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OVERVIEW OF THE HEMATOPOIETIC HORMONES AND THEIR ROLE IN HOST DEFENSE AND CANCER. D.W. Golde

Hematopoiesis is the process by which mature blood cells are produced as a result of proliferation, differentiation, and maturation of precursor cells. Initial steps in dissecting the regulatory influences on the process of hematopoiesis have involved the characterization of humoral modulators able to stimulate in vitro colony formation by myeloid progenitors (colony-stimulating factors, CSFs). A family of CSFs has been molecularly characterized and produced in quantity by recombinant DNA technology. The CSFs are potent stimulators of the production of mature neutrophils, monocytes, eosinophils and, in some cases, platelets and red cell precursors. The CSFs also have important direct actions on mature blood cells. Thus, G- and GM-CSF prime neutrophils for enhanced function with regard to oxidative metabolism, phagocytosis, and cytotoxicity. Similarly, IL-3, GM- and M-CSF have direct stimulatory actions on macrophages. The hematopoietic growth factors act through surface receptors on target cells which are of two large families: Those with and those without intrinsic kinase domains. the receptors define which cells will respond and their distribution suggests important actions of the hematopoietic hormones on nonhematopoietic tissues. The structure and signal transduction mechanisms of GM-CSF receptor will be discussed as a model of CSF action.

Since the blood cells are primarily concerned with host defense, the introduction of the CSFs as therapeutic agents offers the opportunity to develop unique therapeutic strategies designed to enhance overall host defense, particularly with relevance to cancer and AIDS. Administration of CSFs is associated with profound changes in cellular function, and treatment strategies will need to consider the potential deleterious effects of heightened host cell activity and potential effects on nonhematopoietic cells.

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ARE GROWTH FACTORS OBLIGATORY FOR DIFFERENTIATION?

T.M. Dexter

The survival and proliferation of haemopoietic stem cells, induced by growth factors, occurs concomitantly with differentiation and development of the stem cells and their progeny, into mature blood cells. Are the growth factors simply permissive for these processes? Or do they have an inductive role to play in lineage commitment of stem cells and subsequent maturation into phenotypically mature cells? From various experiments, it is clear that the outcome of the response (that is the types of mature cells produced) is a reflection of the range of growth factors to which the cells are exposed - suggesting that combinations of growth factors may well influence the choice of lineage options taken by multipotent cells. The problem, of course, is that in all systems studied to date, no detailed examination has been made of cell death, and that even in growth factor combinations where, for example, no erythropoiesis is found, it is possible that erythroid progenitors are being produced as a consequence of differentiation of the multipotent cells but that these cells are then dying due to lack of availability of the appropriate survival (growth factor) stimulus. We have recently been able to circumvent some of these problems and have shown that, provided the cells receive a survival stimulus, differentiation can occur in the absence of added growth factors and also that proliferation it not a prerequisite for acquisition of a mature cell phenotype. In other words, the growth factors may act primarily as survival and mitogenic stimuli and not as "inducers" of differentiation.

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CYTOKINE REGULATION OF LYMPHOHEMOPOIETIC PROGENITORS IN CULTURE M. Ogawa and F. Hirayama

The majority of hemopoietic stem cells in the steady state marrow are dormant in the cell cycle. Using serial observation (mapping) of blast cell colony formation from bone marrow cells of mice that have been treated with a high dose 5-fluorouracil (5-FU), we have identified a number of cytokines that appear to control the cell cycling of the primitive hemopoietic progenitors. The early-acting cytokines may be divided into three groups. The first group would consist of IL-3, GM-CSF, and IL-4. The second group consists of IL-6, G-CSF, IL-11, IL-12, and LIF/DIA. The third group consists of steel factor (SF). According to our studies in culture, cytokines in each group can interact with those in other groups to initiate cell division in the cell cycle dormant primitive progenitors.

While studies using retrovirally-labeled murine stem cells demonstrated unequivocally the presence of lymphohemopoietic progenitors that are capable of producing both lymphoid and myeloid progenies, it has not been possible to identify and quantitate these progenitors in culture. Recently, we have developed a two-step methylcellulose culture method to quantitate murine lymphohemopoietic progenitors that are capable of producing myeloid cells and and pre-B-cells. After establishing the primary culture system initially with medium conditioned by pokeweed mitogen stimulated spleen cells, we characterized combinations of cytokines that are able to maintain the B-lymphoid potentials of the primary colonies. We observed that two-factor combinations including SF such as SF plus IL-6, SF plus G-CSF, SF plus IL-11, SF plus IL-12 were effective in maintaining the proliferation of B-cell progenitors. Somewhat less effectively IL-4-based combinations such as IL-4 plus IL-6 and IL-4 plus IL-11 also supported the B-cell potentials of the the primary colonies. Interestingly, IL-3-based combinations were unable to maintain the B-cell potentials of the primary colonies even though the cells in myeloid lineages proliferated strongly. We also found that addition of IL-3 to an effective two-factor combination such as SF plus IL-11 inhibit the B-cell potentials of the primary colonies. Our cell culture for the murine lymphohemopoietic progenitors may provide an important tool for studying the mechanisms regulating the early process of lymphopoiesis.

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THE p210BCR/ABL ONCOGENE SHARES SOME SIGNAL TRANSDUCTION PATHWAYS WITH THE IL-3/GM-CSF RECEPTOR IN MYELOID CELL LINES. J.D. Griffin, Nadia Carlesso, Ursula Matulonis, Matthias Eder, Timothy Ernst, Brian Druker. Dana-Farber Cancer Institute, Boston, MA.

Expression of p210BCR/ABL by transfection converts interleukin-3 (IL-3)-dependent cell lines to factor independence and transforms immature hematopoietic cells in vitro. We tested the hypothesis that p210 BCR/ABL may induce factor-independence by constitutively activating signal transduction pathways which are normally regulated by IL-3/GM-CSF. In both the IL-3-dependent murine myeloid cell line, 32Dcl3, and the IL-3/GM-CSF-dependent human line, MO7e, p210 BCR/ABL induces rapid factor-independence despite continuous growth in IL-3-containing medium. One- and two-dimensional antiphosphotyrosine immunoblotting showed that most proteins tyrosine phosphorylated by p210BCR/ABL are different that those phosphorylated in response to IL-3. Several signaling molecules have been found to be activated or phosphorylated by both IL-3/GM-CSF and p210BCR/ABL, including Raf-1, MAP kinase, SHC, vav, and probably PI3K. Other signal transducing proteins were found to be phosphorylated only by p210BCR/ABL (p120rasGAP, two rasGAP associated proteins, and c-fes), or only by IL-3 (p21ras). In order to better define the biochemical activities of p210BCR/ABL which lead to mitogenesis, a series of cell lines were constructed in which the functional expression of p210BCR/ABL was inducible. The uninduced cell lines had a wild-type phenotype while the induced cell lines displayed markedly reduced apoptosis in the absence of growth factor, and some were hyper responsive to growth factors. The phenotypes of these cell lines have been stable in culture, and the lines should be useful to define biochemical activities of p210bcr/abl which are important for mitogenesis.

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THE *IN VIVO* ADMINISTRATION OF RECOMBINANT METHIONYL HUMAN STEM CELL FACTOR (SCF) EXPANDS THE NUMBER OF HUMAN MARROW HEMATOPOIETIC STEM CELLS (HSC). R. Hoffman, J. Tong, M.S. Gordon, E.F. Srour, R.J. Cooper, I. McNiece.

The effects of SCF on HSC were tested in a phase I SCF trial in 8 breast cancer patients. SCF was administered for 14 days by daily subcutaneous injection at dosages of 10, 25, or 50 μg/kg/day. $CD34^+$ $HLA\text{-}DR^-$ $CD15^-$ cells, previously shown by our laboratory to be enriched for various classes of primitive hematopoietic progenitor cells and to have *in vivo* marrow populating ability in an *in utero* sheep transplantation model, were quantitated in BM samples on day 0 (pre-treatment) and day 15 (post-treatment). Isolated $CD34^+$ $HLA\text{-}DR^-$ $CD15^-$ cells were assayed for several classes of primitive hematopoietic progenitor cells including the high proliferative potential-colony forming cell (HPP-CFC), the burst forming unit-megakaryocyte (BFU-MK), and the long-term bone marrow culture-initiating cell (LTBMC-IC). SCF administration resulted in a 2.4-fold (1.1-29.3-fold, $p<0.01$) increase in $CD34^+$ $HLA\text{-}DR^-$ $CD15^-$ cells, an 8.8-fold (2.1-12.4-fold, $p<0.01$) increase in HPP-CFC and a 2.3-fold (1.6-2.8-fold, $p<0.03$) increase in BFU-MK. LTBMCs initiated with $CD34^+$ $HLA\text{-}DR^-$ $CD15^-$ cells from post-treatment marrow had greater longevity (11.5 vs. 9.6 weeks), and produced a 1.8-fold (1.6-2.9-fold, $p<0.04$) increase in the numbers of total cells and a 2.8-fold (2.1-18.4-fold, $p<0.04$) increase in the number of progenitor cells. We conclude that SCF administration results in a significant expansion in numbers and proliferative capacity of human HSC. These studies suggest that *in vivo* SCF administration may be useful for improving the quality of bone marrow grafts to be used for transplantation.

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STK-1: THE HUMAN HOMOLOG OF FLK-2/FLT3 IS EXPRESSED IN THE STEM CELL ENRICHED FRACTION OF BONE MARROW AND MAY BE INVOLVED IN STEM CELL PROLIFERATION

D. Small, M. Levenstein, E. Kim, C. Carow, S. Amin, J. Garg, C. Civin, A. Gewirtz, P. Rockwell, L. Witte

We have cloned the cDNA for STK-1 (**S**tem cell **T**yrosine **K**inase **1**), a human growth factor receptor tyrosine kinase, and investigated its expression in bone marrow, leukemias, and leukemic derived cell lines. STK-1 expression is restricted to the CD34+ fraction of normal human bone marrow, the fraction containing all of the hematopoietic stem cell activity of marrow. Experiments in which CD34+ cells grown on irradiated bone marrow stromal feeder layers were exposed to STK-1 antisense oligonucleotides resulted in inhibition of colony formation. STK-1 is also expressed in most cases of AML, B lineage ALL, and T cell ALL. A number of hematopoietic tissue culture cell lines which express STK-1 have been identified, including KMT2, KG1a, KG1, ML-1, HL-60, Nalm-16, and REH. ML-1 cells stop growing and differentiate after exposure to phorbol esters and other agents. STK-1 expression is completely shut off by this treatment. Anti-peptide antibody generated against several regions of STK-1 identifies a doublet of proteins of 155kD and 130kD, probably corresponding to different degrees of glycosylation, in several of the cell lines.

These data imply a possible role for the STK-1 receptor in the normal proliferation of hematopoietic stem cells and the abnormal proliferation of leukemic cells. Further antisense experiments and the isolation of the growth factor for this receptor will be necessary to fully understand its role in hematopoiesis and leukemia.

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STEM CELL PROLIFERATION FACTOR (SCPF) IS A PROLIFERATIVE GROWTH FACTOR CAPABLE OF SUPPORTING CD34+ CELLS IN BOTH SHORT- AND LONG-TERM CULTURE

R.E. Donahue, M.R. Kirby, P.D. Lawman, S.E. Sellers, S.W. Kessler, and M.J.P. Lawman

A novel factor has been purified to homogeneity from a cell line derived from a human mixed germ cell tumor. By Western blot analysis, using a polyclonal rabbit antibody raised to the purified native protein, SCPF was found to be expressed both as a 32kD secreted and as a 37kD membrane-bound protein. To further evaluate SCPFs' ability to support CD34+ cell growth in culture, SCPF was used in short- and long-term cultures using immunoselected CD34+ cells. For short-term culture studies, CD34+ cells were evaluated prior to and subsequent to a six day exposure to either media alone or media supplemented with IL-3, SCPF, or IL-3+SCPF. The greatest expansion of CD34+ cells was in those expressing CD38. Compared to pre-culture, cultures maintained in SCPF, IL-3, or IL-3+SCPF had, respectively, a 0.7, 2.9, or 0.7-fold increase in CD34+CD38+ numbers. There was also a consistent increase in the ratio of large CD34+38+ cells to small CD34+CD38+ cells. Presumptively, this change represents an increase in the number of cells in either the G2/M or S phase of the cell cycle. Of the cytokine combinations evaluated, only the combination of IL-3+SCPF led to a 1.8-fold expansion of CD34+CD38- Cells above baseline values. By themselves, SCPF and IL-3 led to a 0.4 and 0.7-fold reduction in CD34+CD38- numbers. When compared, however, to the number of CD34+CD38- cells present in media alone after the 6 days in culture, SCPF, IL-3, and IL-3+SCPF hat respectively a 3.0, 5.7, and 12.3-fold greater number of CD34+CD38- cells. In long-term culture assays in the absence of SCPF, the cultures deteriorated rapidly and were lost by day 18. in the presence of SCPF, cell numbers were maintained over the initial 8-14 days in culture, with proliferation becoming evident 16 days post-culture. At day 21, some of the cells were removed from culture media containing SCPF and replated in culture media alone. After an additional 30 days in culture the cells that were no longer exposed to the SCPF had differentiated. Interestingly, the cells that were cultured in SCPF continued to proliferate. After 50 days in culture, these cells were predominantly CD34+, CD33+, CD38+, CD45+, CD71+, and HLA-Dr+, and failed to express Thy 1, CD4, CD8, CD14, CD20, or CD56. Karyotypic analysis demonstrated that these cells hat multiple chromosomal aberrations.

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DOES STEM CELL EXHAUSTION RESULT FROM COMBINING HEMATOPOIETIC GROWTH FACTORS WITH INTENSIFIED CHEMOTHERAPY? IF SO, HOW DO WE PREVENT IT?

M.A.S. Moore

Murine long term repopulation and double transplantation assays have clearly demonstrated irreversible damage to early stem cells by repeated doses of alkylating agents or anti-metabolites. The ability to protect from the short term effects of chemotherapy-induced myelosuppression by administration of hematopoietic growth factors has obscured the potential problem of long term stem cell insufficiency. Indeed in murine models involving repeated cyclophosphamide administration, CSF administration has been reported to protentiate stem cell damage. The development of techniques for isolation of human hematopoietic stem cells in a $CD34^+$ Lin^{-ve} fraction of marrow and blood, together with long term culture-initiating assays on marrow stroma or long term ex vivo expansion with cytokine combinations, permits quantitative analysis of human stem cell proliferation potential. It is becoming apparent that extensive chemotherapy treatment gravely compromises the population of primitive hematopoietic stem cells as reflected in their impaired capacity to peripheralize and to be represented in the blood $CD34^+$ population following CSF treatment with or without cytoxan. Five strategies are currently under evaluation: 1) Upfront harvesting of marrow and/or elicited peripheral blood prior to onset of chemotherapy with subsequent "rescue" following chemotherapy; 2) Fine tuning of cytokine and chemotherapy administration to take advantage of "rebound quiescence" of stem cells; 3) Administration of negative regulators to suppress stem cell proliferation. Transforming growth factor ß, macrophage inflammatory protein α and tumor necrosis factor have all proved protective in preclinical models. 4) Utilize cytokines, eg IL-1, that protect stem cells by increasing drug enzymatic inactivation, decreasing drug influx, and/or increasing drug efflux, and inducing DNA repair or decreasing DNA damage. 5) Utilize gene therapy to introduce into hematopoietic stem cells drug resistance genes such as mutated dihidrofolate reductase that confers methotrexate resistance or enhance the expression of the multi-drug resisting gene (MDR) expression.

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NF-JUN/C-JUN: ROLE IN REGULATION OF PROLIFERATION OF NORMAL AND MALIGNANT MYELOPOIESIS. F. Herrmann and M.A. Brach

An optimal proliferative response is achievable in both normal CD34+ hematopoietic progenitor cells and blast cells of patients with acute myelogenous leukemia (AML) by combinational treatment with Interleukin (IL)-3 and tumor necrosis factor (TNF)-α. Analysis of the molecular mechanisms mediating this synergism revealed that TNF-α, but not IL-3, enhanced binding activity of the c-jun/AP-1 transcription factor. In order to further elucidate the importance of c-jun/AP-1 for the capacity of TNF-α to synergize with IL-3, the antisense-technique was employed. Treatment of AML blasts with an antisense oligomer directed against the translation initiation site of c-jun, but not with the corresponding sense or an unrelated nonsense oligonucleotide resulted in intracellular RNA/oligomer duplex formation followed by efficient inhibition of c-jun/AP-1 protein synthesis. Elimination of c-jun/AP-1 by antisense oligomers relieved TNF-α/IL-3-mediated synergism, while IL-3-induced growth stimulation remained unaffected. Molecular analysis of the mechanisms governing TNF-α-induced c-jun/AP-1 binding revealed that TNF-α induced a signalling cascade leading to posttranslational modification of pre-existing c-jun/AP-1 protein and thereby allowed binding of c-jun/AP-1 to its recognition site, while IL-3 did not. Moreover, TNF-α transcriptionally activated the c-jun gene by enhancing binding activity of the NF-jun transcription factor which recognizes a palindromic sequence within the c-jun promoter located immediately 5' of the SP-1 binding site. Activation of NF-jun and thus expression of c-jun/AP-1 is a prerequisite for TNF-mediated growth-stimulation of IL-3 treated hematopoietic progenitor cells in that deletion of the NF-jun recognition sequence abolished TNF-mediated ativation of a reporter gene linked to the c-jun promoter. Moreover, accumulation of c-jun mRNA was achievable in TNF-stimulated progenitor cells but not in cultures that had received IL-3 only. In contrast, more mature myelopoietic cells - though responding to TNF with functional activation - failed to synthesize DNA on exposure to TNF and also did not exhibit NF-jun binding activity.

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PRECLINICAL BIOLOGY OF HUMAN IL-10 T.L. Nagabhushan, S.K. Narula and M.I. Siegel

Human IL-10 (huIL-10) is a 160 amino acid polypeptide synthesized by a number of different cell types. It is a pleiotropic factor with both immunosuppressive and immunostimulatory activity. Recombinant huIL-10 expressed in CHO cells is not glycosylated, and when expressed in E. coli the protein retains the biological activity of the CHO-derived product. In the presence of antigen presenting cells, huIL-10 inhibits cytokine synthesis in T cells. HuIL-10 downregulates γ-IFN and IL-4 induced MHC Class II antigen expression on monocytes-macrophages. In contrast, it has no effect on Class II expression on purified tonsillar and peripheral B cells. HuIL-10 also inhibits the synthesis of IL-1α, IL-1β, IL-6, IL-8, TNF-α, GM-CSF and G-CSF at the protein and RNA levels in monocytes activated by LPS or LPS and γ-IFN. The γ-IFN or GM-CSF induced phagocytosis of opsonized yeast particles by human peripheral blood-derived macrophages and granulocytes is downregulated by huIL-10. IL-4 induced IgE synthesis by PBMNC is inhibited by huIL-10. The protein also potentiates a strong LAK activity in human PBMNC. These results will be discussed along with some early in vivo biology in rodents.

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Interleukin-11: A novel hematopoietic cytokine possessing multiple biological activities *in vitro* and *in vivo*. J.P. Leonard and S.J. Goldman, Genetics Institute, Cambridge, MA.

Interleukin-11 is a multifunctional hematopoietic cytokine which was originally identified in the conditioned medium from an IL-1 stimulated primate stromal cell line. The human cDNA was subsequently cloned from a fetal fibroblast cell line enabling the expression and purification of the human protein. The *in vitro* biological activities of rhIL-11 result predominantly from synergistic interactions with other growth factors. In combination with other cytokines, rhIL-11 has been shown to support the formation of primitive human blast cell colonies from bone marrow, promote erythroid burst formation and stimulate both early and late stages of megakaryocyte proliferation and differentiation. In addition, rhIL-11 alone directly increased the size and ploidy of enriched megakaryocytes. Although rhIL-11 has no inherent B cell growth factor activity, rhIL-11 has been shown to stimulate immunoglobulin producing B cells both *in vitro* and *in vivo*.

rhIL-11 is biologically active in mice, rats, dogs and primates when administered as a single agent *in vivo*. The predominant effect of rhIL-11 in naive animals was on cells of the megakaryocyte lineage, increasing the number of bone marrow megakaryocyte progenitors, stimulating megakaryocyte endoreplication and increasing peripheral platelet counts in a dose dependent fashion. In a variety of murine models of myelosuppression, the effects of rhIL-11 were multilineage, stimulating the recovery of megakaryocyte, erythroid, and granulocyte and macrophage progenitors in the bone marrow. rhIL-11 administration reduced the platelet and hematocrit nadirs and the overall duration and severity of thrombocytopenia and anemia in these models. In a murine bone marrow transplant model, rhIL-11 also accelerated neutrophil recovery. The results from ongoing preclinical studies continue to confirm the broad spectrum of biological activities possessed by rhIL-11 *in vitro* and suggest this cytokine may be an effective agent in the treatment of myelosuppression associated with cancer chemotherapy and bone marrow transplantation.

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EFFECTS OF SMALL SYNTHETIC PEPTIDES ON HEMATOPOIESIS IN VITRO AND IN VIVO. D. Løvhaug, W.M. Olsen, O.P. Veiby, S. Bergseth, A. Høgset, H.I. Tøn, and P. Bhatnagar*.

A monomeric pentapeptide (pEEDCK) inhibits murine hematopoiesis in vitro and in vivo while its dimer counterpart $(pEEDCK)_2$ formed through a disulphide bridge has a stimulatory effect in the same systems. Stable peptides of the dimer have been made by substitution of the disulphide bridge with a dimethylene bridge. In vitro the dimer seems to have no direct effect on GM-CFC or on purified Lin-Sca1+ cells in the HPP-CFC assay (400 cells per culture, 15-20% PE). The monomer does not affect GM-CFCs, but inhibits approximately 50% of the purified HPP-CFCs. The dimer stimulates human or mouse stromal cells to produce M-CSF and possibly other cytokines which augment colony formation in vitro and also activate human PBL as measured by an increased expression of CD11b. In ex vivo experiments it was found that the dimer increases and the monomer decreases cell cycle rate of CFU-GM and CFU-S in the bone marrow. Intraperitoneal injection of the dimer (1 to 100 ng/kg) into mice, led to increased progenitor cell number in the bone marrow and also increased survival of mice given a lethal dose of cyclophosphamide (550 mg/kg). At present receptors of the peptides have not been identified. These studies have shown that the dimer has an indirect effect on hematopoiesis as a stimulator of cytokine production, while the monomer seems to act both as an antagonist of dimer action and is also able to directly inhibit early myeloid progenitor cells. The possibility that these two compounds have therapeutic efficacy in diseases involving a myelosuppressed bone marrow is indicated.

