent systemic chemotherapy. In the other four patients the frequency of pleurocenteses was significantly reduced after intrapleural mitoxantrone.

133 Differentiation of Endothelial Cells in Normal and Malignant Human Tissues*

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In malignancies, infiltration of tumor cells is supposed to rely on vascular growth with proliferation of specific endothelial cells. Surface-marker analysis of blood vessels was performed on various epithelial tumors (ten hypernephromas, two mamma cell carcinomas, 1 colon carcinoma, 1 seminoma) in comparison to normal adult and fetal tissue to investigate the specificity of endothelial cells in malignant cell proliferation. Monoclonal antibodies directed against different vascular antigens (TE 1, TÜ 70, TÜ 72) or against the HLA-class II molecules DP, DR and DQ were applied on cryostat sections using the immunoperoxidase technique. In normal adult tissue the endothelia of large veins and vessels of intermediate size stained with TE 1, TÜ 70 and TÜ 72, whereas big arteries lacked these determinants. Endothelia of all microvessels expressed TÜ 70^+ antigens. TÜ 72, however, did not bind to sinus endothelia of liver and spleen, and TE 1 was not reactive or only weakly reactive with glomerular capillaries. Labelling of fetal tissue of 21 weeks' gestational age indicated the sequential expression of TÜ 70, followed by TÜ 72 and/or TE 1 determinants on vascular endothelia in liver and kidney during organospecific angiogenesis. Thus far, the staining patterns of these three antibodies have suggested different subsets, as well as distinct maturation stages of normal endothelia. Comparative analysis of various epithelial tumors revealed the presence of TÜ 70, TE 1 and TÜ 72 determinants on endothelia of most vessels surrounding the malignant tissues, but a variable lack of expression of TÜ 72 and/or TE 1 on capillaries within the tumors. TÜ 70^+, TE 1^-, TÜ 72^- microvessels were particularly found in the tumor center. In contrast to the HLA-DR^+ and -DP^+ capillary endothelia of normal adult organs, these blood vessels were also found to be negative for all HLA-class II antigens like specific microvessels in fetal tissue. These tumor vessels may also carry heterogeneous endothelia of an early differentiation stage and contribute to tumor protection against immunological recognition by the lack of expression of HLA-class II antigens.

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134 DNA Stem-Line Heterogeneity in Solid Tumors

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In the present study, DNA analyses by flow cytometry were carried out in 163 malignant tumors to assess the frequency and distribution of heterogeneous DNA stem lines. Of 88 large-bowel carcinomas 72 (82%) were aneuploid, and 29 (22%) revealed heterogeneous DNA stemlines; in the 54 lung carcinomas analyzed and 21 osteosarcomas, the frequencies of DNA aneuploidies were 83% and 86% and of heterogeneous tumor stemlines 37% and 52%, respectively. No correlation was found between the DNA stem-line polyclonality and the morphologic tumor heterogeneity. In lung carcinomas, however, a significantly higher rate of muliclonal DNA stem lines

was observed in squamous cell carcinomas, as compared with the other histologic subgroups ($P < 0.025$). Since DNA stem-line heterogeneity is probably the manifestation of secondary chromosomal aberrations, which determine the biology of malignant tumors, a high incidence of multiclonal tumor cell lines, as revealed by the present study, possibly has a substantial impact on the clinical course of these cancers.

135 F-CB 3 and Fibronectin Concentration in Plasma of Cancer Patients

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Many malignant cells secrete high levels of proteases, which are of central importance in the metastatic process. Some of these proteases possess coagulatory and fibrinolytic properties. In 140 patients with cancer we measured F-CB 3-related antigen, an early degradation product of the α-chain of fibrinogen, as marker for fibrino-(geno)lysis by RIA and fibronectin (FN) by laser nephelometry. In all nonmetastatic cancer patients the F-CB 3 levels (88 ± 31 pmol/ml) were significantly elevated above normal values (up to 40 pmol/ml) without respect to the histological tumor type. Significantly ($P < 0.01$) higher levels of F-CB 3 (191 ± 75 pmol/ml) were observed in patients with metastases. Highest levels were found in patients with gastrocolorectal tumors (216 ± 75 pmol/ml). The elevation of FN values was less significant ($P < 0.05$). A good correlation was observed ($n = 100$; $r = 0.522$) between F-CB 3 and FN values. These results suggest that F-CB 3 especially seems to be a useful parameter in the control of tumor progression. However, the present results do not indicate whether the elevation of F-CB 3 in metastatic tumor patients is only due to the mass of the tumor or whether the development of metastases is due to the selection of metastatic tumor cells that secrete high levels of proteases.

136 Ploidy Patterns of Megakaryocytes in Patients with Metastatic Tumors and Patients with Limited Cancer in Comparison to Control Study Groups A Direct Correlation Between Tumor Volume and Megakaryocyte DNA Content

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An alteration in platelet heterogeneity could be one of the reasons for thrombotic events in malignant diseases. There is evidence that heterogeneity of platelets depends on the different ploidy values of the megakaryocytes. We measured the megakaryocyte DNA content by Feulgen cytometry in bone marrow smears of 38 tumor patients. Of these patients, 15 had no metastases; in 23 metastases were present. Twelve healthy controls were also measured. Tumor volumes of patients with limited cancer disease was measured in the resected tumors. In patients with metastatic cancer the tumor volume was assessed at autopsy. Patients with metastatic tumors had a highly significant increase in ploidy of the megakaryocytes (ploidy index: 3.56) compared with the controls (ploidy index: 3.04, $P < 0.0001$). This shift was less pronounced in patients with limited cancer disease (ploidy index: 3.18). Correlating the logarithm of the tumor volumes with the average megakaryocyte ploidy index of each patient, a highly significant linear correlation was found ($r = 0.7096$, $P < 0.0001$). Our study shows that DNA content of megakaryocytes increases linearly with the exponential growth of the tumor, regardless of the histologic type of tumor. A platelet population altered by increasing ploidy of megakaryocytes could be another cause for thrombotic events in malignancy.

137 Results of Chemotherapy in Germ Cell Tumors Between 1976 and 1985 at the University of Freiburg

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Between 1976 and 1985, 101 patients with germ cell tumors have been treated: 21 had seminoma and 80 nonseminoma tumors. A total of 83 patients primarily received chemotherapy according to multiple protocols after orchiectomy and (some patients) lymphadenectomy. Complete remission (CR) was attained in 60.3% with chemotherapy alone and in an additional 9.8% after resection of the remaining tumor. Patients with "advanced disease" (stages IIc, III, IV and extragonadal tumor) had a worse prognosis. Only 57% reached CR. Eight of 15 (53%) patients treated according to the "Einhorn" protocol and 21 of 26 (80.7%) treated with cisplatin, bleomycin and etoposide reached CR. High-doseage cisplatin, in combination with a podophyllotoxin, improves the prognosis of these patients even in very advanced disease; there is significant, but tolerable toxicity.

138 Delay in Diagnosis of Testicular Cancer

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The prognosis and amount of treatment required for testicular tumours are both related to the stage at presentation. Delay in diagnosis may affect the stage and prognosis. We therefore undertook a partially prospective study to measure the delay(s) in diagnosing testicular cancer among a large patient series currently being monitored in our in- and outpatient clinic. To date 100 consecutive patients (age at the time of diagnosis: mean 27.6 years, range 16–57 years) with testicular cancer (18 seminomas, 82 non-seminomas) have been interviewed personally by one of us. The results are given in the table.

Delay[a] (s)	Patient	Physician	Hospital[b]	Total
Mean	4.31 m	29.7 d	3.6 d	4.95 m
Median	2.50 m	1 w	1 d	2.5 m
Range	1 d–3 y	1 d–9 m	1 d–5 w	1 d–3 y

[a] (d day, w week, m month, y year)
[b] (1st visit to orchiectomy)

The causes of delay(s), symptoms, histological type, stage, and a 1-foetoprotein and β-HCG levels at presentation were also documented. The impact of the delay on stage, amount of treatment and prognosis are discussed. We also found that young men generally do not know that testicular tumours can occur at their age. Testicular self-examination can probably lead to early diagnosis; it can, however, cause alarm, offense, and anxieties. Furthermore, self-examination is less practicable and, in view of the low incidence of testicular tumours, a somewhat questionable preventive measure. As symptoms were reported by the majority of patients, seeking prompt-medical advice for any changes in the testicles seems as to be a more realistic approach to early diagnosis.

139 Membrane Antigens on Human Small Cell Lung Cancer

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Small cell carcinoma of the lung (SCLC) is distinguished from other types of lung cancer by its property for early development of distant metastases and its rapid, fatal clinical course. The effect of therapy of SCLC is still not satisfactory and has not improved in recent years. After the initial success of chemotherapy, resistance of the tumor cells usually develops within a few months. Experimental studies are necessary to understand the biological properties of SCLC. Original SCLC tumor cells and tumor cells growing in nude mice were tested by the immunoperoxidase method with monoclonal antibodies (Mo'Abs) defining human leukocyte antigen (HLA class 1), common leukocyte antigen (HLe-1), tumor-associated antigen (BAS 10, SAM 12, BA_2), epithelial membrane antigen (EMA), as well as carcinoembronic antigen (CEA), transferrin receptor (OKT 9) and neuronspecific antigen (CEA), transferrin receptor (OKT 9) and neuronspecific enolase (NSE polyclonal). All SCLC cells were negative for HLe-1 and EMA antigen and most of the cells had lost HLA antigens. On the other hand, there was a strong reaction with the following Mo'Abs: BA_2, SAM 12, OKT 9, BAS 10 and NSE. No tumor-specific reactivity was found for SCLC with the antibodies tested. However, there seems to be a characteristic pattern of Mo'Abs defining SCLC, which allows differentiation from other lung tumors.

140 Papillomatosis of the Bile Duct

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In patients with signs of obstructive jaundice, rare diseases such as biliary papillomatosis also need to be taken into consideration. These primarily benign epithelial tumours show a tendency towards recurrence and malignant degeneration and therefore have to be regarded as precancerous. An early diagnosis is essential, preferably preoperatively, because extensive surgical treatment is required. Today this diagnosis can be made by ultrasound, endoscopic retrograde, cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC). This study describes two cases of progressive biliary papillomatosis observed in 1978 and 1985 (one female 48 years of age, one male 53 years). The first case was only diagnosed after relaparotomy and several palliative operations were carried out without satisfactory results (malignant degeneration into adenocarcinoma). In contrast, in 1985 the diagnosis was suspected by ultrasound and confirmed by ERCP and biopsy. A liver transplant, the first in the world, was performed successfully. Six months later the patient showed no clinical symptoms and the ultrasound findings of the bile duct and the liver were normal.

141 High-dosage MTX/5-FU and Adriamycin in Advanced Gastric Cancer Experience in Tübingen

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Fourteen patients with nonresectable or metastatic gastric carcinoma (undifferentiated adenocarcinoma: $n = 10$; well-differentiated: $n = 2$; unclassified: $n = 2$) were treated with combination chemotherapy consisting of high-dosage MTX, 1.5 g/m², given 1 h prior to 5-FU, 1.5 g/m², as bolus injections. Leucovorin rescue, 15 mg/m² q 6 h×12, was started 24 h after MTX administration. Adriamycin, 30 mg/m² was given on day 14. Response rate was 57% with one complete remission (CR) and one partial remission (PR) of 13 and 24 months duration, respectively. Six patients had stable disease according to the WHO classification, lasting from 3 to 8+ months.

To date, median survival of the whole group is 10 months; two patients are still alive although they had recurrences at 24 and 30 months. Toxicity was acceptable with grade 3 and 4 (WHO) leukopenia in 4, gastrointestinal side effects in 3, and impairment of kidney function in 3 of 64 treatment courses. We conclude that some, although only a minority of patients with advanced gastric carcinoma, clearly benefit from treatment with sequential MTX/5-FU and ADM, and that the efficacy of the protocol compares favorably with that of other adriamycin-containing regimens.

142 Comparative Immunohistochemical and Serological Investigation of TPA and CEA on Patients with Carcinoma of the Colon and Rectum

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TPA and CEA were localized in resected tissue specimens of 31 patients with adenocarcinoma of the colon and rectum, using the indirect immunoperoxydase technique. The intensity of specific staining was recorded as 0, 1, 2, 3 and compared with histological grading, clinical stage according to Duke, and the preoperative serum TPA and CEA concentration. Concerning the immunohistochemical investigation, only 74% ($n = 23$) of the TPA-marked tissue specimens showed staining degree 1 and 2, while 81% ($n = 25$) of the CEA-marked tissues were characterized by staining degree 2 and 3. In the cytoplasm of well-differentiated cylindrical tumor cells, TPA and CEA staining was mostly localized close to the apical cell surface; the other tumor epithelials were characterized by diffuse intracytoplasmatical staining. Strong TPA staining was almost limited to moderately differentiated adenocarcinoma, whereas strong CEA staining was seen in moderately and well-differentiated tissues. Necrotic intraluminal tissue showed virtually no TPA staining but strong staining for CEA. There was no definite correlation between the clinical stage according to Dukes, stages A, B, C, D and the immunohistochemical staining of TPA and CEA. A positive correlation was seen between increased serum TPA concentration and poorly differentiated tumors, as well as a high serum level of CEA and moderately to well-differentiated carcinoma.

143 Influence of Tumour Differentiation on Prognosis of Carcinoma of the Colon

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The influence of tumour differentiation (grading) on prognosis was studied in the histories of 393 patients, who were operated upon for carcinoma of the colon between 1977 and 1985. The percentage of well-differentiated adenocarcinomas (G 1) amounted to 37%, of moderately differentiated tumours (G 2) to 42%, and of poorly differentiated tumours (G 3) to 21%. Half of the well-differentiated tumours were found in early stage T_{1-3} No Mo compared to 24% of the G 2 and 10% of the G 3 tumours. The percentage of incurable cases in G 2 tumours was twice as high and in G 3 tumours three times as high as in patients with well-differentiated carcinomas. The recurrence rate increased significantly with decreasing tumour differentiation (up to 50% in the group with G 3 tumours). Poorly differentiated tumours relapsed in 80% within the 1st postoperative year. The recurrence-free interval of well-differentiated carcinomas surpassed 2 years in 23%. Twenty-six per cent of the patients with recurrent G 1 tumours were free of lymph node involvement or metastases at secondary operation (G 2: 11%; G 3: 12%). Five-year survival (operation between 1977 and 1980) was 52% for patients with well-differentiated tumours, 32% for patients with G 2 and 17% with G 3 tumours. The differences in prognosis were caused by the higher percentage of incurable cases at primary and secondary operation and the higher recurrence rate for the moderately and poorly differentiated tumours.

144 Alpha-2C Interferon (IFN 2C) in Combination with 5-Fluorouracil (5-FU) for Refractory Colorectal Carcinoma (CC)

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Prior studies with clonogenic assay systems have suggested synergistic effects of IFN and various cytostatic agents. The authors undertook a phase I/II study in patients with refractory CC in order to determine the toxicity and efficacy of IFN and 5-FU. Six patients with progressive disease under 5-FU chemotherapy were entered into the trial. Both IFN 2C (Boehringer, Ingelheim), 2×10^6 E s.c., and 5-FU, 10 mg/kg i.v., were administered twice per week for at least 3 months. All patients could be evaluated for toxicity and 5 for response. One patient had a partial remission, 3 achieved stable disease, and 1 showed progressive disease. Three of six patients experienced transient fever; diarrhea occurred in 1; 2/6 suffered from pain. Hematologic toxicity was minimal (WHO grade 1). No patients were withdrawn due to toxicity from this regimen. In conclusion these preliminary data suggest that the safety and toxicity IFN and 5-FU levels of the combination in this schedule are tolerable. It is too early to evaluate the remissions definitely, however.

145 5-Fluorouracil Plus Mitoxantrone in the Treatment of Advanced Colorectal Cancer Initial Experiences

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In colorectal carcinomas, in human tumor cell lines, and in nude mouse transplant tumors, mitoxantrone has shown a clear dose-related response (K. H. Link et al. 1986). We therefore studied the combination of 5-FU and mitoxantrone in patients with progressive metastatic colorectal carcinoma. The treatment schedule was as follows: mitoxantrone, 10 mg/m^2 on day 1, i.v.; 5-fluorouracil, 1000 mg/m^2 on days 1–5 as continuous infusion. On day 29 the treatment was repeated. We treated eight patients (4 males, 4 females) with a median age of 68 years (range 51–76 years). Three patients were pretreated by irradiation and 5-FU/MTX, one patient was pretreated by the Falkson scheme (5-FU, DTIC, vincristine, BCNU), and two patients were pretreated by liver perfusion with FUDR. Two patients were not pretreated. No complete and no partial remissions were obtained. All six patients pretreated showed progressive disease. The two patients not pretreated had stable disease (NC) for 4 months. No toxicity was observed – especially no hair loss and no vomiting. No significant myelosuppression was noted.

146 In Situ Study of Lymphocytic and Monocytic Cells in Cryostat Sections of Breast Carcinomas Using Monoclonal Antibodies

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Cryostat sections of 44 breast carcinomas (biopsy material resected during surgery) were examined for the presence and infiltration density of leukocytic and monocytic cells by use of a panel of monoclonal antibodies. Sixty-six percent of the patients (29/44) exhibited moderate or strong lymphocytic infiltration; remarkable monocytic infiltration density was found in 48% (21/44). The majority of the infiltrating lymphocytic cells were T-cells, and in most cases a pre-

dominance of T-helper-cells was shown. No significant correlation could be demonstrated between lymphocytic infiltration and lymph node involvement, but in the group with high lymphocytic as well as monocytic infiltration density, 4 of 5 patients were free of axillary metastasis. Patients with low ER and PR values showed a stronger lymphocytic infiltration density than those with high values. Protein products of the oncogen c-myc were studied by the use of monoclonal antibodies and these results compared with the infiltration density of "reactive cells," especially regarding possible correlations with the clinical course.

147 Aminoglutethimide in Advanced Breast Cancer with Secondary Resistance Against Tamoxifen

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Aminoglutethimide (AG) has been shown to be effective in postmenopausal women with breast cancer with and without secondary resistance against tamoxifen. The usual daily dose is 1000 mg, but there is evidence that lower doses of AG are equally effective and better tolerated. The aim of the multicenter study reported will be to evaluate the clinical efficacy (rate and duration of remission) and the tolerance to 3 times 250 mg AG plus 37.5 mg cortisol acetate daily in postmenopausal women without and premenopausal patients with ovarectomy who are under treatment for advanced breast cancer (stage IV) and secondary resistance against tamoxifen. Sixty patients are projected for the study. The AG treatment will be continued until renewed progress of the disease becomes evident. The results will be presented after 6 months.

148 Image Analysis of Melanoma with Digitizer Tables

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It is well known that quantification of the infiltration of melanoma into the cutis is of great prognostic importance (Breslow 1975). Moreover, an estimation of the prognostic index calculated from the depth of invasion and the mitotic index is urgently recommended (Schmoeckel and Braun-Falco 1978). Based on these reports retrospective investigations were carried out to check the possibilities of using semiautomatic devices and record data uptake with a digitizer table. All measurements used are functions available in common hardware and software. Infiltration into the cutis, exophytic growth, the compactness of the malignant infiltrations, and the frequency of mitoses can be measured. Data reduction can be carried out by the function "center of gravity," with which the coordinate for the reference level of the perpendicular growth and the distribution of the aggregates of the tumor can be obtained. With appropriate computer aids it seems possible to implement the method for clinical routine. However, eventually such semiautomated devices will not be as successful as fully automated methods.

References:
Breslow A (1975) Ann Surg 182: 572
Schmoeckel Ch, Braun-Falco O (1979) Arch Dermatol 114: 871

149 Myc-Oncogene Expression in B-Cell Lymphatic Leukemia and in Hairy Cell Leukemia

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Previous studies have demonstrated a change in expression of the myc-oncogene in myeloid cells, depending on their differentiation status. We investigated myc-expression in B-cell chronic lymphatic (B-CLL) and in hairy cell (HCL) leukemia. The latter should be more mature than B-CLL according to surface-marker studies. We also studied myc-expression in B-CLL after incubation with the phorbolester TPA, because B-CLL develops both phenotypical and biochemical characteristics of HCL when incubated with TPA. Twenty cases of HCL, 17 of B-CLL and 3 healthy blood donors were tested for myc-expression. In all cases of HCL, myc-expression was low and no difference in myc-expression was recorded between the HCL cases and the controls. However, in 3 cases of B-CLL myc-expression was found to be four- to five-fold higher than in the other B-CLL cases, HCL cases, and the controls. Six cases of B-CLL were incubated with 2 nM TPA at 37°C for 96 h. After incubation with TPA, myc-expression remained unchanged and, furthermore, myc was overexpressed in the three B-CLL cases mentioned above. In conclusion, (1) HCL is characterized by a low myc-expression similar to healthy controls; (2). In 3 of 17 cases of B-CLL, myc was overexpressed; (3) Myc-expression in B-CLL is not altered by 2 nM TPA.

150 Treatment of Hairy Cell Leukemia with Interferon-α(IFN-α): Before or After Splenectomy?

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There is increasing evidence that malignant diseases can be effectively treated with interferon-α. This is demonstrated by the large scale of disorders that will possibly, most likely or almost definitely respond to IFN-α therapy. Hairy cell leukemia (HCL) is the first malignant disease in which the efficacy of IFN-α treatment has been accepted. Since HCL can be treated efficiently by splenectomy (Sx), it should be discussed whether or not Sx should be replaced by IFN-α as primary treatment for HCL. Seventy percent of all HCL patients are eligible for Sx regardless of spleen size; 70% of splenectomized patients benefit from this treatment. Median survival after Sx is 5 to 10 years. The mortality of Sx is 1%. In contrast, almost all HCL patients are eligible for IFN therapy, even after Sx; 80% of HCL patients benefit from IFN-α therapy. Median survival after IFN therapy is not yet known. Although IFN-α induces complete remissions (30% – 50%) in HCL patients in contrast to Sx, we still consider Sx the primary treatment for the following reasons: (1) it has not been shown that HCL is cured by IFN alone; (2) it is not clear if IFN induces longer phases of stable disease than Sx; (3) it is unknown if resistance to IFN might develop in HCL; (4) eligibility for Sx decreases with age and progression of the disease.