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BASIC FIBROBLAST GROWTH FACTOR IN NORMAL AND MALIGNANT HEMATOPOIESIS

J.L. Gabrilove and E.L. Wilson

There is evidence that basic fibroblast growth factor (bFGF) plays a role in the regulation of normal blood cell proliferation and differentiation. Basic FGF is produced by and is a potent mitogen for human stromal cells. It is found in megakaryocytes and cells of the granulocyte lineage, in vivo, and it enhances megakaryopoiesis and myelopoiesis in human long term bone marrow cultures. It stimulates progenitor cell growth and augments the proliferation of progenitor cells when added in conjunction with other hematopoietic growth factors. In addition, it counteracts the suppressive effects of transforming growth factor beta. Basic FGF also synergizes with stem cell growth factor to augment granulocyte macrophage colony stimulating factor stimulated progenitor cell growth. Based on the observations in normal hematopoiesis, the role of bFGF in malignant hematopoiesis is presently an area of active investigation.

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NATURAL KILLER CELL STIMULATORY FACTOR/INTERLEUKIN-12 (NKSF/IL-12): THE MISSING LINK BETWEEN NATURAL RESISTANCE AND ADAPTIVE IMMUNITY G. Trinchieri

NKSF/IL-12 is a heterodimeric cytokine produced by monocyte-macrophages, B cells and other accessory cells in response to various stimuli including bacteria and bacterial products. NKSF/IL-12 acts on T cells and NK cells inducing cytokine production, proliferation, and enhancement of cytotoxic activity. NKSF/IL-12 is a particularly efficient inducer of IFN-γ production, acting alone or in synergy with other IFN-γ inducers such as IL-2, antigens, anti-CD3 or anti-CD16 antibodies, mitogens, and phorbol diesters. NKSF/IL-12 appears to play a major role in regulation of natural resistance: when produced by monocyte-macrophages, it directly activates the cytotoxic activity of NK cells and induces both NK and T cells to produce IFN-γ and other cytokines with important effects on activation of phagocytic cells. The important role of NKSF/IL-12 in response to bacterial products is clearly demonstrated by the ability of anti-NKSF/IL-12 antibodies to inhibit *in vivo* in mice the IFN-γ production induced by LPS in a toxic shock model. NKSF/IL-12 is also an important factor in the regulation of adaptive immune response, by inducing the differentiation and growth of T helper cells type 1 (Th-1) and by preventing the differentiation of IL-4 producing Th-2 cells. A possibly obligatory role of NKSF/IL-12 for Th-1 cell differentiation can be demonstrated *in vitro* in the human lymphocyte response to allergens or bacteria-derived antigens and both *in vitro* and *in vivo* in the murine system, e.g. in the immune response to Leishmania infection. Production of NKSF/IL-12 by accessory cells is stimulated by IFN-γ, a product of Th-1 cells and suppressed by IL-10 or IL-4, products of Th-2 cells. These results suggest that NKSF/IL-12 represents an important link between natural resistance and adaptive immunity and is at the center of a cytokine network that regulates the equilibrium between cellular and humoral immunity.

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PHASE I TRIAL OF RECOMBINANT METHIONYL HUMAN STEM CELL FACTOR (SCF) IN WOMEN WITH LOCALLY ADVANCED OR METASTATIC BREAST CANCER TREATED WITH CYCLOPHOSPHAMIDE (C) AND DOXORUBICIN (A). M.S. Gordon, J. Costa, G. Sledge, D Hayes, S Galli, R Hoffman, E Merica, W Rich, B Harkins, B McGuire, and G Demetri.

SCF is one of the earliest-acting hematopoietic growth factors. Pre-clinical studies have demonstrated its multi-lineage hematopoietic effects. We have conducted a phase I trial of SCF and report now on the first 21 patients (pts) with stage IIIB (n=7) or IV (n=14) breast cancer. The study was designed to evaluate the tolerability and biologic effects of SCF. Pts received SCF pre-chemo (cycle 0) and following cycles 2-6 of C/A chemotherapy. Cohorts of 5 pts were randomized in a 4:1 ratio to receive either SCF (n=17) by subcutaneous injection at dosages of 10, 25, and 50 μg/kg/day for 14 days or no SCF as a parallel C/A control group (n=4). Various physiologic, biochemical, pharmacokinetic, and hematologic parameters were studied. Bone marrow (BM) and peripheral blood (PB) progenitors were assayed. SCF administration was associated with PB progenitor mobilization in cycle 0 at all dose levels. Absolute neutrophil counts demonstrated modest dose-related increases over baseline ranging from median values of 20% (10 μg/kg/d) to 120% (50 μg/kg/d). No reproducible effects were seen on red blood cells or platelets. The principal adverse events were dermatologic in nature. They included mild to moderate reactions at the injection site in all pts, and moderate to severe reactions distant to the site in 10 pts (primarily at higher doses). At 50 μg/kg/d, 4/10 pts experienced dose-limiting respiratory symptoms including cough, hoarseness, and laryngospasm. Because of SCFs known mast cell effects, prophylaxis with H1/H2 blockers and bronchodilators is being evaluated. No pts developed antibodies to SCF. Evaluation of effects following chemotherapy is ongoing. We conclude that SCF is an active hematopoietin capable of stimulating production of BM and PB progenitor cells as well as peripheral neutrophils.

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BIOLOGIC THERAPY OF CML: CLINICAL AND LABORATORY UPDATE FROM M.D. ANDERSON CANCER CENTER STUDIES

M. Talpaz, R. Kurzrock, Z. Estrov, M. Wetzler and H. Kantarjian.

Work with alpha-interferon in CML started at MDACC in 1981 and has evolved over the years to allow us interpretation of response rate in a large number of patients with relatively long follow up. Newly diagnosed patients (<1 year from diagnosis) were treated with a starting dose of 5 MU/m^2 of interferon. Of 313 such patients followed in 6 different studies, we noted a 77% complete hematologic remission; 56% cytogenetic response, and 35% complete and partial cytogenetic response (<35% Ph^1). In long-term analysis of 190 patients, 101 achieved cytogenetic response (53%) of which 49 (26%) were complete; 14 (7%) were partial, and 38 (20%) were minor 56 (55%) of the cytogenetic responses are durable. Of major interest is the fact that 92% of the complete cytogenetic remissions are durable (45 of 49) but only a minority of the partial and minor cytogenetic responses are durable (36% and 16%, respectively). This clearly set our goal on improving the incidence of complete cytogenetic remission. Median survival ranges between 60-65 months. However, of particular interest is the excellent survival rate of the complete cytogenetic responders (46 of 49 alive at time of follow-up). Current studies are focusing on idealizing combination therapies and will be discussed. CML is a paradigm of tumor progression; therefore, we were looking for characteristics associated with disease progression and identified evidence of autocrine growth factor loop formation with disease progression and the overproduction of IL-1ß. Finally, we are also focusing on the study of minimal residual disease in patients with complete cytogenetic remissions and have identified residual Ph^1 progenitor cells in the majority of these patients, including patients with unmaintained remission. The nature of such a remission will be discussed as well.

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THE MXA-PROTEIN: A NEW AND EXCELLENT MARKER FOR ENDOGENOUSLY PRODUCED TYPE-I-IFN

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The human MxA-protein is a recently identified human IFN-induced protein, which is synthezised by peripheral blood cells specifically and dose-dependently upon stimulation with type-I-interferons both in vitro and in vivo. Mononuclear cells (MNC) both from healthy persons and cancer patients (n=55) are usually (in the absence of a virus infection) Mx-negative. Patients starting an IFN-α-therapy become MxA-positive (N=96) within 24 hours and remain positive throughout their therapy. Furthermore, two groups of patients are known to have frequently measurable serum IFN-titer: SLE-patients and AIDS-patients (N=76) studied expressed significant MxA-levels in their MNC. While all patients with measurable IFN-titer had MxA-levels above 1 U/20.000 MNC, also the vast majority of serum IFN-negative patients possessed detectable MxA-levels indicating that also these patients produce endogenous IFN, although due to its rapid clearence from the circulation it may not be measurable in serum.
In addition, 55 patients with acute hepatitis were investigated for the MxA-protein. Surprisingly large difference in the endogenous IFN-production were observed. Only in the first two weeks after the onset of clinical symptoms hepatitis A patients (N=21) had both high MXA-protein and 2-5-A-Synthetases levels, but not thereafter. The levels of both IFN-induced activities reached concentrations found in cancer patients treated with 3-5- Mill. IU rIFN-α per dose. In acute hepatitis B and C (N=20;N=14) only marginally elevated MxA and 2-5-AS levels were found. In summary, the MxA-protein is a new and excellent marker for endogenously produced type-I-IFNs.

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100 ISOLATED LIMB PERFUSIONS WITH TNFα,gamma-IFN AND MELPHALAN FOR STAGE III MELANOMA OR IRRESECTABLE SOFT TISSUE SARCOMAS OF THE LIMBS

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100 Patients with melanoma in transit metastases (stage III) or irresectable soft tissue sarcomas of the limb have been treated in multicenter studies in the institutions listed above.
Material and Methods: 70 Patients (pts) with stage III a/ab melanoma and 30 pts with irresectable primary or recurrent soft tissue sarcomas of the leg (25) or arm (5) have been treated sofar. Patients were pretreated with 0.2 mg gamma-IFN s.c. on the 2 days prior to the ILP. The ILP consisted of a 1 1/2 hour long perfusion with 0.2 mg IFN, 4 mg TNF (leg) or 3 mg TNF (arm), and 10-13 mg/L limb volume of Melphalan at mild hyperthermia (39-40 °C). There is extensive haemodynamic and cardiopulmonary monitoring (peri)operatively.
Results: 63/70 Melanoma pts had a CR (90%), 7 pts a PR (10%). Median duration of response 14++ months. In the sarcoma pts the limb salvage rate has been very high: 27/30 (90%). In 14 pts a CR was obtained and in 16 pts a PR. In most pts the tumor became resectable and was subsequently resected. Toxicity has been severe (Shock, ARDS, renal failure) in some pts with leakage > 10 % with 2 treatment related deaths. In most patients (leakage 1-2%) only fever with chills and some mild hypotension occurred. Local toxicity is the same as after ILP with Melphalan alone.
Conclusion: Isolated limb perfusion with high dose TNF, IFN and Melphalan is very effective in achieving limb salvage in patients with melanoma stage IIIa/ab or irresectable soft tissue sarcoma.

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CLINICAL DEVELOPMENT OF RECOMBINANT HUMAN INTERLEUKIN 4 (Schering 39400) IN PATIENTS WITH MALIGNANCY. M.E. Rybak

Interleukin 4 (IL-4) is a cytokine with pleiotropic immunomodulatory activities. These actions include B cell stimulation and co-stimulation, enhancement of IL-2 mediated generation of cytotoxic T cells, increase in the expression of HL-A class II antigens by monocytes and the inhibition of the production of a number of monokines including IL-6, IL-8 and TNFα. Human IL-4 has been cloned and expressed in E. coli. The mature protein has 129 amino acids, MW 14,957 D. Studies of rHuIL-4 in malignancy were prompted by in vitro data demonstrating the ability of IL-4 to inhibit the proliferation of chronic myelomonocytic cells, Non-Hodgkin's lymphoma and myeloma cells in culture, and to inhibit the IL-2 induced proliferation of chronic lymphocytic leukemia. In vivo models utilizing murine IL-4 demonstrate an immune response to tumors (e.g lung, sarcoma and melanoma) following systemic administration of IL-4 or the administration of tumor cells transfected with the IL-4 gene. Initial phase I trials of rhuIL-4 have been completed in 57 patients. Forty-seven patients received daily subcutaneous (s.c.) rHuIL-4, 0.25-5.0μg/kg. Side effects seen in $\geq$ 10% of patients included headache, fatigue, fevers (<38°C), myalgias and reversible elevation of SGOT, SGPT and alkaline phosphatase. Nausea, pedal and periorbital edema, and rhinitis were also seen. The MTD for rHuIL-4 was approximately 2μg/kg/d s.c. Activity has been seen in Hodgkin's disease, non-small cell lung cancer, chronic lymphocytic leukemia, and multiple myeloma. Current studies include Phase II investigations in advanced non-small cell lung cancer, refractory Hodgkin's disease, and relapsed multiple myeloma. An update on these studies will be presented.

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THE ROLE OF THE COLONY STIMULATING FACTORS IN MYELOID LEUKEMIA DEVELOPMENT D. Metcalf

This lecture will review the evidence concerning the possibility that sustained overstimulation by growth factors might provoke or accelerate myeloid leukemia development.
The regulatory factors likely to be relevant are the four colony stimulating factors although a possible role for SCL and IL-6 probably also needs consideration.
In general, prolonged overstimulation by growth factors in the mouse, either using transgenic mice or engraftment of hemopoietic cells engineered to produce growth factors leads to hyperplasia but not leukemia. In contrast insertion of CSF genes into immortalized, but non-leukemic, cell lines leads to prompt leukemic transformation. If FDC-P1 cells are engrafted in preirradiated animals, these can undergo delayed leukemic transformation often by activation of CSF genes by inserted IAP particles.
Normal cells can be transformed to leukemic cells by retroviral insertion of a combination of a CSF gene with Hox 2.4, the apparent function of the Hox 2.4 being to dysregulate the process of self-generation in early hemopoietic precursor cells. Thus two distinct changes appear to be necessary for leukemic transformation - abnormal self-generation and an acquired capacity for autocrine growth factor production.
All the above studies involve autocrine growth factor production but we have recently completed a study indicating that sustained stimulation by exogenous growth factors can also accelerate leukemic transformation. This study involved FDC-P1 cells engrafted in GM-CSF transgenic mice. Again the actual transformation process often involved rearrangement of CSF genes with autocrine CSF production.
The latter experiments raise the potential clinical hazard of prolonged CSF treatment in myelodysplastic patients but this possibly should not be overinterpreted.

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CLINICAL APPLICATIONS OF HAEMOPOIETIC GROWTH FACTOR (HGF)

J.H. Scarffe

Since granulocyte (G-CSF) and granulocyte macrophage (GM-CSF) colony stimulating factors were first introduced in to clinical trials in 1987, there have been a vast number of clinical trials demonstrating the utility of these agents in reducing neutropenia, and therefore infection in patient groups ranging from congenital neutropenia to bone marrow transplantation. The discovery that a large number of progenitors are released into the peripheral blood by these agents either alone or after chemotherapy was not one of the major indications predicted in 1987. The rapid reconstitution of both neutrophils and platelet counts when these peripheral blood progenitors (PBPC) are used as autologous rescue after myeloblative treatment, is encouraging many centres to use this type of rescue instead of ABMT. Interesting studies are at present indicating that these PBPCs can be expanded in vitro prior to clinical use. Attention is now moving to the stimulation of platelet productions with IL-3, IL-6, and IL-11. There is increasing evidence from experimental haematology that combinations of HGFs give optimal stimulation often of more primitive cells than previously thought. Clinical studies are now being performed, but the logistic problem of testing all the possible combinations is daunting. As the use of haemopoietic growth factors successfully protects the patients against haematological problems, other organs such as the GI tract, become dose limiting, often keeping the patient in hospital after haemopoietic recovery. Repeated high dose treatments also deplete the haemopoietic stem cells. Great interest is now being found in protecting the normal haemopoietic and other organ stem cells from the effects of radiochemotherapy. This may be possible using molecules such as MIP 1 alpha or TGF beta. Other workers have used the timing of the use of stimulatory HGFs to induce quiescence of the haemopoietic stem cells. The optimum use of HGFs would probably be to in sequence, prime the haemopoietic progenitors, then protect them from radiochemotherapy, followed by rapid expansion under the influence of probably a mixture of HGFs.

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OPTIMIZATION OF THE SCHEDULE OF FILGRASTIM (r-metHuG-CSF) ADMINISTRATION IN PATIENTS RECEIVING CHEMOTHERAPY

G. Morstyn

Extensive phase II studies have shown a reduction in the severity and duration of neutropenia when Filgrastim (r-metHuG-CSF) is administered soon after chemotherapy. Two phase III studies demonstrated that Filgrastim, given to small cell lung cancer patients from the day after chemotherapy, reduced the incidence of febrile neutropenic episodes by approximately 50% (Crawford et al, 1991; Trillet-Lenoir et al, 1993). These studies administered Filgrastim from the day after chemotherapy, and they suggest that Filgrastim is highly effective when used as an adjunct to chemotherapy to prevent fever and neutropenia. In a recent study (Maher et al, in press), Filgrastim administration was delayed until after the onset of fever and antibiotic therapy. Patients enrolled in this study were a median of 10 days after their last dose of chemotherapy. It was found that this later commencement of Filgrastim could significantly accelerate neutrophil recovery, produce a shorter duration of febrile neutropenia, and decrease the risk of prolonged hospitalization; however, maximal benefit appears to be derived from adjunctive use of Filgrastim prior to the onset of fever and neutropenia.

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CLINICAL TRIALS UPDATE ON THE USE OF IL-3

T.C. Jones

IL-3 is the first cytokine available for human use which was predicted to have both a activity on early hematopoietic progenitor cells, as well as specific action leading an increase in the number of circulating platelets. Phase I/II clinical trials have confirmed these predictions and have demonstrated, in addition, moderate activity on the late myeloid cell lineage leading to increased numbers of circulating neutrophils and eosinophils, events predicted from monkey studies, but not from in vitro and rodent studies. Phase I studies provided a clear definition of the maximum tolerated dose (10 μg/kg daily IV or s.c.), since at 15 μg/kg an unacceptable frequency of severe headache, flushing and tissue swelling were observed. At doses between 2.5-10 μg/kg, depending on the bone marrow reserve, cytotoxic chemotherapy and malignant disease, IL-3 prevented or reduced the severity of neutropenia and thrombocytopenia, reduced the number of platelet transfusions needed and allowed adherence to myelotoxic chemotherapy. In combination with other cytokines, such as GM-CSF or G-CSF, enhanced myeloid responses were seen including increased numbers of peripheral blood progenitor cells. These observations have led to now ongoing Phase III clinical trials using IL-3 in conjunction with cytoxic chemotherapy in several malignancies and in conjunction with autologous bone marrow transplantation. There appears little doubt at present that IL-3 will be an important contribution to management of the patients with oncologic or hematologic diseases.

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RECOMBINANT HUMAN INTERLEUKIN-6 (RhIL-6) BEFORE AND AFTER CHEMOTHERAPY. Vries de EGE, Gameren v MM, Vellenga E, Limburg PCB, Mulder NH, Willemse PHB.

From in vitro and animal studies it is known that IL-6 is a stimulator of thrombopoiesis, a stimulatory factor for T and B cells, that it stimulates hepatocytes and can have antitumor activity. An ongoing phase I-II study with rhIL-6 before and after chemotherapy (CT) is performed. Patients (pts) with breast cancer or non-small cell lung cancer receive E. Coli derived rhIL-6 (Sandoz, Basle) in doses of 0.5, 1, 2.5, 5 and 10 μg/kg/d, with $\geq$ 3 pts per dose step. Before CT rhIL-6 is given d1 iv and d2-7 sc. CT (mitoxantrone 10 mg/m^2 and thiotepa 40 mg/m^2, iv d1) starts d15, q 3 wks. After CT rhIL-6 is given d4-15 sc in all cycles (c). Before CT 16 pts were treated. RhIL-6 related side effects were: fever WHO grI-II (100%), headache (56%), myalgia (25%), local erythema (100%) and starting at 2.5 μg nausea (56%), increase of liver enzymes WHO grI-II (44%) and anemia (33%). Symptoms were controllable with acetaminophen (n=16) and antiemetics (n=2). Upto 10 μg there was a dose response related platelet increase compared to d1 ($r=0.75$, $p<0.002$), with highest count after rhIL-6 cessation. At 0.5 μg max. increase was 25.7 ± 5.6 (SE), at 10 μg 114.0 ± 38.0%. On d3 a leukocyte increase compared to d1 was observed starting at 2.5 μg upto 10 μg (d1 5.80 ± 0.51, d3 8.76 ± 0.45, $p<0.001$) mainly due to neutrophil increase ($p<0.001$). At d8 increase of lymphocytes occurred compared to d1 (d1 $1.02+0.19$, d8 $1.24+0.19$, $p<0.05$). There was a reversible hemoglobin reduction especially at 5 and 10 μg, starting d3 with a max. decrease at d8 (5 μg 24.0 ± 7.4 g/l, 10 μg 23.3 ± 5.5 g/l), at d15 initial values were almost reached without intervention. A dose related acute phase response was observed, with max. value of CRP (mg/l) 67 ± 11 at 0.5 μg and 602 ± 69 at 10 μg ($r=0.87$, $p<0.002$), and max. value of Serum Amyloid A (mg/l) at 0.5 μg of 258 ± 61 and at 10 μg 838 ± 143 ($r=0.80$, $p<0.002$). After CT 2 c (no c=22) were evaluable upto dose 5μg, in these c rhIL-6 related side effects were: fever (82%), headache (73%), myalgia (27%), local erythema (68%), nausea (27%) and increase in liver enzymes (14%). Upto c2 there was no difference in platelet nadir, moment and duration of platelets <100 x10^9/l between 0.5-1 μg and 2.5-5 μg doses. The same was observed for leukocytes. In conclusion, rhIL-6 upto 10 μg/kg/d has a acceptable toxicity profile and results, before CT, in dose dependent platelet and acute phase proteins increase. After CT, evaluable now for the first cycle, there is a faster platelet recovery at higher dose steps.

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G-CSF IN ACUTE LYMPHOBLASTIC LEUKEMIA. D.Hoelzer, O.Ottmann rhG-CSF given in acute lymphoblastic leukemia (ALL) after induction/consolidation therapy or allogeneic/autologous BMT can shorten the recovery time of neutrophils by 4-9 days, with reductions of the infection rate in some studies. Granulocytopenia is the major dose limiting toxicity not only after but also during chemotherapy. For example, severe neutropenia occurs in 66% of patients during the intensive 8 wk induction regimen of the German ALL trials. Therefore, the effect of parallel administration of G-CSF (Filgrastim) and chemotherapy was explored. rhG-CSF (5µg/kg) was given concurrently with cyclophosphamide, araC, 6-mercaptopurine, prednisone and prophylactic cranial irradiation. rhG-CSF was continued until neutrophil counts exceeded 1000/µl, but for at least seven days following the last dose of chemotherapy. In a pilot study of 15 pts, the median duration of granulocytopenia < 500/µl was 6 days compared to 12 days in a historical control group. When rhG-CSF was applied throughout the entire 8 wk induction treatment (Scherrer et al) in 14 pts, the CR rate was 93%, the time of neutrophil recovery to > 500/µl after the end of chemotherapy was reduced from 29,5 days to 14,5 days, and the infection rate was lower in the G-CSF treated patients. From these studies it was concluded that the concomitant administration of rhG-CSF and intensive chemotherapy in adult ALL is feasable, well tolerated and without major side effects; in particular, there was no evidence of prolonged neutropenia due to stimulation of hemopoietic stem cells by G-CSF and their subsequent elimination by chemotherapy. The clinical effects of rhG-CSF given concomitantly to chemotherapy require confirmation in a randomized trial. In an ongoing randomized multicenter trial, an interim analysis of 40 pts revealed a highly significant reduction of the total duration of severe neutropenia (< 500/µl) in the patients receiving G-CSF. Further analysis of the potential clinical benefits of this treatment modality is in progress.

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TREATMENT OF APLASTIC ANEMIA PATIENTS WITH GRANULOCYTE COLONY-STIMULATING FACTOR, FILGRASTIM: AN OVERVIEW OF JAPANESE EXPERIENCES. A.Shimosaka

Aplastic anemia is a chronic disease with severe pancytopenia and treatment had been very difficult. When Japanese first study started in early 1988, it was a pilot trial and the effect had not been expected so highly. Because serum concentrations of G-CSF in those patients are high with endogenous production. Knowing potential activities and almost no severe side effects of G-CSF, Kojima et al started the study at 100 µg/m^2 intravenous infusion with dose escalation every two weeks up to 400 µg/m^2 until response in neutrophil count observed. The patients whose neutrophil count over 100 responded to filgrastim treatment.[1] Five patients who did not respond to 400 µg/m^2 filgrastim treatment, were further treated up to 1200 µg/m^2 and 3 patients responded to the treatment.[2] Long term treatment with subcutaneous filgrastim revealed the recovery of erythrocyte [3] and the further study indicated that long term filgrastim treatment may support neutrophil and even erythrocyte and platelet. Long term follow up showed the benefit of filgrastim for patients with aplstic anemia. [4]

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RESTORATION OF HEMOPOIESIS BY INFUSION OF AUTOLOGOUS FILGRASTIM (G-CSF) -MOBILISED PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) AFTER HIGH DOSE CHEMOTHERAPY (HDC). W. P. Sheridan[1], C. G. Begley[1,2], C. A. Juttner[3], E. de Luca[2], L. B. To[3], J. Szer[4], D. Maher[5], D. Watson[5], A. Grigg [1], J. Cebon[5], G. Morstyn [5,6], K. M. McGrath[1], M.D. Green[1], P. Simmons [3], P. A. Rowlings[3], D. Tomita[6], E. Hoffman[6], R. M. Fox[1]. Royal Melbourne Hospital[1], Walter and Eliza Hall Institute[2], Royal Adleaide Hospital / Hansen Centre for Cancer Research [3], Alfred Hospital [4], Ludwig Institute[5], Amgen Inc [6].

We performed a phase II trial to assess the ability of G-CSF - mobilized PBPC to rapidly and completely restore hemopoiesis after high dose chemotherapy in the absence of bone marrow infusions, with selection for PBPC-only infusions based on yield of granulocyte - macrophage colony - forming cells (GM-CFC) after G-CSF treatment. Twenty-nine adults with acute lymphoblastic leukemia, non-Hodgkin's lymphoma, or Hodgkin's disease who were eligible for autologous bone marrow transplantation were treated. G-CSF was given at 12 or 24 µg/kg/d for 6-7 d and mononuclear cells collected by apheresis on days 5,6,7 or 4,6,8. Yield of PBPC was assessed by assay for granulocyte-macrophage colony forming cells (GM-CFC). High yield was defined as total GM-CFC collected >30 x 10^4/kg. High dose busulfan (4mg/kg/d x 4d) and cyclophosphamide (60mg/kg/d x 2d) were administered and hemopoietic cells infused, and recovery of hemopoiesis monitored. Progenitor cell yield was high in 11 of 29 patients. Patients given infusions of G-CSF-mobilized PBPC, but without bone marrow infusion, experienced recovery of hemopoiesis in all cases. No patient given PBPC alone required bone marrow infusion in up to 24 months of follow up. Kinetics of recovery of both the platelet and neutrophil counts were more rapid in the high yield (PBPC-alone) group than in the low yield group (PBPC plus bone marrow). Platelets recovered to >50 x 10^9/l at a median of 11 days and neutrophils to >0.5 x 10^9/l at 9 days in the high yield group, compared with 37 days and 10 days in the low yield group (p=0.0016 and 0.0308 respectively). Fewer platelet transfusions were required in the high yield group (median 11 packs per patient vs. 39, p=0.0138). We conclude that in patients with a high yield of PBPC after G-CSF therapy, infusion of G-CSF-mobilized PBPC results in rapid and sustained restoration of hemopoiesis. Use of G-CSF mobilised PBPC to support multiple cycles of HDC for treatment of high-risk stage II and III breast carcinoma is now being examined in a phase II study pursued by our group. Our data on PBPC moblisation without chemotherapy allows consideration of G-CSF-mobilised PBPC for haemopoietic cell allotransplantation and for gene therapy trials.

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DURABILITY OF HEMATOPOIESIS AFTER TRANSPLANTATION OF PERIPHERAL BLOOD HEMATOPOIETIC PROGENITORS ELICITED BY CANCER CHEMOTHERAPY AND CYTOKINE(S)

S. Siena, M. Bregni, M. Di Nicola, F. Peccatori, and A.M. Gianni

The hematopoietic system irreversibly damaged by potentially curative, although myeloablative, high-doses of chemoradiotherapy can be reconstitued by transplantation of autologous hematopoietic progenitors (CPCs) retrieved from peripheral blood. Successful recovery of early marrow functions can be achieved by transplantation of optimal numbers of CPCs, while it remains unknown if hematopoiesis is sustained at later times after such therapeutic procedures. Twentyeight pts (18 lymphoma, 7 myeloma, 3 breast cancer), not eligible to marrow harvest, were treated with 10-12.5 Gy TBI plus 120 mg/m^2 L-PAM (lymphoma and myeloma) or thiotepa 160 mg/m^2 plus 200 mg/m^2 L-PAM (breast cancer) and transplanted with CPCs (>8 x 10^6 CD34+ cells/kg) harvested at the time of recovery from 7 g/m^2 CTX- or 2 g/m^2 VP16-induced pancytopenia and during i.v. rhGM-CSF, rhG-CSF, or rhIL3 combined with rhGM-CSF or rhG-CSF. In 17 evaluable pts (not evaluable: 4 too early, 7 disease progression), numbers of pts with hematologic toxicity (WHO Scale) are:

Months after Transplant:		1	3	6	12	24	30
No. of Patients at Risk:		17	16	16	16	8	5
WBC toxicity	I-II	5	3	1	2	1	0
	III-IV	0	2	1	0	0	0
PLT toxicity	I-II	5	3	2	1	0	0
	III-IV	0	1	0	0	0	0
Hb toxicity	I-II	4	3	2	1	0	0
	III-IV	0	0	0	0	0	0

Transitory cytopenias occurred in a minority of pts during boost-radiotherapy 1-3 months after transplantation. Thus, hematopoietic reconstitution after transplantation of CPCs elicited by cancer chemotherapy and rhGM-CSF or rhG-CSF is rapid, complete, and, at present time of follow-up, durable in all patients.

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PERIPHERAL BLOOD PROGENITOR CELLS: MOBILIZATION, CLINICAL USE AND EX VIVO EXPANSION

L. Kanz, W. Brugger, R. Henschler and R. Mertelsmann

Our approach for high-dose (HD) chemotherapy is to first treat patients eligible for dose intensification with a standard dose chemotherapy (VIP: VP26 = etoposide: 500 mg/m², ifosfamide: 4 g/m², cis-platinum: 50 mg/m²) followed by the application of colony stimulating factors (G-CSF, GM-CSF or IL-3 + GM-CSF) in order to combine a regimen with broad anti-tumor activity with the recruitment of peripheral blood progenitor cells (PBPCs). PBPCs are reinfused into the patients after high dose intensification chemotherapy (according to underlying disorder: HD-VIP, VIC-E or BEAM).

Highest numbers of PBPC were recruited by the sequential administration of IL-3 + GM-CSF (median of 420 $CD34^+$ cells/μl and 24.000 total progenitors/ml, including multilineage as well as megakaryocytic progenitors). CSF-mobilized PBPCs include lineage negative $CD34^+$ cells, 4-HC resistant precursors as well as long term culture initiating cells (LTC-IC).

So far, a total of 47 patients were supported with PBPCs and CSFs following HD-chemotherapy. The period of severe neutropenia (<100/μl) as well as thrombocytopenia (<20.000/μl) was reduced to a median of 4 or 5 days respectively.

In order to decrease the number of potentially contaminating tumor cells in the leukapheresis preparations, we have started to positively select $CD34^+$ cells by an avidin-biotin immunoadsorption column (provided by R. Berenson, CellPro, USA). In all these patients, recovery data were comparable to the patients who received unseparated cells.

To provide sufficient numbers of PBPCs for repetitive use or in patients with low progenitor cell yield as well as to possibly avoid leukapheresis, we investigated the ability of hematopoietic growth factor combinations to expand PBPCs ex vivo. Chemotherapy + G-CSF recruited $CD34^+$ cells from 18 patients were enriched by immunoadsorption (purity: 89.2 ± 4.8) and cultured in suspension. A combination of stem cell factor, erythropoietin, interleukin-1ß, IL-3, and IL-6 was identified as the optimal combination for the expansion of clonogenic progenitors. Proliferation peaked at day 14 with a mean 190-fold increase (range 46-930) of clonogenic cells. Interferon-gamma synergized with the 5-factor combination, whereas the addition of GM-CSF or G-CSF decreased the number of clonogenic cells. Large-scale expansion of $CD34^+$ cells in autologous plasma supplemented with the 5-factor combination resulted in an equivalent expansion. Our data indicate the feasibility of large-scale progenitor cell expansion in cancer patients, starting from small numbers of $CD34^+$ cells. The number of cells generated ex vivo might be sufficient for use in high-dose chemotherapy.

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RECOMBINANT HUMAN ERYTHROPOIETIN AFTER BONE MARROW TRANSPLANTATION: INTERIM ANALYSIS OF A PROSPECTIVE PLACEBO CONTROLLED TRIAL.

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The aim of this study is to evaluate the effects of recombinant human Erythropoietin (rhu EPO) on hematopoietic regeneration after allogeneic or autologous bone marrow transplantation. Patients were randomized to receive either 100 U rhu EPO/kg body weight or placebo as continuous intravenous infusion from day 1 after BMT until independence from erythrocyte transfusions for 7 days or until day 42. The randomization was performed per each center and strafified according allogeneic or autologous BMT and major ABO-blood group incompatibility. At the time of the planned interim analysis with 205 patients treated, the time to erythrocyte transfusion independence after allogeneic BMT was shorter in group A (n = 52) than in group B (n = 55). After autologous BMT no difference between group A (n = 49) and B (n = 49) could be detected so far concerning time to transfusion independence or the number of transfusions applied, considering either erythrocyte or thrombocyte transfusions. There were no major differences in side effects between groups A and B. As of October 1992, the study was finished with a total of 329 patients.

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Cost-Benefit Relation in the Application of Hemopoietic Growth Factors

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Given the pressures on health care resources new health technologies need to be assessed to establish whether their benefits justify their costs. G-CSF is an important new technology that has profound impacts on cancer chemotherapy and patients outcome. As clinical studies show, the main economic benefits of G-CSF come from

* a significant reduction of hospitalisation during chemotherapy,
* reduced expenditure on inpatient and outpatient i.v. and oral antibiotic treatment,
* and reduced platelet and RBC transfusions.

In addition, it is expected that a therapy with G-CSF during cancer chemotherapy shows intangible and non-monetary benefits:

* increase in the rate of treatment as intended (time, number of cycles)
* increase of quality of life of patients
* reduction of drop-outs
* ability to intensify dosage

Recently coducted economic cost-cost studies show that the cost of managing neutropenia and infection and the cost of chemotherapy are lower for patients receiving G-CSF than those who do not. Future studies have to focus mainly on the quality-of-life cost-effectiveness of G-CSF.

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RISK FACTOR ANALYSIS AND MECHANISMS FOR SPONTANEOUS REGRESSION AND RESPONSE TO BIOLOGICAL THERAPY OF RENAL CELL CANCER

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Since 1978 164 RCC patients have been screened for entry into clinical trials and entered onto surveillance as first management policy until symptoms or serial radiology indicated tumour progression. 3+4(4%) have demonstrated unexplained "spontaneous" CR+PR(median duration 17 mths), and 7(4%) "stable" disease(median 28mths). None of 19 not nephrectomised regressed, while CR+PR was 11% of 40 nephrectomised at diagnosis of metastasis, 1% of 76 developing metastases 1-17 mths after nephrectomy and 10% of 29 developing metastases more than 18mths after nephrectomy. It was 12% of 47 with lung only verses 1% of 117 with other sites. Possible evidence for relief of potentially immunosupressive influences have been demonstrated in 4 of 7 patients demonstrating unexplained "spontanous regression. Study of HLA class I and II antigen abnormally on these tumours and their influence on tumour infiltrating lymphocytes will be reviewed.

80 patients seen to progress on surveillance were entered into treatment trials, the remainder being too old, sick or having bone or brain metastases needing radiotherapy. 2+6(10%) achieved CR+PR. 2+5 of 14(50%) of patients with lung only verses 0+1 of 66(1.5%) of those with other sites of metastasis. Although these results suggest that no harm has come from a period of preliminary surveillance, the fact that the therapeutic benefits including durable complete remission from therapy are confined almost entirely to the good risk small volume asymptomatic patients makes it difficult to justify a policy of surveillance for such patients.

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RECOMBINANT IL2 (rIL2) AFTER AUTO BMT FOR ACUTE LEUKEMIA IN CR1 : FOLLOW UP OF 41 PATIENTS. D.Blaise, A.M.Stoppa, D.Olive, M.Attal, J.L.Pico, C.Bellanger, J.Reiffers, M.Legros, M.Michallet, R.Gabus, M.Brandely, D.Maraninchi.

Auto BMT, as alternative of allo BMT, lacks immunotherapical effect delivered by allo graft. However immune reconstitution after auto BMT shows features recalling the post allo setting. Futhermore in vitro and in vivo in rIL2 is able to stimulate the proliferation and the lytic functions of NK and T cells, major effectors of this GVL effect. For these reasons from 1990, we have so far treated 41 patients with acute leukemia (AML:17;ALL:24) in CR1 with auto BMT followed with rIL2. All conditioning regimen included TBI. Median time between diagnosis and BMT was 5.2 mths (4-9 mths). rIL2 was started as soon as platelets reach $50x10^9/l$ and ANL $0.5x10^9/l$. rIL2 was given in 5 cycles of 5 days (C1) and 2 days (C2 to C5), starting on D1,D15,D29,D43,D57. rIL2 was given as a continuous infusion at a median of 16M IU/m^2/D (12M-24M). No patient died of rIL2 related toxicity. Platelet toxicity was obvious during the starting cycle but did not impair the long term hematological recovery. Immune stimulation was major and intense for both NK and T cells and both LAK and NK activity (all $p<0.05$). Long term infusion conducts to privilegiate NK stimulation. With a median follow-up of 24 mths, relapse and survival probabilities are respectively 45% and 65% fo ALL and 27% and 89% for AML comparying favorably with historical control ; these data invited us to set up a randomised study in CR1 AML and ALL after auto BMT. Started in 1991 in France, Italy and England, 135 pts have been so far included.

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ALPHA-INTERFERON, INTERLEUKIN-2 AND 5-FLUOROURACIL AS A PROMISING BIOCHEMOTHERAPY REGIMEN FOR THE MANAGEMENT OF ADVANCED RENAL CELL CARCINOMA

J. Atzpodien, H. Kirchner, E. Lopez Hänninen, M. Fenner, M. Deckert, and H. Poliwoda.

Recent clinical trials for the biological therapy of solid tumors have used recombinant human cytokines in combination with conventional chemotherapy.

In patients with metastatic renal cell carcinoma, we established a 3-drug combination of subcutaneous recombinant human interferon-α (IFN-α), interleukin-2 (IL-2) and 5-fluorouracil (5-FU) as outpatient regimen. Treatment consisted of eight weeks each of IFN-α (10 million U/m^2 x3 per week SQ) combined sequentially with IL-2 (5-20 million IU/m^2 x3 per week SQ for four weeks) and 5-FU (750 mg/m^2 IV weekly for four weeks).
Among 39 consecutive patients treated, there were 6 complete (15.4%) and 12 (30.8%) partial responders, with an overall objective response rate of 46.2% (95% confidence interval, 30-63%). Median response duration was calculated at 10+ months, and no relapse has occurred among complete responders.
Systemic toxicity was mild to moderate, with no severe 5-FU related mucositis or diarrhea. There were no dose limiting adverse effects of SQ IL-2 and no toxic deaths.

In summary, this outpatient biochemotherapy was as effective as the most aggressive inpatient IV IL-2 regimen; it appeared to significantly improve the therapeutic index in patients with metastatic renal cell carcinoma.

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Inhalative natural Interleukin-2 (IL-2) in combination with low dose systemic IL-2 and Interferon Alpha (INFa): Longterm follow up of patients with pulmonary metastasis of renal cell carcinoma (RCC)

Edith Huland, Hans Heinzer, Burkhard Falk, Hartwig Huland

We have shown that local, inhalative IL-2 application in combination with low dose systemic IL-2 and IFNa is highly effective and has low toxicity (J Urol 147,344, 1992). Here we report about longterm follow-up from 4 to 28 month of an outpatient schedule in 17 patients with pulmonary metastasis of renal cell carcinoma. Treatment protocol based on 5 time daily inhalation of IL-2 (5 x 200.000 U) combined with low dose i.v. IL-2 ($4x10^6$ U/4 days every 2 weeks) in 5 patients or low dose IL-2 s.c. injection of 100.000 U per day in 12 patients. All patients received 5 x 10^6 s.c. IFNa three times weekly. Median treatment duration was 17 month (range 1-24 month). Toxicity of IL-2 inhalation was low, no fever, no vasculary leakage. Even after up to 24 month of continuous inhalative treatment no evidence for irreversible pulmonary damage due to IL-2 induced immunomodulation occured in 17 patients. In pulmonary metastases 1 complete response (6 month), 9 partial response (4,5,8,13, + 18, 18, 24,24,24) and 7 stable disease (1,6,7,10 + 16, 20, 23, +) were achieved. 4 of 17 patients responded with nonpulmonary metastasis. Overall tumor response considering both, pulmonary and nonpulmonary metastases was 1 complete response, 7 partial responses 6 stable diseases, 2 mixed responses and 1 progressive disease. While IL-2 per inhalation had to be stopped because of grade II toxicity in 1 patient only, IFNa systemically had to be stopped in 10 of 17 and IL-2 i.v. cycles in 5 of 5 patients. 13 patients are alive with median actual survival of all patients of 16.9 month which seems to be prolonged compared to risk factors.

Inhalative treatment with IL-2 combined with low dose systemic cytokines represents a highly valuable model for the low toxicity of local IL-2 application, resulting in an effective long term treatment schedule with long term responses.

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Metastatic melanoma: Immunotherapy with IFNα and IL-2, DTIC/IFNα as second line treatment, IFNα/IL-2 + DTIC or CDDP.

U Keilholz, C Scheibenbogen, W Tilgen, D Maclachlan, P Brossart, Th Möhler, W Hunstein.

This abstract summarises our 3-year experience in the treatment of metastatic melanoma with sequential or combined chemo-immunotherapy. Patients with progressing metastatic melanoma have been treated with IFNα and IL-2. 10 mill U/sqm IFNα were given s.c. on days 1-5, and a new decrescendo regimen of IL-2 was used: 1mg/sqm/6hours, followed by 1mg/sqm/12 hours, and 1mg/sqm/24 hours, and 0.25mg/sqm/24 hours x 3. The current response rate is 33% (3 CR, 12 PR, 16 MR/SD, 14 PD). Patients not responding to IFNα/IL-2 (SD and PD) were eligible for subsequent chemotherapy with DTIC, 850 mg/sqm day 1, followed by IFNα, 3 Mill U/day 2-6. The response rate for this second line regimen is 18% (1 CR, 3 PR, 5 SD, 13 PD, n=22). Using this sequential approach, the overall response rate in this cohort is 51%, and the median survival is 17 months.
In preparation of a randomized trial comparing chemo-immunotherapy and immunotherapy a pilot study was performed. Patients not responding to the standard IFNα/IL-2 regimen received a single dose of DTIC, 850 mg/sqm (n=6) or CDDP, 100 mg/sqm (n=7) on day one, followed by IFNα/IL-2 according to the identical protocol as previously without chemotherapy. In the case of CDDP, grade 3 nephrotoxicity was observed in 2/7 patients. Pharmacokinetics of IL-2 were not influenced by previous chemotherapy, except in the patients with CDDP-associated nephrotoxicity. Induction of secondary mediators (TNFα, IFNγ, neopterin, sCD25) by IL-2 was not diminished by previous chemotherapy. 3 patients unresponsive to immunotherapy alone showed tumor shrinkage upon chemo-immunotherapy.
Conclusions: With initial immunotherapy followed in nonresponders by chemotherapy a response rate above 50% is achieved. Combined chemo-immunotherapy is feasible, and the immunologic response to IL-2 is not diminished by previous chemotherapy. A randomised trial is necessary to determine, whether combined chemo-immunotherapy is superior to immunotherapy alone.