151 Lethal Infectious Complications in Patients with Hairy Cell Leukemia under Treatment with hum-r-Interferon α_2C (arg)

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During the period from 1st January 1985 to 15th April 1986, 96 patients from 31 different institutions were considered for a multicenter phase-II study on the effects of alpha-2-interferon on

hairy cell leukemia. In 4 patients, hairy cell leukemia could be excluded. Six patients have so far needed no treatment; one patient died before treatment started. Eighty-five patients were treated SC daily with interferon at a dose of 1.2 million IU/m² BSA or less. Of the group, 34 had had splenectomy before treatment with interferon. To date, 8 patients have died in this group during treatment with interferon; none of the patients died who had not had a splenectomy. One patient died from thrombocytopenic CNS bleeding. Seven patients died from severe infections (1× miliar tuberculosis, 1× pneumonia with candida, 2× septicemia with salmonellae, 3× pneumonia without evidence of infectious agents). In all patients, immediate antibiotic or antimycotic therapy was unsuccessful. We presume that treatment with interferon has a nonspecific effect on lymphatic and myelomonocytic cells that reduces specific immunity responses. This appears to be particularly dangerous for patients who have undergone splenectomy. This newly recognized danger for splenectomy patients must be considered in our initial discussion of the primary treatment of hairy cell leukemia. Thus, an increase in morbidity and mortality in possible relapse therapy is an additional risk for patients with hairy cell leukemia after splenectomy.

152 The Dosage of α-Interferon in the Treatment of Hairy Cell Leukemia

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Alpha-Interferon (α-IFN) is known to be effective in the treatment of hairy cell leukemia (HCL), yet the optimal dose required and the duration of IFN treatment remain to be established. Seventeen patients with progressive HCL were treated with recombinant α2B-IFN. First, α-IFN was given to 9 patients in an initial dose of 4×10^6 U/m² every second day; the 8 following patients were treated with an initial dose of 2×10^6 U/m² every second day. Thirteen patients are available for the evaluation of response. Normalization of complete bloods counts was observed in both groups of patients. In both groups, response was observed between 1 and 6 months of treatment. Subsequently, the dosage was reduced to $0.5-1\times10^6$ U α-IFN for maintenance therapy. In conclusion, a dose of 2×10^6 U/m² α-IFN seems to be sufficient in the initial treatment of HCL. The effect of long-term treatment will be determined in a prospective randomized study.

153 Low-Dosage Interferon-α Therapy of Hairy Cell Leukemia

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Nineteen patients (36–71 years of age; 11 women and 8 men) with cytologically and histologically proven hairy cell leukemia were treated with low dosage IFN-α therapy, which consisted of 1 Mio IU given daily s.c. for 1 month. Thereafter, the dosage was reduced to 1 Mio IU IFN-α2b three times weekly. All patients exhibited at least one of the major cytopenias defined as follows: Hb $<$ 10 g/dl, thrombocytes $<$ 100,000/μl, granulocytes $<$ 1000/μl. Bone marrow infiltration by hairy cells was present in all patients. Six of 19 patients had been splenectomized; 2/19 received chemotherapy prior to the IFN treatment. The IFN therapy was very well tolerated. Only 3 of 19 patients had mild fever after the first injection. One patient experienced somnolence and apathy after 2 months of therapy; 16/19 patients are evaluable at present; 2/16 showed complete response with complete normalization of peripheral blood and bone marrow; 10/16 patients experienced a partial response (PR) with overall improvement in the cytopenias, but hairy cell infiltration was still observable in their bone marrow. The peripheral blood parameters improved in 2/16 patients under therapy without reaching a PR. Two of 16 patients did

not benefit from the IFN treatment. They are at present being treated with 5 Mio IU IFN-α three times weekly. The data indicate that at this very low dose of 3 Mio IU/week, IFN-α is an effective treatment for hairy cell leukemia.

154 Recurrence of Hairy Cell Leukemia upon Discontinuation of IFN Treatment

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In several clinical trials, IFN-α has recently been shown to be effective for the treatment of hairy cell Leukemia (HCL). Although more than 80% of HCL patients responded to IFN, only a minority achieved complete remissions. Furthermore, it remains unclear whether these IFN-induced remissions are stable or if maintenance therapy will be necessary to obtain long-term remissions. Addressing this problem, we stopped IFN treatment in three of our patients who achieved complete or partial remissions (two PR, one CR) 7–12 months after starting IFN therapy. In contrast, six patients were maintained on IFN treatment for 18–29 months. In the three patients, in which IFN treatment was discontinued, disease relapse or progression occurred after 9–12 months. This was documented by progressive hairy-cell infiltration in the bone marrow and peripheral blood in two patients, respectively. Because of recurrent infections in one of these patients, IFN therapy was reinstituted and proved to be successful. The other six patients held on maintenance therapy for 18–29 months remained stable or showed continuous improvement of the disease (six PR, one MR). In none of these cases was IFN resistance encountered. Despite this long-term treatment no evidence for chronic toxicity was noted. From these preliminary data we conclude that some form of long-term maintenance or reinduction therapy will be necessary in most patients with hairy cell leukemia.

155 Stable Partial Remission After Interferon-α (IFN-α) Treatment in Patients with Hairy Cell Leukemia (HCL)? Immunocytological Demonstration of Hairy Cells in Peripheral Blood

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Numerous clinical trials have demonstrated that human recombinant IFN-α is highly active in HCL. In leukemic patients a rapid decrease in circulating hairy cells is observed. Despite the severity of pancytopenia in most patients, blood counts improve rapidly. In contrast, in most cases hairy cell infiltration of the bone marrow decreases only slowly with continued IFN-α therapy. A German multicenter trial of low-dosage human recombinant IFN-α in patients with HCL was started in December 1984. Patients received 2×10^6 IU rIFN-α-2 carg (Berofor) s.c. daily for at least 28 days. During a 15-month period we analyzed the frequency of hairy cells in the peripheral blood of 48 patients. Hairy cells were identified by their very strong expression of the B-cell-specific antigen detected by our monoclonal antibody HD 39 (B 3, CD 22). The HD 39 antigen (a glycoprotein of 130/140 kD) is present in the cytoplasm of all normal and neoplastic B cells and is expressed on the cell surface only in mature B-cells. The strongest expression is found in normal activated B-cells and in malignant hairy cells. Hairy cells were stained with HD 39, using indirect immunofluorescence or the APAAP technique. In 24 of the 26 patients followed over a longer period, circulating hairy cells could be detected after rIFN-α-2 therapy. Despite normal blood counts, most patients had residual hairy cells when analyzed by HD 39 (B 3) staining. This stresses the importance of immunocytological evaluation for monitoring IFN therapy. Moreover, results suggest that the rate of "complete remission" is very low or nonexistent. However, IFN-α therapy may result in biological control of the disease.

156 Identification of Subtypes of Hairy Cell Leukemia

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Three subtypes of hairy cell leukemia are known (Bartl et al. 1983): the "ovoid," the "convoluted," and "indented" types, each of which has a different clinical course and prognosis. Sixteen patients with hairy cell leukemia were examined by light and electron microscopy (aged 25–74; 14 males, 2 females) for periods of several months up to 6 years. In 6 cases a transformation from the "ovoid" type to the "convoluted" or "indented" type was observed during the course of the disease. It seems possible that the subtypes belong to a definite stage of the disease. The fact that the prognosis for the "indented" and "convoluted" types is worse may be due to their representing an advanced courses of the disease.

157 Endogenous Peroxidase Activity in Hairy Cells and in B-CLL Cells After Differentiation Induction by TPA

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Immunological and molecular biological studies on hairy cells support the assumption that hairy cell leukemia represents a disorder related to the B-cell lineage. However, the ultrastructural demonstration of a platelet-like peroxidase (PLP) in the nuclear envelope and in the endoplasmatic reticulum of hairy cells raises doubts about that lineage affiliation, because PLP has been negative in all lymphatic cells studied to date. Recent studies on B-CLL cells incubated with TPA have revealed an immunological phenotype characteristic for that of hairy cells and, therefore, the question arose whether TPA could induce PLP in those B-cells. We compared PLP activity in 6 cases of untreated B-CLL prior to and after differentiation induction by TPA with PLP activity in hairy cells. After 3 or 6 days in vitro the immunological phenotype of the B-CLL cells became similar to that of hairy cells; in 4/6 cases tartrate-resistant acid phosphatase was found. However, in none of the cases studied could PLP be detected before or after the culture, clearly indicating a difference between hairy cells and the TPA-induced B-CLL cells. Thus, it is questionable if the immunological marker profile alone, without the demonstration of PLP activity, is sufficient to characterize these cells as hairy cells; the meaning of PLP is still unclear.

158 Staging of 252 Patients with Hodgkin's Disease (HD) by Laparotomy and Splenectomy (LAP): Diagnostic Value of Operative and Non-Operative Measures

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Since the introduction of combined radio- (RT) and chemotherapeutic (CT) modalities for low stages of HD with risk factors the use of LAP has been controversial for such patients. We compared the results of clinical staging (CS) and pathological staging (PS) in 252 patients who underwent LAP between July 1980 and February 1986 when evaluated for inclusion in a multicenter trial. The diagnostic value of noninvasive variables as well as the impact of LAP on therapeutic strategies has been analyzed. In 81 cases (32%), LAP resulted in a change of stage: 26/55 CS I, 45/144 CS II, and 4/46 CS III patients; 7 further cases underwent LAP for therapeutical reasons. Patients with constitutional symptoms or extranodal disease had no higher frequency

of occult disease (31 and 32%, respectively). In patients with a large mediastinal mass (> one-third of the thoracic diameter), infradiaphragmal disease was somewhat less frequent (20%, $P = 0.10$). Patients with CS I and II, with or without infradiaphragmal disease showed no significant differences with respect to histological subgroups, ESR, alkaline phosphatase, eosinophils, or lymphocytes. In 73 cases (29%), the results of LAP influenced the therapeutic approach: 71 CS I–II patients received combined modality treatment instead of RT alone; only 2 of the CS III-A patients received more extensive CT because they rose to PS stage IV. If, in addition to a large mediastinal mass, extranodal disease and diffuse spleen involvement, a high ESR or involvement of three or more lymphatic areas were considered to be risk factors qualifying for combined CT + RT, LAP could be omitted in 128 of the cases (48%). Only 17 of these would have had stage IV undetected by noninvasive diagnostic methods. In contrast, patients with CS I–II and no such risk factors should undergo LAP, because in our analysis 36% of them had occult infradiaphragmatic disease.

159 Expression of the C-ABL Oncogene in Malignant Non-Hodgkin Lymphomas (NHL) – A Marker of Dissiminated Disease?

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Ten reactive lymphatic tissues and 180 NHL were investigated for the presence of the protein product p 150 of the cellular oncogene c-abl. A polyclonal antibody and an indirect peroxidase staining procedure were used for this in situ examination. In reactive lymphatic tissues, cells brightly positive for the c-abl antigen were usually localized within the mantle zone and, to a lesser extent, within the germinal center. In peripheral blood, about 60% of the circulating B lymphocytes expressed the p 150 antigen. In NHL, positive staining of the cell membrane was observed predominantly in histological entities of putative mantle zone origin (i.e., centrocytic NHL). However, a minor percentage of c-abl-positive cases was also observed in other histological entities of the Kiel classification. In about 70 cases, clinical data were available on the patients. c-abl-positive tumor cells were almost exclusively seen in patients with disease stages III and IV ($P < 0.0005$), independent of their proliferative potential. Although the clinical outcome was not influenced by the expression of the c-abl oncogene, we nevertheless conclude that the presence of this antigen indicated dissemination of the malignant disease.

160 Comparison of the Working Formulation and the Kiel Classification According to Histological and Prognostic Factors

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A total of 244 lymphomas were reclassified according to the Working Formulation and the Kiel Classification and correlated with survival data. Our special interest was focused on comparison of histological and prognostic factors. We found that with a few exceptions the two classifications are not equivalent. Within the Working Formulation, diffuse mixed cell lymphomas, diffuse small cleaved cell lymphomas, and diffuse large cleaved cell lymphomas show the greatest diversity. Within the Kiel Classification the CB-CC, the CB, and the CC lymphomas were the most heterogeneous lymphoma types. In conclusion, we think it is impossible to transform the different lymphomas from one classification to the other easily, as maintained in the Working Formulation. The low-grade and high-grade malignant lymphomas of the Working Formulation correspond more or less to the low- and high-grade malignant lymphomas of the Kiel Classifica-

tion. However, about half of the 83 intermediate grade malignant lymphomas of the Working Formulation could be grouped into the low-grade and the rest with the high-grade malignant lymphomas of the Kiel Classification. The few survival data available on the diffuse large cell lymphomas do not support the proposal of the Working Formulation that this type of lymphoma should be grouped with the intermediate grade.

161 Immunohistological Analysis of Bone Marrow Biopsies for Diagnosis, Prognosis, and Follow-up of Non-Hodgkin Lymphomas (NHL)

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A total of 151 bone marrow biopsies from patients with NHL of the B-cell type were investigated by the use of cryostat sections and the immunoperoxidase technique. Neoplastic involvement was found in 75% of cases and the tumor cells could be phenotypically characterized. The histological pattern of infiltration (diffuse vs nodular/interstitial) as well as reactivity with monoclonal antibodies, e.g., percentage of cells stainable with the proliferation marker Ki-67, were correlated with the clinical course. A considerable number of T-lymphocytes was found among neoplastic B-cells with a prevalence of T-helper subtype. During treatment, changes in T-cell content and in T helper to suppressor ratio were observed within marrow infiltrates.

162 Monoclonal Antibodies Against Hodgkin-Derived Cell-Lines

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Monoclonal antibodies against the Hodgkin-derived cell line, L 428, were developed and screened immunohistologically. The two monoclonal antibodies (MOABs) H-SR-1 and H-SR-2 stained all Hodgkin and Sternberg-Reed (H and SR) cells and a subgroup of non-Hodgkin's lymphomas. In other tissues the two MOABs stained only a small subpopulation of cells, which morphologically resembled histiocytic elements. Despite the similar reactivity pattern of these two MOABs and the earlier described Ki-1 MOAB, inhibition experiments with biotinylated MOABs suggest that these MOABs detect different epitopes on the same antigen. The significance of these MOABs with respect to diagnosis and therapy of Hodgkin's disease is discussed.

163 Ki-B1 – A Monoclonal Antibody for Detection and Differentiation of Low-Grade Non-Hodgkin Lymphomas

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The murine monoclonal antibody Ki-B 1 recognizes a cell surface antigen that is found on follicle mantle cells and dendritic reticulum cells in lymphatic tissue. In peripheral blood it reacts with less than 5% of normal blood lymphocytes, which could be identified as a small B-cell fraction (about 20% of B lymphocytes). In patients with chronic B-lymphocytic leukemia or LP immunocytoma, Ki-B 1 detects more than 90% of neoplastic lymphocytes. However, leukemic cells in patients with centrocytic lymphoma, B-prolymphocytic leukemia, hairy-cell leukemia, or lymphoblastic lymphoma lack Ki-B 1 antigen. Thus, monoclonal antibody Ki-B 1 is a sensitive and almost specific reagent for the identification of even small numbers of leukemic cells in patients with chronic B-lymphocytic leukemia or LP immunocytoma and is recommended as useful reagent for the classification of low-grade non Hodgkin lymphomas.

164 Clinical Analysis of Ki 1 Lymphoma

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From the Kiel lymph-node register (1981 to 1985), 98 cases of unclassifiable high-grade malignant lymphoma were identified by the monoclonal Ki 1 antibody. By morphological criteria they could be further differentiated into three groups: primary Ki 1 lymphoma (prim. Ki 1-L., $n = 62$), secondary Ki 1 lymphoma (sec. Ki 1-L., $n = 15$) and Ki 1-positive non-Hodgkin lymphoma (Ki 1^+ NHL, $n = 21$). Retrospective analysis of the clinical data of 95 of these patients revealed differences with respect to age distribution, mean survival probability, rate of complete remissions achieved, and the mean duration of remissions. In prim. Ki 1-L. the age distribution showed a peak in the 2nd decade, sec. Ki 1-L. and Ki 1^+ NHL occurred more frequently at a more advanced age. The rate of complete remissions obtained and the mean survival probability were higher in prim. Ki 1-L. (approximately 60% and 43 months, respectively) than in sec. Ki 1-L. (approximately 45% and 10 months, respectively) or Ki 1^+ NHL (approximately 50% and 19 months, respectively). In comparing the different therapeutical approaches, a prognostic advantage seemed to be connected with the application of pediatric high-grade non-Hodgkin lymphoma treatment protocols in 11 patients with prim. Ki 1-L. who were treated accordingly (100% complete remissions, 73% without relapse, mean duration of remissions 14 months). In 54 of all of the cases, the Ki 67 antigen was also determined. So far, however, there has been no distinct correlation with clinical parameters.

165 Simplified Purification of Immunoglobulin Fragments Using "Fast Protein Liquid Chromatography" (FPLC) Production of Monoclonal Anti-idiotype Antibodies in B-Cell Lymphomas

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Anti-idiotype antibodies are tumor-specific in B-cell lymphomas. They can be used as sensitive probes for monitoring malignant cells in the peripheral blood and bone marrow. Preparation of light chains and Fab fragments after surface digestion of malignant B-cells with papain is facilitated by the use of FPLC. We have used these immunoglobulin fragments to immunize mice. Antibody responses were seen in several animals. The technique and results are demonstrated.

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166 Enzymes of Purine Metabolism in B-Cell Neoplasia and Their Therapeutic Significance

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The purine degradative enzymes, adenosine deaminase (ADA), purine nucleoside phosphorylase (PNP), and ecto-5 '-nucleotidase (5 'NT), play an important role in the development of lymphocytes. Investigations of these enzymes are of value in defining subsets of lymphoid malignancies. Pharmacological inhibition of ADA with deoxycoformycin (DCF) has been found to be an effective and specific treatment for some lymphoid malignancies. We have studied the ac-

tivities of these enzymes in the circulating malignant cells of 25 patients with B-chronic lymphocytic leukemia (CLL), 4 patients with B-prolymphocytic leukemia (PLL), 7 patients with leukemic centrocytic lymphoma (CC), 18 patients with hairy cell leukemia (HCL), and 16 patients with immunocytoma (IC). For comparison, the normal circulating T ($n = 12$) and B ($n = 8$) lymphocytes were also investigated. Despite morphologic similarity, the leukemic cells of these B-cell malignancies demonstrate different enzyme patterns. B-CLL is characterized by very low activities for all enzymes ADA, PNP, and 5 'NT. In HCL cells, the highest PNP values are found. The leukemic cells of IC are characterized by low levels of ADA but moderate levels of PNP and high levels of 5 'NT. Thus some of the entities of B malignancies show typical enzyme patterns, which are of importance in defining maturation stages of the disease. The differences in these enzyme patterns may be relevant to therapy with purine enzyme inhibitors such as deoxycoformycin.

167 Multicenter Randomized Trial of the Polychemotherapy of Advanced Centrocytic Lymphoma: No Prognostic Advantage by Anthracycline

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In patients with advanced centrocytic (CC) lymphoma (stages III and IV) the therapeutic efficiency of the COP vs the CHOP regimen was investigated in a randomized multicenter trial. By April 1986, 59 of the 82 patients who had entered the study fulfilled all criteria for randomization. The initial parameters and risk factors identified in 87 patients analyzed in a prospective observation study of the Kiel Lymphoma Study Group were similarily represented in the 35 COP- and 24 CHOP-treated patients: low Karnofsky index (17% vs 20%), B symptoms (42% vs 46%); lymph node (100% vs 95%), bone marrow (65% vs 63%), peripheral blood (37% vs 38%), or spleen involvement (54% vs 50%); male sex (66% vs 83%). Comparing the two regimens (COP vs CHOP) no significant differences were obtained with respect to rates of complete (39% vs 44%) or partial remissions (55% vs 52%), median probability of survival (36 vs 37 months), or median duration of remission (11 months each). Immediate initiation of therapy, however, resulted in a significantly higher probability of survival as compared with the patients of the prospective observation study in whom treatment was not started before disease progression became evident (strategy of "watch and wait"). The continuous decline of the survival curves without plateauing and the failure to obtain stable remissions strongly suggest that so far, advanced CC lymphoma must be considered an uncurable disease, thus demonstrating characteristics of other lymphomas classified as low-grade by histological criteria.

168 Treatment of Intensively Pretreated Low-Grade Malignant Non-Hodgkin Lymphomas with Low Dosage, Continuous Infusion of Vincristine, Doxorubicin, and High-Dosage Dexamethasone (VAD)

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Thirteen patients with intensively pretreated low-grade malignant non-Hodgkin lymphomas received 2–7 courses of a 4-day continuous infusion of 9 mg doxorubicin (adriblastine)/m^2 daily, 0.4 mg vincristine daily and 40 mg dexamethasone orally on days 1–4, 9–12, and 17–20. Treatment cycles were repeated every 28 days. Twelve of 13 patients are available for evaluation; 10/13 showed a response: 1 patient had no response, 1 patient had progressive disease, and 1 patient

cannot be evaluated. Of the 10 patients with a response, 3 have only had a small residual form of the disease for more than 6 months (+), five had partial remission, and 2 had only minimal response. Toxicity and side effects were minimal except for 1 patient in whom accidental extravasation of Vincristine and Doxorubicin occurred. Two patients experienced congestive heart failure, but it was treated successfully. None of the patients showed relevant myelosuppression. Low-dosage, continuous infusion of Doxorubicin and Vincristine, in combination with oral high-dosage dexamethasone seems to be an effective regimen for pretreated low-grade malignant NHL, and the toxicity is only minor.

169 Response-Oriented Therapy in High-Grade Malignant Non-Hodgkin's Lymphomas (NHL) with CHOP and VIM-Bleomycin

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Twenty-four patients (male 10, female 14, median age 52 years) with high-grade malignant NHL (centroblastic 12; immunoblastic 6; high-grade malignant, not further defined 6) were treated in a multicenter study. Eight patients had stage II, 4 stage III, and 12 stage IV disease. Treatment was initiated according to the CHOP protocol. Patients reaching at least a partial remission after 2 cycles and a complete remission after 4 cycles were continued on CHOP to a total of 9 cycles. Patients not meeting these criteria were switched to combination therapy with etoposide, ifosfamide, methotrexate and bleomycin (VIM-Bleo). With the CHOP treatment, 16 patients (67%) achieved complete remission. Of the remaining 8 patients, 7 were treated with VIM-Bleo, 1 patient died with progressive disease before change of treatment. In 2 patients, VIM-Bleo has just started. Of the 5 patients on VIM-Bleo who are available for evaluation, 3 had a complete remission. Thus, the overall complete remission rate was 19/24 (79%). With a median follow-up of 15 months, 5 patients had relapses. The projected 2-year disease-free survival rate is 64%, with an overall survival of 87%. All relapses occurred in patients with CHOP as the only form of therapy. With this therapy, good remission rates can be achieved; however, there is a significant rate of relapses.