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CYTOKINES IN INFAMMATION: INTRODUCTORY REMARKS

K. Resch

Inflammation is characterized by an accumulation of leucocytes at the site of injury. It hab been well established that the disease associated lesions are caused by a plethora of soluble mediators released by both the infiltrated leukocytes as well by tissue cells, including many degrading enzymes, lipid mediators, reactive oxygen species, or cytokines. Among those, cytokines play a crucial role in the inflammatory process as their release appears to represent the initial response which mediates the secretion of subsequent effector molecules responsible for the pathophysiological effects (e.g. changes of vascular resistance) in an autocrine or paracrine fashion. This holds true for the acute inflammation during which circulating granulocytes or monocytes are activated directly by a noxious agent; an example being the pivotal function of tumor necrosis factor (TNF) in septic shock. Chronic inflammatory diseases are initiated and perpetuated by immune reactions. Cytokines represent an important link between immune reactions and the recruitment and activation of blood borne infiltrating inflammatory cells. Thus interferon τ and tumor necrosis factor β, secreted by activated T lymphocytes, are potent stimulators of mononuclear phagocytes. The activated macrophages themselves secrete cytokines such as Interleukin-1 (IL-1), TNF α or Interleukin-8 (IL-8) which have been termed collectively as inflammatory cytokines. IL-1 and TNF again in an autocrine or paracrine way upregulate the synthesis of the ultimate pathogenic mediators in macrophages - e.g. the secretion of degrading enzymes as in rheumatoid arthritis -. Equally important they induce functional changes in vascular and autochthonous tissue cells which are thus recruited to contribute to the inflammatory lesions. IL-8, and similarly the lymphocyte derived RANTES, chemotactically attracts to the site of lesion and activates granulocytes, which then participate in the inflammatory reaction. While this rapidly recruits circulating phagocytic cells, the secretion of colony stimulating factors by activated T lymphocytes and macrophages also increases the pool of granulocytes and monocytes by stimulating their synthesis in the bone marrow. In turn IL-1 an TNF α synthesized by monocytes (and also some tissue cells) are coactivators of T lymphocytes, and thus contribute to al local immune reaction. Besides this general role of cytokines in inflammation their involvement in specific situations becomes increasingly apparent, an important example being the allergic inflammation. Cytokines synthesized by T lymphocytes such as Interleukin-4 or Interleukin-5 not only regulate immunoglobulin synthesis of B lymphocytes, including IgE, but equally important modulate the functions of mast cells or eosinophilic leukocytes to become effective effector cells of allergic reactions. The detailed functions of cytokines in different acute or chronic inflammatory diseases will be discussed in the subsequent contributions.

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SOLUBLE CYTOKINE RECEPTORS AS POTENTIAL THERAPEUTIC ANTAGONISTS

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The cytokines IL-1 and TNF play central roles in inflammatory responses which can lead to tissue injury. Naturally occurring antagonists such as soluble cytokine receptors or receptor antagonists are also produced during inflammatory responses. These soluble receptors and antagonists may act as a buffer system to reduce and limit tissue injury induced by cytokines. However, in some disease states this naturally occurring buffer system may not be sufficient to inhibit the detrimental actions of an inflammatory response. Responses to IL-1 are mediated via the type I receptor (IL-1R, type I). The type II receptor (IL-1R, type II) has never been shown to signal, but instead appears to be shed as an IL-1 antagonist. Both recombinant soluble IL-1R, type I an IL-1R, type II are capable of binding IL-1 and inhibiting responses in vitro. In a Phase I clinical study evaluating soluble IL-1R (type I) in modifying cutaneous allergic responses, IL-1R was a potent inhibitor of allergen induced late-phase inflammation in the skin, with a high safety profile.

Responses to TNF are mediated via the p80 or p60 TNF receptor. Soluble forms of both the p80 and p60 TNFR occur naturally and are increased in many inflammatory states. Dimeric constructs of the p80 TNF have been engineered to form a soluble Fc fusion protein with two monomeric TNFR extracellular portions contained on a truncated Ig heavy chain (TNFR:Fc). The TNFR:Fc possesses higher affinity than a monomeric soluble receptor and the Ig-like Fc structure imparts a longer half life.

Animal studies have shown that soluble IL-1R, type I and TNFR:Fc are effective antagonists of inflammation. In animal models for arthritis and pulmonary inflammation, IL-1R and TNFR:Fc reduced inflammatory cell infiltration and tissue damage. Both molecules are currently in clinical trials and hold promise for treatment of autoimmune and allergic diseases such as rheumatoid arthritis and asthma.

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CYTOKINE ANTAGONISTS IN COMPLICATIONS OF ALLOGENEIC BONE MARROW TRANPLANTATION - RATIONALE AND FIRST CLINICAL RESULTS

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Experimental as well as clinical studies suggest dual involvement of proinflammatory cytokines as TNFalpha and IL1 in complications and GvHD following allogeneic BMT: Nonspecific activation of recpient tissues during pretransplant conditioning results in early release of TNFa which strongly enhances donor T cell activation; in the effector phase, T cell stimulation is amplified by IFNgamma and LPS mediated release of TNF and IL1. The role of these mediators as critical effectors of GvHD related tissue damage could be confirmed by application of cytokine antagonists early after murine BMT: Both, anti-TNFa and IL1-receptor-antagonist (IL1ra, as shown by J Ferrara, Boston) resulted in a >50% reduction of GvHD related mortality in mice, while pentoxifylline proved to be ineffective. In human BMT, phase I/II studies analyzing anti-TNFa and IL1ra in refractory GvHD report substantial improvement in 50 - 70% of patients, though reoccurrence of symptoms after cessation of treatment in most patients indicates a late effector function of cytokines at least in advanced GvHD. Contribution of TNFa to recipient related induction of GvHD is further suggested by a recent phase I/II study performed in our institution as prophylactic application of anti-TNFa during pretransplant conditioning suppressed occurrence of early GvHD in high risk patients as compared to historical controls. Though these data indicate a future role of a variety of cytokine antagonists in management of clinical complications of BMT their impact on long term survival has to be evaluated and compared to classical strategies (e.g.the use of corticosteroids and T-cell-manipulation) in randomized studies.

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EFFECTS OF PENTOXIFYLLINE IN ACUTE AND CHRONIC CYTOKINE RELEASE SYNDROMES

P. Zabel, F.U. Schade and M. Schlaak

Recently, evidence was raised that pentoxifylline (POF) is able to suppress the synthesis of tumor necrosis factor-alpha (TNF) in cell cultures, in vivo, and to protect experimental animals against endotoxin shock. It was found that POF selectively inhibits the formation of TNF but not interleukin-6 (IL-6).

We could confirm these pharmacological effects of POF in humans under controlled conditions of endotoxinemia. Ten healthy volunteers recieved a bolus injection of endotoxin (100 ng of LPS of S.a.e.), which caused a transient increase of circulating TNF and IL-6. 3 weeks later the volunteers were again injected with endotoxin and POF was also infused. Due to POF administration, there was no rise in circulating TNF, whereas IL-6 levels rose in parallel with body temperature, comparable to those seen in the first part of the study.

Treatment of allograft transplant recipients with the murine anti-CD3 monoclonal antibody OKT3 leads to an acute cytokine release syndrome. Especially TNF seems to play a pivotal role in the pathophysiology of the OKT3 first-dose reaction. Pretreatment of 8 patients with POF prior to OKT3 administration was able to reduce the endogenous TNF formation significantly as compared to controls and, thus, prevents severe clinical side effects, whereas IL-6 formation and febrile response were not affected.

Severe pulmonary tuberculosis is associated with a chronic cytokine release syndrome (elevated levels of circulating TNF and IL-6). In 8 patients POF treatment inhibited chronic TNF release selectively, and, thus, reduced TNF-dependent cachexia without affecting chronic IL-6 formation and related symptoms, such as fever and night sweat.

In conclusion, we suggest that POF may improve therapeutic strategies in cases of acute and chronic cytokine release syndromes.

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EXPRESSION OF TNF-RECEPTORS, TNF-α AND TNF-ß IN RHEUMATIC DISEASES, B. Heilig, A. Pezzutto, C.Fiehn, B. Berke, W.Hunstein, Department of Internal Medicine, University of Heidelberg, D-6900 Heidelberg, Germany

Tumor Necrosis Factor (TNF) plays a central role in the maintenance of the inflammatory events in rheumatoid arthritis. We evaluated the expression of p75 and p55 TNF receptors (TNFR) and of TNF-α and TNF-ß on the surface of synovial fluid mononuclear cells in patients with rheumatoid arthritis (RA) (n = 9), spondylarthropathy (SpA) (n = 11), and traumatic effusions (n = 3). Synovial T-lymphocytes from RA patients express in all cases the p75 TNFR on the cell-membrane, in 4/9 cases also a weak expression of the p55 TNFR is detectable, both mRNAs can be detected by polymerase chain reaction (PCR). Synovial macrophages also express the p75 TNFR and low amounts of the p55 TNFR. Patients with active RA also have circulating p75 TNFR positive T-lymphocytes in their blood. High concentrations of soluble TNFR (sTNFR) are found in the joint effusions of RA patients: up to 40 ng/ml of p75 sTNFR and up to 54 ng/ml p55 sTNFR. Significantly lower sTNFR levels are found in SpA effusions. Both receptors are also more elevated in the serum of RA patients (2.59 ± 0.28 ng/ml p75 sTNFR and 4.49 ± 0.55 ng/ml p55 sTNFR) as compared to SpA patients (1.41 ± 0.09 ng/ml p75 sTNFR and 1.78 ± 0.08 ng/ml p55 sTNFR, $p < 0.001$). TNF-α could be detected in the synovial fluid of RA patients (up to 140 pg/ml), but not in the serum. The soluble TNFR are biologically active and neutralize the effects of TNF-α in a cytotoxicity assay. The high levels of soluble TNFR in the inflammatory effusions may reflect TNF neutralizing activity in an environment where enhanced TNF synthesis has occured. We have generated several anti-sense-TNF-α oligonucleotides (as-TNF), in order to down-regulate TNF biosynthesis at the mRNA level. With as-TNF-3 we could achieve more than 90% inhibition of TNF secretion in PHA-stimulated peripheral blood or synovial fluid lymphocytes. The effects of as-TNF-3 on the expression of TNFR and on the synthesis of other cytokines are currently being investigated.

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MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) IS A CYTOKINE SECRETED BY THE PITUITARY AFTER ENDOTOXIN STIMULATION. J. Bernhagen, T. Calandra, R. A. Mitchell, W. Voelter, A. Cerami, and R. Bucala.

The systemic regulation of the host response during acute inflammatory states remains poorly understood. Among the regulatory systems that are likely to play a role in controlling host responses to bacterial infection is the neuroendocrine axis. The pituitary for example, is ideally situated to integrate central and peripheral stimuli and among other effects initiates the systemic increase in glucocorticoid production that accompanies host stress responses. We studied the secretory response of the murine pituitary cell line AtT-20 *in vitro* and whole pituitaries *in vivo* after endotoxin (LPS) stimulation. We identified macrophage migration inhibitory factor (MIF), a previously described T-cell cytokine, as a major secreted protein of AtT-20 cells that were stimulated by sub-nanogram amounts of LPS. To study the expression of pituitary-derived MIF *in vivo*, BALB/c mice were injected ip with sub-lethal amounts of LPS. Pituitaries and serum were collected at intervals and pituitary MIF mRNA and pituitary and serum MIF protein were measured. Pituitary MIF mRNA specifically increased with time and reached a plateau 16-24 hr after LPS challenge. MIF protein, which is present constitutively in the pituitary, decreased within 20 hr. Determination of serum MIF in normal, athymic and hypophysectomized BALB/c mice indicated that the pituitary is an important source of serum MIF that appears in the post-acute phase (8-20 hr), whereas T cell MIF contributes primarily to the hyper-acute rise in serum MIF (2 hr). These data suggest that MIF plays a central role in the systemic response to endotoxin and that pituitary MIF is likely to reflect the interplay of diverse neurohumoral stimuli that regulate acute and chronic inflammation.

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THE EFFECT OF RECOMBINANT GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) ON NEUTROPHIL KINETICS AND FUNCTION IN NORMAL, YOUNG AND ELDERLY SUBJECTS. DC Dale, TH Price, GS Chatta, RC Allen, and P Stevens.

To investigate the effects of G-CSF (r-metHuG-CSF, Amgen, Thousand Oaks, CA) on neutrophil production, blood distribution, survival and function, daily subcutaneous injections of G-CSF were administered to healthy, young (Y) (20-30 years) and healthy elderly (O) (70-80 years) subjects for 14 days at three dose levels, 0 mcg (n = 7), 30 mcg (n = 10), and 300 mcg (n = 10). Daily CBCs and serial measurements of neutrophil oxidative activity by chemiluminescence were made. In addition, blood neutrophil kinetics were determined (day 6), transit time of the marrow neutrophil post mitotic pool (NTT) (days 7 to 14), neutrophil migration to skin chambers (days 0 and 5), and blood colony forming cells (CFU-GM) (day 0 and 5), as well as routine marrow morphology studies (days 0, 5 and 15) were performed. Baseline neutrophil counts were similar in the Y and O subjects and counts increased similarly for the two age groups, with peak counts of 51.6 ± 6.7 for the 300 mcg dose. G-CSF significantly shortened the NTT from 5.7 ± 1.6 days (control) to 4.2 ± 0.3 days (30 mcg) and 2.9 ± 0.4 days (300 mcg) ($p < 0.05$). A concomitant of the shortened NTT was a dose dependent increase in the chemiluminescence responses, reflecting higher myeloperoxidase activity. The shortened NTT was also reflected by a decreased proportion of marrow cells in the post mitotic pool (metas, bands and PMNs) and apparently lengthened blood half life of the circulating PMNs. Neutrophil migration to cutaneous inflammatory sites was also decreased in a dose dependent fashion. Comparison of the age groups showed the only significant difference to be in the mobilization of blood colony forming cells with blood CFU-GM increasing 80 fold in the young versus 40 fold in the elderly over 5 days (300 mcg). No significant toxicities were observed in these normal subjects. These studies demonstrate the dose dependent stimulation of neutrophil production with G-CSF administration, which is not affected by the age of the subjects. The neutrophils released into the peripheral blood have enhanced oxidative responses but somewhat reduced migratory capacities, probably reflecting the accelerated production and early release of the developing neutrophils. These changes are remarkably similar to changes in neutrophil production and function observed with bacterial infections in hematological normal individuals.

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HAEMOPOIETIC GROWTH FACTORS AS MODULATORS OF EXPERIMENTAL ENDOTOXIC SHOCK IN RODENTS.
A. Wendel, J. Barsig, S. Uhlig and G. Tiegs

Pretreatment of mice or rats with granulocyte colony-stimulating factor (G-CSF) protected against endotoxin-induced lethal shock or against endotoxin-induced liver injury in galactosamine-sensitized animals. In the animals protected by such pretreatment, the systemic tumor necrosis factor α (TNF) bioactivity was significantly suppressed. Various macrophage populations taken *ex vivo* from G-CSF-pretreated animals showed an attenuated TNF release following LPS stimulation. However, G-CSF had no such effects on macrophages when directly added *in vitro* to the cells. These findings show that G-CSF protects against septic shock *via* TNF suppression in a way requiring the participation of additional circulating cells or factors.

Pretreatment of rodents with granulocyte macrophage colony stimulating factor (GM-CSF) greatly enhanced the susceptibility of the animals to endotoxin and led to a tremendous increase of the systemic TNF found following an LPS challenge. An anti-GM-CSF antibody significantly protected animals against septic organ failure. Considerable amounts of endogenous GM-CSF are released following endotoxin challenge with a maximum at 2 h. The enhancement of LPS-inducible TNF release from macrophages takes also place *in vitro.* It is concluded that GM-CSF is a pivotal mediator of sepsis as well as a directly acting enhancer of LPS-inducible TNF release.

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ABNORMALITIES IN NEONATAL PHAGOCYTIC IMMUNITY: THE ROLE OF HEMATOPOIETIC GROWTH FACTORS IN THE TREATMENT OF NEONATAL SEPSIS. Mitchell S. Cairo

Neonatal hematopoiesis and host defense are developmentally immature in the newborn (NB) compared to the adult. We have previously demonstrated that protein production and mRNA expression of GM-CSF, G-CSF, and IL-3 were decreased in activated MNC from umbilical cord (C) compared with adult (A) peripheral blood (Cairo, et al, *Pediatr Res* 30:362, 1991, and 31:574, 1992). RhG-CSF ± recombinant rat Stem cell factor (rrSCF) administration in newborn rats has, however, resulted in a significant induction of neutrophilia, an increase in bone marrow post-mitotic pool, and is synergistic with antibiotics during experimental group B streptococcal sepsis (Cairo, et al, *Blood* 76:1788, 1990, and 80:96, 1992). To assess the toxicity and efficacy of rhG-CSF in newborns with presumed sepsis, 42 NB ≤3 days (26-40 wks) with a diagnosis of presumed sepsis were randomized to either placebo (P), 1.0, 5.0, or 10.0 μg/kg q 24 hrs, 5.0 or 10.0 μg/kg q 12 hrs of IV rhG-CSF x 3 days. CBC, differential, and platelet counts were obtained at time 0, 2, 24, 48, 72, and 96 hrs. Tibial bone marrow aspirates were performed at 72 hrs and bone marrow NSP, NPP, CFU-GM, and CFU-GEMM were determined. Serum for G-CSF levels was obtained before and 2, 6, 12, 14, 16, 18, and 36 hrs after rhG-CSF dosing and measured by a sandwich ELISA assay. RhG-CSF induced significant neutrophilia at 24 hrs vs. placebo (85 ± 19%) following both 5.0 (203 ± 40%, $p<0.04$) and 10.0 μg/kg/d (351 ± 89%, $p<0.02$). Significant neutrophilia continued at 48 and 72 hrs at both 5.0 ($p<0.03$ and <0.013) and 10 μg/kg/d ($p<0.05$ and <0.014) respectively. RhG-CSF also significantly induced a dose-dependent increase in the BM post-mitotic pool (P vs. 10 μg/kg/d) (18 ± 3.7 vs. 63 ± 7.5%) ($p<0.005$). $T^1/_2$ of G-CSF in the NB was 4.42 ± 0.44 hrs ($r= -0.9$) with peak G-CSF levels occurring within 2 hrs. To date there has been no evidence of acute or chronic toxicity secondary to rhG-CSF. Future studies are required to determine the clinical implications of the biological efficacy of rhG-CSF in newborns with presumed sepsis.

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RECOMBINANT HUMAN (RH) MACROPHAGE INFLAMMATORY PROTEIN (MIP)-1α DOES NOT MODULATE CLONAL GROWTH OF HUMAN NON-HEMATOPOIETIC TUMOR CELL LINES UNDER OPTIMAL GROWTH CONDITIONS IN VITRO.

A Korfel, Z von Marschall, D Oberberg, E Thiel, and WE Berdel

MIP-1α is a member of a family of proinflammatory cytokines produced by activated macrophages which has been shown to be a negative regulator of early hematopoietic stem cell progenitors. Our group investigates the interactions between hematopoietic cytokines and non-hematopoietic malignant cells. Here, we describe results testing rhMIP-1α (rh stem cell inhibitor, SCI; kindly provided by Dr. Wolpe, Genetics Institute, Cambridge, MA, USA) on the clonal growth of different human non-hematopoietic tumor cell lines in vitro. Cell lines tested included the following histologies: glioblastoma 6x, neuroblastoma 1x, head and neck carcinoma 2x, lung carcinoma 1x, colorectal carcinoma 2x, gastric carcinoma 1x, pancreatic carcinoma 1x, breast carcinoma 1x, bladder carcinoma 1x, prostate carcinoma 1x, choriocarcinoma 1x, ovary carcinoma 1x, osteosarcoma 1x, melanoma 3x. MIP-1α (0, 2, 20, 200 ng/ml) was tested in human tumor cloning assays (HTCA) in mixtures of methylcellulose and agar. HTCA has previously been shown to detect positive and negative growth control by cytokines. Plating efficacy of the cells in the controls was > 2 % (median 8 %, range 2.2 - 46.2 %) in this series of experiments. Tumor cells were continuously exposed to the cytokine for the complete assay period. Clonal growth of none of the cell lines was significantly and reproducibly stimulated or inhibited by MIP-1α. Since MIP-1α may enter clinical trials for indications such as protecting hematopoietic stem cells from damage caused by cytotoxic chemotherapy in tumor patients, further experiments should be performed in vitro and in vivo.

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EFFECTS OF TRANSFORMING GROWTH FACTOR-ß (TGF-ß) ON GROWTH OF CLONOGENIC CELLS FROM FRESHLY EXPLANTED HUMAN TUMORS IN VITRO S. Kreill, H. Depenbrock, T. Block, H. Vogelsang, Ch. Fellbaum, J. Rastetter, A.-R. Hanauske

TGF-ß is known to function both as inhibitory and stimulatory factor depending on the type of cell line investigated. The purpose of our study was to determine the effects of TGF-$ß_1$ and TGF-$ß_2$ (concentrations: 0.1 - 1 - 10 ng/ml) on soft agar colony formation of freshly explanted human tumors in vitro. Of 89 specimens, 6 had to be excluded from further analyses (4 confirmed benign, 2 bacterial/fungal contamination). 50 of the remaining 83 tumors showed evaluable growth in control capillaries (60%). At 10 ng/ml, TGF-$ß_1$ inhibited colony formation (≤ 0.5 x control) in 12 specimens (24%): 5/19 renal, 4/6 Non Hodgkin's lymphoma, 1/7 breast, 1/2 ovarian, 1/2 melanoma (median: 0.37 x control, range: 0.22 - 0.49). At 10 ng/ml, TGF-$ß_2$ showed inhibition of colony formation in 17 specimens (34%) with a similar spectrum of activity (median: 0.36 x control, range 0.06 - 0.48). Stimulation (colony formation ≥ 1.5 x control) was observed in only 1/50 specimens. Combination of TGF-$ß_1$ with Epidermal Growth Factor (EGF) reversed the inhibitory activity in 4/5 specimens (80%). Combination of TGF-$ß_1$ with Platelet Derived Growth Factor (PDGF) reversed the inhibitory activity in 2/5 specimens (40%). Similar results were observed for TGF_2. We conclude that TGF-$ß_1$ and TGF-$ß_2$ inhibit a subgroup of freshly explanted clonogenic tumor cells. Their activity, however, appears to be modulated by other growth factors.

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PRECLINICAL ACTIVITY OF COMBINATIONS OF HEMATOPOIETIC GROWTH FACTORS ON FRESHLY EXPLANTED HUMAN TUMOR COLONY FORMING UNITS

H. Depenbrock, B. Strahlhuber, B. Heinrich, M. Rotter, A. Lehmer, H. Nekarda, J. Rastetter, and A.-R. Hanauske

Clinical studies have demonstrated the activity of single hematopoietic growth factors (HGF) in restoring bone marrow function after chemotherapy. These studies have prompted clinical trials using multiple growth factors to promote the maturation of precursor cells at various stages of differentiation. However, HGF also have the capability to stimulate growth of non-hematopoietic tumor cells at least in long-term cell cultures. We have assessed the growth-modulating activity of combinations of various HGFs on freshly explanted human tumor colony forming units in vitro. A total of 86 tumor specimens were exposed for 21 - 28 days to IL-3, GM-CSF, G-CSF, SCF (all at final concentrations of 100 ng/ml) and IL-1ß (final concentration: 10 ng/ml) or combinations of these HGFs using a capillary soft agar cloning system. 54 specimens (63%) showed evaluable growth in control capillaries. Stimulation of colony growth was observed in 17/1026 tests (1.7%) with 6/54 (11%) specimens expressing sensitivity to individual HGFs, 3/54 (6%) to combinations of two HGF, and 6/54 (11%) to combinations of more than two HGFs. Inhibition of colony growth was observed in a total of 24/1026 tests (2.3%) with 1/54 (2%) specimens expressing marginal sensitivity to individual HGFs, 6/54 (11%) to combinations of two HGF, and 7/54 (13%) to combinations of more than two HGFs. For inhibitory effects, median colony survival for combinations > 2 HGFs was 0.46 x control (range 0.31-0.49). For stimulatory effects, median colony survival for combinations > 2 HGFs was 2.0 x control (range: 1.6 - 2.5). Our data indicate that combinations of HGF will not substantially alter the pattern of clonal proliferation of the majority of freshly explanted tumor cells in vitro. However, growth modulation may occur in a minority of tumors.

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IS GRANULOCYTE COLONY-STIMULATING FACTOR AN ANGIOGENIC FACTOR IN HUMAN GLIOBLASTOMA?

S. Corbacioglu, K. Welte and T. Pietsch

Granulocyte-colony stimulating factor (G-CSF) belongs to a family of glycoproteins which regulate growth, differentiation and function of hematopoietic cells of the myelomonocytic lineage. In addition, G-CSF induces migration and proliferation of endothelial cells *in vitro*. To investigate the effects of G-CSF on vascularization of glioblastoma *in vivo* we transplanted the human glioblastoma cell line U-87 MG which is capable of producing G-CSF in high amounts. After s.c. inoculation of these cells into the backs of athymic mice, they developped highly vascularized solid tumors. In order to block the effect of G-CSF directly or to inhibit the production of G-CSF by tumor cells neutrolizing monoclonal antibody (mAb) 75A against G-CSF or dexamethasone were injected intravenously. At 2-day intervals the tumor volumes were measured. After seven days the mice were sacrificed and the tumors were explanted. Blood was collected for differential blood counts and serum was tested for G-CSF. Fresh frozen sections of the tumors were specifically stained for capillaries using Bandeiraea (Griffonia) Simplicifolia Lectin I Isolectin B_4 (BSL B_4). Morphometric studies of the stained sections were performed in order to quantitate the vascularization of the tumors. The differential blood counts showed significantly increased neutrophils due to the effect of human G-CSF produced by the glioblastoma cells. This effect was inhibited by anti-G-CSF mAbs or dexamethasone. However neither G-CSF mAbs nor dexamethasone could inhibit tumor growth compared untreated tumor bearing mice. Dexamethasone significantly decreased the tumor vascularization whereas anti-G-CSF mAbs did not have any effects on tumor vascularization. These findings suggest that G-CSF is not an essential angiogenic factor *in vivo*.

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EFFECTS OF INSULIN-LIKE-GROWTH-FACTORS [IGF] ON CLONOGENIC PROLIFERATION OF FRESHLY EXPLANTED HUMAN TUMOR CELLS IN VITRO

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IGF is known to be mitogenic for a variety of cell lines in vitro. We have studied the effects of Insulin-like-Growth-Factor I [IGF-I] and Insulin-like-Growth-Factor II [IGF-II] on 92 freshly explanted human tumors using a capillary soft agar cloning system. 6 specimens had to be excluded from further analyses (4 confirmed benign, 2 bacterial/fungal contamination). 51/86 specimens (59%) showed adequate growth in controls (17 renal, 7 breast, 7 lymphoma, 6 colorectal, 14 miscellaneous) with a median colony formation of 18.7 colonies/capillary (range: 4.2 - 120.0). At final concentrations between 10^{-11} M and 10^{-8} M, IGF-I significantly stimulated colony formation ($\geq$ 1.5 x control) in 10/51 evaluable specimens (20%) with a median of 2.05 x control (range: 1.57 -3.22) and inhibited colony formation ($\leq$ 0.5 x control) in 4/51 specimens (8%, median: 0.33 x control, range: 0.08 - 0.49). IGF-II stimulated 8/51 specimens (16%, median: 2.66 x control, range: 1.50 - 3.71) and inhibited 8/51 specimens (16%, median: 0.32 x control, range: 0.05 - 0.47). The optimal concentration for stimulation was found to be 10^{-9} M for IGF-I and IGF-II. Of 39 specimens not significantly stimulated by either IGF-I (10^{-9} M) or Epidermal Growth Factor (EGF, 10^{-10} M), 4 (10%) were significantly stimulated by the combination of the two factors. 1/39 (3%) specimen was stimulated by a combination of IGF-II (10^{-9} M) and EGF. We conclude that IGF modulates the clonogenic growth of a subgroup of freshly explanted human cancer cells in vitro.

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SPGM1: A NOVEL CELLULAR MODEL FOR IL-3 INDUCED LINEAGE SWITCH

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The SPGM1 cell line was established from a transplantable mouse progranulocytic/promacrophage tumor (Dührsen U et al. 1989, Leukemia 3: 796-803). Extensive phenotyping of this mouse progenitor line revealed the properties of a typical CD5 positive pre-B cell, SPGM1 being positive for PB76, B220 (CD45R), and the pre-B cell immunoglobulin receptor complex, comprising μ-heavy chains, λ5- and v_{preB}-surrogate light chains and the IgMα and Igβ co-receptor molecules. Southern Blot analysis revealed clonal rearrangement of the μ-heavy chain locus and germline light chain loci.

Interestingly, SPGM1 readily formed blast cell, macrophage and occasional granulocytic colonies in soft agar in the presence of Interleukin 3 (IL-3). In suspension cultures IL-3 also induced macrophage differentiation, the cells becoming larger, adherent and independent of 2-mercaptoethanol in the culture medium. IL-3 induced an initial burst of proliferation during differentiation, accompanied by loss of self renewal capacity, subsequently followed by a decrease and cessation of proliferation. The earliest changes were detectable at 24 hours by Northern Blot analysis. IL-3 treatment increased Mac1 mRNA, induced *cfms* mRNA (M-CSF receptor) and down regulated mRNA for μ, λ 5, v_{preB}, and mb1. After 2 to 4 days the cells morphologically, phenotypically and functionally resembled macrophages, expressing strongly Mac1 and F4/80, and phagocytosing latex beads (Martin M et al. 1993 J.Immunol. in press).

Thus, IL-3 induced the CD5 positive pre-B cell line SPGM1 to switch its differentiation program in a coordinate fashion from a pre-B cell to a macrophage.

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IS THERE AN ASSOCIATION OF MONOSOMY 7 AND LEUKEMIC RESPONSE TO MYELOID GROWTH FACTORS?

D. Haase, M. Freund, K. Welte, B. Zabel and C. Fonatsch

The myeloid growth factors G-CSF and GM-CSF are increasingly introduced in therapy trials in neutropenic disorders and in MDS. In a series of 4 therapy trials 12.5% up to 36.4% of patients treated with GM-CSF displayed a stimulation of blast cells (Antin 1988, Estey 1991, Ganser 1989, Vadhan-Raj 1987). Recently, we could demonstrate a growth advantage for monosomy 7-cells in GM-CSF containing bone marrow cultures (Haase, 1990) which is supported by results from others (Andreeff 1991). Now, further data are available corroborating an association of monosomy 7 and leukemic blast stimulation by myeloid growth factors. In a cytogenetic follow-up study of 13 patients with MDS under GM-CSF (in preparation) we could observe a cytogenetic, clinical, and cytologic progression in a patient with monosomy 7 within 34 days. In a patient with Kostman's syndrome, receiving G-CSF, we performed sequential cytogenetic analyses. The patient's disease progressed to MDS and finally to AML. He initially had a mosaic karyotype of normal and monosomy 7 cells but displayed a rapid karyotype evolution with supersession of normal cells and gain of additional abnormalities. A recent publication from a Japanese group adds further information to a possible association of monosomy 7 with stimulation of leukemia in 14% of neutropenic children treated with G-CSF (Kojima, 1992). Besides the need for further cytogenetic follow-up studies in growth factor therapy trials, data are accumulating that monosomy 7 is a risk factor in GM-CSF and G-CSF therapy.

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CYTOGENETIC IN VITRO RESPONSE TO IL-3 IN MDS AND ANLL

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Il-3 is a cytokine with multilineage activity and stimulates proliferation of immature hemopoietic progenitors (Yang, 1989). Recently, IL-3 has been introduced in clinical trials in MDS (Dunbar, 1990; Ganser, 1990). As in GM-CSF therapy one major concern attributes to an IL-3 mediated stimulation of leukemic cells, since a rise in blast counts has been observed in two of 32 patients reported (Ganser, 1992). However, as yet it is not known whether the blasts stimulated belong to the normal or leukemic population.
We performed cytogenetic analyses of bone marrow cultures with and without addition of 100 ng/ml IL-3 (Behringwerke, Marburg). The influence of this cytokine on the clonal compostion in 7 patients (3 ANLL, 4 MDS) with mosaic karyotypes was examined. In all 7 patients independent from diagnosis and chromosome abnormality the normal cell population was promoted by IL-3 with different intensity. Individual data are outlined on the table:

Dg.	Abnormality/ies	n*	% normal metaphases + IL-3	- IL-3
RA	1p-,t(2;11),5q-	53	3.8	0
RAEB	dup(1q)	40	96.8	88.9
RAEB	complex	194	62.2	20.3
RAEBT	-7	127	19.6	10.6
M2	t(8;21),-X	23	9.1	0
M6	+8	102	42.1	20.3
M7	complex	70	82.4	52.8

n*= total number of metaphases analyzed

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STEM CELL FACTOR CAN PROMOTE T CELL PROLIFERATION

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Stem cell factor (SCF) is known to promote proliferation of hematopoietic progenitors and mast cells. However, the spectrum of its biological effects is not completely understood. Since SCF may be able to accelerate hemopoietic recovery after chemotherapy or in other situations where severe immunosuppression is present, we were interested in its effects on T cells. Thus, we have studied the influence of human recombinant SCF on T cell proliferation in-vitro.

When cultured for 6 days in serum-free medium, 3H-thymidine incorporation of unstimulated peripheral blood mononuclear cells (PMNC) resulted in 1480±689 cpm without growth factors, 1189±119 cpm with IL-3 (50ng/mL), 2386±1149 cpm with SCF (10ng/mL), and 3828±1660 cpm with both IL-3 and SCF (n=6). Addition of SCF to one way mixed lymphocyte cultures (MLC) increased thymidine incorporation by 27% (0-182%); addition of SCF plus IL-3 increased thymidine incorporation by 324% (48-734%; IL-3 alone 0%, n=5). After depletion of monocytes and the majority of B cells from PMNC, the proliferation of the remaining cell fraction, which consisted mainly of T cells, was not enhanced by IL-3, SCF or IL-3+SCF.

We conclude that SCF can promote proliferation of unstimulated or allostimulated T cells in the presence of IL-3. Since this effect requires monocytes or other accessory cells, a direct influence of SCF on T cells does not become evident from our data.

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EFFECTS OF STEM CELL FACTOR (SCF) ON B-CLL-CELLS IN SERUM FREE SUSPENSION CULTURES

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The effect of Stem Cell Factor (SCF) on peripheral blood B-CLL-Cells were studied in vitro by bromodeoxyuridine/propidiumiodide (BrdU/PI) cell-cycle-analysis. Peripheral blood cells from 41 patients with B-CLL were examined with CD-markers and prepared as follows: after Ficoll centrifugation and lysis of monocytes by leucine-methyl-ester (LME) T-Lymphocytes were depleted with a CD3 monoclonal antibody and magnetic cell sorting. The cells were grown in serum free culture medium (CG-medium) containing 10 µmol/l BrdU, and 0.1 ng/ml up to 100 ng/ml SCF. Controls were grown without cytokines. Samples were drawn repeatedly at 20, 68, 92, 116, and 140 hours. Cell-cycle-analysis was performed after double DNA staining with propidiumiodide and anti-BrdU-antibodies and determinated by flow cytometry.
Minimal alterations were observed with T-cell depletion, the B-CLL cells from 31 patients were stimulated by SCF during the first 92 hours reaching a maximum of ~2.77 % compared with the controls after 68 hours in cultures containing 100 ng/ml SCF. On the other hand, cultures without T-cell depletion showed no effect for SCF. We conclude that SCF has only a minimal stimulatory, early effect in inducing the proliferation of B-CLL cells.

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LEUKOTRIENE B_4 TRANSCRIPTIONALLY ACTIVATES INTERLEUKIN-6 EXPRESSION INVOLVING NF-κB AND NF-IL6 IN A H_2O_2-DEPENDENT PATHWAY.

M.A. Brach, S. de Vos, C. Arnold, H.J. Gruss, and F. Herrmann.

Leukotriene B_4 $(LTB)_4$ is a notable participant in inflammation and chemotaxis. It is, however, still unclear whether LTB_4 acts in this regard directly or indirectly by stimulating the release of chemotactic and inflammatory cytokines. Here we report that LTB_4 induces synthesis of interleukin (IL)-6 by human blood monocytes through transcriptional activation of the IL-6 gene. We furthermore demonstrate that this process involves activation of the transcription factor NF-κB and, to a lesser extent, of NF-IL6, while the activity of the transcription factor AP-1, shown to otherwise confer IL-6 inducibility, appeared to be unaffected by LTB_4. Involvement of NF-κB and NF-IL6 in induction of IL-6 transcripts by monocytes was demonstrated using deleted forms of the IL-6 promoter. Activation of the IL-6 promoter by LTB_4 was not only associated with accumulation of the respective transcripts but resulted in synthesis of functional IL-6 protein as well. In addition, LTB_4 mediated transcactivation of a heterologous promoter construct containing the NF-κB or the NF-IL6 enhancer, but not the AP-1 enhancer. The signalling events mediating this effect appeared to involve the release of H_2O_2, since LTB_4 failed to induce NF-κB or NF-IL6 in the presence of the scavenger of H_2O_2, N-acetyl-L-cysteine.

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IL-3 AND GM-CSF INDUCE RAPID SERINE-PHOSPHORYLATION OF THE SMALL STRESS PROTEIN (hsp27) INVOLVING ACTIVATION OF THE MAPKAP-KINASE 2. A. Ahlers, K. Engel, M. Gaestel, F. Herrmann, and M.A. Brach

Both Interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) have been previously identified to induce rapid phosphorylation of the MAP-kinase (Blood 79:2880,1992). However, little is known about signalling events initiated by IL-3/GM-CSF which occur downstream of the MAP-kinase. MAP-kinase has been shown to phosphorylate the AP-1 transcription factor and to activate two kinases designated insulin-stimulated protein kinase-1 (ISPK-1) and MAPKAP-kinase 2. We here report that IL-3 and GM-CSF induce MAPKAP-kinase 2 activity in the human megakaryoblastic leukemia cell line MO7E and phos-phorylate the human small heat shock protein hsp27 on serine residues. In contrast, neutrophils failed to phosphorylate hsp27 upon IL-3, while GM-CSF induced hsp27 phosphorylation in a similar range as observed in MO7E cells suggesting that MAPKAP-kinase 2 mediated hsp27 activation occurs independently of proliferation. Hsp27 phosphorylation is dose-dependent and occurs as early as 5 minutes following exposure to IL-3 or GM-CSF. Moreover, hsp27 phosphorylation is inhibited by tyrosine kinase inhibitors such as genistein or herbimycin A. In addition, we show that protein tyrosine phosphatase and protein phosphatase 2A (PPA2) interfere with the ability of IL-3 or GM-CSF to induce serine phosphorylation of hsp27. Taken together, our findings indicate that tyrosine phosphorylation of MAP-kinase is a prerequisite for serine phosphorylation of hsp27 mediated by MAPKAP-kinase 2. Hsp27 is localized in the nucleus and has been linked to the cellular stress response. Its precise function, however, is largely unknown. Our data identify hsp27 as a target of IL-3/GM-CSF stimulation via MAP-kinase and MAPKAP-kinase 2. Further-more, our results indicate that hsp27 may also exert phosphorylation-activation functions involved in growth signalling pathways in unstressed cells.

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IONIZING RADIATION INDUCES EXPRESSION OF IL-6 BY HUMAN FIBROBLASTS INVOLVING ACTIVATION OF NUCLEAR FACTOR-κB

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We here report that human lung fibroblasts respond to X-ray treatment (XRT) with release of Interleukin (IL)-6. Synthesis of IL-6 upon ionizing radiation is preceded by an increase of IL-6 transcript levels resulting from transcriptional activation of the IL-6 gene. Analysis of deleted fragments of the IL-6 promoter indicated that transcriptional activation of the IL-6 promoter was due to enhanced binding activity of the transcription factor nuclear factor (NF)-κB. Although activation protein (AP)-1 did not participate in the rapid induction of the IL-6 promoter, its binding activity was also enhanced by XRT. In contrast to binding kinetics observed with NF-κB, AP-1 binding following XRT was biphasic with the second peak being dependent on de novo protein synthesis. In contrast, however, NF-IL-6 activity was not enhanced by XRT in fibroblasts. The introduction of both the NF-κB- and the AP-1 recognition sequence, confered inducibility by XRT to a heterolgous promoter, with reporter gene activity being maximal 24 hours or 48 hours following XRT, respectively. Sequential acitvation of two distinct transcription factors might thus contribute to synchronize transcriptional activation of different genes participating in the X-ray (XR) response.

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SPONTANOUS DEGRADATION PRODUCTS OF CEFTAZIDIME, A BETALACTAM ANTIBIOTIC, CAUSE GREATER SUPPRESSION OF MYELOPOIESIS IN LONG-TERM BONE MARROW CULTURE

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In recent years it has been shown that the mechanism of betalactam antibiotic-induced agranulocytosis involves a direct inhibition of the replicative DNA polymerases alpha, delta, and epsilon. In this report we examine, as a representative of these antibiotics, the effect of freshly prepared ceftazidime (CEF) and degradation products of CEF on myelopoiesis. We investigated freshly dissolved CEF and CEF incubated for 96 hours at 37° (CEF_d) on the production of myeloid cells in the supernatant (SN cells) and colony stimulating activity (CSA) by the stroma. One week after drug treatment of the mLTBMC a significant, dose-dependent inhibition of the myeloid cell production ($x10^6$±SEM) and CSA (assessed by CFU-GM assay) occured, as summarized in the following table:

	day 7		day 8		day 14		day 15	
	SN cells		CSA		SN cells		CSA	
control culture	2.61±0.2		33.4±4.3		1.99±0.2		21.2±2.0	
CEF fresh 500 µg/ml	0.62±0.1		12.8±4.2		0.25±0.0		14.0±2.5	
CEF_d 100 µg/ml	1.20±0.1		25.5±4.4		0.61±0.1		19.9±3.2	
CEF_d 250 µg/ml	0.85±0.1		15.4±3.8		0.33±0.0		14.8±3.0	
CEF_d 500 µg/ml	0.45±0.1		4.6±2.3		0.23±0.0		9.2±1.6	

Four weeks after the treatment all values returned to controls, except for the cell production in CEF_d 500 µg/ml treated cultures. These results indicate that degradation products of CEF result in a reversible myeloid toxicity that is greater then freshly prepared CEF.

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HEMATOPOIETIC STROMAL MICROENVIROMENT PROMOTES RETROVIRUS-MEDIATED GENE TRANSFER INTO HEMATOPOIETIC STEM CELL

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Using Southern blot analysis and ADA probe, we demonstrated that prestimulation of bone marrow cells over an in vitro culture of stromal adherent cells (ACL) provides favourable conditions for gene transfer in the absence of exogenous growth factors. It was also found that the developmental fate of hematopoietic stem cells varied with the presence of exogenous growth factors or an ACL in the prestimulation phase. Use of an adherent cell layer may be advantageous for successful hematopoietic stem cell gene therapy.

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ROLE OF THE STROMA IN THE REGULATION OF HEMOPOIESIS

K.M. Abdulkadyrov, V.I. Rugal

On the basis of study of functional and morphological characteristic of bone marrow stromal tissue of human fetuses, 8-27 week gestation, and of adults aged 16-60 years, and in experiments on animals, the role of the sinusoidal endothelium, reticular, fat and endosteal cells in hemopoiesis regulation has been concretely defined.

The endothelium of sinuses forms the histo-hematic barrier "bone marrow-blood", ensures the transcellular migration of stem cells and mature blood cells, releases hemopoiesis-regulating factors and is involved in the erythroid cell maturation. Bone marrow reticular cells participate in the formation of intramedullar supporting frame-work, regulate the transvasal and intramedullar cell migration, form the extracellular matrix, produce humoral regulators of hemopoiesis, effect the cell differentiation by way ot their intercellular contacts with hemopoietic precursors and give origin to adipocytes. Intramedullar adipocytes present an energetic depot of hemopoietic and stromal tissues and in the stage of preadipocytes they effect, by means of contacts, the granulocyte development. The endosteal cells of bone marrow are the source of intramedullar stromal tissue, they participate in the anchorage of the hemopoietic stem cells and form the microenvironment of the latter, regulate the endosteal Ca ion levels and might be a possible source of hemopoietic tissue (population of cells of the residual embryonal mesenchyma). The thesis on the mechanism causative of the impaired regulation of precursor proliferation and differentiation in hematologic diseases is proposed.

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CD34-POSITIVE SELECTION IS EFFECTIVE TO SEPARATE FUNCTIONALLY NORMAL HEMOPOIETIC STEM CELLS FROM PATIENTS WITH NON-HODGKIN'S LYMPHOMA TREATED WITH rhG-CSF AND CHEMOTHERAPY.