170 Treatment of Adult Patients with Disseminated High-Grade Lymphomas of the B-Type

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The poor prognosis of disseminated high-grade B-NHL has been improved in children as a result of the aggressive protocol BFM 81/83 developed by the BFM study group for childhood lymphomas (containing MTX 500 mg/m^2 as 24-h infusion with leucovorin rescue, cyclophosphamide, VM 26, Ara-C, adriamycin and prednisone in addition to MTX intrathecally and prophylactic CNS irradiation). On the basis of these results, we treated 6 adult patients [1 female, 5 males; median age 25 (18–47) years] according to this protocol, modified as follows: beginning leucovorin rescue earlier (8 h after the end of MTX infusion), prolongation of course interval (mostly 3–4 weeks), moderate reduction of doses. The histologies were: 3 NHL lymphoblastic, Burkitt's type; 2 polymorphic B-centroblastic; 1 B-immunoblastic. All patients had stage III–IV lymphomas (Murphy). The results were that five of six patients achieved complete remission (27+, 19+, 3+, 3+, 2+ months) 1/6 PR (10 months). Toxicity: Hematotoxicity was prominent: 11 of 41 patients developed agranulocytosis (WHO grade 4), 7 patients had short-term fevers (WHO grades 2–3); no severe thrombocytopenia (WHO grade 4) occurred.

171 Failure to Cure Secondary High-Grade Non-Hodgkin Lymphoma by Aggressive Polychemotherapy

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Eleven patients with secondary high-grade non-Hodgkin lymphomas emerging from low-grade lymphoma entities (6 centroblastic lymphomas in previously diagnosed centroblastic-centrocytic lymphomas, 5 immunoblastic lymphomas in preexisting LP immunocytomas) were treated with aggressive polychemotherapy according to the CHOP, CHOP + bleomycin, ProMACE-MOPP, ProMACE-CytaBOM and MACOP-B regimens. Two patients achieved complete remissions lasting, however, only 2 and 6 months respectively. Two patients attained only partial remissions of short duration, and 7 patients did not respond to treatment. Our results are in contrast to the hypothesis raised by DeVita and Hubbard (1982) which suggested that nodular, poorly differentiated, lymphocytic (NPDL) lymphoma according to the Rappaport classification (corresponding predominantly to centroblastic-centrocytic lymphoma of the Kiel classification) might become curable by aggressive polychemotherapy after progression from low-grade to high-grade malignant histology.

172 Frequency and Spectrum of Autoimmunological Derangements (AID) in Lymphoproliferative Disorders (LPD)

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In a retrospective analysis of 655 cases of LPD and 355 cases of myeloproliferative diseases (MPD), AID of the following kind were found in 51 patients with LPD (7.8%) and 6 patients with MPD (1.7%): Coombs-positive hemolytic anemia, autoallergic thrombocytopenia, Evans' syndrome, lupus anticoagulant, discoid lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, Behçet's syndrome, iritis, Hashimoto's disease, Graves' disease, pernicious anemia, primary biliary cirrhosis, chronic active hepatitis, insulin-dependent diabetes mellitus, nontropical sprue, Crohn's disease, ulcerative colitis, glomerulonephritis, Guillain-Barré syndrome, and myasthenia gravis. In 3.2% of patients with LPD (1.4% in MPD) the AID preceded the neoplastic disease, the frequencies observed in Hodgkin's disease (3.6%), low-grade non-Hodgkin lymphoma (NHL) (3.5%), high-grade NHL (3.6%) and multiple myeloma (2.1%) being comparable. In 4.6% of patients with LPD (only 0.3% in MPD) AID developed during the subsequent course of the disease. Here we found marked differences between Hodgkin's disease (1.5%), low-grade NHL (7.7%, almost exclusively lymphocytic and immunocytic lymphomas), high-grade NHL (1.2%), and multiple myeloma (2.1%). We conclude that AID occur by far more frequently in LPD than in MPD. The frequency of AID developing during the course of LPD correlates with the prognosis of the NHL entities: the more favorable the prognosis, the higher the likelihood of autoimmunological complications.

173 Nodular Sclerosing Hodgkin's Disease: Prognostic Relevance of a Histological and Cytological Subtyping

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In order to find out whether prognostic relevance can be related to the different cytologic features of nodular sclerosing Hodgkin's disease (NS), thus justifying a subtyping, we analysed the

cytologic features of 69 cases with NS according to the general subtyping of NS: (1) lymphocyte predominant form ($n = 15$); (2) mixed cellularity form ($n = 32$); (3) mixed cellularity form with transition to lymphocyte-depleted form and lymphocyte-depleted form ($n = 14$); (4) in addition – as an early manifestation – the so-called cell-predominant phase was defined. The correlation of these morphological parameters with the clinical features, course and prognosis resulted in the following: at the time of diagnosis, there was no significant difference in mean age (26 years), sex ratio or stage (mainly I or II) between the groups. The frequency of mediastinal lymph node involvement (40%–65%) was independent of the cytologic features; a bulky disease occurred more frequently in mixed cellularity form (significant at a low level). The rate of complete remissions (75%–93%), the relatively high frequency of relapses (27%–50%) and the survival rate after 5 and 8 years (82%–100%) and after 9 years (61%–87%), was independent of the cytologic features. Also, the grade of fibrosis did not correspond to the clinical data. We conclude that morphological criteria, such as cytologic features and fibrosis of NS are not prognositc parameters and therefore subtyping does not seem to be justified.

174 Combination of Non-Hodgkin Lymphoma with Hodgkin's Disease

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Nineteen cases with a morphological picture apparently indicating a combination of NHL and HD were investigated. Three groups were identified. Group 1 ($n = 9$) consisted of the following NHL components: 7 cases of chronic lymphocytes leukemia of B-type (B-CLL), 1 LP-immunocytoma (LP-IC), and 1 cb/cc. The NHL components showed either a monotypic Ig distribution pattern and/or a leukemic blood picture. In addition to the NHL, typical HD was present, in which H- and SR-cells were positive for both Ig light chains, IgG, 3 C 4 and LeuM 1. Group 2 ($n = 7$) consisted exclusively of cb/cc in combination with HD, in which H- and SR-cells were rare and negative for 3 C 4 and LeuM 1. Group 3 ($n = 3$) consisted of 3 B-CLL not in combination with HD because, apart from the presence of typical H- and SR-cells which were 3 C 4 and LeuM 1 positive, further characteristics of HD were absent. The following conclusion was drawn from the results: the monoclonal antibodies 3 C 4 and LeuM 1 can neither confirm nor exclude the possibility of HD because, on the one hand, they do not recognize H- and SR-cells in all cases of HD and on the other they recognize H- and SR-cells in B-cell NHL.

175 True Histiocytic Lymphoma – Clinical Course and Treatment of Four Patients

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True histiocytic lymphoma originating from the macrophage lineage is a rare disease. Its therapy is not well defined. Histopathologically there is no unequivocal difference from malignant histiocytosis, but the expression of cell-surface antigens (Tac, Ki-1, OKT 9, MAX.26 and Ia) is helpful in characterizing the disease. We report on four patients with histologically proven histiocytic lymphoma. Two of our patients achieved remission (12 and 15 months) by a treatment with CHOP and VIM-Bleo (vincristine, ifosfamide, methotrexate, bleomycin). One patient died as a result of leukemic meningeal involvement during induction therapy. Another patient with meningeal involvement partially responded to chemotherapy (including high-dosage methotrexate, etoposid, adriamycin, ifosfamide and cytarabine) but never achieved continuous remission. Patients with true histiocytic lymphoma that responds to CHOP treatment seem to have a better prognosis. However, they fare worse when they present with CNS involvement; prophylactic cerebrospinal treatment may be indicated.

176 Prognostic Significance of Skin and Bone Marrow Findings in Generalized Mastocytosis

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Mastocytosis is usually subdivided into purely cutaneous variants and generalized forms with polytopic infiltration of bone marrow, liver, spleen, and lymph nodes. A survey of clinical and histopathological data from 66 patients revealed that the occurrence of skin lesions resembling urticaria pigmentosa in cases of mastocytosis has significant prognostic implications. Thus, patients with generalized mastocytosis and primary skin involvement (systemic mastocytosis) show a significantly longer survival time than patients without skin involvement (malignant mastocytosis). The short survival period in malignant mastocytosis is chiefly due to the more frequent appearance (70% of cases) of myeloproliferative disorders, especially acute and chronic myeloid leukemias and overt mast-cell leukemias. The bone marrow histology in systemic mastocytosis exhibits patchy, often peritrabecular mast-cell infiltrates with intact hematopoiesis. In contrast, bone marrow findings in malignant mastocytosis show, apart from the disseminated mastocytic infiltrates, severe alteration of the architecture with extreme hyperplasia of granulocytopoiesis or sheets of blast cells. True mast-cell leukemias show a diffuse infiltration of the bone marrow by atypical mast cells, concomitant with a marked depletion of fat cells and blood-cell precursors.

177 Gastrointestinal Lymphomas: A Retrospective Analysis of 43 Cases

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Forty-three patients with localized gastrointestinal lymphomas, who were treated during the period 1976–1984, were analyzed retrospectively. The median age was 59 (14–81) years. The chemotherapy protocols were CHOP or comparable regimens. Radiation therapy was performed as an "abdominal bath," partly with local booster doses. After 8 years the probability of survival was 71%, with a disease-free survival of 50%, irrespective of age, sex, and site. The presence of B-symptoms was a bad prognostic sign ($P = 0.003$), whereas there was no difference between stages IA and II (survival probability 80% and 74%, respectively). Patients with resection had better results, although this difference can be attributed in parto the additional risk factors of the group not operated upon, such as large tumor mass. Within the resection group there was a slight but not significant advantage in favor of additional chemotherapy plus radiation. In view of the good results, it seems justified to continue with this approach.

178 Preliminary Results of the MACOP-B Regimen in Patients with High-Grade Malignant Lymphomas

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Since November 1985, 14 patients with high-grade malignant lymphomas have been subjected to MACOP-B chemotherapy (Klimo and Connors, Ann Intern Med 102: 596). Pretherapeutic staging indicated stadium II E for 2 patients, stadium III in 4 cases and stadium IV for 8 patients. Therapy has so far been completed in 8 patients, 7 of whom achieved complete remissions and 1 a partial remission. The other patients are presently still on therapy. Toxicity and side effects were minor, including myelosuppression and mucositis. These preliminary data con-

firm the previously reported positive results, possibly superior to those of other presently used regimens, and argue in favor of this protocol because of its efficacy in high-grade malignant lymphomas and its short duration (12 weeks).

179 Etoposide, Ifosfamide, Methotrexate and Bleomycin in CHOP-Resistant Non-Hodgkin's Lymphomas

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In an ongoing study, 29 patients with non-Hodgkins lymphoma (NHL) (centrocytic, 3; centroblastic-centrocytic, 6; centroblastic 11; immunoblastic, 6; high-grade malignant, not further defined, 3), which was resistant to therapy with CHOP, were treated as follows: ifosfamide, 1.0 g/m^2 i.v. on days, 1–5, with mesna for prophylaxis of cystitis; etoposide, 100 mg/m^2 i.v., on days 1–3; methotrexate, 30 mg/m^2 i.v., on day 3; bleomycin, 10 mg i.v., on days 7 and 14. Cycles were repeated at 3-week intervals. Twelve patients (41%) achieved complete remission, 3 with a NHL of low- and 9 with a NHL of high-grade malignancy. Six patients had a partial remission. With a median follow-up of 18 months, median relapse-free survival is 10 months. Median survival is 16 months with no significant difference regarding the grade of malignancy. Survival for patients in complete remission was longer (median not yet reached) than for those less responsive (median 6 months). Toxicity was usually mild. In 8.3% of 109 cycles analysed so far, leucocytes fell below 2000/μl. In 6.4%, platelets dropped to less than 75 000/μl. Fever was seen in 6.4% of courses. Severe stomatitis developed in 1.8%. We conclude that this treatment is effective and well tolerated in CHOP-resistant NHL. Updated results will be presented at the meeting.

180 Cytotoxic-Induced Testicular and Ovarian Dysfunction in Patients Treated for Hodgkin's Disease with Chemotherapy

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As a consequence of the therapeutic success in patients with Hodgkin's Disease (HD), concern has arisen about late adverse sequelae of chemotherapy (CT). Most of these patients are young adults and concerned about their subsequent reproductive potential, pregnancy outcome, and sexual activity. We undertook a prospective study in order to examine the effect of CT on reproductive and endocrine gonadal function in patients with HD. Fourteen women and 19 men successfully treated for HD were studied 1–17 years after COPP-CT. Diagnostic procedures to establish gonadal toxicity included an interview, sperm evaluation and hormone analyses by RIA (follicle-stimulating hormone, LH, testosterone (T), estradiol (E), progesterone and prolactin). All men showed azoospermia and marked increased FSH levels (median: 1095 ng/ml) 1–12 years after CT. In all men LH levels were within normal limits (median: 48 ng/ml) as well as T levels (median: 578 ng/100 ml). Eighteen of nineteen men reported having normal sexual function, but no men had fathered children after CT. Eight of fourteen women reported having premature menopausal symptoms, revealing marked elevated FSH levels (median: 3700 ng/ml) and decreased P values (median: 0.24 ng/ml). In only 3/8 of these patients were LH increased and E decreased. The incidence of amenorrhea in patients aged over 24 years was 86% versus 14% under 24 years ($P = 0.009$). Three of fourteen women aged under 24 years became pregnant after CT, delivering 5 healthy children. Our data suggest: (1) irreversible infertility in all men; (2) normal endocrine testicular function; (3) age-related ovarian failure in women; (4) no increased risk of fetal malformations in patients successfully treated for Hodgkin's disease with COPP chemotherapy.

181 The Diagnostic Usefulness of Blood and Serum Tests During Follow-up of Hodgkin's Disease

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The value of a number of laboratory tests performed during the 12 months preceding and at the time of diagnosis of 94 recurrences of Hodgkin's Disease (between 1968 and 1984) was evaluated. Laboratory tests were abnormal in 91 cases. The ESR (2 h) was elevated in 88%, ESR (1 h) in 82%, haptoglobin in 84%, and serum copper in 69%. A further 15 laboratory tests provided no additional information. The ESR and haptoglobin concentration increased during the 4–6 months preceding the diagnosis of recurrence, which provides a possibility to advance the date of diagnosis if all other diagnositc tools were then applied. A number of laboratory tests could be omitted. As long as more specific blood tests are not available, for follow-up we recommend the determination of ESR, haptoglobin, blood picture, and alkaline phosphatase. This cost-saving laboratory programme will be discussed in detail.

182 Recognition of Relapse of Patients with Malignant Lymphomas: Efficiency of Different Methods

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The study is based on the observation of 584 patients with malignant lymphomas (Hodgkin's disease 248, non-Hodgkin's of favorable histology 203, non-Hodgkin's of unfavorable histology 133), who had regular follow-ups from 1974 to 1983. Of these, 315 patients attained a complete remission (169, 64, 82, respectively). Of these patients 68% had symptoms indicating the relapse (62%, 77%, 76%). Thirty-three percent palpated the tumor themselves (36%, 41%, 20%). Twenty percent showed specific symptoms like local pain or lymphedema (13%, 13%, 44%). Fifteen percent suffered from systemic symptoms (13%, 13%, 12%). As far as the 32% of the patients without symptoms are concerned, the relapse was diagnosed by clinical examination in 10% (11%, 9%, 8%). A routine chest X-ray revealed the relapse in 8% (10%, 9%, 4%). In 6% the relapse was discovered by other investigations (7%, 0%, 8%). At the time of the relapse, the following laboratory findings were striking: 49% of patients with Hodgkin's disease had leukocytosis, in 70% an elevated ESR, and in 58% the α-2 globulin level was increased by more than 10%. In 60% of patients with unfavorable NHL elevated ESR and LDH were found, and in 46% the α-2 globulin fraction was increased. Our study shows that history and clinical examination are the most important parameters in recognizing a relapse. Chest X-ray and sonography show the relapse in only 8% or 7%. The laboratory findings are less important, except for the ESR, LDH and the α-2 globulin level.

183 Monoclonal Gammopathy Mimicking Systemic Lupus Erythematosus

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A circulating anticoagulant of the "immediate type" (CA) is clinically often associated with recurrent abortions, thromboses, neurological abnormalities, thrombocytopenia and false-positive serological test results for syphilis. The syndrome is mainly observed in women with systemic lupus erythematosus (SLE) and is possibly caused by auto-antibodies against phospholipids (Br Med J 287: 1088, 1983). Of 11 patients with a CA seen between 1980 and 1985, only 7 suffered from primary auto-immune disease (AID). The other 4 patients had monoclonal

gammopathy (MG) ascribable to multiple myeloma or lymphoplastoid immunocytoma. Six patients with AID and 3 patients with MG showed the characteristic clinical correlates of the CA. The patients with MG, however, differed from those with AID by androtropy, higher age at the onset of disease and a lesser frequency of serological auto-immune phenomena. During the course of the disease in one patient the concentration of the monoclonal protein correlated with the alteration of coagulation assays and the clinical symptomatology. It is tempting to speculate that the monoclonal proteins are responsible for both the coagulation defect and the clinical findings. Although the specificity of monoclonal immunoglobulins for phospholipids and the interference with coagulation assays have repeatedly been demonstrated, the full clinical picture associated with the CA in MG has yet to be described.

184 Vincristine, Cyclophosphamide, Melphalan, Prednisolone (VCMP) Chemotherapy of Multiple Myeloma After Ineffective Melphalan, Prednisolone (MP) Therapy

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The escalation of chemotherapy for progressive multiple myeloma during MP treatment is a matter of controversy. Some of the chemotherapeutic regimens, like the VCMP combination, are of low toxicity, which is advantageous in older patients. We therefore treated 13 patients who had not profited from the MP regimen with the VCMP combination. Nine of these patients showed a remission which was of longer duration (mean 9 months, range 3–24 months). Four patients did not respond; two of them later died. The VCMP regimen is a low-risk form of chemotherapy which can be tried in older patients, even if MP treatment has been ineffective.

185 Treatment of Advanced Multiple Myeloma with Vincristine, Adriamycin and Dexamethasone (VAD)

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Previous studies have suggested the specific efficacy of high and frequent doses of glucocorticosteroids combined with long-term application of vincristine (VCR) and adriblastine (ADR), thus taking account of the slow proliferation of plasma cells. With 4-day continuous infusion of VCR 0.4 mg/day and ADR 9 mg/m^2/day and dexamethasone 40 mg i.v. for 4 days beginning on days 1, 9, 15, Barlogie et al. (N Engl J Med 1984, 310) achieved a 75% reduction of tumor mass in about 70% of patients with resistant myeloma. We treated ten patients according to this protocol. In accordance to South West Oncology Group (SWOG) criteria there was one objective response (A); two patients improved (B); four showed a progression of their disease and three remained in a stable condition (C). Apart from infectious complications in 5 of 30 courses, no serious side-effects occurred. Our conflicting results need further evaluation to identify subsets of patients who could benefit from this protocol.

186 Diagnosis of Meningeal Myeloma Diagnosed with Monoclonal Antibodies

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A 64-year-old woman with an IgG-κ-myeloma diagnosed 14 months previously was examined because of bone pain, confusion and dysarthric speech. The right arm and leg were weak, with

brisk tendon reflexes. Repeated bone marrow examination showed small sheets of immature plasma cells for the first time: late evidence of systemic disease. A lumbar puncture yielded 113 cells/μl: 20% lymphocytes and ~ 60% heavily proliferating plasma cells. CSF did not contain a monoclonal band in the same position as the band in the serum. The diagnosis of neoplastic meningitis was confirmed by monoclonal antibody testing. We used a panel of ten monoclonal and three polyclonal antibodies comprising T, B markers and antibodies recognizing complete Ig and L-chain proteins by means of PAP immunochemistry staining. Some 75% of all clearly non-lymphocytic large cells stained intensively for κ-L-chains only. The cytoplasmic stain for IgG in the accelerated phase of the disease was slightly positive in $< 3\%$. The mechanism underlying the expression of a sole κ-antigen and the biological significance of our findings remain unclear. Irradiation treatment of the cranium and intrathecally administered methotrexate produced some improvement, but the patient died shortly thereafter of septicemia.

187 Inhibition of the In Vitro Growth of Human Megakaryocytic Progenitor Cells by Recombinant α- and γ-Interferons

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While interferons are becoming more important in the treatment of hematological and non-hematological disorders, no information is yet available concerning the effect of IFNs on the in vitro growth of megakaryocytic progenitors (CFU-Mk) and in vitro differentiation of pluripotent progenitors (CFU-GEMM) along the megakaryocytic lineage. Therefore, the effect of recombinant IFN-α (Genentech, USA) and recombinant IFN-γ (Biogen, USA) on normal human bone-marrow derived CFU-Mk and CFU-GEMM was tested in a clonal culture system containing 30% human plasma in IMDM, 5% PHA-leukocyte conditioned medium, 0.9% methylcellulose, 50 μM 2-ME, and 1 U erythropoietin/ml. Addition of IFNs ($10\text{-}10^4$ U/ml) to unseparated bone-marrow cells resulted in a dose-dependent inhibition of CFU-Mk (24% – 100%) and CFU-GEMM (34% – 100%), which could be selectively blocked by respective monoclonal antibodies. Removal of T-lymphocytes and/or adherent cells from the target cells had no effect on the inhibition exerted by IFN-α; however, the suppressive effect of IFN-γ was significantly reduced. The percentage of multilineage colonies containing megakaryocytes was not influenced by either IFN. It is concluded that (a) rIFN-α and rIFN-γ markedly suppress in vitro megakaryopoiesis; (b) the effect of rIFN-γ is largely mediated through accessory cells; (c) the inhibition takes place at an early level of progenitor cell proliferation and not at later stages of in vitro maturation.

188 Modulation of In Vitro Production of Tumor Necrosis Factor, Lymphotoxin, and Interferon-γ Induced by Recombinant IFN-γ In Vivo

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Both the production of tumor necrosis factor (TNF) and lymphotoxin (LT) as well as their effects on cellular functions are subject to complex regulation which can be effectively modulated by IFN in vitro. The secretion of these cytotoxins in response to inducing agents such as PHA or IL-2 can be significantly enhanced by rIFN-γ in vitro. This study demonstrates that rIFN-γ is capable of modulating the production of TNF, LT, and IFN, when administered in vivo. Changes observed during in vitro production of IFN by the blood mononuclear cells (MNC) of patients receiving rIFN-γ were related to the dose of rIFN-γ administered. At relatively low in vivo dosages (0.1 mg/m^2) there was a rapid increase (5- to 10-fold of baseline value) in the amount of IFN-γ secreted upon stimulation in vitro. With higher doses (0.25 mg/m^2) we initially observed a hyporeactivity in response to PHA, followed by an 5- to 10-fold increase after

24–36 h. The in vitro induction of cytotoxins in MNC using the same in vivo doses of rIFN-γ was moderately enhanced by different inducers without any hyporeactivity. IFN-γ induced low titers of cytotoxins in MNC from treated patients, but not in MNC from untreated patients. This mechanism may play an important role in the antitumor activity of IFN-γ.