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CD34+ cells contain early hemopoietic stem cells which are capable of sustaining hemopoiesis. The *in vitro* expansion of these cells would be beneficial in supporting the chemotherapy of hematological/oncological malignancies. We report on first data concerned the evaluation of the hemopoietic potential of the CD34-positive enriched fraction from patients with non-Hodgkin's lymphoma treated with rhG-CSF and chemotherapy. The number of the colony-forming units (CFUc) in CD34+ fraction was compared with those in the native preparation of peripheral cells, in mononuclear cells after ficoll density separation (MNC), and in the fraction of non-adherent cells (NAC) after CD34-positive selection by using the AIS MicroCELLector T-25 cell culture flasks (Applied Immune Systems, USA).

8 samples from 4 patients were studied. The outcome of CD34+ fraction was in the range from 1.8% to 3% of the MNC prepared (6 experiments). The methylcellulose cultures of all studied fractions were established in 24-well plates in 0.5 ml of Gibco stem cell growth medium. 10,000 cells per well of the native cells, MNC, NAC and 1,000 cells per well for the CD34-enriched fraction were stimulated for colony formation by the addition of 50 ng rhSCF (Amgen, USA), 10 U rhIL-3 and 1 U rhEpo (Boehringer Mannheim, FRG). Colonies were counted after 14 days of incubation.

A significant enrichment of the CFUc was obtained. Comparing with MNC the CD34-positve fractions were 24.1 to 61.1-fold enriched for CFUc and 18.5 to 64.2-fold for CFUc + clusters. This enrichment was most prominent for CFUmix which contained many immature blast cells. In contrast, there were only few or no CFUmix among the native cell-preparation as well as MNC and NAC. These data suggest that CD34-positive selection is effective for obtaining stem cells to be expanded *in vitro*.

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MOBILIZATION OF CFU-S INTO CIRCULATION FOLLOWING rhG-CSF AND EPO TREATMENT OF MICE

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The self-renewal capacity of CFU-S (spleen colony-forming unit) following the treatment of (CBAxC57B1)F1 female mice with recombinant human granulocyte colony-stimulating factor (rhG-CSF) was investigated. The possibility of synergism between erythropoietin (EPO) and rhG-CSF in blood CFU-S mobilization was also studied. During the investigation the following observations were made: 25 and 250 mkg/kg/day injection of rhG-CSF expended the number of CFU-S-11 in circulation 4- and 32-fold, accordingly. The self-maintenance potential of peripheral blood CFU-S-11 did not change significantly. The treatment of mice with 250 mkg/kg/day of rhG-CSF resulted in two fold increase of spleen cellularity and 15 fold augmentation of CFU-S-11 number, without noticeable changes in their self-renewal capacity. Moderate changes in CFU-S-11 number were observed after EPO administration in spleen and peripheral blood, however no significant synergistic effect of EPO with both doses of rhG-CSF was detected. The multifold increase of CFU-S-11 number in peripheral blood following rhG-CSF administration with no reduction in their self-maintenance potential suggests that mobilized with rhG-CSF blood stem cells provide an appropriate source for reconstitution of the hematopoietic system.

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EFFECTS OF IL-2 AND IL-4 AND ITS COMBINATION ON B-CLL-CELLS

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We used the BrdU-Incorporation method to show the effects of IL-2 (100, 1000, 3000 U/ml),IL-4 (0.1, 1, 10 ng/ml) and IL-2 (3000 U/ml) plus IL-4 (10 ng/ml) on B-CLL-cells. After Ficollseparation, lysis of the erythrocytes (NH_4Cl) and lysis of monocytes (1-leucin-methyl-ester), cells were divided into two groups. Group 1 was cultured in a serum free medium (+BrdU +cytokine) without any T-cell depletion. Group 2 was cultured in a serum free medium (+BrdU +cytokine) after T-cell ($CD3^+$) elimination by the MACS (Magnetic Activated Cell Sorting). Samples were taken after 20h, 68h, 92h, 116h and 140h. After staining with anti-BrdU FITC and propidiumiodide (PI) proliferation was measured by flow cytometry (FACS scan).

IL-2	G1	G2	IL-4	G1	G2	IL-2+ IL-4	G1	G2
100 U/ml	P=6 N=3 I=0	P=2 N=7 I=0	0,1 ng/ml	P=1 N=16 I=0	P=0 N=15 I=1	IL-2 3000 U/ml	P=10 N=0 I=0	P=10 N=0 I=0
1000 U/ml	P=7 N=2 I=0	P=7 N=2 I=0	1 ng/ml	P=1 N=12 I=4	P=1 N=13 I=2	IL-4 10 ng/ml	P=1 N=4 I=5	P=1 N=4 I=5
3000 U/ml	P=8 N=1 I=0	P=7 N=2 I=0	10 ng/ml	P=4 N=11 I=2	P=6 N=8 I=2	IL-2 + IL-4	P=0 N=3 I=7	P=1 N=1 I=8
	n=9	n=9		n=17	n=16		n=10	n=10

P=proliferation N=no effect I=inhibition

Conclusions: IL-2 shows a proliferative effect on B-CLL-cells independent of T-cells. IL-4 shows heterogeneous effects. By itself it has most often no effect on proliferation, but sometimes it inhibits or increases the proliferation. This effect does not seem to depend on T-cells. It could depend on the dosage or some unknown patients' characteristics. Further on IL-4 inhibits IL-2 induced proliferation in nearly all cases independent of T-cells.

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STIMULATION AND DIFFERENTIAL MODULATION OF G-CSF AND GM-CSF PRODUCTION BY INTERLEUKIN-1 AND TUMOR NECROSIS FACTOR IN HUMAN VASCULAR ENDOTHELIAL CELLS

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Il-1 and TNF are inflammatory cytokines with overlapping biological activities. Human vascular endothelial cells express Il-1 and TNF receptors and respond to Il-1 and TNF stimulation by elaboration of colony stimulating factors such as G-CSF and GM-CSF. However quantitative data are required in order to evaluate the contribution of Il-1 and TNF to the activation of inflammatory hemopoietic cells such as granulocytes and macrophages by CSFs. Therefore we quantified the production of G-CSF and GM-CSF in endothelial cell cultures subsequent to treatment with IL-1α (0.1-200U/ml) and TNF(10-2000U/ml) or Il-1 in combination with TNF. A dose-dependent stimulation of G-CSF and GM-CSF secretion was found following Il-1 and TNF treatment of endothelial cells. Il-1 was a more potent inducer of G-CSF secretion than was TNF using approximately equipotent doses of IL-1 (100U/ml) and TNF (1000 U/ml) regarding the induction of GM-CSF. In addition significant superadditive stimulation of G-CSF and GM-CSF production was found with a low dose of Il-1 (1U/ml) and a saturating dose of TNF(1000U/ml) in combination. However the costimulatory effect of Il-1(1U/ml) was significantly more pronounced with G-CSF than with respect to GM-CSF. In summary the differential modulation of G-CSF and GM-CSF production in endothelial cells by Il-1 and TNF may indicate a disparate role of Il-1 and TNF in vascular inflammatory processes and atherosclerosis regarding recruitment and activation of inflammatory leukocytes.

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STEM CELL FACTOR (SCF; C-KIT LIGAND) AS INFLAMMATORY MEDIATOR: DIFFERENTIAL REGULATION OF SCF EXPRESSION IN HUMAN ENDOTHELIAL CELLS BY VARIOUS BACTERIA

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Production of hematopoietic growth factors by endothelial cells play a pivotal role during inflammatory processes. Stem cell factor (SCF) is known to be expressed constitutively in endothelial cells. In order to investigate the regulation of this cytokine expression by inflammatory stimuli, we cocultured human umbilical vein endothelial cells with various gram-negative bacterial strains (Escherichia coli, Yersinia enterocolitica, and Chlamydia trachomatis, respectively). Experiments were performed with bacterial concentrations ranging from 10^2 to 10^7 infectious units/mL for three hours. SCF specific mRNA expression was studied using Northern blot analysis. The results demonstrated that stimulation with both enteropathogen bacterial strain Y. enterocolitica or with E. coli, led to a significant concentration dependent increase of SCF mRNA expression. Kinetic experiments with E. coli showed also a time dependent accumulation of SCF transcripts. In contrast coculture of human endothelial cells with the intracellular gram-negative strain C. trachomatis had no effect on SCF mRNA expression. As a component of gram-negative bacteria we stimulated human endothelial cells with lipopolysaccharide (LPS). LPS induced a maximal SCF mRNA accumulation within two hours followed by decrease of SCF specific transcripts to the basis level after 24 hours. From these data we conclude that SCF expression is regulated by bacterial stimuli, suggesting an important role of its expression in human endothelial cells during inflammation.

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IFN-γ INDUCED EXPRESSION OF THE $CD16_{TM}$-RECEPTOR COMPLEX ON INTRINSIC HUMAN GLOMERULAR MESANGIAL CELLS (HMC)

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Glomerular deposition of immune complexes, followed by activation of complement and generation of chemoattractants, may be involved in the initiation and exacerbation of human glomerulonephritis. With primary cultures of HMC from three different donors we determined the capacity of these mesenchyme-derived cells to express receptors for IgG. We employed polymerase chain reaction from reverse transcribed RNA (RT-PCR) of HMC, which have been growth arrested (48 hrs serum-free) or were kept under cycling conditions (medium with 10% FCS). Resting HMC were stimulated for additional 20 hrs with recombinant interleukin-1β, transforming growth factor-β (TGF-β), and both resting and proliferating HMC with interferon-γ (IFN-γ), lipopolysaccharide E.coli (LPS) and combinations thereof. Primer pairs specific for the three types of human Fcγ receptors (CD16, CDw32 and CD64) were used and the identity of the resulting RT-PCR products controled by Southern blot hybridization with independent [α^{32}P]-labeled FcγR cDNA-probes. Our studies showed that in contrast to control cells, such as human monocytes, granulocytes or HL-60 myeloid tumor cells, mRNA for FcγRI (CD64) and FcγRII (CDw32) was not detectable in resting or unstimulated proliferating HMC. IFN-γ in combination with LPS but not LPS alone elicited a significant transcription of FcγRIII mRNA in growth arrested HMC, while other stimuli tested failed to induce any of the three Fcγ receptor types. Proliferating HMC showed a basal transcription of FcγRIII mRNA, which was significantly enhanced by IFN-γ in combination with LPS. Slot blot analysis with specific oligonucleotides distinguishing between the FcγRIII-A and the FcγRIII-B gene indicates that only the FcγRIII-A gene encoding the transmembrane isoform of FcγRIII ($CD16_{TM}$) is expressed in HMC. Transcripts for the γ subunit of the FcεRI receptor were detected in HMC, and presumably γ proteins are co-associated with $CD16_{TM}$ in these cells. The $CD16_{TM}$-receptor complex were functionally active as we demonstrated stimulation of HMC IL-6 mRNA synthesis with heat aggregated IgG (50 μg/ml) only after induction of FcγRIII-A transcription by IFN-γ alone or in combination with LPS.

In conclusion the $CD16_{TM}$-receptor complex expressed on the surface of intrinsic glomerular mesangial cells may play a critical role within the pathogenesis of human glomerulonephritis.

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DIFFERENTIAL EXPRESSION OF CYTOKINES IN HUMAN LYMPHOMAS AND LEUKEMIA AND THEIR RELATION TO P56LCK,

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Cytokines may regulate the growth and differentiation of normal hematopoietic cells and are possibly involved in the biology of malignant lymphoma and leukemia. The expression of cytokines is mainly regulated by extracellular factors via specific signal transduction pathways. P56lck is an tyrosine kinase involved in specific signal transductions from the cell surface especially for IL2 and IFN-gamma.

To analyze the role of cytokines and p56lck in human lymphoid neoplasia, we have studied as a first step the transcription of interleukins in acute lymphoblastic leukemias, Burkitt's lymphoma and Hodgkin's disease derived cell lines using the polymerase chain reaction. The results are summerized in the table:

	IL2	IL3	IL4	IL5	IL7	IL8	IL9	IL10	IL11	IL12
c-ALL	2/13	5/12	5/12	0/19	10/19	17/19	0/19	17/19	1/12	9/13
preB/B-ALL	2/6	0/6	2/6	0/6	3/13	11/13	0/13	12/13	5/6	5/6
preT/T-ALL	2/6	0/6	1/5	0/6	0/13	11/13	0/13	2/13	4/6	2/6
Burkitt's lymph.		1/18	4/18	4/18	2/18			12/18		
Hodgkin' disease		2/8	1/8	2/8	3/8	1/8		4/8		
activ.PBL	+						+	+	+	+
PBL	+	-	-	-	+/-	+	-	-	-	+/-

Thus these data demonstrate that certain interleukins are differentially transcribed in lymphoid neoplasia.

We have shown the expression of the protein of p56lck using Western blotting and immunoprecipitation in cell lines derived from T-ALL and Burkitt's lymphoma. Now we are analyzing in more detail the crossregulation of IL2/IFN-gamma in T-cell neoplasia and IL10 in B-cell neoplasia with p56lck in dependence from different stimulations and inhibitions (Herbimycin, Polymyxin-B).

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CYTOKINE PRODUCTION BY HUMAN BONE MARROW: EFFECTS OF UVB-IRRADIATION

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Cytokines are known to be involved in the pathophysiology of graft *versus* host disease (GHVD) and to effect lympho-hematopoetic progenitor cell growth after bone marrow transplantation. The use of T-cell depleted marrow in human transplantation is associated with suppression of GVHD but decreased rates of engraftment. We have shown in rodent models that UVB irradiation (UVB) of donor inoculum inhibits GVHD while preserving engraftment. To determine the effects of UVB on cytokine production by cells associated with GVHD, human marrow mononuclear cells isolated by ficoll density gradient were UVB-irradiated at doses of 10-200 J/m2 and then stimulated with PHA/LPS or allogeneic cells. ELISA assays were used to measure the production of GM-CSF, IL-3, LIF, IL-1beta, IL-2, IL-6, TNF-alpha, and IFN-gamma by stimulated cells . Two week methylcellulose cultures were used to determine viability of CFU-GM, BFU-E and CFU-Gemm progenitor cells after UVB. All results were compared to non-UVB-irradiated marrow and to marrow depleted by soybean agglutination and sheep erythrocyte rosetting. Progressively increasing doses of UVB produced progressively decreasing levels of all cytokines except IL-6, which remained unchanged. T-cell depleted marrow produced decreased levels of all cytokines except IL-6. UVB at 75 J/m2 resulted in higher levels of GM-CSF and IL-3 as compared to T-cell depleted marrow. This same dose of UVB essentially preserved CFU-GM, BFU-E, and CFU-Gemm colonies. We conclude that UVB may inhibit the cytokine cascade active in GVHD while preserving progenitor cell growth at UVB 75 J/m2. UVB may serve as GVHD prophylaxis in clinical marrow transplantation and *in vivo* studies on non-human primates are in preparation.

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BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF CYTOKINES FROM QUARTZ DUST (DQ12) TREATED HUMAN MACROPHAGES

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A large number of substances from the working place and in the general environment such as quartz and coal mine dusts are causing silicosis and leading to lung fibrosis. Alveolar macrophages are the primary target for the noxious effect of quartz dust.
Quartz dust incubated human macrophages release in vitro a cytokine, which stimulates cell proliferation of human lung fibroblasts (Wistar 38). In recent studies we found that beside fibroblasts also epithelial cells of the alveolar unit, such as pneumocyte type II cells (A-549) respond to the cytokine with strong proliferation.
Supernatants of quartz dust exposed macrophages were concentrated by ultrafiltration and thereafter fractionated by gel-filtration (Sephadex G150, Pharmacia). Biological activity of the factor eluted within a molecular range of 75-102 kD. Furthermore, the factor was purified by anion exchange chromatography (Q-Sepharose). Fractions revealing biological activity were further analysed by SDS-gel electrophoresis (PAGE) and showed two protein bands with apparently molecular masses of 76 and 79 kD,respectively.
After addition of the supernatant initially spindle shaped pneumocytes were detected, followed by epithelial-like cells when cell proliferation progressed.
Induction of spindle-shaped pneumocyte type II cells could also be seen after addition of pure Tumor Necrosis Factorα. However, in this case no cell proliferation was observed. We assume that beside the cytokine, which is responsible for the induction of cell proliferation TNFα is present in supernatant.

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THE ROLE OF AUTOCRINE MOTILITY FACTOR AND ITS RECEPTOR AS A TUMOR DERIVED CYTOKINE IN BLADDER CARCINOMA

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The ability to migrate is fundamental for the acquisition of invasive properties by tumor cells. A tumor derived cytokine was identified by its ability to induce direct and random migration via a receptor mediating signal pathway, i.e. autocrine motility factor (AMF). We identified the receptor for AMF (HAMF-R) and found that hAMF-R plays a role in invasiveness and metastasis in human bladder carcinomas.

We investigated the expression pattern of hAMF-R in 83 fresh frozen bladder cancer specimens by immunofluorescence technique on the translation level and found a strong correlation ($p<0.01$) to tumor stage and grade. Furthermore we have shown that patients who were found to be hAMF-R positive have an increased risk for early tumor progression ($p<0.05$).

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COMPARISON OF SERUM LEVELS AND CONCENTRATIONS OF INTERFERON–γ AND IL-1–α AFTER STIMULATION OF PERIPHERAL WHOLE BLOOD IN SARCOIDOSIS

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Patients with pulmonary sarcoidosis show characteristic differences in a variety of immunologic parameters. In peripheral blood however differences to healthy persons have hardly been described. In this investigation we compared serum levels and concentrations of Interferon–γ (IFN–γ) and IL-1–α in stimulated samples of whole blood. 50 µl whole blood of 38 healthy controls (REF) and 39 patients with sarcoidosis, 27 without treatment (POT) and 12 under corticoid medication (PUT), was incubated in medium at 37°C and 5% CO_2. Con A was used for stimulation of IFN–γ and LPS for IL-1–α. Concentrations were determined with an ELISA. Results: Without stimulation no measurable amounts of IFN–γ could be found. After stimulation REF showed median concentrations of 127 ng/ml, POB 10 ng/ml and PUT 2 ng/ml. The difference between REF and PUT respectively POT was statistically significant. IL-1–α: without LPS the differences were not significant. Under stimulation POT had significantly higher values (220 pg/ml) compared to REF (170 pg/ml) and also to PUT (155 pg/ml). In conclusion we were able to demonstrate that in contrast to serum levels stimulation of peripheral whole blood reveals significant differences in concentrations of IFN–γ and IL-1–α between patients with sarcoidosis and healthy references.

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SEMIQUANTITATIVE ASSESSMENT BY PCR OF CYTOKINE mRNA-TRANSCRIPTS OF HUMAN MONONUCLEAR CELLS AND CELL LINES IN VITRO

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We established a modified polymerase chain reaction protocol for the detection and semiquantitative assessment of mRNA-transcript levels for IL-2, IL-4, IL-5, IL-6, IFN-γ, TNF-α, GM-CSF, TGF-β and IL-2-receptor-α (IL-2R). The method was shown to distinguish 10-fold differences in template concentration after 4 rounds each of amplification in the range from 20 to 40 cycles; the lower threshold of sensitivity was at 10 copies per PCR-reaction. Reproducibility was >95% for a positive result after 32 rounds of amplification; it decreased to 80% after 36 rounds. This corresponded to the detection of 1,000 and 100 template copies, respectively, per PCR-reaction.
Human peripheral blood mononuclear cells (PBMC) and tumor cell lines were evaluated for mRNA-expression with or without stimulation and these results were compared to secretion of the corresponding cytokine. For PBMC, constitutive mRNA-expression was found positive for TNF-α, IFN-γ, IL-4, IL-6, TGF-β and IL-2R, whereas detectable expression of IL-2, IL-5 and GM-CSF was induced only after stimulation. Using β-actin as an internal standard, the PCR could demonstrate relative differences in cytokine transcripts after stimulation with (A) 100 IU/ml IL-2, (B) 10% lymphocyte-culture conditioned media (CCM) and (C) PMA (10ng/ml) plus A23187 (100ng/ml). For IL-2 transcripts no detectable expression was found without stimulus or after addition of IL-2, whereas CCM resulted in a 1,000- and PMA/A23187 in a 10,000-fold increase. Other mRNA-transcripts increased 10-fold (TNF-α) up to 10,000-fold (GM-CSF) with or without differences between the stimulating agents.
The cell lines CAKI-1 (renal cell carcinoma) and Daudi (Burkitt lymphoma) also expressed comparable levels for cytokine transcripts, with a strong induction after stimulation with PMA/A23187. The relative IFN-γ mRNA-content in CAKI-1 increased from 0 to 1,000, for GM-CSF from 0 to 10,000.

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THE REDUCED DNA SYNTHESIS AND IL-2 PRODUCTION OF MONONUCLEAR CELLS FROM PATIENTS WITH NON-HODGKIN-LYMPHOMAS IN VITRO IS NOT INDUCED BY TGF-ß

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Recent studies implicating a deficiency of interleukins in several diseases have underlined the importance of measuring in vitro the DNA synthesis and the cytokine production (IL-1, IL-2, IL-6, TNF-alpha) in the same cell system. Previously had found that normal peripheral blood mononuclear cells (MNC) of patients suffering from high-malignant Non-Hodgkin lymphomas showed a decreased capability to proliferate after mitogenic stimulation (PHA, Con A, PWM). Here we have studied the DNA synthesis and the production of different cytokines (IL-1, IL-2, IL-6, TGF-ß and TNF-alpha) by pokeweed mitogen (PWM) stimulated MNC from 15 healthy control subjects and from 14 patients with NHL. The IL-2 production of PWM-stimulated MNC of patients with NHL was found to be significantly decreased, wheras the IL-1, IL-6 and TNF-alpha release were not changed significantly. These data showed a good correlation with the reduced capability of MNC from patients with NHL to proliferate after mitogenic stimulation. The multifunctional cytokine Transforming Growth Factor-ß (TGF-ß) is known to inhibit the DNA synthesis, as well as the IL-2 production of mitogen-stimulated MNC. However, TGF-ß release was not significantly changed in cell culture supernatants from patients with NHL in comparison to healthy controls. We conclude that the suppressed DNA synthesis and IL-2 production of MNC from patients with NHL is not the consequence of a deceased TGF-ß level secreted by these cells.

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EFFECTS OF CYTOSTATIC DRUGS ON INTERLEUKIN-6 PRODUCTION BY HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS IN VITRO.

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The influence of different cytostatics on IL-6 production by peripheral blood mononuclear cells (PBMC) was studied. PBMC were pre-incubated with or without phytohemagglutinin P (PHA-P), then pulse exposed during 1 hr to different cytostatics in their therapeutical concentrations, washed three times and incubated additionally during 24-72 hrs. Then the supernatants were collected and IL-6 biological activity was measured in MTT-modified B9-cells biological assay.