189 Immunomodulatory Effects of Recombinant α-Interferon, γ-Interferon, and Interleukin-2 In Vitro*

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The immunomodulatory activities of recombinant α-IFN, γ-IFN, and IL-2 were studied. We tested in vitro parameters of B- and T-cell functions as well as NK-cell activity: immunoglobulin (Ig) secretion and lymphocyte proliferation in cultures of peripheral blood mononuclear cells after stimulation with mitogens or antigens and NK activity against the human myeloid cell line K 562. All cultures were set up with and without the addition of α-IFN, γ-IFN, IL-2, and combinations of these substances. B-cell function: Ig synthesis was strongly inhibited by α-IFN and by combinations including α-IFN, whereas γ-IFN and IL-2 and their combinations had no or only a marginally suppressive effect on Ig synthesis. Lymphocyte proliferation responses were also depressed by α-IFN and α-IFN including combinations, whereas γ-IFN and IL-2 showed no effect on lymphocyte proliferation capacity. These inhibitory activities of α-IFN were related to the time the substance was added but were not dose-dependent over a longer period. NK activity was augmented by α-IFN, γ-IFN, and IL-2, α-IFN being the most effective substance in this regard. Combinations of these biologically active agents were able to potentiate the enhancing effect on NK-cell function. Our data demonstrate strong suppressive as well as enhancing effects of α-IFN, γ-IFN, and IL-2 on certain immune functions. We conclude that during clinical trials with these biological response modifiers the immunomodulation by these substances should be carefully monitored.

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190 Treatment of Malignant Diseases with Interferon-α: Sources, Doses, and Frequency of Treatment

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The antineoplastic activities of interferon-α (IFN α) have been demonstrated in several malignancies. For example, high response rates (80%) have been shown in the treatment of hairy-cell leukemia (HCL) and lower but relevant response rates (25%) have been reported in some studies on metastatic renal cell cancer (RCC). These two disorders are discussed here in relation to the effects of IFN α source, daily doses, number of treatments/week (t/w), and time to response (ttr). In HCL, natural (n) and recombinant (r) IFN α preparations are effective; the daily dose ranges from 0.1 to 5 million IU; two to three t/w are sufficient; the time to first response varies from 1 to 4 weeks. In RCC, also n and r IFN α preparations seem to be effective but the necessary daily dose of r IFN α is probably 10-fold higher than of n IFN α; it is not yet clear if less than five t/w will be sufficient; the ttr varies from 2 to 12 weeks. These clinical observations show that completely different regimens are required for IFN α therapy of HCL and RCC. Concerning the source of IFN α, it seems that n IFN α contains a particular subtype which is more effective – at least in RCC – than the tested r subtypes. Therefore, higher doses of r than of n IFN may be required for equal therapeutic effects. The minimal t/w has not yet been determined. The lack of in vitro production of IFN α in the HCL patients and their low basal 2–5 oligoadenylsynthetase (2–5 AS) suggest either low or a lack of IFN production in these patients. Substitution of low doses of IFN α shows clinical effects and increases 2–5 AS levels

in HCL patients. In RCC the same low doses are not effective, which suggests differing effects of IFN α in HCL and RCC. In conclusion, it is difficult to predict effective sources and doses of IFN α unless the mechanism of IFN α action is known in the particular disease. For experimental treatment of new indications it is recommendable to start with the maximal tolerable doses. Accordingly, we suggest adjusting IFN doses for treatment of HCL to the actual platelet counts (Blut 51: 73–82, 1985).

191 Intralesional Versus Systemic IFN Therapy in Advanced Malignant Melanoma

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Fifty-one patients with advanced malignant melanoma were treated either with highly purified natural IFN-α (3×6 million IU/week) or recombinant IFN-α_2 (3×6 million IU/week) intralesionally. Even when a patient had several skin metastases, only one lesion was injected during the IFN treatment. If progress was noted after 28 days, the treatment was stopped. If the disease remained stable or regressed, treatment was continued. The intralesional injections were well tolerated and did not induce any observable inflammatory reaction. Tumor response was measured according to the WHO recommendations for the determination of overall efficiency. In addition, the size of the injected skin lesions was measured to determine the local efficiency of the IFN treatment. After the intralesional injections IFN was detectable in the serum at titers similar to the following i.m. or s.c. IFN injections, as reported previously by others. In or at the injected skin lesions, the concentration of IFN was up to 100-fold higher than in the serum. Of the 51 patients, 3 experienced a complete and 6 a partial response (17%). This shows clearly that intralesionally administered IFN-α has systemic antitumoral activity in malignant melanoma. Of all injected lesions, 45% showed at least a 50% reduction in size. In addition to their injected skin lesion, 42 patients had other skin metastases, which were situated in other lymphatic drainage regions than that of the IFN injection. In 21% of non-injected skin metastases there was a reduction in size, whereas 45% of the injected lesions in the same patients decreased in size. The difference is statistically highly significant at a P level of < 0.01. It is tempting to speculate that the high local IFN concentrations measured are responsible for the higher local therapeutic efficiency of IFN-α.

192 Abnormalities in Production of Interferon (IFN) Caused by Long-Term α-IFN Therapy in Patients with Laryngeal Papilloma

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α-Interferon (IFN) treatment was given to 8 patients with permanently recurrent laryngeat 2 papillomas (LP) who had been receiving conventional therapy for periods of 2–7 years. By determining the induction kinetics of $'-5'$-oligo-A-synthetase activity it was possible to find out for each individual patient which dose of α-IFN would be adequate for effective therapy without inducing the well-known side-effects. The patients received native α-IFN, $5-20\times10^4$ U/kg 2–3 times/week i.m. During immunological control of the long-term α-IFN therapy, serum titres of α-IFN, spontaneous and induced IFN production in leucocytes of the patients were analysed before the start of therapy and then every 3-b months. Up to now we have surveyed the results of 4 patients after 3 years of treatment and more. After about 12 months of therapy, they developed severe depression of endogenous α-IFN production, and after a further 6–12 months of therapy, severe depression of γ-IFN production was noted. In spite of these important abnormalities in the IFN system, no atypical courses or accumulation of infections occurred. Thus long-term α-IFN therapy can be considered as invasive, but without recognizable detrimental consequences for the patients.

193 In Vitro Colony Inhibition of Tumor Necrosis Factor in 14 Human Tumors

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The colony inhibition of recombinant-produced tumor necrosis factor (TNF) was studied in 14 human tumors. The donor tumors grew in serial passage in athymic nude mice. The colony assay performed was a modification of the method of Hamburger and Salmon. TNF was considered to be active if the colony number was $< 30\%$ of the controls. Counting of colonies was performed by an automatic feature analysis system. Tumor cell suspensions ($1-5\times10^5$ cells/dish) were incubated continuously throughout the experiment (6–18 days). In the investigated dose range of 0.01 to 1 μg/ml a clear dose-response relationship was found. Three human tumors were sensitive at a dose of 0.01 μg/ml (1 large cell cancer of the lung, 1 renal cancer and 1 osteosarcoma). TNF was effective in 6 of 14 tumors at a dose of 0.1 μg/ml and in 6 of 10 tumors at 1.0 μg/ml. Two small cell cancers of the lung were resistant even at the highest dose of TNF. The colony inhibition of TNF will be studied in 40 human tumors in order to determine target tumors for the clinical phase II studies.

194 Phase-I Study of Intratumoral Application of Recombinant Human Tumor Necrosis Factor in Patients with Malignant Diseases

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After the completion of preclinical trials, we started a phase-I trial of intratumoral application of recombinant human tumor necrosis factor (rhuTNF; provided by Asahi Chemical Inc.) in patients with malignant diseases. The plasma levels of rhuTNF were determined immunologically by ELISA, using a monoclonal antibody against rHuTNF, and functionally by the L-cell assay. The starting dose was 1×10^5 U/m^2. By April 1986 we had treated 14 patients with a maximum dose of 7×10^5 U/m^2. The main side-effects were chills, followed by fever of up to 40.5°C. These reactions were seen in nearly all of the patients and appeared to occur independent of the dose of rHuTNF. In some patients we observed circulatory disturbances (a brisk rise in blood pressure followed by a decline to subnormal levels), mild nausea or vomiting and pain at the injection site. A small decrease of platelets could be detected in most cases, while only a few patients had a slight transient increase of transaminases. All side-effects were fully reversible. The maximum tolerated dose of intratumoral rHuTNF has not yet been reached. This phase-I study will be continued.

194 Phase-I Trial of Recombinant Tumor Necrosis Factor Alpha in Advanced Malignancy

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Patients with advanced malignancies resistant to standard therapy are being treated according to a phase-I tumor necrosis factor (TNF) protocol, administered i.v. for over 30 min b.i.d. Doses are increased in cohorts of six patients: 2.5, 5, 12.5, 25, 37.5 to 50 μg b.i.d. TNF is administered for 5 days every 2 weeks for a total of 8 weeks. So far, 20 patients with various kinds of solid tumors have been treated including 3 with multiple myeloma. Side-effects have included fever

and chills of short duration (30–45 min) in the majority of patients and were well tolerated. No other significant kinds of toxicity have been encountered with the current dose level of up to 25 μg b.i.d. Two patients with multiple myeloma experienced minor subjective and objective improvements in disease parameters. Although largely ineffective at the dose and schedule used, with the possible exception of multiple myeloma, it is hoped that further dose increases will not only yield toxicological and pharmacological information of interest but will also allow definition of disease categories which might benefit from TNF.

196 Phase-I Study of 24-Hour Infusion of Recombinant Human Tumor Necrosis Factor in Patients with Malignant Diseases

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In 1985 we started a phase-I study of intratumoral application of recombinant human tumor necrosis factor (rHuTNF, provided by Asahi Chemical Inc.) in patients with malignant diseases in order to gain experience with the clinical toxicities of rHuTNF at certain plasma levels. The plasma levels of rHuTNF were determined immunologically by ELISA and functionally by the L-cell assay. As no inacceptable kinds of toxicity were observed at plasma levels of 10 U/ml we believed if would be safe to start a phase-I study involving 24 h continuous infusion of rHuTNJF, starting with a dose of 2×10^5 U/m^2. By April 1986, 8 patients were included in the study, receiving a maximum dose of 5×10^5 U/m^2. The main side-effects were chills, fever and blood pressure reactions. These side-effects were seen in nearly all the patients without there being a clear-cut correlation with the dose given. A short period of increased blood pressure was followed by a decrease to a level of moderate hypotension, which lasted throughout the period of application. Moreover, a slight fall in platelet counts was observed in most patients. All side-effects were fully reversible. No liver or kidney toxicity was observed. The maximum tolerated dose has not yet been reached. The study will be continued.

197 Effects of Interferon on HLA Class-I Gene Expression in Transfected Mouse L-Cells*

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Genes coding for different HLA class-I antigens (HLA-A 2, HLA-B 7, HLA-B 27, HLA-Cw 3) were integrated in mouse L(tk$^-$) cells by DNA-mediated gene transfer. Southern blot analysis showed that 5–200 copies of the transfected gene were present per cell. Using monoclonal antibodies against subtypical and supertypical 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. Isoelectric focusing analysis also revealed no differences between the gene product on transfected and human cells. Exposure of the transfected cells to 10^4 IU of mouse interferon/ml medium resulted in accumulation of the HLA-specific mRNA after 24 h and enhanced expression of the antigen at the cell surface after 36 h. Comparing the induction pattern of the different HLA genes, we were able to show that the HLA-B 7 gene was 2–3 times more inducible than the other genes. These results were confirmed with L-cells transfected with two different HLA genes (HLA-A 2 and HLA-B 7).

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198 Endogenous Production of Tumor Necrosis Factor in a Patient with Hepatocellular Carcinoma

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Endogenous tumor necrosis factor (TNF) was detected in a 61-year-old female patient with hepatocellular carcinoma. The carcinoma was diagnosed in March 1984 and resected. A local relapse occurred in October 1984. In June 1985, the patient developed systemic symptoms (fever, night sweats and loss of weight). Endogenous levels of TNF were detected immunologically by ELISA and functionally by the L-cell assay when therapy with recombinant human TNF (rHuTNF, provided by Asahi Chemical Inc.) was being planned. The plasma levels of TNF displayed a circadian rhythm with maximal levels (approx. 20 IU/ml) appearing between 8 and 9 a.m. We will report on further investigations presently being performed to identify the TNF-producing cells. The significance of endogenous TNF production in patients with malignant disease will also be discussed.

199 Demonstration of Tumor Necrosis Factor in MOLT-4, NC-37 and HL-60 Cultures

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By induction with lipopolysaccharide (LPS) from Escherichia coli cell lines of NC-37, HL-60 and MOLT-4 were caused to produce tumor necrosis factor (TNF). By ammonia-sulfate precipitation the protein was isolated from the medium, then separated by liquid chromatography. Characterization of TNF was possible by isoelectric focusing and determination of cytotoxic activity (in units: 50% killed cells of 10^4 cells during 48 h, vital stain). The cytotoxic activity of TNF (measured in IU) decreases from HL-60 down to MOLT-4 and NC-37. In contrast, TNF obtained from rat blood after induction with zymosan and LPS has less activity than TNF from cell cultures. The molecular weight of in vivo induced TNF (52 000 daltons) is higher than that of said cell lines (17 000 daltons). Initial in vivo examinations (nude mice with neuroblastomas) have confirmed the cytotoxicity observed in vitro.

200 In Vitro Generation of Lymphokine-Activated Killer Cells in Whole Blood from Healthy Donors and Patients with Neuroblastoma

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Lymphokine-activated killer cell (LAK) transfer is a new approach for the treatment of different tumors. In this method, isolated mononuclear cells from blood are incubated in vitro with IL-2. Thereby, they become able to kill autologous and allogeneic tumor cells. For this purpose, however, a great number of lymphocytes has to be isolated under strict sterile conditions. In order to avoid the disadvantages of direct IL-2 administration (short half-life of IL-2 in the body) as well as of the cultivation of large amounts of lymphocytes, we propose that LAK be generated in vitro in whole blood. The following conditions for the optimal in vitro stimulation of killer cells in whole blood were investigated: influence of the concentration of IL-2 and interferon (IFN) and combinations of both; influence of temperature and incubation time; influence of

repeated applications of IL-2 and IFN. K 562 cells, established human neuroblastoma cell lines and tumor cells freshly prepared from patients were used as target cells. We suggest that the in vitro incubation of whole blood with IL-2 and IFN and its subsequent reinfusion may represent a very effective form of supporting therapy in cases of neuroblastoma and other tumors. This procedure can be carried out without major side-effects for the patients.

201 Natural History and Prognosis of Chronic Myelomonocytic Leukemia

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Chronic myelomonocytic leukemia (CMML) is a rare disorder of uncertain etiology and prognosis. In a retrospective study, the clinical and hematological findings, natural history and frequency of overt leukemia were examined in 19 patients (13 males, 6 females). The median age at diagnosis was 69 years (range 31–85 years). Eleven patients (57%) presented with hepatomegaly and/or splenomegaly, while skin infiltrates were observed in only one case. Laboratory findings at the time of diagnosis were: high white blood cell count ($> 10\,000/\mu l$) in 68% absolute monocytosis ($> 1000/\mu l$) in 100%, normal or elevated LAP in 95%, anemia (Hb < 10 g/dl) in 52% and thrombocytopenia ($< 100\,000/\mu l$) in 62% of the patients. The marrow blast percentage was lower than 20%; the median percentage of marrow monocytes was 6–53 (median 23%). During the observation time (max. 6 years), 11 patients died [causes of death: AML (M 5) 2, infection 2, bleeding 2, unknown or not related to CMML 5]. By statistical methods the prognosis of patients was determined and compared with that of chronic myelocytic leukemia (CML; $n = 24$) and refractory anemia with excess blasts (RAEB) ($n = 34$). Whereas the 3-year survival rates were not found to be different in the three groups (CMML 38 ± 17%, CML 25 ± 12%, RAEB 19 ± 12%), the cumulative rate of acute leukemia was significantly lower in the CMML group (3-year rates of acute leukemia: CMML 12 ± 5%, CML 58 ± 12%, RAEB 62 ± 12%). From these results we conclude that CMML has a poor prognosis although its outcome is usually not determined by transformation to overt leukemia.

202 Lymphocyte Subpopulations in the Peripheral Blood of Patients with Myelodysplastic Syndromes

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Lymphocyte subsets were analysed from the peripheral blood of 26 patients with recently diagnosed myelodysplastic syndromes (MDS) (FAB 1980: refractory anemia (RA) 14, refractory anemia with ringed sideroblasts (RAS) 5, refractory anemia with excess blasts (RAEB) 7). Eight patients were also analysed after treatment with prednisone. An absolute lymphopenia under 1.5/nl was found in 24 of the 26 patients (92%), 23 of them showing an absolute decrease of B-lymphocytes (HLA-DC and Leu 14) (0.005–0.13/nl; median 0.04). The absolute number of T-lymphocytes (T 3 and T 4 plus T 8 positive cells) was decreased to under 1.3/nl in all 24 cases (0.1–1.08; median 0.66). By subtyping it was possible to differentiate between two groups: patients with a parallel decrease of T 4-helper cells and T 8-supressor/cytotoxic cells (ratio T 4/T 8 < 3.1: 1.3–2.7; median 1.97; $n = 14$) and such cases with a predominant decrease of T 8-cells (ratio T 4/T 8 > 3.1: 3.4–20.7; median 6.5; $n = 10$). In the second group, clinical/serological autoimmunologic phenomena were often observed (7 of 10; 3 of 14 in group I). No correlation was found for FAB subtype, age or transformation risk. After treatment with prednisone, the absolute number of B- and T-cells increased considerably; however, there was no change in the T 4/T 8 ratios. The constancy of these results allows the conclusion that the observed disturbance is an inherent part of MDS. Further analyses on subpopulations and functional tests only will allow hypotheses regarding the pathogenesis of these disturbances.

203 Essential Thrombocythemia: Platelet Function Tests and Clinical Characteristics

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The blood of 16 patients with essential thrombocythemia (ET), 9 patients with reactive thrombocytosis (RT) and 13 healthy persons was used for platelet aggregation studies. Platelet-rich plasma with sodium citrate as anticoagulating substance was used. When the aggregation was induced with adenosine diphosphate (0.01 μM), collagen (0.1 μg/ml) or platelet aggregating factor (PAF: 0.5 μM) the plasma of the patients with ET showed significantly decreased aggregation (35%–44% of the value for the control groups). Under the influence of inhibitors of platelet aggregation, patients with RT reacted much more than patients with ET and healthy controls. Independent of inhibitors of platelet aggregation, thrombin (0.05 IU/ml) caused similar aggregation in healthy controls and patients with ET; patients with RT showed a greater increase in aggregation. The adrenalin-induced aggregation best showed the difference between patients with ET and control groups. Adrenalin in concentrations ranging from 0.01 μg/ml to 100 μg/ml showed comparable dose-related amounts of aggregation in healthy controls and patients with RT. Over the whole concentration range, patients with ET showed significantly decreased aggregation (28%–34% of the value for the control groups). This difference proved to be independent of the influence of inhibitors of platelet aggregation. The clinical course of a total of 54 patients with ET was followed. At diagnosis, 82% presented with thromboembolic problems (mostly disturbances of the microcirculation), 8% with thromboembolic and hemorrhagic problems, 2% only with hemorrhagic problems. In 8% the diagnosis was made by chance. The maximum number of platelets ranged from 500000/μl to 3000000/μl with an average of 1200000/μl. The bone marrow ($n = 50$) always showed increased megakaryopoiesis. Bone marrow histology ($n = 38$) showed megakaryocytic myelosis in 25 cases, chronic megakaryogranulocytic myelosis in 7 cases, polycythemia vera in 4 cases and myeloproliferative disease without exact classification in 2 cases. Whenever determined, the Philadelphia chromosome was negative ($n = 12$). Three additional cases showed no growth. Bleeding time was normal in 91%, increased in 9%. The lamina aggregation test (Breddin) always showed results outside normal standards. At present, 42 persons (78%) are still alive. Of the 12 deceased patients, 10 most probably died of vascular causes and 2 of acute leukemia.

204 Chronic Myeloproliferation: A Comparative Study of Cytological and Histological Bone Marrow Examination

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A direct comparison of bone marrow (BM) analyses was performed within a clinically well-defined group of 122 patients suffering from chronic myeloproliferation (CMP). Out of 25 morphological criteria, 15 were directly comparable, being judged in general by a ranking of 5 semiquantitative categories. The quantitative morphological parameters such as BM cellularity, fat content, GE index and percentage of blast cells displayed identical results in 40%–60% of cases. The discrepancies did not usually exceed one category (in either direction), thus showing a good and useful correlation between BM cytology and BM histology. The extent of the other discrepancies, however, indicates that there are considerable problems involved in histological BM analyses. There is a general tendency for the histologically defined proportion of blast cells (erythropoesis and granulopoesis) and the quantity of erythropoesis to be overestimated (as compared with differential counts of cytological specimens). Furthermore, there are considerable differences concerning the analysis of megakaryopoesis, the quantity of which is better defined histologically. The data presented confirm the observation that CMP can only be accurately diagnosed on the basis of a combined (histological, cytological, clinical) analysis.

205 Mithramycin: An Inducer of Differentiation in Hemopoetic Stem Cell Disorders?

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Mithramycin, an antibiotic isolated from actinomycete cultures, is known to be a potent inhibitor of RNA synthesis and a putative inducer of differentiation in hemopoetic precursor cells. Recently, Koller et al. (J Clin Invest 76: 365) achieved complete remission in a blast crisis of CML using 1.25 mg mithramycin given in 8 doses every other day, accompanied by accumulation of more mature granulocytes and a decrease of c-abl and c-myc protooncogene m-RNA. Although we showed that mithramycin may represent a potent in vitro inducer of differentiation in cell lines such as HL 60, WEH I 3 B and some fresh leukemic blasts, we were unable to confirm these promising results in our own in vivo studies, when an identical protocol as that proposed by Koller et al. was administered to 8 patients (3 cases of myeloid blast crisis of CML, 1 patient with myelofibrosis emerging from CML and 4 patients with myelodysplastic syndrome). Since, no serious side-effects occurred, dose escalation studies are possible and warranted considering the present bad prognosis of these stem cell disorders.

206 Treatment of Chronic Myelogenous Leukemia with Recombinant Interferon α2

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For a long time there have been no new aspects concerning the treatment of chronic myelogenous leukemia (CML) except for the introduction of allogeneic bone marrow transplantation. The availability of recombinant interferon $\alpha 2$ for CML has now led to a new therapeutic concept. We have treated 12 patients (7 males, 5 females; age 44 years; range 18.4–57.9 years) with previously treated and newly diagnosed Philadelphia-chromosome-positive CML with recombinant interferon $\alpha 2$. The median leukocyte count prior to therapy was 101 000/μl (32 100 to 446 000/μl). One patient received 3×10 million U/week, the others 3×5 million U/week. The median follow-up period is presently 169 days (70–243 days). There was a marked reduction of leukemic cells in all patients. Five of six patients had $<$ 10 000 leukocytes/μl after 170 days of treatment. Thrombopenia $<$ 100 000/μl was observed in three patients; fever was constantly observed after the initial administration of interferon. The dose was reduced in four patients. We conclude that a new treatment concept has been found for CML by the administration of interferon $\alpha 2$. Whether the Philadelphia-chromosome-positive cell clone can be significantly reduced will have to be seen in our patient population.