Though significant individual variations of the IL-6 production were found, all the studied drugs can be separated on several groups:

1) adriamycine and methotrexate induced late increase of IL-6 production (48-72 hrs);

2) cytarabine strongly increased the early as well as the late IL-6 production ;

3) the pretreatment with etoposide and rubomycine suppressed subsequent production of IL-6 by PBMC during 24-48 hrs, the late production was increased;

4) in contrast, the cyclophosphamide pretreatment stimulated the early production and strongly suppressed the late one.

The changes of IL-6 production by PBMC was not due to the cellular death. The PHA-P stimulated PBMC produced usually more IL-6 that unstimulated cells did. These data suggest that the cytostatics possess the different effects on IL-6 production by PBMC that could be important in the therapy of malignancies.

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G-CSF AND IL-6 SERUM LEVELS IN PATIENTS WITH HEMOPOIETIC MALIGNANCIES RECEIVING CHEMOTHERAPY

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Myelosuppression resulting in leukopenia and thrombocytopenia is a common problem after chemotherapy and requires supportive care until normal hemopoiesis has recovered. To study the importance of endogenously produced cytokines for regeneration of bone marrow progenitors we measured serum levels of G-CSF and Il-6. Blood samples were obtained before and 24 hours after chemotherapy, during the stage of leukopenia (<1000/ul) and recovery (>1500/ul)of leucocyte counts. In 6/7 patients we found a more than 5-fold increase in serum G-CSF levels at the stage of leukopenia. Highest amounts were observed in two patients with lethal outcome. There was no correlation between thrombocytopenia and levels of G-CSF or IL-6.

	serum G-CSF (pg/ml, mean, range)	
	before (leuko>1500)	after (<1000/ul)
chemotherapy		
AML DAV	25 (5-100,n=14)	2000 (50-8000,n=7)
ALL 'Hölzer'	100 (10-300,n=4)	1000 (150-2000,n=2)
NHL CEVED	120 (10-200,n=4)	nd

	serum IL-6 (pg/ml, mean, range)	
AML DAV	30 (5-90,n=14)	200 (20-700,n=6)
ALL 'Hölzer'	40 (20-70,n=4)	nd
NHL CEVED	10 (5-20,n=4)	nd

Our results suggest that after chemotherapy endogenous release of G-CSF and, to a lower extent, of IL-6 into the serum is closely related to myelosuppression in patients with hemopoietic malignancies.

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LEVELS OF HEMATOPOIETIC CYTOKINES IN CORD BLOOD AND AMNIOTIC FLUID, E. Weimann, G. Reisbach

We measured different hematopoietic cytokines as G-CSF, GM-CSF and IL-6 in amniotic fluid and cord blood to clearify their physiological and pathophysiological role in fetal and neonatal development. Amniotic fluid was available from the 15th to the 21st week of gestation (n=53), cord blood from the 26th to the 42nd week of gestation (n=60). Activity levels of cytokines were determined by stimulation of the cell lines NFS-60, TTD1, and TF-1, which are responding specifically to G-CSF, IL-6, and GM-CSF. Specificity has been proved by neutralizing antibodies. Calculation of cytokine levels was done by standards of recombinant growth factors. Sensivity for G-CSF and IL-6: 5 pg/ml; for GM-CSF: 100 pg/ml. In amniotic fluid G-CSF ranged from 40 to 123.000 pg/ml and IL-6 from 20 to 12.000 pg/ml, whereas GM-CSF was not detectable. In cord blood G-CSF was between 5 and 23.000 pg/ml and IL-6 between 5 and 30 pg/ml. In most of the samples (90%) GM-CSF was beyond the sensivity limit. In 92% (11 of 12 cases) G-CSF levels were elevated over 200 pg/ml and associated with amnion infection syndrome, while green amniotic fluid alone during delivery did not stimulate the production of G-CSF. The levels of the hematopoietic cytokines showed no influence by the gestational age. Identical twins without maternal infection showed the same G-CSF levels. - Inflammation of the amniotic membrane and maternal sepsis is associated with elevated G-CSF levels in cord blood. GM-CSF can normally not be detected in cord blood and amniotic fluid. Detectable levels of GM-CSF in cord blood and amniotic fluid maybe give a hint for a pathological situation during pregnancy.

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SERUM LEVELS OF sCD8, sCD25, AND sCD23 IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA: CORRELATION WITH DISEASE ACTIVITY AND TUMOR LOAD

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Soluble forms of CD8 and CD25 (sCD8/sCD25) are found to be elevated in patients (pts) with active autoimmune disorders as well as with malignant lymphomas. In addition, CD23 and its soluble form (sCD23) is thought to be involved in the regulation of B-cell proliferation. Therefore, we examined sCD8, sCD25, and sCD23 in pts with B-cell chronic lymphocytic leukemia (B-CLL) in order to assess their role as indicators of disease activity. Fifty-five pts with B-CLL were studied so far. Staging was performed according to the classification systems of Rai and Binet, respectively. Serum samples were freshly stored in liquid nitrogen until further processing. Levels of sCD8, sCD25, and sCD23 were measured by a sandwich ELISA technique using commercially available assays (Biermann, Germany). Advancing Rai stages were associated with a progressive increase of all the three serum factors (sCD8: Rai 0 556$\pm$165 U/ml vs Rai IV 1310$\pm$150 U/ml; sCD25: Rai 0 1326$\pm$190 U/ml vs Rai IV 8850$\pm$1330 U/ml; sCD23: Rai 0 1265$\pm$455 U/ml vs Rai IV 6500$\pm$1800 U/ml). This progression was also evident when Binet's classification was applied. Occurrence of B-symptoms was associated with high levels of sCD25 ($p<0.001$), whereas sCD8 and sCD23 were found also to be increased but without statistical significance. High levels of all the three factors strongly correlated with a lymphocyte doubling time <12 months, a lymphocyte count $>50.000/\mu l$, and with the presence of hepato- and splenomegaly. Interestingly, occurrence of bulky lymph nodes (i.e. at least one nodule of >8 cm in diameter) was linked with high levels of sCD23 only ($p<0.002$). In summary, (1) progressive serum levels of sCD8, sCD25, and sCD23 correlate with advancing stages of disease in B-CLL. (2) B-symptoms were associated with high levels of sCD25. (3) We found sCD23 to be the more sensitive marker of the total tumor load than sCD8 and sCD25. Thus, sCD23 may be useful in monitoring pts with B-CLL.

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TNF, IL-1, IL-6 PRODUCTION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

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Total number of 110 children with ALL, 68 boys and 52 girls, aged from 5 to 15 years were included to the study. TNF production was studied acc. the method based on growth inhibition of sensitive to TNF L 929 mice fibroblasts, IL-1 production acc. method based on inhibition of autologous rosette formation by thymocytes of CBA mouse and IL-6 production acc. to conventional ELISA Genzyme-test. Twenty five healthy children served as the control group. It was found that in children with ALL during the whole period of therapy the IL-1 and IL-6 production, was significantly lower than that observed in the control group of healthy children (p 0.005). The TNF production in ALL children before therapy was lower in comparison with the control group values. During cytostatic therapy was higher and grew up above the normal limits after cessation of the therapy. IL-1 production grew up after the end of the therapy but never reached the value of the control group. EFS at 54 mth in ALL children with IL-1 production $<10\ \mu$ before therapy was higher than that in children with IL-1 $<10\ \mu$ (EFS 90 % v 70 %). The IL-1 production seems to be a good prognostic marker.

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MODULATION OF INTRACELLULAR ARA-C METABOLISM IN AML BLASTS BY GM-CSF PRIMING IN VIVO. C. Reuter[1,3], U. Auf der Landwehr[2], U. Auf der Landwehr[2], B. Woermann[1], Th. Buechner[2], & W. Hiddemann[1].

Cytosine arabinoside (Ara-C) is one of the most active single agents in the treatment of acute myeloid leukemia (AML). Its cytotoxic activity mainly depends on its phosphorylation to Ara-CTP and on its incorporation into the DNA. Based on recent in vitro studies showing that hematopoietic growth factors like GM-CSF and IL-3 enhance the cytotoxicity of Ara-C on clonogenic leukemic cells, the GM-CSF priming concept is currently explored in clinical phase II and III studies. In an ongoing study at the Universities of Muenster and Goettingen GM-CSF ($250\mu g/m^2/d$) is started 24 hrs before induction chemotherapy (TAD9/HAM) until recovery of blood cell counts. This study provided a means to assess the effect of GM-CSF on the intracellular Ara-C metabolism in vivo in 23 pts with AML. Enzyme activities of deoxycytidine kinase (dCK), thymidine kinase (TK), deoxycytidine deaminase (DCD), DNA polymerase (Pol) and DNA polymerase alpha (Pola) were determined before therapy, 24 hrs after the administration of GM-CSF and 48 hrs after the administration of Ara-C. In addition, Ara-C incorporation into the DNA was measured after 48 hrs Ara-C administration. Enhancement of enzyme activity was observed in 11/15, 9/17, 10/16, 6/16 and 7/16 cases for Pola, Pol, TK, dCK and DCD, respectively. Increases ranged from 13-630% for Pola (median 127%), 13-188% for Pol (median 77%), 14-820% for TK (median 113%), 10-670% for dCK (median 43%) and 29-1350% (84%) for DCD. Inadequate blast cell reduction after TAD9 (>5% blast cells on day 10 or 21) was associated with significantly higher DCD blast cell activities compared to the DCD activity values obtained for pts with adequate blast cell clearance (median values: 5.12 vs 0.6 nmol/min x mg, $p<0.01$). Cases with DCD activities <5 nmol/min x mg showed significantly higher Ara-C incorporation into the DNA compared to pts with DCD activities >5 nmol/min x mg (5.2 vs 0.65 ng/10^7 cells). Furthermore, inadequate blast cell clearance was associated with lower Ara-C incorporation into the DNA (median 0.8 vs 5.3 ng/10^7 cells) and lower Pola activities (median 1.3 vs 3.1 pmol/min x mg). In 11 pts we investigated simultaneously the effect of GM-CSF pretreatment on Ara-C metabolism in vitro. Enzyme activities of Pola, TK and Pol correlated significantly in vivo and in vitro ($r^{Pola}=0.84$, $r^{TK}=0.64$, $r^{Pol}=0.54$, $p<0.05$). These data demonstrate that GM-CSF enhances DNA synthesizing enzyme activities in vivo and in vitro. Furthermore, these data suggest that GM-CSF might improve the therapeutic response to induction chemotherapy by increasing DNA polymerase alpha activity and thereby increasing the Ara-C incorporation into the DNA.

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RECOMBINANT HUMAN INTERFERON-ALPHA AND INTERFERON-BETA2/INTERLEUKIN-6 BUT NOT INTERFERON-GAMMA INDUCE MEGAKARYOCYTIC DIFFERENTIATION OF ACUTE MEGAKARYOBLASTIC LEUKAEMIA BLAST CELLS

H.T. Hassan, S. Grell, U. Borrmann-Danso and M. Freund

The effects of Interferon-alpha (IFN-alpha), Interferon-beta2/Interleukin-6 (IL-6) and Interferon-gamma (IFN-gamma) in inducing megakaryocytic differentiation of blast cells from acute megakaryoblastic leukaemia (AMegL) patient determined by the increase in CD41 and CD42b expressions using monoclonal antibodies in APAAP technique were investigated in liquid suspension culture. After six days of culture, the percentage of CD41 and CD42b positive cells increased in control cultures from 15,2 % and 10,6 % on day 0 to 32,0 ± 4,3 % and 22,1 ± 2,5 %, respectively. The addition of IFN-alpha significantly increased the number of CD41 and CD42b positive cells by about two to three fold compared to control cultures, $p < 0,01$ and by about four to six fold compared to day 0, $p < 0,001$. Similarly, IFN-beta2/IL-6 induced a significant increase in CD41 and DC42b positive cells. On the other hand, IFN-gamma failed to increase the number of CD41 and CD42b positive cells in comparison to control cultures on day 6 and instead induced a significant increase in the number of monocytes/macrophages from only 3,7 ± 1,9 % in control cultures to 47,9 ± 2,5 %, 52,3 ± 1,4 %, 54,5 ± 2,9 % and 57,5 ± 2,3 % in 1, 10, 100 and 1000 Units/ml IFN-gamma-treated cultures, respectively, $p < 0,001$. The present results suggest that megakaryocytic differentiation of blast cells in AMegL could be induced by IFN-alpha and IL-6 and support a clinical role for IL-6 in the treatment of AMegL patients. Also, the present results showed that monocytic differentiation of blast cells in AMegL could be induced by IFN-gamma, supporting the multipotent stem or progenitor cell origin of the AMegL subtype of acute myeloid leukaemia.

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A LINEAGE MODULATORY EFFECT FOR INTERLEUKIN-4 IN HUMAN MYELOID LEUKAEMIA CELLS

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The effects of six recombinant human cytokines:Erythropoietin, GM-CSF, G-CSF, Interleukin-3, Interleukin-4(IL-4),Interleukin-6(IL-6) on the differentiation of a human multilineage myeloid leukaemia cell line 225, established from an AML(M7) patient in our laboratory determined by changes in antigen expressions using monoclonal antibodies in APAAP technique were examined in liquid suspension culture. The 225 cells has been growing exponentially without cytokines or conditioned media and are negative for CD3,CD7,CD19,CD20,TdT and sudan black. Only 6-7% of 225 cells are positive for CD13,CD14,CD15,CD34 and CD42b antigens whereas 28.3%, 87.2% and 57.6% are positive for the glycophorin A, CD33 and CD41 antigens, respectively.After five days of treatment with Erythropoietin, GM-CSF, G-CSF or IL-6 no change was observed in 225 cell antigens expressions. 100 U/ml Interleukin-3 induced a moderate increase in only CD13 and butyrate esterase positive cells from 6.5±1.9% and 5.7±2.4 in control cultures to 21.6±3.0% and 19.1±2.8%, respectively. On the other hand, 100 U/ml IL-4 significantly increased the number of CD13,CD15 and butyrate esterase positive cells to 48.9±5.0%, 47.2±3.6% and 46.1±3.0%, respectively. Also, 100 U/ml IL-4 decreased the number of CD41 positive cells from 57.6±2.8% to only 25.9±3.6% and did not change the number of CD33 or glycophorin A positive cells. The present results showed that out of the six myelopoietic growth factors tested, IL-4 was the only one to modulate the lineage-specific marker phenotype of 225 leukaemia cells by increasing the CD33+CD13+CD15+ myeloblast proportion and decreasing the CD33+CD41+ megakaryoblast proportion while the CD33+glycophorin A+ erythroblast proportion remained the same. Therefore, the present findings suggest that IL-4 may have a lineage marker modulatory effect in favour of a myeloblastic at the expense of a megakaryoblastic amplification in human leukaemia cells.

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LEUKEMIA INHIBITORY FACTOR IS INCREASED IN LONG-TERM ADHERENT LAYER CULTURES FROM LEUKEMIC PATIENTS AND CAN BE MODULATED BY INTERLEUKIN-1β, TUMOR NECROSIS FACTOR-α AND INTELEUKIN-4.

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A monoclonal antibody-based ELISA and bioassay were used to measure leukemia inhibitory factor (LIF) protein levels, activity and the functional role of LIF in supernatants of cultured stromal cells derived from patients with acute and chronic myelogenous leukemia, myelodysplastic syndrome, and hairy cell leukemia and from normal controls. We demonstrate that biologically active LIF protein is constitutively produced and secreted by cultured bone marrow stromal cells from all subjects studied. In addition, adherent-layer conditioned-media LIF protein levels were significantly elevated in samples from patients with all hematologic malignancies studied as compared to samples from normal controls.

Confluent adherent layers exposed for 24 hours to interleukin (IL) 1β or tumor necrosis factor-α (TNF-α) showed a significant increase in LIF protein levels, whereas exposure to IL-4 (Sterling Drug Inc., Great Valley, PA) resulted in a dose-dependent decrease in LIF levels.

	Pre/Median Range(ng/ml)	Post/Median Range(ng/ml)	No. of samples	p value
IL-1β (50 U/ml)	3.2 (1.4-4.9)	9.3 (4.9-14.2)	6	<0.036
TNF-α (200 U/ml)	2.65 (2.6-2.7)	9.95 (7.7-12.2)	2	N/A
IL-4 (10-1000 U/ml)	3.0 (1.6-8.0)	2.5 (0.9-5.8)	6	0.036

Interestingly, neutralizing antibody against LIF caused a 25% reduction in normal progenitor proliferation derived from the supernatant but not from the adherent layers, and this effect was reversible by the addition of recombinant LIF protein. We conclude that (i) biologically active LIF protein is constitutively produced by adherent layers from normal donors, (ii) TNF-α and IL-1β increase and IL-4 decreases adherent layers LIF protein levels, (iii) the steady state levels of LIF protein produced by adherent layers from leukemic patients is significantly elevated, and (iv) LIF may participate in the interaction between adherent layers and hematopoietic progenitors to maintain normal hematopoietic colony growth.

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THE ROLE STROMAL CELLS IN REMISSION INDUCTION IN ACUTE MYELOID LEUKEMIA

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It is well known that bone marrow stromal cells have crucial impact on haemopoietic cell proliferation. Little is known about stromal humoral factors leukemic cell proliferation. The aim of this study is to evaluate the effect of stromal cell conditioned media (SCCM) on the 3H-thymidine uptake by normal and leukemic target cells. 21 patients with AML were studied treated with "7+3" based regimens. Long term bone marrow cultures were established in non-leukemic and leukemic patients (before and during treatment). Target cells for SCCM were normal haemopoietic cells and leukemic blasts. The results are the comparison of the effects of leukemic and non-leukemic stromal cells. A part of the patients revealed high stimulative activity upon non-leukemic cells (+125 ± 2 %, $p<0.001$) and inhibited proliferation of leukemic cells (-70 ± 3 %, $p<0.001$) This group entered complete remission. SCCM of another group of patients inhibited proliferation of non-leukemic cells (-84 ± 3 %, $p<0.001$) and stimulated blast cell proliferation (+146 ± 5 %, $p<0.01$). The magnitude of the figures was even more profound later: during treatment, bone marrow aplasia, recovery. This group of patients failed to achieve remission. It seems that stromal cells has significant on impact on restoration of normal or leukemic hemopoiesis after chemotherapy.

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THE INFLUENCE OF INTERLEUKIN 3 ON THE IN VITRO-PROLIFERATION OF BONE MARROW CELLS IN REFRACTORY ANEMIA WITH EXCESS BLASTS

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The simultaneous detection of immunophenotype and DNA-content by flow cytometry permits the evaluation of the proliferative activity in various subpopulations of the hematopoiesis.
We have investigated the influence of interleukin 3 (IL 3) on bone marrow cells in short term cultures. Bone marrow cells of patients with refractory anemia with excess blasts (RAEB) were incubated in 90% Iscoves modified Dulbecos medium , 10% autologous plasma and IL 3 for three days. Thereafter cells were immunophenotyped with anti-CD 34, anti-CD 15 or anti-CD11b. The DNA-synthesis phase (S-phase) of antigenpositive or negative cells were determined with the flow-cytometer by cell cycle phases analysis .
After IL 3 incubation the part of CD 34 positive blasts in the S-phase was 2.97-fold (1,03-fold to 4.45-fold; 8 cases) compared with the control culture. The percentage of CD 34 negative cells in S-phase was only 1.46 times higher (0.84 fold to 2.9-fold) in the IL 3 culture. The part of CD 15 or CD 11b-positive (more mature) cells in the S-phase is only the 1.19-fold in comparison with the control. But the CD 11b negative cells have an 1.92 times higher proliferative activity after IL 3.
These results show that the early proliferation inducing effect of IL 3 is limited on CD-34 positive myeloid progenitors in RAEB.

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RESTORATION OF NORMAL MONOCYTE/MACROPHAGE FUNCTION IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES DURING THERAPY WITH HEMATOPOIETIC GROWTH FACTORS (IL-3, GM-CSF, G-CSF). A. Ganser, A. Maurer, R. Buhl, O.G. Ottmann, G. Seipelt, G. Geissler, D. Hoelzer

Functional defects of the monocyte/macrophage system probably contribute to the increased rate of severe infections in patients with myelodysplastic syndromes (MDS). Therapeutic trials with hematopoietic growth factors (HPGF) have resulted in substantial improvement of cytopenia, especially neutropenia. However, little is known about the alterations of the monocyte/macrophage system during these therapeutic interventions. It therefore was the aim of the present study to analyze the capacity of monocytes/macrophages from MDS patients prior to and after HPGF therapy to secrete IL-1ß, TNFα, IL-6, and IL-8 upon in vitro stimulation with lipopolysacharide (LPS). Sixteen patients were studied: 11 had a refractory anemia, 5 had a refractory anemia with excess of blast cells. Prior to therapy, the capacity of adherent monocytes/macrophages to secrete IL-1ß, TNFα, IL-6, and IL8 was significantly reduced by 50-70 percent as compared to normal controls. On the other hand, oxygen radical release was normal in 11 MDS patients tested. Treatment with GM-CSF (15-250 μg/m2/d SQ x 7-14; n=7), IL-3 (60-500 μg/m2/d SQ x 15-18; n=3), and G-CSF (1-3 μg/kg/d SQ x 84 in combination with all-trans retinoic acid; n=6) normalized the potenital of monocytes/macrophages to secrete IL-1ß, TNFα, and IL-6. IL-8 secretion was only improved by IL-3 or GM-CSF dosages ≥250 $\mu g/m^2$. Oxygen radical release was significantly stimulated by both GM-CSF and IL-3. These results indicate that treatment of MDS patients with GM-CSF, IL-3, and G-CSF (the latter in combination with all-trans retinoic acid) can restore deficient monocyte/macrophage secretory function to normal.

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THE PROLIFERATING EFFECT OF IFNα AND TNFα ON PERIPHERAL B-CLL-CELLS IS DEPENDENT UPON GLUCOCORTICOID PRE-TREATMENT.