207 Hematological and Genetic Findings in Chronic Myelogenous Leukemia Patients Treated with Recombinant Interferon-α2

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Eight patients with Philadelphia chromosome (Ph_1)-positive chronic myelogenous leukemia (CML) in the benign phase of the disease were treated with human recombinant interferon (IFN) $\alpha 2$ (hur-IFN-alpha-2). All of the patients responded to IFN treatment with a continuous improvement in peripheral blood counts, a decrease in serum lactate dehydrogenase and a reduction in organomegaly. Cytogenetic analysis was performed to evaluate the size of the Ph^{1+} leukemic cell clone before and throughout IFN treatment. In parallel, c-abl oncogene expression in peripheral blood and bone marrow was determined by Northern blot hybridization before and 3 months after the start of IFN treatment. Data from these investigations will be presented and compared with the clinical results.

208 Chronic Myelocytic Leukaemia: Reduction of In Vitro Proliferation of Haemopoietic Progenitor Cells in Patients Treated with Recombinant α-Interferon (IFN Alpha-2 B)

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In chronic myelocytic leukaemia (CML) there is a typical change in the in vitro growth pattern of haemopoietic progenitor cells. Compared with normal individuals there is an increase in myeloid (CFU-C) and erythroid (BFU-E) and a decrease in pluripotent (CFU-GEMM) progenitor cells. In a clinical trial, CML patients were treated with IFN α-2 B at an initial dose of 4×10^6 U/m^2 daily. In vitro analyses on haemopoietic progenitor cells were carried out using the CFU-GEMM assay. Before IFN therapy the median for BFU-E was 134 colonies (range $0-2000)/2\times10^5$ MNC, for CFU-C 60 colonies (range $4-1450)/2\times10^5$ MNC (16 patients tested). After an average treatment time of 8–12 weeks with IFN α-2 B the median for BFU-E was 20 colonies (range $6-350$ 2×10^5 MNC and for CFU-C 30 colonies (range $8-98)/2\times10^5$ MNC (10 patients tested). With the exception of one patient, preliminary results showed a decrease of CFU-GEMM, too. From these data, we may conclude that IFN α-2 B therapy in CML reduces the in vitro proliferation of haemopoietic progenitor cells.

209 Treatment Results of 79 Patients with Blastic Transformation of Chronic Myelocytic Leukemia (CML)

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Seventy-nine (48 males, 31 females) patients (mean age 47 ± 14 years) with blastic transformation of CML were treated from December 1976 to April 1986. The mean duration of the chronic phase was 936 ± 774 days. Of 69 patients tested in the chronic phase, 60 (87%) were Philadelphia-(Ph)-chromosome-positive and 9 were negative. Further cytogenetic analysis at the time of blastic transformation revealed additional chromosome changes in 34 (71%) of 48 patients tested. Of 73 patients tested, 11 (15%) had a lymphoid morphology. Bone marrow fibrosis was detected in 28 (50%) of 56 evaluable patients. Fifty-one patients received vincristine/prednisone as first-line therapy, followed by vincristine/prednisolone/adriamycin in responding patients. Non-responders received adriamycin/ARA-C. Of 44 evaluable patients 15 (34%) responded (CR/PR > 14 days). Mean survival for the whole group was 116 ± 122 days (range 1–600 days). Better survival was related to: Ph-chromosome as the only detectable chromosome change; lymphoid morphology; no bone marrow fibrosis, and response to therapy (only response to therapy proved to be statistically significant). These data confirm the poor prognosis of patients with blastic crisis of CML treated with conventional chemotherapy.

210 Myelodysplastic Syndrome: Multiparameter Analysis Concerning the Relevance of the FAB Nomenclature

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In a clinically well-defined group of 35 patients suffering from various myelodysplastic syndrome (MDS) entities, the following parameters were analysed: (1) semi-quantitative determina-

tion of 31 morphological criteria in the cytological and histological specimens for cluster and relevance analyses; (2) clinical and haematological parameters to establish the preliminary diagnosis; (3) morphometric analyses of megakaryocytopoesis; (4) differential counting of bone marrow specimens. The only MDS entity characterized by a stable cluster was CMMoL, which was also the only MDS form with a significantly lower space density of megakaryocytes in the histological preparation. The other MDS entities showed a considerable overlap of criteria, thus leading to diagnostic problems: 30% of BM specimens could not be categorized by histological techniques alone. Only the combination of the two BM analysis techniques, at least in these cases, allows a correct classification of the disease following the FAB MDS proposals.

211 The Clinical Picture of Essential Thrombocythemia Applying Stringent Diagnostic Criteria

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When we applied stringent diagnostic criteria, including histomorphologic and cytogenetic studies similar to the proposals of the Polycythemia Vera Study Group (1982), we found essential thrombocythemia (ET) in only about 30% of cases, with thrombocytes above 800 000/μl. Therefore, other variants of the myeloproliferative disorders with thrombocytosis seem to be more frequent than ET. In 21 cases of ET, so far there have been no deaths related to active disease. Signs and symptoms differed in part from those described in the literature. The risk of venous thrombosis was rather small and the risk of hemorrhage after injuries and operations somewhat high, although not life-threatening. Diffuse and rapidly changing disturbances of the cerebral circulation were often suspected, and similar problems were observed in the upper and lower extremities. Much attention must be paid to rheological disturbances in patients with ET. Therapeutic consequences are discussed.

212 Blast-Crisis Phenotypes in Chronic Myelogeneous Leukemia May Delineate Novel Hematopoetic Precursors

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Mononuclear cells from peripheral blood or bone marrow of 23 patients with chronic myelogeneous leukemia in blast crisis (CML-BC, $>$ 80% leukemic cells) were tested for their surface marker distribution by flow cytometry. In 15 samples, 25 monoclonal antibodies (MOAB) of the differentiation clusters CD 2, 3, 4, 7, 8, 10, 19, 20, 23, w 11, w 14, 15, 16, 25 as well as human leukocyte antigens HLA-DQ, -DR, -DP were tested. Another 8 samples were tested with 40 MOAB, including CD 17, 18, erythroid and megakaryocytic markers and a panel of MOAB of our own laboratory (TÜ 2, 3, 6, 9, 13). The expression of certain antigens specific for the myeloid lineage as recognized by MY 9, as well as other myeloid/monocytic or even megakaryocytic antigens, occurred in addition to binding of either certain T- or B-cell-specific MOAB. In contrast, however, simultaneous expression of T- and B-cell markers on CML-BC cells was never seen. Because of these findings, simply assigning the CML-BC cell phenotypes to the classical differentiation lineages was difficult. When evaluating our results, we attempted to define novel precursor populations that may represent minor subpopulations of hematopoetic progenitors in normal bone marrow. Our findings suggest that at least part of the myeloid and T-cells were derived from a common progenitor. Also, another common progenitor of myeloid and B-cells seems to exist. Experiments applying in vitro differentiation of leukemic blast and concurrent analysis by double-label flow cytometry are being designed to confirm the role of such novel precursor clusters in normal hematopoesis.

213 CML Blast Crisis of Early T-Lymphocytic Differentiation*

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Although lymphoid blast crisis of chronic myelogenous leukemia (CML) occurs quite frequently, little evidence has been provided for T-cell involvement in CML. Recently we characterized cells from a patient with CML blast crisis by modern immunological and genetical techniques and could demonstrate early T-cell differentiation. The Philadelphia chromosome-positive blasts did not react in peroxidase and esterase staining but had high levels of TdT. While little reactivity with antibodies to myeloid antigens was seen, T-cell antibodies 3 A 1 and TH 69 were positive. Typical rearrangement of the breakpoint cluster region was found, while the T-cell receptor β-gene was in germ-line configuration, consistent with early T-cell differentiation.

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214 Low-Dosage Cytarabine as Monotherapy and in Combination with 13-Cis-Retinoid Acid in Myelodysplastic Syndromes

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Interest in the use of differentiation-inducing agents in myelodysplastic syndromes (MDS) is increasing. The studies reported here were based on this principle and were sequential. In the first study we treated 14 patients with low-dosage cytarabine (Ara-C; 10 mg/m² per 12 h SC) for 14 to 24 days. Even though 2 complete remissions (CR) and 2 partial remissions (PR) were achieved, this regimen was associated with severe and sometimes fatal complications. In the second study we applied thymostimulin 300 mg/day (daily in the first week, 3×per week in 2nd to 4th week and once weekly from 5th to 12th week). Only one temporary improvement in a total of 8 patients was attained. Based on the experimental data showing a synergistic interaction between Ara-C and 13-Cis-retinoid acid in the sense of differentiation induction in leukemic cells, we have started a third study. The regimen consists of: Ara-C 5 mg/m² per 12 h SC and 13-Cis-retinoid acid, 60 mg/m² per day orally, from days 1 to 14. To date, 2 improvements and 2 PR have been achieved out of 11 patients. As a whole, the results of treatment in MDS are still not satisfactory.

215 Antileukemic Effect of rIFN-α in CML: Comparison In Vitro and In Vivo

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In an ongoing phase-II interferon trial we aimed at predicting the clinical responses of Ph_1+ CML patients in vitro. To date, 16 patients with Ph_1+ CML have been entered into this study. Eight of them presented with chronic phase CML (group A); the remainder showed disease acceleration (group B), as defined by the criteria of Canellos et al. Group A patients were treated with 1–4 Mill. units of rIFN-α 2 daily, given subcutaneously; group B patients received the same dosage and, in addition, single-agent chemotherapy comprising busulfan and 6-mercaptopurine, respectively. In normal controls and group A patients, in vitro colony formation in CFU-GM, BFU-E, CFU-E and CFU-MEG cells was inhibited by IFN in a dose-dependent manner. BFU-E and CFU-MEG proved to be the most sensitive cell lineages, whereas CFU-E and CFU-GM were about 10 times less sensitive. In contrast, group B patients exhibited marked in vitro resistance to IFN-α. Even at IFN concentrations of 1000–10 000 units per ml, 30%–50% residual colony formation was observed in CFU-GM and CFU-MEG cells. Corresponding to the in vitro results, all CML patients in the chronic phase of the disease responded to relatively

low doses of IFN-α, whereas only 4 of the group B patients were successfully treated with a combination of IFN and single-agent chemotherapy. From these preliminary data we conclude that the colony-forming assay might be a proper tool to predict clinical responses to IFN in CML patients.

216 Blast Crisis in CML Confined to the Lymph nodes

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In a 23-year-old woman, a diagnosis of Ph 1-positive CML was established 15 months before she presented with disseminated lymphoma and clinical evidence of leukemia. Pretreatment consisted of busulfan. The blood picture was inconspicuous except for moderate anemia. Further abnormalities present were gross splenomegaly, enlargement of even the abdominal lymph nodes, and a deficiency of humoral antibodies. Cytochemistry and marker findings of lymph node aspirates revealed three different populations of blast cells:

- 10% POX^+, but $VIM2^-$, $VIM\text{-}D5^-$
- 80% $WT1^+$, $Lyt2^+$, the major part TdT^+, isolated $CALLA^+$, IA^-
- 10% $WT1^+$, OKM^+, $Lyt2^-$, POX^-, $VIM\text{-}D5^-$

These findings indicate the existence of a predominant T-lymphoblastic population and of a minor myeloblastic population as well. Even the possibility of a blastic clone with hybrid differentiation must be considered. Blastic transformation of the marrow was excluded by means of light microscopy. Cytogenetics did not reveal further chromosomal aberrations in either marrow or lymph nodes. Early relapse appeared after an 8-week remission, obtained by the ViDAP schedule and extended by MTX for CNS prophylaxis. In contrast to a recently published case [1], remission was achieved promptly by an ALL-designed regimen of the German ALL Study Group and has lasted for 4 months now.

Reference

1. De Hogge et al (1984) Unusual karyotypic changes and B cell involvement in a case of lymph node blast crisis of chronic myelogenous leukemia. Blood 64: 123

217 Plasma Cell Dyscrasia in Idiopathic Myelofibrosis

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Out of 49 patients treated for idiopathic myelofibrosis (IMF) from 1980 to 1985, 3 had additional monoclonal gammopathy (MG). In 1 patient, progressive osteolytic lesions were primarily suggestive of coexistent multiple myeloma (MM); in the other 2 patients, however, MG appeared only 11 and 13 years, respectively, after the onset of IMF and could not be further elucidated. After many years of a benign clinical courses 1 of these patients suddenly developed massive hyperproteinemia, which was paralleled by rapid deterioration of his general condition. So far, the clinical course of the other patient is compatible with benign gammopathy. In comparison to the general prognosis associated with IMF and MM the patients showed an unusually favorable course (survival: 6.2 to 21 years). In these cases MG suggests the presence of a B-cell neoplasm in addition to IMF. Though rare, the coexistence of a lymphoproliferative and a myeloproliferative disorder has repeatedly been described. Various hypotheses have been offered to explain this situation, the most attractive one assuming neoplastic clonal proliferation of pluripotential stem cells, which have retained the ability to further differentiate into both lymphoid and myeloid cells. The late manifestation in 2 of the patients presented here might signify that MG is just another variant of the terminal stage of myeloproliferative disorders.

218 Hypereosinophilic Syndrome: Successful Treatment

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Empiric threefold criteria have been established for the idiopathic hypereosinophilic syndrome: persistent peripheral eosinophilia of 1500 eosinophils/mm^3 for at least 6 months, lack of evidence for parasitic, allergic or other recognized causes of eosinophilia, and signs and symptoms of organ system involvement or dysfunction, either directly related to eosinophilia or unexplained in the given clinical setting. We present the case of a 43-year-old patient admitted to our hospital with marked eosinophilia ($>$ 4000/mm^3) and signs and symptoms of involvement of several organs, mainly affecting the heart (Löffler endomyocardial fibrosis, mitral and tricuspid regurgitation, arrhythmias) and the central nervous system (TIAs and one stroke). We started treatment with hydroxyurea, prednisolone and anticoagulation with vitamin K antagonists combined with dipyramidole, under which the cardiac arrhythmias and thromboembolic complications have already been well controlled for 1 year.

219 Long-Term Results in Acute Myeloid Leukemia Treated with Conventional Daunorubicin, Ara-C (DA) and Thioguanine, Ara-C, Daunorubicin (TAD) Polychemotherapy

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Between 1978 and 1982, 56 patients have been treated with DA polychemotherapy. The induction dosage was: AraC, 2×100 mg/m^2 i.v., for days 1–7; DNR, 45 mg/m^2 i.v., for days 1–3. First and second consolidations were: AraC, 2×100 mg/m^2 i.v., for days 1–5; DNR, 45 mg/m^2 i.v., for days 1–2. Maintenance was: 6-TG, 80 mg/m^2 orally, for days 1–4; AraC, 60 mg/m^2 i.m., on day 5; 1× weekly for 1.5 years. Between 1982 and 1984, 45 patients have been treated with TAD polychemotherapy. Induction was: AraC, 100 mg/m^2 civi, for days 1–7; DNR, 45 mg/m^2 i.v., for days 1–3; 6TG, 2×100 mg/m^2 orally, for days 1–7. First consolidation was: AraC, 2×100 mg/m^2 i.v., for days 1–7; DNR, 45 mg/m^2 i.v., for days 1–2. Second consolidation was: AraC, 100 mg/m^2 civi, for days 1–7; DNR, 45 mg/m^2 i.v., for days 1–2. There was no maintenance therapy given. The treatment results were as follows: DA yielded 55% complete remission (CR), TAD 65% CR. Probability for total survival (± SD) was calculated according to Kapland and Meier. DA: 24 months, 0.29 (± 0.086); 60 months, 0.18 (± 0.080). TAD: 24 months, 0.05 (± 0.031). The long-term results for disease-free survival for patients treated with TAD polychemotherapy without maintenance were remarkably dismal. However, to date the total survival has been identical in both groups.

220 COAP for First- or Second-Remission Induction Therapy in Patients with Acute Leukaemia

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Eightheen patients (11 males, 7 females) 19–77 years of age ($\bar{x}$ = 52.6 years) with acute leukaemia (17 myeloblastic, 1 lymphoblastic) and contraindications for anthracycline therapy were treated with cyclophosphamide, vincristine, Ara-C and prednisone (COAP) for induction of

first or second remission. After 1 to 6 courses of COAP, 3 patients (2 AML, 1 ALL) achieved complete remission; 1 of these AML patients attained a continuous remission. Partial remissions were obtained in another 3 patients, and 11 patients were non-responders, whereas 1 early death occurred on day 20 of chemotherapy. Treatment results are demonstrated and the benefit of COAP chemotherapy, as a second-line protocol in patients with contraindications for anthracyclines, is discussed.

221 High-Dosage Cytosine Arabinoside and Mitoxantrone (HAM) in Refractory Acute Myeloid Leukemia (AML): Results of a Phase I/II Study

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In a clinical phase I/II study, 36 patients with refractory AML were treated with the combination of high-dosage Ara-C, 3 g/m² every 12 h for days 1–4, and mitoxantrone at a dose of initially 12 mg/m² per day for days 3–5, which was subsequently escalated to 4 and 5 doses of 10 mg/m² per day for days 2–5 and 2–6, respectively. All patients were recruited from the multicenter AML study and had received daunorubicin and Ara-C at a conventional dosage. Based on previously defined criteria, they were considered to be refractory regarding the previous treatment. Of the 36 patients, 19 (53%) obtained a complete remission and one additional case a partial remission. In 4 cases the AML was refractory against HAM; 10 patients died during bone marrow aplasia because of infectious complications; two additional patients succumbed to acute cardiomyopathy and pericardial effusion because of leukemic infiltration. Of the 19 responders 10 are still alive at 2^+ to 15^+ months, with a median survival of 7 months.

222 Neurotoxicity of Combination Therapy with High-Dosage Ara-C and Mitoxantrone in 35 Patients with Acute Leukaemia

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In two multicenter studies, 35 patients with acute leukaemia were treated with high-dosage Ara-C (3 g/m² every 12 h for 4 days) and mitoxantrone (10 mg/m² for 4 days). Eighteen patients with AML received this combination as one primary course in their induction chemotherapy. Seventeen patients with relapsing acute leukaemia (15 AML relapses, 2 ALL relapses) received this combination as relapse therapy. The 2 patients with ALL relapse had previously been treated with CNS radiation, intrathecal methotrexate, Ara-C and dexamethasone. These two patients developed severe central nervous symptoms about 5–8 days after the beginning of therapy and died within 3 weeks. There were no signs of CNS involvement and no bleeding. There was toxic damage to the brain with cell necrosis. The neurotoxicity of high-dosage Ara-C after CNS radiation and intrathecal treatment with methotrexate, Ara-C and dexamethasone seems to be much higher. The remaining 33 patients showed no signs of CNS toxicity. However, in the multicenter study a total of about 13% of patients exhibited reversible neurological symptoms.

223 Intermediate High-Dose Ara-C (I-HiDAC): Toxicity and Remission Induction in Poor-Risk AML

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The aim of this study was to define toxicity and antileukaemic activity of I-HiDAC combined with aclarubicin (ACM). Treatment consisted of AMC, 9 mg/m² i.v., and Ara-C, 600 mg/m², as 2-h infusions every 12 h. The first 6 patients received ACM for 5 and Ara-C for 4 days (5 + 4); the following 7 patients were administered both drugs for 5 days (5 + 5) and one patient for 6 days (6 + 6). Fourteen patients with a median age of 44 years (23 – 75) were entered into this study: 4 with preceding "preleukaemia", 1 with secondary AML, 2 with subacute AML, 1 in first relapse of a TdT+ AML, 2 in their 2nd or 4th relapse, 2 with refractory AML and 2 with CML blast crisis. The toxicity level was acceptable. Bone marrow regeneration occurred rather rapidly (granulocytes $> 500/\mu l$ after 17 and platelets $> 25\,000/\mu l$ after 20 days). Four of 13 patients available for evaluation obtained a continuous remission of 2+ to 9+ months duration. One patient with CML blast crisis reentered the chronic phase for 3 months. The combination of I-HiDAC with ACM is useful in the treatment of AML and the toxicity is acceptable. Incorporation of this regimen into future treatment programs for AML seems to be warranted.

224 The Value of Estimation of Intracellular Thymidine-Kinase Activity for Follow-up of Patients with Acute Leukemia and the Diagnosis of Early Relapse

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Thymidine-kinase (TK) is an essential key enzyme in the biochemical pathways of DNA synthesis. It catalyzes the first phosphorylation step to thymidinemonophosphate, which is integrated into the DNA molecule after further phosphorylation steps. In comparison to 26 specimens from healthy donors, a total of 328 bone-marrow cell populations from patients with acute leukemia were assayed for TK activity in the cytosol. At first diagnosis of acute leukemia ($n = 87$), the mean values of TK were 61.3 ± 60.1 nmol/min$\times 10^{10}$ cells. It was 4.9 times higher than TK activity in normal bone marrow ($P < 0.0001$). The sensitivity level for finding such high TK activities in acute leukemias was 83%. In complete remission ($n = 172$) TK values were as low as 19.0 ± 16.1. During the course of remission, e.g., 1–2 months before a relapse ($n = 13$), TK values rose again to 33 ± 28.6. In overt relapse ($n = 57$, TK was as high as at first diagnosis (60.3 ± 45.5). In summary, TK is a very sensitive marker for characterizing the cell populations of leukemic bone marrow. An increase in TK activity during remission may predict a relapse and a follow-up bone-marrow smear is indicated.

225 The Effects on Gonadal Functions of Intensified Therapy in Acute Lymphoblastic and Undifferentiated Leukemia (ALL/AUL) in Adults

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The improved survival rate of patients with ALL/AUL, particularly young adults in their reproductive years, has increased interest in the effects of intensified chemotherapy (CT) on fertility, mutagenicity, and teratogenicity. This investigation was conducted to evaluate the effects of

antileukemic therapy on reproductive potential in both women and men. Seven women and 20 men were studied during and after maintenance therapy (MT) in complete remission. All patients had received intensified induction and reinduction chemotherapy according to the protocol of the multicenter trial of AUL/ALL. To assess the grade and duration of gonadal toxicity after CT, hormone analyses by RIA [FSH, LH, testosterone (T), prolactin, progesterone (P), estradiol (E)], interviews, and sperm evaluations were carried out. In 7/8 men FSH levels were elevated (median: 976 ng/ml) and showed azoospermia in the first year of MT. In contrast, 10/11 men showed normal FSH values (median: 545 ng/ml) and oligo- or normospermia in the seconde year or after MT. In 19/20 men T and LH levels were normal. In 7/7 women during and after MT, levels of FSH, LH, P, and E were within normal limits, and no women suffered from premature menopausal symptoms. In conclusion, our preliminary data suggest: (1) no evidence of acute or chronic gonadal toxicity in women; (2) acute gonadal toxicity with germ-cell hypoplasia in all men; (3) recovery of spermatogenesis in the second year of maintenance therapy; (4) normal endocrine gonadal functions after chemotherapy in all men.

226 Unusual T-Cell Leukemia in Ataxia Teleangiectasia (AT)

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A 27-year-old male patient with AT developed atypical chronic T-cell leukemia with increasing leukocytosis and bone marrow infiltration in the absence of organomegaly. Phenotypically, three cell subsets were found: 60% of the cells had a $T3^+$ $T4^+$ $T8^+$ $T6^-$ $T10^-$, 30% a $T3^+$ $T4^-$ $T8^+$ $T6^-$ $T10^-$ phenotype and 7% showed positivity for Leu-11. Despite an eightfold increase of the WBC the relative size of these subsets remained unchanged throughout the observation period. The cells proliferated after stimulation with PHA, ConA and sepharose-bound anti-T3. The response to stimulation of the T11-receptor with anti-$T11_2$ plus anti-$T11_3$ was characteristic of thymocytes, proliferation being only achieved in the presence of exogenous IL-2. ADCC and NK activity were not detectable, and there was no response in the MLR. Chromosome analysis showed complex clonal aberrations, including a loss of one chromosome 20, a loss of the Y chromosome and an interstitial del 14 (q21; q31). In this patient leukemia was apparently due to monoclonal proliferation of an early T-cell, which had kept the ability to further differentiate into different phenotypes. While proliferation was uncontrolled, differentiation still responded to some regulatory mechanism. Since our data reveal striking similarities with three previously reported cases of T-CLL in AT, this type of leukemia may be a frequent complication of AT.

227 Sweet's Syndrome in a Patient with Acute Myelomocytic Leukemia

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A 66-year-old female patient is described who was treated for a relapse of heavily pretreated acute myelomocytic leukemia (AMML). She developed fever of up to 40°C, associated with painful indurated erythematous areas on the skin of her arms, legs and upper chest wall, which could not be attributed to microorganisms or pure allergic phenomena. These considerable skin

changes began approximately 7 days after amsascrine (m-AMSA) treatment had been terminated, i.e., in a phase of inadequate granulocyte reserve with low leukocyte counts ~ 1000/μl. Skin-biopsy specimens were taken and corresponded to the diagnosis of acute febrile neutrophilic dermatosis (Sweet's syndrome). Antibiotic therapy had no effect. She was successfully treated with high-dose corticosteroid injection therapy and became afebrile within 24 h. Complete skin remission followed after a few more days. A similar dermatological picture appeared after the second m-AMSA course and she again responded to corticosteroids shortly before her hematological state began to deteriorate. Dermatological symptoms, specific or non-specific, are quite common in patients with acute leukemia; however, Sweet's syndrome is said to be extremely rare and may be associated with 10% of all cases with malignant hematological disorders.

228 Extramedullary Myelogenous Leukaemia (Chloroma)

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Although the histological definition of chloromas is well known, they are often wrongly diagnosed. Three cases are described. The primary diagnosis was in two cases malignant lymphoma, and in one case undifferentiated carcinoma. All specimens indicated an undifferentiated tumor. Striking activity of the naphthol-ASD-Cl-esterase was the only evidence for the myelogenous origin of the tumors. Treatment consisted of local irradiation in two patients. After complete remission generalized relapse occurred 6–22 months later. The following conclusions can be made: (1) to avoid confusion with other undifferentiated tumors, naphthol-ASD-Cl-esterase should be stained mandatorily for respective specimens; (2) with respect to the high incidence of generalized relapses, chloromas should be treated with a combined modality of primary radiotherapy followed by chemotherapy for AML.

229 Mitoxantrone and High-Dose Cytosine Arabinoside in Acute Nonlymphocytic Leukemic (ANLL)

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A total of 17 patients with relapsed ($n = 9$), primarily refractory leukemia ($n = 4$), with blast crisis of chronic myeloid leukemia (CML-Bc; $n = 2$) and refractory anemia with an excess of blasts in transformation (RAEB-T; $n = 2$) were treated with mitoxantrone in combination with high-dose cytosine arabinoside (ara-c). The regimen consisted of: high-dose ara-C 3 g/m^2 every 12 h by 3 h continuous infusion, days 1–4; mitoxantrone 10 mg/m^2 per day i.v. (30 min infusion), days 2–6. Of these 17 patients, 12 (70%) – not including 12 of 15 CML-BC patients (80%) – have achieved complete remission including 2 with RAEB-T and 2 with primary resistance. No case of early death was observed. Treatment failures were due to refractory leukemia. Toxicity was moderate, consisting of nausea, vomiting, diarrhea and mucositis. In one patient moderate, but reversible CNS toxicity was observed. This study demonstrates that the combination of mitoxantrone and high-dose ara-C is a very effective and well-tolerated induction regimen for patients with ANLL of poor prognosis. These results are among the best reported for this group of patients and suggest evaluation of this combination as front-line therapy for ANLL.

230 High-Dose Cytosine Arabinoside and Mitoxantrone in Relapse Leukemia

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Three children with acute leukemia (AML, AEL, ALL) in second relapse were treated with two or three courses of high-dose cytosine arabinoside (ara-C) and mitoxantrone in an attempt to induce a further remission. In each course 2×3 g/m^2 per day $\times 4$ of ara-C and 10 mg/m^2/day $\times 4$ of mitoxantrone were given simultaneously. After the first course of therapy, one complete but transient, and two partial remissions were achieved. Repeated courses of this combination prolonged the remissions and converted one partial into a complete remission. The combination of high-dose ara-C and mitoxantrone was well manageable and there were only minor complications. These complications included stomatitis and conjunctivitis, requiring repeated flushing of the eyes with distilled water. Neither cardiotoxicity nor liver or kidney damage were observed. Myelosuppression was severe. We conclude that the combination of high-dose ara-C and mitoxantrone can induce remissions in heavily pretreated children with acute leukemia. At present, we would offer this drug combination to patients with acute leukemia in their second relapse.

231 Noncardiogenic Pulmonary Edema in Acute Myeloid Leukemia after Treatment with High-Dose Cytosine Arabinoside and m-Amsacrine (mAmsa)

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Infection, hemorrhage, intravasal coagulation and the acute respiratory distress syndrome are pulmonary complications that occur after treatment of acute myeloid leukemia (AML). The aim of this study was to investigate the incidence of these causes by roentgenogram and clinical and laboratory tests in 15 patients with relapsed AML after treatment with high-dose cytosine arabinoside (ara-C) and mAmsa. Out of 15 patients, 8 had pulmonary symptoms, one of them with massive hemoptysis and radiographic signs of pulmonary bleeding. Two patients had pulmonary infections that were detected by bacteriological tests and X-ray and five patients suffered from acute respiratory failure 5–15 days after the end of therapy. In these latter patients, noncardiogenic pulmonary edema was diagnosed by X-ray. There was no evidence of cardiogenic, infectious or metabolic reasons. Four of these patients recovered within a few days; one patient died despite controlled ventilation support. In conclusion; the most frequent pulmonary complication of treatment with high-dose Ara C and mAmsa is non-cardiogenic, toxic pulmonary edema.

232 Avascular Necrosis of the Hip Following Combination Chemotherapy of Acute Lymphoblastic Leukemia (ALL)

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Three adult patients with acute lymphoblastic leukemia were treated according to a protocol which was a modified form of a successful therapy regimen for childhood ALL. The protocol includes the drugs daunorubicin, vincristine, L-asparaginase, prednisolone, cyclophosphamide, cytarabine, dexamethasone and (for maintenance therapy) methotrexate and mercaptopurine. During the initial 4-week induction period, the dosage of prednisolone is 60 mg/m^2 daily, for reinduction dexamethasone (10 mg/m^2) daily is used, again for 4-weeks. Prednisolone and dexamethasone are then reduced over 9 days. Corticosteroid treatment lasts a total of 74 days. All

three patients obtained a complete remission. Some 6–12 months following induction chemotherapy, all the patients complained of increasing pain of the hips. Radiographic examinations, bone scans and computer tomography revealed avascular necrosis of both hips. Two patients underwent total hip replacement, one of which was bilateral. The third patient was also advised to undergo this procedure. There is little doubt that in all three patients the avascular necrosis of the hip was caused by the corticosteroids included in the treatment protocol. The precise incidence of avascular necrosis, in this multicenter therapeutic trial, which now includes several hundred patients with ALL, is not yet known. Personal communication suggests that there are more patients affected than the three reported here. Reduction of the corticosteroids should be considered in future protocols to prevent this severe complication, which significantly reduces the quality of life following complete remission.

233 Intralesional Application of Interferon (IFN) Combined with Chemotherapy for the Treatment of Refractory Cutaneous Relapse of Acute Myelomonocytic Leukemia

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There is increasing evidence that IFN treatment may positively influence the course of various hematological malignancies and solid tumors. This effect may sometimes be more pronounced with intralesional application of IFN than with systemic application, as recent studies in malignant melanoma suggest. A 52-year-old woman who had a refractory cutaneous relapse of acute myelomonocytic leukemia was recently treated by intralesional application of IFN, combined with chemotherapy. A chemotherapeutic regimen consisting of m-AMSA and etoposide, similar to that previously instituted without success for the forth skin relapse was used for the treatment of rapidly progressing skin infiltrates. After completion of 4 days' daily treatment with 100 mg m-AMSA and 150 mg etoposide, daily intralesional injections of β-IFN (0.5×10 IE/nodule) were administered for 5 days. We observed the complete disappearance of skin infiltrates after 2 weeks. Experimental systemic maintenance therapy with α-IFN (2.0×10 IE twice weekly) was instituted. During the following weeks, rapidly progressing cutaneous and subcutaneous infiltrates were noted at sites which had not previously been injected with IFN. We conclude that intralesional application of IFN combined with systemic chemotherapy may induce longer local remissions than chemotherapy alone in patients with extramedullary relapse of acute myelomonocytic leukemia. Low-dosage systemic IFN, however, does not seem to be an effective treatment for the prevention of new relapses at sites which have not previously been intralesionally injected with IFN.

234 Cyclophosphamide, Vincristine, Cytarabine and Prednisone (COAP) Regimen Successful Treatment Approach in Patients with Acute Myeloblastic Leukemia (AML) Without Complete Remission after TAD Induction Therapy

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One year after our first report we are now able to present 15 patients with AML who had not reached complete remission after three courses of induction therapy according to the anthracycline-containing TAD regimen (thioguanine, cytarabine, daunorubicin: R. P. Gale et al.) and were subsequently treated according to the COAP protocol (cyclophosphamide, vincristine, cytarabine, prednisone: E. J. Freireich et al.). Twelve patients achieved complete remission last-

ing 4+ to 28+ months (median: 11 months), whereas 1 patient attained only a partial remission of 6 months' duration, and 2 patients did not respond to the COAP regimen. In 2 patients, complete remission allowed successful allogeneic bone marrow transplantation, and in one patient autologous bone marrow transplantation was successful.

235 Hypertransfusion in Childhood Acute Lymphoblastic Leukemia (ALL) During Induction Therapy: Results of a Randomized Prospective Study

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A randomized prospective hypertransfusion study was performed an 61 children with acute lymphoblastic leukemia (ALL), all treated with the BFM protocol ALL 79. There was no difference in the times for recovery of granulocytes or thrombocytes, if the hemoglobin was maintained above 12 g/dl during remission-induction therapy. However, significantly fewer patients within the hypertransfused group developed severe infections ($P < 0.05$). The bone marrow erythrocyte to granulocyte precursor ratio was significantly ($P < 0.05$) reduced in the hypertransfused children (median 0.65 : 1) compared with the control group (median 1.9 : 1). The remission rate and remission duration were in the same range for both groups.

236 Low-Dosage Cytosine-Arabinoside in the Treatment of Acute Myeloblastic Leukemia and Myelodysplastic Syndrome

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From May 1984 to March 1986, 13 patients (7 men, and 6 women) were treated with cytosine-arabinoside (Ara C) 10 mg/m^2 s.c. twice daily, or 20 mg/m^2/24 h by continuous infusion for over 7–19 days. The average age of the patients was 60 years (23–76 years). Eight patients had AML, 2 had AML in relapse, and 1 had secondary leukemia following Hodgkin's disease. Two patients had the diagnosis RAEB, and three patients had RAEB in transformation. Seven patients were treated primarily with low-dosage Ara C, 4 had previously received different forms of induction therapy. Two patients achieved a complete remission, 1 of whom relapsed after 8 months. Two patients attained a partial remission lasting longer than 7 months. Eight patients relapsed within 1 month; 1 patient died of infectious complications because of bone marrow aplasia. All patients showed bone marrow insufficiency.

237 Pseudohyperkalemia in Acute Lymphoblastic Leukemia (ALL)

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A 20-year-old male with severe ALL (number of blast cells in the peripheral blood up to 622 000/μl) showed an elevated concentration of serum potassium (up to 7.5 mM) during the initial phase of the disease. After the application of vincristine sulfate (vincristine) and Decortin-H, a dramatic increase in the potassium concentration was noted. Since corresponding alterations in the ECG were absent, massive lysis of blast cells during blood aspiration was suspected as the cause of the hyperkalemia. No signs of hemolysis were detected. Moreover, blood which had been obtained without aspiration exhibited subnormal concentrations of potassium. Clinicions must be aware of the possibility of pseudohyperkalemia to avoid the administration of potentially fatal therapy.

238 Prolonged Temporary Microembolisation with Enzymatically Degradable Starch Microspheres and Metachronous Intra-Arterial Chemotherapy in the Treatment of Solitary Liver Metastases: Clinical Results

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A method for prolonged temporary peripheral arterial embolisation of the liver using a starch microsphere infusion under angiographic control was developed. Periods of ischaemia of up to 4-h duration were attained. Intra-arterial chemotherapy with 5×30 mg 5-flourouracil/kg body weight was administered every 4 weeks metachronously to embolisation ischaemia. Thirteen patients with inoperable liver metastases (11 colorectal, 1 carcinoid, 1 malignant melanoma) were treated. Signs of ischaemic tumour necrosis were observed as early as 5 h after embolisation. These comprised bleeding into the metastases and transient (up to ten-fold) increases in carcino-embryonic antigen (CEA) levels. Signs of ischaemic liver damage (SGOT, SGPT elevations) were transient. Complete occlusion was not possible in 2 patients due to arteriovenous shunting. Tumour regressing, with decrease in CEA levels over 50% and over 50% reduction in size of the metastases, as demonstrated by computed tomography were observed in 9 of 13 patients. One patient showed stable-disease, 3 patients tumour regression, of whom 1 had malignant melanoma, and another had inadequate implantation of the arterial catheter, showing regression of metastases in the treated right lobe of the liver with progression in the left lobe. The mean survival was 14+ months. Two patients are still alive more than 28 months following therapy. Prolonged temporary arterial micro-embolisation with ischaemia times of up to 4 h leads to marked tumour necrosis. The advantages compared with permanent occlusion are discussed. In combination with metachronous chemotherapy, a high primary response rate can be achieved.

239 Comparative Studies on the Pharmacokinetics of 5-Fluorouracil During Isolated Liver Perfusion and After Intravenous Administration*

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In order to investigate the mode of action of isolated liver perfusion (ILP) better, as well as the possible local advantages of ILP vis-a-vis systemic administration, we studied the effects of both therapeutic modalities on 5-fluorouracil (5-FU) levels. Determinations of 5-FU concentration in the liver perfusate revealed that ILP leads to very high levels of 5-FU, with T_0 values of 1.5–3 mM. Due to the rapid flow of 5-FU out of the perfusates, the levels drop to $1/20$–$1/1000$ of the T_0 values within 60–90 min. The time course of the 5-FU perfusate demonstrates two phases: initially, the 5-FU outflow reveals zero-order kinetics, with a constant extraction of 8–12 mg 5-FU per minute. When a lower threshold concentration of 1.5–4.3 mM has been reached, further decrease occurs in accordance with first-order kinetics, with very short half-lives of 2.5–5 min. One hour after initiation of ILP, 80%–90% of the 5-FU has disappeared from the perfusate. During ILP, only 2%–12% of the substance enters the systemic circulation, so that sytemic side effects are not to be expected. In the case of i.v. injection of 8–15 mg/kg, the serum levels after distribution are in the region of 0.1 mM, and then decrease steeply with half-lives of 6–12 min. After 30–40 min, the serum levels are already 20–1000 times lower than the corresponding perfusate levels seen in ILP. These studies show that a very high local 5-FU extraction occurs in ILP, and thus confirm the advantage of high local concentrations of 5-FU vis-a-vis systemic administration.

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240 Treatment of Metastatic and Relapsed Neuroblastoma with ^{131}I-Metaiodobenzylguanidin (MIBG) in Childhood

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Between April 1984 and December 1985 we treated ten children suffering from stage III or IV neuroblastoma with 1–5 courses of MIBG treatment, with a total dose of 85–596 mCi. The reasons for use of this treatment were the failure of conventional chemotherapy and relapse of the disease. Eight of the children had large solid tumors and/or bone marrow involvement. In six of eight patients there were decreases in solid tumor mass, severity of bone marrow involvement and elevated catecholamine levels in serum and urine. These children, who suffered from fever and pain at the beginning of therapy, became free of pain and fever during the first 3 days of therapy. Of children with manifest tumor disease, seven of eight died from the disease 55–350 days after the end of treatment. The most important toxic effect was a reversible bone marrow depression. We also investigated the stability and kinetics of the radiolabeled pharmacon and free iodine. We found a dissociation of half-life between MIBG-bound and free iodine. Dosimetric measurements showed tumor doses up to 120 Gy.

241 Comparable Efficacy of the New Thioetherlipid BM 41.440 and the Alkyllysophospholipid ET-18-OCH_3

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The antitumor effect of the new thioetherlipid 1-hexadecylmercapto-2-methoxymethyl-3-propyl-phosphoric-acid-mono-cholinester (BM 41.440) was compared to the reference analogue ET-18-OCH_3 (rac. 1-octadecyl-2-methyl-sn-glycero-3-phosphocholine, ALP) in a modified colony assay introduced by Hamburger and Salmon. Tumor cell suspensions were obtained from 21 different human tumor xenografts growing in athymic mice and from the mouse leukemia L 1210. Efficacy was defined as $\geq$ 70% suppression of colony formation compared to controls. Overall, both compounds demonstrated a similar in vitro activity. Continuous drug exposure of the cells showed no efficacy at a dose of 3 μg/ml for either drug, but efficacy was observed at 10 μg/ml in 4/21 (BM) and in 5/21 (ALP) respectively, at 30 μg/ml in 12/19 (BM and ALP) tumors and at 100 μg/ml in 15/15 (BM and ALP) tumors. Furthermore, a comparison of efficacy within tumor groups gave no hint of any difference between BM and ALP. Effective in vitro cytotoxicity could also be obtained at 10 μg/ml for both compounds in experiments with L 1210 cells. In vivo efficacies were studied by daily i.p. injections (3, 10, and 30 μg/kg) in 6 mice per group starting 1 day after i.p. inoculation of 10^5 L 1210 cells. Neither drug achieved an increase in survival compared to 8 untreated L 1210-injected mice. In spite of the in vitro results there is no in vivo efficacy in L 1210 cells, so the therapeutic benefit of BM and ALP seems rather doubtful.

242 Enhancement of the Activity of Immunotoxins Directed Against Human Ovarian Carcinoma Cells

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The present study was planned to evaluate whether the activity of immunotoxins (ITs) directed against human ovarian carcinoma cells could be enhanced by verapamil, dansylcadaverine or

trifluoperazine. The following ITs, constructed with either ricin A chain (RTA) or *Pseudomonas* exotoxin (PE), were used: 454 A 12-rRTA, 260 F 9-rRTA and 454 C 11-RTA (all three ITs were gifts from Cetus Corp., Calif., USA), and HB 21-PE. 454 A 12-rRTA and HB 21-PE are directed against the human transferrin receptor. The effect of the ITs on protein synthesis was studied in human ovarian carcinoma cell lines (kindly provided by Drs. Ozols and Hamilton, National Cancer Institute, Bethesda, USA) with and without the modulating agents. Verapamil was found to enhance the activity of all four ITs. For example, the activity of 454 A 12-rRTA was enhanced sixfold by verapamil (20 μg/ml) in OVCAR-3 cells. The activity of HB 21-PE, 454 A 12-rRTA and 260 F 9-rRTA could be enhanced by dansylcadaverine or trifluoperazine. These enhancing agents did not decrease the specificity windows of the ITs. Enhancement by these drugs could help to overcome some of the problems associated with the low activity of ITs.

243 Phase I Trial of Monoclonal Antibody Conjugates to f-MET-LEU-PHE (fMLP)*

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We have previously shown that IgG directed against tumor-associated antigens conjugated to the chemotactic peptide fMLP retains antigen recognition and chemotactic functions in vitro (Int J Immunopharmacol 5: 307, 1983) and increases intratumor macrophage numbers in an animal model (Cell Immunol 84: 169, 1983). In a phase I trial of fMLP conjugates, side effects and immune responses were evaluated in ten pretreated consenting patients with metastasizing tumors. Patients were given 1, 10, 100, 1000 and 2500 μg of an endotoxin-free fMLP conjugate of the antimelanoma MOAB 9227. Blood pressure, pulse rate, temperature, hematological and biochemical blood tests, and urinalysis were monitored at weekly intervals. No fever, allergic reactions or other signs of clinical toxicity were observed in the dose range tested, nor were any abnormal laboratory values due to these conjugates determined. No antimouse response occurred, possibly due to the low doses administered or blockage of the most immunogenic sites on the Fc region by fMLP. A phase II clinical trial is feasible. MOAB 9227 was a gift of Hybritech Inc., San Diego, California.

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244 Lipid Conjugates of 1-β-D-Arabinofuranosylcytosine: Antineoplastic Activity in Vitro and Therapeutic Effects against 3-Lewis Lung Carcinoma in Mice

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Four different lipid conjugates of 1-β-D-arabinofuranosylcytosine (Ara-CDP-L-Dipalmitin; Ara-CDP-D, L-PBA; Ara-CDP-D, L-PCA; Ara-CDP-D, L-MBA) were tested in comparison with Ara-C, the ether-lipid ET-18-OCH_3, and their equimolar mixtures. The compounds were tested in vitro for cytotoxicity (by trypan blue dye exclusion test) against cells from three different leukemias, one glioblastoma and two bronchogenic carcinomas of human origin. The compounds were given in vivo to assess their therapeutic activity against 3-Lewis lung carcinoma (3-LL) of syngeneic $C_{57}Bl_6$ mice. Although the conjugates have shown cytotoxic activity in vitro against the cell samples tested, they have not revealed higher cytotoxicity than ET-18-OCH_3, Ara-C or their equimolar mixtures. In these experiments, the LC_{50} values for ET-18-OCH after 48 h of incubation were $\leqq 8$ μM for the leukemias and approximately 20 μM for the solid tumors. The

order of effectiveness against leukemias was Ara-C > equimolar mixtures > ET-18-OCH_3, >conjugates (Ara-CDP-D, L-MBA), and against the solid tumors was ET-18-OCH_3, > equimolar mixtures > conjugates (Ara-CDP-D, L-MBA) > Ara-C. The conjugates produced a remarkable tumor growth inhibition and increase of surviving animals in $C_{57}Bl_6$ mice bearing i.p. implanted 3-LL. In these experiments Ara-CDP-D, L-PBA, Ara-CDP-L-Dipalmitin and Ara-CDP-D, L-PCA were more active than the parent compounds Ara-C or the ether-lipid ET-18-OCH_3, alone. The order of effectiveness among the conjugates was Ara-CDP-D, L-PBA > Ara-CDP-L-Dipalmitin ≧ Ara-CDP-D, L-PCA > Ara-CDP-D, L-M. Furthermore, some of the conjugates could inhibit the development of metastasis when given after the surgical removal of the primary tumor, as demonstrated by increases of median survival times and numbers of surviving animals. Although their mode of action still remains unclear, these conjugates, especially Ara-CDP-D, L-PBA, are strongly recommended for further investigation as experimental drugs in cancer therapy.

245 Control of Cell Proliferation in Malignant Tumors: Effect of the Extracellular Concentration of H^+-Ions on the Survival of Clonogenic Malignant Cells and their Rate of Proliferation and Cell Cycle Phase Distribution in Vitro

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It has been demonstrated in several studies that the median pH in malignant tumors of both animal and human origin is lower by about 0.4 units than the median pH in normal tissues, and large regional variations have been found in tumor tissues (extreme values as low as 5.8; Vaupel et al. 1981). Since various normal cells are characterized by a transient rise of intracellular pH after the initiation of cell proliferation, we analyzed the effect of an acidic microenvironment on survival, proliferation rate, and cell cycle distribution of clonogenic malignant rat cells in culture. Rat mammary carcinoma cells, BICR-$MlR_{k\text{-}d}$ and malignant rat neurogenic cells, BTlC were incubated as monolayers in culture media of varying pH. The population doubling time of both cell lines increased with increasing extracellular H^+-ion concentration (BICR-$MlR_{k\text{-}d}$, pH 7.4 at 18 h; pH 6.5 at 70 h). In both cell lines growth was completely inhibited at pH 6.1. A pH shift from 7.4 to 6.1 simultaneously arrested the cell cycle progression of BTlC and BICR-$MlR_{k\text{-}d}$ cells in all phases of the cell cycle, as demonstrated by flow cytometry. In contrast, malignant rat kidney cells, KNRK preferentially accumulated in the G_1 phase under these conditions. After incubation in acidic culture media, the survival of clonogenic BTlC and BICR-$MlR_{k\text{-}d}$ cells is inversely related to the extracellular H^+-ion concentration and the exposure time. Survival of BTlC cells at an extracellular pH of 6.1 was reduced to 25% and 5% that of control BTlC cultures at pH 7.4 after 24 h and 72 h respectively. These results indicate that both survival and proliferation rates of malignant cells are generally well adapted to a slightly acidic microenvironment. However, the microenvironmental pH may be one of the factors limiting cell survival and proliferation in certain parts of malignant tumors.

246 Bone Marrow Toxicity of Oral Benzo(a)pyrene

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The Ah-locus regulates the induction of cytochrome P-450-mediated mixed-function oxygenase systems in inbred strains of mice. Genetic predisposition at this locus (responders, versus non-

responders) determines the hematotoxicity of oral benzo(a)pyrene (BaP) in inbred strains of mice. In this experimental model system the continued oral administration of BaP produced severe bone marrow depression in non-responders, affecting all hematopoietic lineages, but produced only moderate bone marrow depression in responders, affecting CFU-S cells and erythropoiesis only. All non-responders were killed by the continued oral administration of BaP. Hematopoietic malignancies developing late after the limited administration of oral BaP are also strictly correlated to the Ah-locus: only non-responders developed hematopoietic malignancies after limited administration of oral BaP. Pharmakokinetic studies showed marked accumulation of BaP in the bone marrow of non-responders.

247 Interaction of 5 Fluorouracil (5 FU) with Tumor Ischemia

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In the local treatment of liver metastases several clinical studies have employed intra-arterial 5 FU with concomitant reduction of the arterial blood flow. As 5 FU is activated in the tumor cell, ischemia and reduction of metabolic activity might have a protective effect. Consequently, the increased metabolic activity after ischemia might enhance the drug sensitivity of the tumor. An undifferentiated mammary adenocarcinoma (AT 7) was subcutaneously implanted in isogenic C 3 H mice. The tumor regrowth delay i.e. the times necessary for the tumor to return to and to double its pre-treatment size were calculated. No treatment was given to one group of mice. Two groups were treated with either intraperitoneal 5 FU or ischemia alone. The remainder received 100 mg/kg 5 FU intraperitoneally every 4 h for 48 h. After 20–60 min, ischemia was induced by clamping the tumor for 90 min. Serum pharmacokinetics after intraperitoneal application were measured. All groups showed tumor regression after 5 FU. The administration of 5 FU prior to ischemia leads to less regrowth delay than 5 FU alone, whereas 5 FU administration after the end of ischemia causes a markedly longer regrowth delay than 5 FU alone. Tumors in animals receiving 5 FU 20 min prior to ischemia had reached their pre-treatment size after 7.3 days, whereas regrowth delay with 5 FU given 20 min after the end of ischemia was 11.9 days. Ischemia plays a major interactive role in chemotherapy with 5 FU. Clinical studies must include awareness of potential interactions with ischemia.

248 Results of a Pilot Study with Hyaluronidase as an Additive Drug to Cytostatic Therapy in Malignant Diseases

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We report the results of treatment of 134 patients with either 7500 IU or 200 000 IU hyaluronidase, either systemically or intraperitoneally. The only side effects which occurred were a few reversible anaphylactic reactions. The following results have been achieved in patients, who did not respond to a certain type of chemotherapy, and subsequently continued to receive the same chemotherapy, with the addition of hyaluronidase: Myelomas, complete remission (CR) 2/17, partial remission (PR) 3/17, subjective improvement 10/17; non-Hodgkin lymphomas, CR 3/19, PR 9/19; Hodgkin's disease, CR 2/7, PR 3/7; mammary carcinoma, CR 2/14, PR 5/14 (including 1 PR of a cerebral metastasis); squamous cell carcinoma of the auditory or upper respiratory tracts, CR 8/16, PR 3/16; hypernephroma CR 1/5. In the rest of the patients, either hyaluroni-

dase was added from the onset of chemotherapy, or the chemotherapy was changed at the commencement of hyaluronidase treatment. Among these patients, the results were as follows: cerebral metastases of mammary carcinomas, PR 3/3; glioblastomas 1 PR and 1 no change (NC); 1 astrocytoma NC (all over a period of several months). Intraperitoneal treatment with hyaluronidase of 8 patients with peritoneal carcinomatosis from tumors led to good toleration of cytostatic agents like adriamycin, cis-platin, and 5-fluorouracil administered intraperitoneally. In all patients, suppression of ascites lasting between 1 and 16 months was achieved.

249 Clinical Pharmacology of Mitoxantrone in Patients with Breast Cancer and Leukemia

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In our previous publications the pharmacokinetic parameters of mitoxantrone after a single dose of 14 mg/m^2 were described by a three-compartment model. The terminal half-life was 214.8 h., and the steady-state volume of distribution (Vdss) was 3, 792 l. The total body clearance was 358 ml/min and the renal clearance was 26 ml/min. These results suggest that mitoxantrone is sequestered in a deep tissue compartment and is only slowly released. A pharmacokinetic study was performed as part of a phase II trial in leukemic patients to investigate the body distribution and elimination of mitoxantrone after repeated doses (5×10 mg/m^2). The terminal half-life was 175 h ($n = 6$) and the Vdss was 3, 841 l ($n = 3$). The urinary excretion of mitoxantrone, expressed as a percentage of the daily dose was unchanged from days 1–5. The capacity of the tissues to bind mitoxantrone thus seems to be large. There was no evidence of binding saturation, which would have been demonstrated by a decrease in the Vdss or half-life values. While the long elimination half-life indicates that significant tissue accumulation can be anticipated after repeated doses, the data demonstrate that the kinetics are not significantly altered in leukemic patients.

250 Cardiotoxicity of Adriamycin and 4′-Epidoxorubicin: Echocardiographic Study with Angiotensin-II Stress

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To study the chronic cardiotoxicity of adriamycin (ADM) and 4′-epidoxorubicin (4′-Epi) we recorded serial M-mode echocardiograms at rest and during afterload stress (induced by the infusion of angiotensin II, 1000–3000 ng/min) in 17 normal individuals, 15 patients receiving ADM and 12 patients receiving 4′-Epi. Ventricular function was estimated at rest by estimation of the diameter shortening (Δ D%) the shortest distance between mitral and septal echoes (ES), and (under stress) by the slope K of the linear regression of systolic cuff pressure and endsystolic diameter. None of the recordings [ADM $\leq$ 595 mg/m^2 ($n = 55$); 4′-Epi $\leq$ 835 mg/m^2 ($n = 36$)] showed abnormal conventional parameters at rest (Δ D%; ES). However, K decreased below the lowest normal value (4.5 mmHg/mm) in 5 patients who received ADM (at 120, 250, 410, 510, and 595 mg/m^2) and in 2 patients who received 4′-Epi (at 530 and 570 mg/m^2). At dosage levels of 400–600 mg/m^2, 40% of stress studies were abnormal with ADM, as opposed to 22% with 4′-Epi. The echocardiographic diagnosis of toxicity due to anthracyclines is facilitated by angiotensin-stress testing. At equivalent dosage levels 4′-Epi is less cardiotoxic than ADM.

251 Doxorubicin-Induced Localized Urticaria in Patients with Hodgkin's Disease

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Doxorubicin has been associated with severe, acute toxic reactions, such as local skin necrosis and deep tissue damage at the site of a drug infiltration. We describe three patients treated for Hodgkin's disease who had unusual reactions at the injection site. The clinical picture is identical with the one described with allergic causes. In patients treated with ABVD or ABV we saw skin changes that mainly consisted of an erythematous streak up the punctured vein, which was immediately associated with urticaria and pruritus, and lasted ~ 30 min. This appeared twice after course 6 and once after course 13 of doxorubicin, respectively. The interval between the first course doxorubicin therapy and the onset of allergic reactions was 3.5 to 7 months. No prophylactic corticosteroids were given since in all cases the local reactions improved rapidly and a second severe allergic reaction did not occur. The possibility that this ill-defined and rare complication might occur should not contraindicate subsequent use of the drug, as shown in our patients.

252 Effects of Harringtonine on the Parasynchronized T-Lymphoblastic Cell Line JM: A Study by Flow Cytometry

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Harringtonine is an alkaloid derived from the South Chinese tree Cephalotaxus fortunei. It has been used for a long time in Chinese folk medicine for the treatment of neoplasia. S-phase parasynchronization was induced in the T-lymphoblastic lymphoma cell line JM by the addition of 0.2 mM thymidine for 20 h. M-phase parasynchronization was performed by incubation with 0.1 μg/ml demecolcine (Colcemid) 11 h later for 6 h. After release 0.1–1.0 μg/ml Harringtonine was added. Concentrations of 0.1 μg/ml slowed the growth of S-phase parasynchronized cells. Concentration ≥ 0.5 μg/ml caused blocking of early S-phase. Concentrations ≥ 0.1 μg/ml blocked the transition of M-phase parasynchronized cells from G 1-phase to S-phase. We conclude that the main effects of Harringtonine are to block transition from G 1-phase to S-phase and inhibit growth in S-phase.

253 Protection of Cultured Malignant Cells from Anthracycline Cytotoxicity by Low Extracellular pH: A Possible Mechanism for Primary Chemoresistance in Vivo

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Several recent studies have clearly demonstrated that in malignant tumors the pH distribution is shifted to lower values than those found in normal tissues or peripheral blood. In a transplanted murine tumor the frequency of pH values < 6.7 was approximately 50% (Vaupel et al. 1981). The present study tested whether an increase of the extracellular H^+-ion concentration influences the response of clonogenic malignant cells to the cytotoxic action of anthracycline antibiotics and mitoxantrone. Rat mammary carcinoma cells BICR-MIR_{k-d} growing in monolayer culture were exposed to adriamycin at concentrations of 0.1–1.0 μg/ml for 24 h in culture

media of varying pH. At an extracellular pH (pH_e) of 7.4 (adriamycin concentration, 0.25 μg/ml) the survival of clonogenic BICR-MIR_{k-d} cells was reduced to 0.1% of that determined for cells without adriamycin. However, at a pH_e of 6.8 survival was only reduced to 10%, indicating a hundred-fold protection of BICR-MIR_{k-d} cells against adriamycin toxicity. In most animal and human tumors the median pH is 6.8–7.0. The protective pH effect is not due to the inhibition of cell proliferation of BICR-MIR_{k-d} cells at low pH_e, since the population doubling time of these cells is only marginally prolonged by a pH shift from 7.4 to 6.8. Similar protective effects of a lower pH_e were observed agains epirubicin, daunorubicin, and mitoxantrone (an anthracenedione with structural similarities to anthracyclines). Reduced cellular uptake of these drugs at low pH_e as described by Skovsgaard (1977) may contribute to the protective effect. The possible relationship of these results to the primary resistance of malignant tumor cells to anthracyclines in vivo is discussed.

254 Potentiation of Cyclophosphamide Cytotoxicity to Clonogenic Malignant Cells by Reduction of Extracellular pH in Vitro: A Possible Strategy for Tumor-Selective Chemosensitization

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The development of tumor-selective modalities of cancer treatment requires the exploitation of cellular properties that distinguish cancer cells from normal cells. Several recent studies have shown that one such property, the aerobic glycolysis of malignant cells, can be exploited to reduce the pH selectively in malignant tissues in vivo. By parenteral administration of glucose, for example, the pH in TVIA rat tumors is reduced to a median value of 6.1 (range 5.5–6.7, Jähde and Rajewsky 1982). As a first approach to the question of whether this tumor-selective modification of the cellular microenvironment could be used to sensitize malignant cells to the action of cytocidal drugs, we analyzed the survival of clonogenic BICR-MIR_{k-d} rat mammary carcinoma cells exposed to 4-hydroperoxy-cyclophosphamide (aCP) as a function of extracellular pH (pH_e) in vitro. At a pH of 7.4 (aCP concentration, 1 μg/ml) survival was reduced to 10% of that determined for cells without aCP. However, at pH_e values of 6.8 and 6.2, survival was reduced to 2% and 0.05%, respectively. The latter value corresponds to a 200-fold increase of aCP cytotoxicity by lowered pH_e. The magnitude of the H^+-ion sensitizing effect increases with extracellular H^+-ion concentration, with aCP concentration, and with exposure time. The effect is dependent on the bis-chloroethylamino-group of the terminal alkylating aCP metabolites, since a similar potentiation of cytotoxicity was also observed with nornitrogen mustard and melphalan, but not, however, with ifosfamide. The potential use of glucose as a biochemical modulator for the tumor-selective potentiation of cytocidal drug activity is discussed.

255 Thermostability of Cytostatic Drugs in Vitro and Thermosensitivity of Cultured Human Lymphoblasts Against Cytostatic Drugs

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The application of hyperthermia in combination with cytostatic drugs for the treatment of solid tumors was studied. Such studies should use cytostatic drugs which are more active in the hyper-

thermic tumor region, but have no increased systemic toxicity. We examined the in vitro stability of cytostatic drug solutions in saline. In a biological test system with cultured human lymphoblasts, the activity of the cytostatic drugs was assayed at 37°C after preincubation without the cells for 1 h at temperatures up to 43°C. A slight increase of activity was found for vincristine and slight decreases for cisplatin and etoposide. The activities of all the other drugs tested were unaffected by hyperthermic preincubation. When the lymphoblasts were incubated with the cytostatic drugs under hyperthermic conditions, there was a clear increase in the activities of ifosfamide (×7), methotrexate (×5), and 4′-epi-doxorubicin (×4). Some other cytostatics showed two- to three-fold incresases in activity. No hyperthermic effect was seen with 5-fluorodeoxyuridine, cytarabine, or etoposide. In summary, ifosfamide, methotrexate and 4′-epi-doxorubicin are favorable candidates for the combination of cytostatic drugs with regional tumor hyperthermia. Increased cytostatic drug activity occurs only at temperatures above 39°C. Thus, with local hyperthermia, in which the whole body temperature does not rise over 39°C, no increase of systemic toxicity (e.g. bone marrow depression) is to be expected.

256 Cytotoxic Drug Testing in Human Leukemias by Multiparametric Flow Cytometry

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Human bone marrow cells from 19 patients suffering from leukemia, from 1 patient suffering from pernicious anemia, and from several human cell lines were assayed by a newly developed in vitro flow cytometric assay. Cells were incubated with or without cytosine arabinoside (Ara-C), L-Asparaginase, daunorubicin, prednisone, or vincristine at different concentrations. After an incubation period of 2–7 days, surviving cells were stained by the esterase dye ADB and dead cells by the DNA-dye PI. Dose-response curves were established according to the percentage of cells surviving, the ratio of high to low esterase and the ratio of vital to dead cells. Bone marrow samples from 16 out of 20 patients could be evaluated; daunorubicin and Ara-C were cytotoxic in samples containing leukemic blasts as well as in samples from patients in clinical remission, whereas vincristine was mainly effective in the leukemic group (P 0.05). The cell lines exhibited different patterns of sensitivity. The myeloblastic line HL 60/16, the T-cell lines CEM, JURKAT and the B-cell line RAJI were sensitive, while the T-cell line MOLT and the B-cell lines RPMI 1788 and DAUDI were more resistant to drug treatment. Vincristine arrested the cells in G2/M-phase, Ara-C increased the number of cells in the S-phase.

257 Therapy-Induced Leukemia and Preleukemia

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Acute myeloblastic leukemia may occur as a late complication of cytotoxic or immunosuppressive chemotherapy. Alkylating agents, procarbazine and nitrosoureas have been most frequently associated with the development of secondary leukemia, which is different from de novo AML with respect to morphology, chromosome studies and prognosis. During the past decade, 19 cases of secondary AML ($n = 15$) and myelodysplasia ($n = 4$) have been diagnosed at the University of Düsseldorf. These patients had received cytotoxic and/or immunosuppressive chemotherapy for various diseases (Hodgkin's disease, $n = 5$; multiple myeloma, $n = 3$; lymphocytic NHL, $n = 2$; solid tumors, $n = 7$; rheumatoid arthritis, $n = 2$). Alkylating agents (cyclophosphamide, dihydroxybusulfan) had been administered in 13 cases. Two patients had been treated with azathioprine for rheumatoid arthritis. The interval between treatment of primary disease

and the development of acute leukemia ranged from 9 months to 32 years (median 6 years). Two patients presented with simultaneous occurrence of AML and relapsing primary disease (Hodgkin's disease, colon carcinoma). The overall incidence of leukemia in patients with Hodgkin's disease treated with polychemotherapy or radiochemotherapy ($n = 307$) was 1.6%. In contrast to other authors, we did not find a prevalence of monocytic or myelomonocytic leukemia. Our findings emphasize the leukemic risk which is associated with antineoplastic chemotherapy and may be of relevance for long-term survivors among these patients.

258 Immunoregulation of Myelopoiesis: the Roll of Soluble Mediators

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Soluble products, which result from the interaction of T-cells, monocytes and NK cells, are decisively involved in the regulatory network, which guarantees homeostasis and adjustment to the requirements of the situation on myeloid effector cells. Positive or negative feedback signals result in a "net" colony stimulating activity (CSA), which quantitatively determines the extent of clonal growth of committed progenitor cells in functional granulocytes/monocytes (G/M). The goal of the present study was to analyze lymphokine/monokine interactions in the generation of colony-stimulating and inhibiting activities. In conclusion, it was possible to conceptualize the following experimental model for the regulation of myelopoiesis. First, interleukin-2 induces secretion of γ-interferon (γ-IFN) in T-cells. Second, γ-interferon regulates: (a) transcriptional secretion of G-CSF as well as colony-inhibiting tumor necrosis factor in monocytes; (b) membrane expression of HLA-DR and DC molecules on myeloid progenitor cells (target structure for prostaglandin-mediated negative signals); (c) production and release of interleukin-1 in monocytes. Third, interleukin-1 recruits, on the one hand, concentration-dependent GM-CSF secretion in T-cells and, on the other hand, mediates in higher concentrations direct, antiproliferative effects on myeloid progenitor cells.

259 Studies of Recombinant-DNA-Derived Interferon-α and Tumor Necrosis Factor as Regulators of Hematopoietic Cell Proliferation*

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Preparations of gene-cloned human interferon-α (rIFNγ) and tumor necrosis factor (rTNF) were assessed for their influence on colony formation of normal human hematopoietic progenitor cells (CFU-GM, CFU-E, BFU-E). Colony growth was inhibited by both cytokines in a dose-dependent manner. Early and late erythropoietic precursors were clearly more susceptible to rIFNγ-mediated inhibition (50% inhibition occurring at about 60 U/ml rIFNγ), while more than 400 U/ml of rIFNγ was required for 50% inhibition of CFU-GM. Competition between rIFNγ and colony-stimulating activity seems to account for this relative resistance of CFU-GM. In contrast, CFU-GM were more responsive to rTNF than CFU-E and BFU-E. CFU-GM formation was completely inhibited by rTNF at doses higher than 20 U/ml, and 50% inhibition was observed in the presence of only 1 U/ml rTNF. TNF was able to directly inhibit progenitor cell proliferation. In contrast, rIFNγ-mediated suppression depends on the presence of auxiliary cells. The simultaneous presence of rTNF and rIFNγ resulted in a dramatic synergistic inhibition of all types of progenitor cells assayed. These data might be of practical importance when designing clinical trials employing combined treatment modalities for rIFNγ and rTNF. Furthermore, both cytokines should be implicated in a hypothetical, pathophysiological concept of factor-mediated bone-marrow failure.

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260 Antigen-Specific Elaboration of Granulocyte-Macrophage Colony Stimulating Factors by Human Alloreactive T-Cell Clones and Its Inhibition by Cyclosporin A

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Human alloreactive T-cell clones were derived after sensitization in MLC by limiting dilution techniques. Culture supernatants were screened for the presence of granulocyte-macrophage colony stimulating factors (GM-CSF) in standard progenitor assays. Normal bone-marrow mononuclear cells or progenitors highly (30-fold) enriched by fluorescence-activated cell sorting employing MAB My 10 served as target cells. Supernatants from 12 of 12 $CD4^-$ helper clones stimulated by specific, but not third-party, antigen were able to support CFU-GM colony growth in a dose-dependent fashion. However, this lymphokine was not produced at detectable levels by 3 of 8 non-helper clones. The presence of 30% – 55% eosinophilic colonies and lack of activity on mouse (BALB/c) targets suggest rather that helper T-cell clones homogeneously produce only GM (α)-CSF. In order to investigate the potential inhibitory effects of cyclosporin A (CSA) on GM-CSF production, T-cell clones were stimulated in the presence of 10 to 0.1 μg/ml CSA. Even the latter concentration consistently resulted in a significant decrease of colony numbers as compared to controls treated with solvent and CSA alone. These findings suggest that if T-cell-derived CSFs play a role as physiological stimulators of myelopoiesis during antigen challenge, the defence mechanisms of patients on cyclosporin A therapy may be impaired in addition.

261 CSF-Like Activity is Produced by EBV-Transformed B-Cell Lines

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Spontaneously developed lymphoblastoid cell lines were established from the mononuclear peripheral blood cells of healthy donors. One line was characterized by immunological phenotyping, HLA typing and EBV-DNA detection. CSF-like activity is constitutively produced by this cell line in a time-dependent manner as tested in a C3H bone-marrow colony assay. De novo production of activity can be inhibited by cycloheximide. Titration of the activity shows a steep dose-dependency gradient similar to that of murine M-CSF. The morphology and cytochemistry of pooled and single bone marrow colonies are consistent with those of macrophages. Crude supernatants also induce maturation of the promyelocytic HL60 cell line into macrophages. Further biochemical characterization was done. Gel filtration with sepharose 4B, even in the presence of 6M guanidine hydrochloride, detected activity in a fraction with an unusually high molecular weight of 200 – 400 kilodaltons. Thus, EBV particles can be excluded as colony-stimulating agents. With DEAE sepharose we find charge heterogeneity. The activity is sensitive to pH conditions below 3 and above 9, can be heat-inactivated at 80°C and is stable at 56°C for 30 min. The lymphoblastoid line RPMI 1788 also exhibits M-CSF-like activity. In addition, however, all of the cell lines that we have analyzed produce a colony-growth inhibitor, especially the EBV-positive Burkitt lymphoma lines DAUDI and RAJI.

262 Regulation of Hematopoiesis by NKTa$^+$ Natural Killer Cells via a Specific Target Antigen (TNK_{TAR}) on Progenitor Cells

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Natural killer (NK) cells to play an important role in the regulation of hematopoiesis have been shown. In previous experiments, various human NK clones were shown to have heterogeneous suppressive effects on bone marrow progenitor cells. In the present study we have utilized different T3$^+$ Ti$^+$ NK clones, to examine the receptor target cell interaction in this regulatory system. Monoclonal antibodies against the Ti-like structure NKTa on the effector cells, and the target antigen TNK_{TAR}, an early-activation antigen on bone marrow progenitor cells, as well as the LFA-1 antigen on both cell types were used to study their blocking effects on the NK inhibition of colony growth (CFU-GM, CFU-E, BFU-E and CFU-GEMM). Normal bone marrow cells were purified 50-fold, preincubated before culture for 18 h with NKTa$^+$ NK cells and NKTa$^-$ control NK clones in the presence of media and anti-NKTa, anti-TNK_{TAR}, and anti-LFA-1 monoclonal antibodies. The results revealed a significant inhibitory effect on hematopoietic colony growth by various NK clones, which was not found in various T-cell clones. This inhibitory effect could be reversed in NKTa$^+$ NK clones when anti-NKTa or anti-TNK_{TAR} against the specific target structure was applied in autologous and allogeneic systems. These antibodies had no influence on NKTa$^-$ NK cells. Anti-LFA-1 had an inhibitory effect and therefore partially reversed the inhibition for all NK clones. It can be concluded that NKTa is a specific T-cell receptor structure and the recognition of specific target structures such as TNK_{TAR} play an important role in the regulation of hematopoiesis by NK cells. LFA-1 appears to be an additional cell-cell binding molecule in this specific recognition system.

263 Evidence for an In Vivo Activated T8$^+$ Cell Type in the Pathogenesis of Non-A, Non-B Hepatitis Associated Aplastic Anemia: Role of γ-Interferon

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There is growing evidence to support the concept that immune mechanisms may cause marrow failure following virus infections, particularly following non-A, non-B hepatitis. We studied one such patient whose circulating lymphocytes were almost entirely T8$^+$ and Ia$^+$, thus indicating that their activation is presumably virus-induced. A new permanent cell line (SMAA) was established using lectins, EBV-transformed irradiated B cells and IL-2 from these cells. It was shown that SMAA cells (or a soluble factor released by SMAA cells) inhibit myeloblast DNA synthesis and their differentiation into early- and late-appearing neutrophils and eosinophil colonies by 90%, whereas monocyte colonies remained unaffected. Similarly, growth into eryhtroid colonies and bursts were completely inhibited. This soluble factor was sensitive to acid (pH 2), trypsin and heat (56°C). Monospecific anti-IFN gamma antibody was able to abrogate the inhibitor effect of SMAA Sup, but more than 10^4 nU/ml had to be added. Moreover, the effect of SMAA could be duplicated by adding 10^4 U/ml purified recombinant γ-IFN to colony and proliferation assays. The concentration of γ-IFN in SMAA culture medium was estimated to be greater than 32×10^4 U/ml by serial dilution assays. These results suggest that γ-IFN released by activated T cells may play a role in the pathogenesis of some cases of bone marrow failure.

264 Kinetics of Hemopoietic Progenitor Cells in Long-Term Human Bone Marrow Microcultures*

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In the presence of a marrow-derived adherent cell layer, human pluripotential hemopoietic stem cells can be maintained in vitro for several weeks and remain transplantable post-culture. However, this liquid culture system is not clonal and requires many cells for initiation. Using human marrow cells, microcultures were established in order to analyse even small cell samples and to propagate clonal stem cell cultures. After 4 weeks of incubation the microcultures showed an adherent layer of fibroblastoid and epitheloid cells, being an inductive environment for early hemopoietic precursors. The microcultures were fed weekly by the removal of half the non-adherent cell suspension and the addition of an equal volume of fresh medium. Each week the non-adherent cell suspensions of each microculture were pooled, counted for nucleated cells, and tested for the presence of granulocyte-macrophage colony-forming cells (GM-CFC). When corrected to the volume of macrocultures, the cyto-kinetics of both the non-adherent cells and the GM-CFC are quite similar to the data reported for long-term human marrow macrocultures. Moreover, both macro- and microcultures maintain hemopoiesis for about 7 weeks. The work in progress aims to establish a stem-cell-free stromal haemopoietic environment which will permit the cloning of fresh pluripotential stem cells by "limiting dilution" analysis.

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265 Analysis of Antigenic Determinants on Human Megakaryocytic Progenitor Cells

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To date, the antigenic phenotype of megakaryocytic progenitor cells (CFU-M) has hardly been investigated. Because these cells are extremely rare in human bone marrow, a detailed antigenic analysis would be facilitated by the enrichment of bone marrow progenitors. We fractionated bone marrow cells by two-step gradient centrifugation to separate cells that give rise to hemopoietic colonies in culture (< 1.077 g/ml) from low-density cells (< 1.05 g/ml). Density centrifugation was followed by indirect immunopanning to negatively select for an enriched population of hemopoietic progenitors: cells of density < 1.077 g/ml were labeled with a panel of monoclonal antibodies (IgM-subtype) detecting myeloid, lymphoid and erythroid antigens. The resulting nonadherent, lymphocyte- and monocyte-depleted cell fraction was subsequently labeled with monoclonal antibodies (IgG subtype) directed against the antigens of interest. Finally, the samples were analyzed on the fluorescence-activated cell sorter (FACS) and positively and negatively staining fractions were isolated and assessed for the ability to form megakaryocytic colonies in culture. Our study demonstrated that CFU-M express HLA-DR and My 10 antigens, but lack HLA-DQ as well as glycoprotein III a, the most sensitive marker so far identified for megakaryocytic cells. In addition to antigenic analysis, the combination of density centrifugation, immunoadsorption and FACS provides a suitable approach to further studies on enriched hemopoietic progenitors.

266 Fibroblast Stem Cells Circulating in Cord Blood

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CFU-F colonies were cultivated in vitro from cord blood, peripheral blood of premature infants, and from the bone marrow of older children and adults. In 14 of 22 cord blood specimens there were 110 ± 70 colonies per 6×10^6 buffy coat cells. A very small minority of stem cells (about 1%–2%) have high proliferation capacities; the appearance of these cells in culture is delayed, and they probably represent CFU-F precursor cells, tentatively called BFU-F. CFU-F were identified by their fibroblast progeny which reacted positively to anti-fibronectin and anti-collagen III monoclonal antibodies. Fibroblast monolayers derived from CFU-F cultivated from cord blood produce CSF (colony stimulating factor), to which fresh cord blood and bone marrow lymphoid cells respond by CFU-GM colony formation in a dose-dependent manner. BFU-F colonies from cord blood discharge round cells into the supernatant that give rise to progeny BFU-F and CFU-GM colonies in secondary cultures. We believe that CFU-F and BFU-F form cellular migration streams in the blood connecting, during ontogeny, the stromata of different hematolymphopoietic organs.

267 Bone Marrow Stromal Cells from Children in Remission from Acute Lymphocytic Leukemia: A Source of Physiological Hematopoietic Stem Cells?

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In vitro CFU-F colony formation from bone marrow was seen to be severely suppressed during active ALL (7 patients) but reverted to normal when successful remission was induced (8 patients) During remission, the stromal cells of bone marrow comprise, in addition to CFU-F, fibroblastic stem cells with a clearly higher proliferation capacity, a delayed appearance in culture, and more rapid growth kinetics. These cells appear to be precursors of CFU-F cells and have tentatively been designated BFU-F. Three- to six-week-old BFU-F, but not CFU-F colonies, have round cells associated with their fibroblasts, i.e. lymphoid/transitional and myeloid cells. These round cells produce secondary BFU-F and CFU-GM colonies in methocel cultures. This suggests that BFU-F are stem cells with a capacity for (limited?) self-renewal which may be involved in the repopulation of leukemic bone marrow with normal hematopoietic stem cells during the induction of clinical remission.

268 The enhancement of Human Neutrophil Granulocyte Function by Natural and Recombinant Hemopoietic Colony Stimulating Factors

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Highly purified natural and recombinant pluripotential colony stimulating factor (nPPO, rPPO or G-CSF) and natural α-pluripoietin (n-PPO-alpha or GM-CSF) are potent stimulators of antibody-dependent cellular cytotoxicity (ADCC) of polymorphonuclear granulocytes (PMN) against leukemia and lymphoma cell lines in vitro. The concentrations of PPO required for maximal enhancement of ADCC of PMN are slightly higher than those required for maximal colony formation from CFU-GM in agar cultures. Bacterial peptide fMLP, human recombinant tumor necrosis factor and γ-interferon also stimulate the ADCC activity of PMN. Synergistic or additive effects of these stimulators together with PPO are observed. By virtue of their stimulatory activities on mature hemopoietic cell function and on hemopoietic progenitor cells, the PPO compounds appear to be potentially clinically useful biological mediators.

269 The Role of Interleukin-2 in Immunoresponsiveness in a New Subtype of Primary Immunodeficiency

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We have investigated the cause of a lack of T-cell reactivity against alloantigens, mitogens and recall antigens in a 6-month-old child. Surface marker analysis revealed normal proportions of B- and NK-cells. The number of T-cells was only slightly reduced, but a lack of cells bearing the T-suppressor phenotype was apparent. In contrast to severe combined immunodeficiency, there was an unusually high count of mature T-helper cells, which required explanation. Direct cell mediated lymphoysis (CML) of allogeneic or NK-target (K 562) cells was not found. However, the patients cells became cytotoxic toward specific allogeneic and K 562 cells after allogeneic stimulation in the presence of exogenous crude or recombinant IL-2. Endogenous IL-2 was not produced following different modes of T-cell activation. Whether the IL-2 gene or the m-RNA expression is affected is presently under investigation. An attempt to reconstitute the immunodeficiency by T-cell-depleted haploidentical bone marrow transplantation (BMT) failed because of primary non-take. These data suggest a new subtype of primary immunodeficiency, characterized by the failure of T-cells to produce IL-2. The role of IL-2 in immunoresponsiveness and in allogeneic BMT will be discussed.

270 Inhibition of Humoral Immune Response by In Vivo Application of Monoclonal Antibodies Against Dendritic Reticulum Cells

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The dendritic reticulum cells (follicular dendritic cells) of lymphoid follicles are considered to be accessory cells of the B-cell immune response. The monoclonal antibodies Ki-M 4 and Ki-M 4 R selectively recognize dendritic reticulum cells in human and rat tissues, respectively. Rats were immunized with the soluble antigen alkaline phosphatase. The antigen was detectable in the sinus lining cells and germinal centers of lymph nodes, the sites of B-cell proliferation, within 3 h. Plasma cells producing specific antibodies against alkaline phosphatase could be demonstrated after 5 days. Rats treated with Ki-M 4 R did not show any binding of alkaline phosphatase to sinus lining cells and germinal centers. The titer of specific IgM against alkaline phosphatase decreased concurrently. These results show that dendritic reticulum cells are not only necessary for the binding of soluble antigen in lymphoid follicles, but also initiate the production of specific antibodies.

271 Suppressor- and Helper Function of T-Cell Clones Derived from Peripheral Blood Mononuclear Cells (PBMC) During Erythropoiesis in Vitro

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T cells are potent regulators of erythropoiesis, via stimulatory effects, such as the production of burst-promoting activity (BPA), and also by the secretion of suppressive factors. Such effects can be quantified by the formation of erythroid colonies (BFU-E) from peripheral blood mononuclear cells (PBMC). Certain donors have been shown to contain increased or decreased activ-

ity in PBMC cultures stimulated with PHA. In the BFU-E indicator system the regulatory effects of 9 T-cell clones derived from a patient with hemochromatosis were evaluated by adding either irradiated T-cell clones or their culture supernatants. All T-cell clones were CD 4-positive and expressed the antigen 4 B 4, which is specifically expressed in helper-inducer-T-cells for immunoglobulin production. Seven of these clones were potent suppressors of BFU-E and of these, two produced this inhibition by the induction of suppressor effector cells in normal resting PBMC. The other two clones increased BFU-E by inducing helper T-cell subpopulations in normal PBMC. The induction of suppressor- and helper-cells by these CD 4-positive clones for BFU-E was not matched by an equal function in assays for T-cell proliferative responses, such as a mixed lymphocyte culture (MLC), since only two clones also suppressed an MLC and the remaining clones had no effect. Also natural-killer like cytotoxicity in five of the clones did not correlate to either suppressor or helper cell induction in the BFU-E or MLC systems. These results show that the induction of help and suppression by CD 4-positive T-cell clones is specific to a defined effector phase, and that the expression of function-related antigens such as 4 B 4 by suppressor-inducer cells in the generation of a B-cell response may be irrelevant to other systems.

272 Studies of the In Vitro Stimulation of Hemoglobin Synthesis by Mitogenic Substances

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Substances known to have a mitogenic effect on lymphocytes were tested as to their influence on erythropoiesis in cell cultures derived from fetal mouse liver. The incorporation of ^{59}Fe in hemoglobin was used to measure the synthesis of hemoglobin. Different concentrations of Con A and β-mercaptoethanol were added to fetal mouse liver cell cultures which had been stimulated by erythropoietin. Both Con A and β-mercaptoethanol significantly increased the synthesis of hemoglobin in the cultured cells. The Con A-induced effect could be seen only in cell cultures which had been previously stimulated with erythropoietin. After simultaneous application of both substances, further stimulation occurred in addition to the stimulation produced by a single addition of β-mercaptoethanol. The mitogenic effects of Con A and β-mercaptoethanol on T-lymphocytes are well recognized. The effects on erythropoiesis could be produced by a similar mechanism.

273 T-Cell/Macrophage Interactions in the In Vitro Production of Humoral Factors Regulating Human Granulopoiesis

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A variety of macrophage functions are modulated by the T-cell lymphokine γ-interferon (γ-IFN). We assessed the capacity of γ-IFN to induce the release of granulocyte-monocyte stimulating factors (CSF-GM) from highly purified monocyte preparations by colony assays and Northern blot analysis using a CSF-GM cDNA probe. Whereas the secretion of CSF-GM by monocytes is negligible in the absence of T cells, it is induced by regulation at the transcriptional level by concentrations of γ-IFN as low as 10 U/ml. This effect could be abrogated by a specific neutralizing monoclonal antibody to γ-IFN, and was restricted to monocytes, as resting T-cells failed to secrete detectable CSF-GM in response to γ-IFN. The response of monocyte preparations to γ-IFN was biphasic, in that concentrations greater than 250 U/ml did not induce detectable CSF-GM activity. However, this was shown to be due to the release of a humoral inhibitor of G/M-progenitor cells, which was identified as tumor necrosis factor-a by monoclonal antibody neutralization.

274 Interleukin 1 (IL 1) Regulates Colony-Stimulating Activity (CSA) Production by Human T-Lymphocytes In Vitro

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The biologically relevant granulopoietic stimulator CSA is derived from a variety of cell types, including monocytes/macrophages, T-lymphocytes, vascular endothelial cells, and fibroblasts. Its production is integrated into a network of interacting soluble messenger molecules and is subject to positive and negative regulatory control. Previous work on the mechanisms of CSA production in steady-state granulopoiesis documented a fundamental role played by monocytes/macrophages, by producing not CSA, but other soluble factors which stimulate cells to produce CSA (Bagby et al., J Clin Invest 68: 56). We now show that the monokine CSA may be identical to IL 1. Non-preactivated (albeit IL 1-binding) T-cells failed to secrete detectable levels of CSA when colony assays and proliferation assays employing purified myeloid progenitor cells were used as a detection system, but T-cells could be induced to secrete CSA if highly purified IL 1 at concentrations as low as 0.5 U/ml was present for 3–5 days during the conditioning of the T-cell medium. Preliminary bioassays lead to the identification of this CSA as GM-CSF, but further work is in progress to characterize the quality of IL 1-induced T-cell CSA on the gene level, using cDNA probes from different CSF-species and Northern blot analysis.

275 Humoral Regulation of Normal Bone Marrow Uni-, Bi-, and Multipotential Progenitor Cell-Derived Colony Formation by Cloned Human NK Cells

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The involvement of NK cells in the regulation of hematopoiesis has been suggested by both in vivo studies of murine bone marrow transplantation and in vitro murine and human colony assays. In order to investigate possible heterogeneity in the recognition and modulation of the proliferation and differentiation of marrow progenitors by NK cells, we studied the effects of a series of cloned human NK cell lines for their effects on the colony growth of early and late CFU-GM, CFU-E, BFU-E, and CFU-GEMM. Normal bone marrow was purified 50-fold to minimize the effects of accessory cells. Eight clones with different phenotypes and target specificities were tested for activities that promoted or inhibited colony growth. None of the NK clones induced or enhanced growth of any of the progenitor cells, while all the clones suppressed growth in subpopulations of progenitor cells in a heterogenous, but clonally stable manner. The generation of this inhibitory activity was not constitutively expressed, and required NK cell contact with hematopoietic cells for 8–18 h. The inhibitory effect of NK clones was at least partially mediated by humoral factors, as similar colony suppression was seen when testing cell-free supernatants generated by NK clones activated upon contact with progenitor populations. The inhibitory effects upon hematopoietic colonies could be separated from the previously described NK cytotoxic γ-factors, and could be partially neutralized by monoclonal antibody to γ-interferon. These results suggest that some of the inhibitory effects produced by NK cells on colony-forming cells are mediated by γ-interferon.

276 Bone Marrow Transplantation and DNA

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During the past several years, the molecular biologists of the Beckman Research Intitute at the City of Hope and the clinical investigators of the City of Hope Bone Marrow Transplant Team have collaborated to develop and employ methods based on DNA technology for diagnostic purposes and possible therapy in various areas of clinical bone marrow transplantation (BMT). First, DNA probes for class II antigens (HLA-DR region) have been utilized to determine whether genotypically histocompatible sibling pairs as determined by standard serologic typing are also identical at the molecular level. Our data indicate that typing with DNA-blotting methods can detect differences which cannot be identified by serologic techniques. The relationship between the DNA disparities and the occurrence of graft-versus-host disease is being investigated. Second, the most abundant and highly immunoreactive matrix protein of human cytomegalovirus (HCMV), a glycoprotein with a molecular weight of 64 000 daltons (gp 64) has been purified to homogeneity and microsequenced. The gene for gp 64 has been cloned. With the pure antigen, it is possible to detect viral DNA by in situ cytohybridization and/or dot-blot analysis in leukocytes and lung specimens obtained from patients with HCMV infection. This protein may also be useful to immunize BMT-donors by taking advantage of the adoptive transfer mechanisms of immunity. Third, the restriction fragment length polymorphism of DNA facilitates the detection of even minimal amounts of donor- or host-type stem-cell-derived cells after BMT. Using sensitive genetic markers, it has been established that 12%–24% of BMT recipients (depending upon the preparative regimen used) carry hemopoietic and/or lymphopoietic cells derived from both the donor and the recipient (mixed chimeras). Mixed chimeras seem to have distinct survival advantages compared to full chimeras. Detailed data will be presented.

277 Perimembranous Glomerulonephritis and Cholestasis Following Allogeneic Bone Marrow Transplantation

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A 20-year-old man with severe aplastic anemia received a bone marrow transplant from an HLA-identical sibling. He was given cyclosporin A as GVH prophylaxis. Four weeks after BMT, a trilinear take was documented. Slight proteinuria occurred 7 months after transplantation. After a further 3 months, the patient developed an overt nephrotic syndrome with proteinuria of up to 30 g/24 h, and cholestasis. Antibodies directed against nuclei were detected in the patient's serum. Renal biopsy showed a perimembranous glomerulonephritis with immunohistologically identified granular subepithelial deposits, and interstitial infiltrates of mature activated T-lymphocytes and monocytes. Furthermore, a segmental increase in HLA-class II antigen expression was observed in tubular and glomerular cells. The liver biopsy demonstrated nonspecific signs of inflammation. Cyclosporin A was withdrawn. Three cycles of alternating corticosteroids and chlorambucil were administered. The cholestasis and proteinuria improved, and the patient is now alive 1 year after this episode. Immune-complex-mediated disease and GVH disease will be discussed as possible causes of the perimembranous glomerulonephritis.

278 The Influence of "Comorbidity" on Survival of Patients Aged Above 70 Years with Hematologic Malignancies

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For some time we have been preoccupied with the effects of "comorbidity" on the prognosis, therapeutic results, and survival of patients with hematological malignancies. In this retrospective study, we examained these effects in 83 patients above 60 years with hematologic malignancies. The number of patients in some of the disease groups studied being rather small, the statistical analysis has not been conclusive in all cases. The only exact statistical result we ascertained is that in patients over 70 years of age comorbidity and survival are well correlated. Indeed, in patients without comorbidity, survival was significantly longer than in those with comorbidity.