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Peripheral B-CLL-cells from 51 patients were investigated upon the proliferating effect of IFNα, TNFα or combination of both in serum free culture medium (CG-medium). Blood cells were drawn from patients and lymphocytes separated by Ficoll Hypaque and monocyte-lysis (Leucine-methyl-ester incubation). T-cells were depleted using αCD3 mAB and MACS (Magnetic Activated Cell Separator, Miltenyi Biotec). At each step heterogeniety of the population was controlled by FACS analysis with 7 different mAB (CD 3, 4, 8, 10, 14, 19, 20, 56). The homogeneous population (contamination less than 1%) was co-incubated with both cytokines (0.1 - 10 ng/ml) and Bromodeoxyuridine (BrdU) in CG-medium.
After 20, 60, 92, 112 and 140 hours cells were harvested an analysed for BrdU-incorporation into the genome. IFNα and TNFα measurings (51 in total) were almost similar: 24 patients were non-responder and showed no stimulatory effect on cells; 5 patients showed an inhibitory effect; cells from 21 patients were responding upon cytokine cultivation. The combination of both IFNα and TNFα produced in these cells an additive effect (14 out of 21).
Best results could be observed when the control population (without cytokine) was minimal proliferating compared to no proliferation. A high correlation was observed between cytokine response and pre-treatment: without glucocorticoid treatment of patients prior to measurements the influence of cytokines on resting B-CLL-cells was significantly higher (with methyl-prednisolone 15%, without pre-treatment 61% were responders).

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G-CSF ENHANCES RECOVERY OF LEUKOCYTES BUT MAY IMPAIR ENGRAFTMENT OF CD6 DEPLETED MARROW IN THE DOG

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Depletion of CD6 positive T cells has been used in human patients for prevention of GVHD. We studied depletion of $CD6^+$ cells from canine marrow for induction of GVH-tolerance across a complete DLA-haplotype difference. Prompt engraftment and fatal GVHD occurs in a littermate combination of DLA-homozygous donors and DLA-heterozygous recipients when undepleted marrow is given. Allogeneic marrow depleted with a crossreactive antibody to human CD6 and immunomagnetic beads was given to 6 dogs. One dog died with haemorrhage on day 48 due to thrombocytopenia, 5 dogs showed complete hematopoietic recovery. 4 dogs became tolerant chimeras and one dog died with GVHD due incomplete depletion. Chimerism was mixed early after transplantation, became complete later and is still complete in 2/4 dogs after 1-2 years. 4 dogs received CD6 depleted marrow grafts and 10μg/kg/d s.c. r-canine G-CSF starting on day 2 after transplantation. Although all dogs had fast recovery of granulocytes, 2 dogs receiving 0.5×10^8 MNC/kg died of marrow aplasia on days 47 and 62 without recovery of thrombopoiesis. Two dogs receiving 1×10^8 MNC/kg had sustained engraftment with delayed recovery of thrombocytes compared to dogs without G-CSF. FACS analysis of depleted marrow showed complete depletion of $CD4^+$ cells but about 2% of $CD8^+$ cells; CFU-C growth and NK-activity was retained after depletion. CD6 depleted marrow inhibited the generation of cytotoxic cells. These experiments indicate that qualitative T-cell depletion is effective, since CD6 recognizes only subpopulations of T-cells. The application of r-canine G-CSF enhanced the recovery of granulocytes but led to graft failure in dogs receiving a low number of marrow cells.

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PROGNOSIS FACTORS FOR THE COLLECTION OF PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) IN PATIENTS WITH MULTIPLE MYELOMA (MM).

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We wished to analyse the factors which may affect the yield of PBPC (CFU-GM) to be collected by leukapheresis following high-dose Cyclophosphamide (HD-CYC: 7g/m^2). We retropectively studied the following criteria in 31 patients with high-risk MM of which 10 received GM-CSF (Sandoz SA/ Schering-Plough) after HD-CYC: time from diagnosis to HD-CYC, number of chemotherapy cycles (CTC) *(Fermand, 1992)*, ß2 microglobulin, bone marrow plasma cell count before HD-CYC, administration of GM-CSF after HD-CYC, "slow" or "fast" rate of platelet and WBC recovery *(Jagannath, Schwartzenberg, 1992)*, "poor" or "good" mobilization of PBPC *(Jagannath, 1992)*, differential WBC count between "day x" and "day x-1" during haematopoietic recovery. Each variable was studied as continuous (regression analysis) and discontinuous (t-or chi-square tests).

When the differential WBC count was < 1300 WBC/µL, 37% of the leukapheresis procedures performed on day x yielded more than 30x 10^4 CFU-GM vs 90% when it was ≥ 1300/µL ($p<0.05$). The infusion of GM-CSF was associated with a higher yield of CFU-GM (BMT, 1993). The patients with "good" PBPC mobilization (≥ 50 x 10^4 CFU-GM in ≥ 2 leukapheresis) could all be transplanted with PBPC alone (vs 47% of those with "poor" mobilization). They had a shorter duration of aplasia after transplantation than the other patients ($p<0.03$). The "fast" WBC recoverers had a higher yield of CFU-GM than the other patients ($p<0.05$). When only the patients who did not receive GM-CSF after HD-CYC were considered, a higher yield of CFU-GM was achieved in patients who underwent < 6 CTC as compared to those who underwent ≥ 6 CTC before HD-CYC ($p<0,04$).

No other criteria had a significant prognosis value.

These criteria will help to define which patients may need the collection of BM or the infusion of GM-CSF (i) to collect PBPC and (ii) after transplantation.

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Haemopoietic cells released into the circulation by G-CSF (Lenograstim) alone or in combination with intense chemotherapy: Assessment of progenitors and stem cells.

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We report results of a Phase I/II recombinant human granulocyte colony stimulating factor trial for dose intensification in advanced breast cancer. Sixteen patients were studied. Seven patients underwent the Phase I G-CSF trial before entering the Phase II dose intensification study plus chemotherapy. Nine patients were entered into the Phase II study with four cycles of dose escalating chemotherapy. The release of progenitor cells into the circulation was determined by clonogenic assays. Two stage long term bone marrow cultures were used to assess the repopulating capacity and therefore the stem cell quality of the mobilised cells. Normal bone marrow from allogeneic transplant donors (with informed consent) were used as controls. We found equivalent total numbers of progenitors in both cycle 1 and cycle 4 of G-CSF plus chemotherapy peaking at day 11-12, respectively. In contrast, we found significant differences between the progenitor cells of cycle 1 and 4 generated from circulating haemopoietic cells when seeded onto preestablished irradiated bone marrow stroma. This indicates that peripheral blood stem collection should be performed in an early stage of chemotherapy due to an increased damage of the stem cell pool with increased levels of chemotherapy. The capacity of haemopoietic cells released by G-CSF to repopulate bone marrow stroma was equivalent to normal bone marrow as recently reported.

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GCSF AND AUTOLOGOUS BONE MARROW TRANSPLANTATION.

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Autologous BMT has been over the last years the major field to investigate dose effect. However secondary aplasia and morbidity remained a limiting toxicity even in this setting. GCSF allows quicker hematological toxicity when given post BMT as well as peripheral stem cells collections when given at hematological steady state. However limited comprehensive impact have been so far reported. We retrospectively analysed 66 patients for whom caracteristics were similar (diagnosis : malignant lymphomas n=37 ; breast carcinoma : n=29 ; conditioning : Novantrone-Cytoxan-Melphalan : n=29 ; BEAM : n=37) and who were transplanted during the last 30 mths. GCSF was used either after BMT, or prior BMT to collect peripheral stem cells or marrow. Initial analysis confirms impact of post graft GCSF on granulocytes recovery as well as impact of primed cells on platelets recovery. More detailed analysis will be presented as well as a 5th group transplanted with cytapheresis alone.

	G1 n=26	G2 n=17	G3 n=11	G4 n=12
GCSF priming	-	-	+	+
.Infused cells	BM	BM	BM	BM and PBSC
.post BMT GCSF	-	+	-	±
.D to reach 500 ANC	17	13	16	11
.D to reach 50 000 platelets	29	26	21	22

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INTERLEUKIN-6 (IL-6), G-CSF, TNF-α AND SOLUBLE INTERLEUKIN-2 RECEPTOR (SIL-2R) LEVELS IN COMPLICATIONS OF HUMAN AUTOLOGOUS BONE MARROW TRANSPLANTATION

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In autologous bone marrow transplantation (aBMT) acute phase responses usually occur between day 5 and day 15 following aBMT, and increases in bilirubin levels are observed in early endothelial complications such as endothelial leakage syndrome and veno-occlusive disease (VOD). IL-6 is the central cytokine modulating acute phase responses. Therefore we performed a prospective study analyzing IL-6, TNF-α, sIL-2R and G-CSF levels in serum samples of 21 patients undergoing aBMT (3 ALL, 4 AML, 8 Hodgkins disease, 5 Non Hodgkins lymphoma, 1 Ewing sarcoma). 10 patients received G-CSF post aBMT, 11 patients were transplanted without G-CSF. During leukopenia <300/µl G-CSF levels of 1612 ± 348 pg/ml (422-3309 pg/ml) were measured in patients not receiving G-CSF. During regeneration, when leukocyte counts exceeded 1.000/µl, the endogenous G-CSF levels declined to the normal range (<50 pg/ml). Severe transplantation associated complications were defined as VOD or interstitial pneumonia. IL-6 levels increased from 26 ± 6 pg/ml before therapy to 404 ± 159 pg/ml during febrile leukopenia ($p<.01$). Patients with complicated aBMT had significantly higher IL-6 levels (1123 ± 398 pg/ml n=7 vs 99 ± 33 pg/ml n=14) ($p<.01$). sIL-2R-levels increased from 130 ± 24 U/ml before aBMT to 383 ± 74 U/ml during leukopenia. In patients receiving G-CSF sIL-2R-levels further increased to 456 ± 121 U/ml during regeneration, while in patients not receiving G-CSF the sIL-2R-levels were 320 ± 71 pg/ml ($p<.05$). TNF-α levels remained unchanged during the course of aBMT; there was no correlation with febrile neutropenia or severe complications.

These data suggest a direct feedback regulation of endogenous G-CSF release by increases in peripheral granulocyte counts. Monitoring of IL-6 serum levels during aBMT may give insights into pathophysiology of aBMT associated complications resulting from activation of monocytes and endothelial cells.

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THE CONDITIONING REGIMEN USED PRIOR TO AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION (ABSCT) DOES NOT EXPLAIN THE DIFFERENCES IN HEMATOPOIESIS RECOVERY. C. Barbot, A. Rice, J. Reiffers.

We have previously reported that the rate of hematopoietic recovery following Autologous Blood Stem Cell Transplantation (ABSCT) could be influenced either by the type of conditioning regimen or by the underlying disease. We also reported that Peripheral Blood Stem Cell (PBSC) growth was sensitive to the stimulation of allogeneic irradiated stromal layers. Thus we undertook a study to examine the quality of the Bone Marrow (BM) microenvironment of patients who had undergone ABSCT for either Malignant Lymphoma (ML) or Multiple Myeloma (MM) after either myeloablative chemotherapy or TBI conditioning regimens. Thirteen patients with ML and eight patients with MM underwent ABSCT using cells collected during recovery phase after intensive chemotherapy. We used the long term culture system (LTC) to investigate the quality of the BM microenvironment from 13 patients (pts) with ML and 8 patients with MM. All BM were collected after ABSCT. Twenty-two LTC established with BM from 13 patients with ML were investigated; 10/13 pts received myeloablative chemotherapy (CBV) as conditioning regimen and 9 out of 10 of these pts developed a complete confluent stromal layer. Three of the 13 ML pts received TBI and 2/3 of these pts developed a complete confluent stromal layer. Eight LTC were established with BM from 8 patients with MM, who had all received TBI prior to ABSCT. Three of the 8 MM pts developed a complete confluent stromal layer. We evaluated the CFU-GM production by the stromal layer from all patients. The type of disease (ML vs MM at D7 p=0,935 and D14 p=0,294), the conditioning regime used prior to ABSCT (TBI vs CBV at D7 p=0,961 and D14 p=0,126), the presence or absence of a complete, confluent stromal layer (at D7 p=0,469 and D14 p=0,650) did not influence the production of CFU-GM by BM stromal layers from autografted patients. Nor was there a correlation between the development of a confluent stromal layer and the duration between LTC and ABSCT or the date of the cytapheresis as compared with the date of diagnosis or the number of nucleated cells and CFU-GM used to engraft the patients. Interestingly, when we examined the ability of confluent and non confluent stromal layer to support hematopoiesis, we observed no difference in the total cumulative production of nucleated cells or CFU-GM, suggesting that the quality of the BM stromal microenvironment after ABSCT cannot explain the differences seen in hematopoetic reconstitution.

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MOBILIZATION OF PERIPHERAL BLOOD STEM CELLS (PBSC) WITH G-CSF DURING STEADY-STATE HEMATOPOIESIS AND FOLLOWING CHEMOTHERAPY IN PATIENTS WITH MULTIPLE MYELOMA (MM)

H. Goldschmidt, R. Erhardt, M. Schmitt, M. Moos, R. Haas and W. Hunstein

Dose-escalated cytotoxic therapy with stem cell support may be considered for patients with stage III multiple myeloma, because of the poor median survival of only 3.5 years with conventional treatment. A threshold quantity of $5x10^6$ CD 34+ cells/kg bw is necessary for a rapid and sustained engraftment following myeloablative conditioning therapy. Since june 1992 six patients (median age: 46 years, range 31-53) with MM received 5µg G-CSF/kg bw (Neupogen®, Amgen) sc. daily at the time of best response with conventional treatment. The content of CD 34+ cells in the peripheral blood was monitored by FACS each day. Leukapheresis were started when a detectable population of CD 34+ cells appeared. In 2 of 9 steady-state leukaphereses, more than $1,0x10^6$ CD 34+cells were harvested. After the therapy with high dose cyclophosphamide (5 pts $4g/m^2$, 1 pt $7g/m^2$) plus G-CSF more than $1,0x10^6$ CD 34+ cells were collected in 11 of 19 leukapheresis.

To date two pts have undergone myeloablative conditoning therapy with hyperfractioned total body irradiation (14,4 Gy) and melphalan (140 mg/m^2). One patient received 200 mg/sqm melphalan as conditoning therapy. After the reinfusion of the G-CSF-mobilized PBSC, a rapid engraftment was achieved with median time of 13 days (range 9-16) to reach 0,5 x 10^9/l neutrophils and 9 days (range 5-14) for $20.0x10^9$/l platelets. No hematopoietic growth factors were given post-transplantation.

In this pilot study, high dose cyclophosphamide and G-CSF is an effective method for harvesting PBSC. Evaluation of the mobilization of hematopoietic stem cells during steady-state hematopoiesis using higher doses of G-CSF is planned.

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PERIPHERAL BLOOD STEM CELLS AND IMMUNOHEMATOPOIETIC RECONSTITUTION AFTER MULTIPLE CONSECUTIVE COURSES OF INTENSIVE CHEMOTHERAPY A. Könneke[1], J. Andres[2], K. Battmer[1], I. Südmeyer[2], L. Arseniev[1], C. Bokemeyer[1], H.-J. Schmoll[1], M. Freund[1], H. Link[1]

The present study was designed to estimate the immunologic and hematopoietic reconstitution after several consecutive chemotherapy courses (Ctx). Patients selection: ALL (n = 3/9 Ctx-cycles), AML (n = 11/21), NHL (n = 6/16), M. Hodgkin (n = 3/4), and non-seminomatous germ cell tumor pts. (NSGCT, n = 7/17). Ctx was performed according to the current treatment protocols used at our institution. The NHL and NSGCT pts. received G-CSF before Ctx for mobilization of stem cells for apheresis. G-CSF application (5^+µg/kg) after Ctx was used in all NHL, ALL and NSGCT and 3 AML pts. The collected PBSC were reinfused after each following Ctx cycle to all NHL and 5 NSGCT pts. The CD3, CD14, CD19, CD25, CD33, CD34, CD56 and HLA-DR positive cells in peripheral blood were analyzed, using dual color fluorescence and flow cytometry. CFU-GM from peripheral blood MNC's were used as control for the clonogenic capacity of $CD34^+$ cells. Samples were obtained before, immediately after Ctx and several times after the WBC's reached $>1x10^9$/l. A positive correlation of the rising and decreasing subpopulation counts within the MNC's were noticed (r = .65-.85), however the $CD3^+$ were in inversed ratio to the $CD14^+$ cells (r = -.66). The percentages of $CD3^+$ and especially of the $CD25^+$ cells showed an increment immediately after Ctx, where the proportions of $CD14^+$ and $CD34^+$ cells tended to fall. There was also a correlation between $CD34^+$ and $CD14^+$ cells (r = .76, p < .001) and between $CD34^+$ cells and CFU-GM growth (r = .82, p < .001). An increased clonogenity was associated with low numbers of $CD34^+$ cells: $CD34^+$: CFU-GM 38:1 before Ctx; 18:1 immediately after Ctx; 38:1 during the regeneration phase. The same phenomenon could be seen by intensively pretreated compared to less intensively pretreated patients. The hematopoietic reconstitution parameters (median) were as follows in the PBSC-rescued vs non-rescued pts: platelet transfusions - 18 vs 28 (p < .02), RBC transfusions - 6 vs 8, days with platelets <50000/µl - 6 vs 13 (p < .01), duration of neutropenia with WBC < 1000/µl - 5 vs 9, days with fever - 6 vs 8.5.

The augmentation of $CD34^+$ cells correlated with rising numbers of MNC's and especially of $CD14^+$, but not with $CD3^+$. The correlation between $CD34^+$ cells and CFU-GM in peripheral blood was convincing.

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MOBILIZATION, COLLECTION AND RETRANSFUSION OF PERIPHERAL BLOOD STEM CELLS FOR THE SUPPORT OF MULTIPLE CONSECUTIVE COURSES OF ESCALATED DOSE CHEMOTHERAPY IN PATIENTS WITH NHL AND NSGCT L. Arseniev[1], J. Andres[2], K. Battmer[1], I. Südmeyer[2], M. Zaki[2], A. Könneke[1], H.-J. Schmoll[1], C. Bockemeyer[1], M. Freund[1], H. Link[1]

Two different escalated-dose chemotherapy protocols were examined in attempt to estimate the optimal timing for collection and the results of retransfusion of peripheral blood stem cells (PBSC). Refractory or relapsed NHL patients (n = 14) were treated with Mtx, Ifo, Ara-C, prednisolone and escalating doses of VP16. Pts. (n = 12) with relapsed or advanced non-seminomatous germ cell tumors were treated with cisplatinum, escalating doses of VP16 and Ifo. The protocol design was similar: G-CSF before Ctx (2x12 µg/kg/die s.c.) with PBSC-aphereses at days 5 to 7 (NHL) or days 5, 6 (NSGCT) followed by 2 to 4 Ctx-courses. Cytaphereses were also performed after Ctx when the total WBC's recovered above $1x10^9$/L. The Ctx-cycles were followed by reinfusion of the previously collected PBSC (n = 44) and application of G-CSF (5 µg/kg/d.; n = 57) up to the last day of the subsequent stem cell collection. The $CD34^+$ cells, the clonogenic peripheral blood progenitor cells (CFU-GM & BFU-E) and light density cell (LDC) counts were determined in 150 cytapheresis samples. The Ctx/G-CSF courses contributed to substantially higher progenitor cell amounts than G-CSF alone (n = 75/21; p < 0.01), without a difference in the collected LDC (1.3 ± 0.2 vs $1.7 \pm 0.1 x10^8$ LDC; 3.9 ± 1.0 vs $0.8 \pm 0.1 x10^6$ $CD34^+$ cells; 10.9 ± 2.1 vs $9.0 \pm 1.5 x10^4$ CFU-GM; 11.6 ± 4.3 vs $6.9 \pm 0.9 x10^4$ BFU-E/kg/apheresis; n = 102/48; mean ± SEM), but with approximately two times lower clonogenity. Two or three leukaphereses were enough to rescue 3 Ctx-courses with a minimal dose of $2x10^6$ $CD34^+$ cells/kg/patient. The optimal time to initiate PBSC-collection after Ctx in the studied patient group was proved to be at the 1^{-st} to 4^{-th} day after reaching leukocyte > 1000 µL. The original total leukocyte, light density cell (LDC) and platelet counts in the peripheral blood at start of leukapheresis played an essential role for the efficiency of the procedure (EFF), as shown by regression analysis: EFF to total leukocyte count correlation was $r^2 = -0.48$ (p < 0.01); EFF to LDC-count $r^2 = -0.55$ (p < 0.001); and EFF to platelet count $r^2 = -0.25$ (p = 0.056). The results of PBSC retransfusion on hematopoietic recovery were as follows: 8 days to reach leukocytes > 1000 µL and 12 days - platelets > 50000 µL; duration of neutropenia was 5 days; and 8 days to become platelet transfusion-independent (median).

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THE EFFECT OF GM-CSF AND G-CSF IN TREATMENT OF SEVERE NEUTROPENIA ASSOCIATED WITH CHEMO-THERAPY IN CHILDREN WITH MALIGNANCIES.

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Total number of 25 children, 17 girls and 8 boys, with neoplasia aged from 1-18 years were treated with GM-CSF-Leucomax Sandoz and G-CSF Filgrastim Hoffmann-La Roche, during severe myelosupression occuring after intensive polychemotherapy. In 4 children GM-CSF was applied twice after 2 consequent courses of chemotherapy. One child received GM-CSF four times after 4 chemotherapy courses. Twenty children with malignancies served as historical control group. GM-CSF was given at dose 5 μg/kg, G-CSF 5-10 μg/kg daily s.c. Duration of therapy ranged from 2-28 days with median 10 days. After cytokines therapy increase of mean and median numbers of total WBS, neutrophils, monocytes and eosinophils was observed. The median time to hematopoietic recovery was shorter in the group of children treated with cytokines when compared with the control group. (9 v 16 days). In 19 of 25 children signs of infection disappeared even before granulocyte count increase. Also shorter median time of febrile days, 4 v 12 days, in comparison with the control group was observed. No serious side effects during cytokines therapy were noticed. Only in one patient local erythema in injection place was observed. In two children transient retrosternal pain was seen. Our results showed that GM-CSF and G-CSF administered in children with neoplasia after chemotherapy shortens the period of neutropenia and infection duration.

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RECOMBINANT HUMAN INTERLEUKIN-3 (RHIL-3) ENABLES CHEMOTHERAPY (CT) DOSE INTENSIFICATION IN OVARIAN CANCER (OC). M.M. van Gameren, P.H.B. Willemse, R. Mull, B. Biesma, E.G.E. de Vries.

Based on previous studies (Cancer Res. 1991:51, 116, Blood, 1992:80, 1141) we know that many patients (pts) cannot receive CT consisting of carboplatin (CBDCA) 300 mg/m^2 and cyclophosphamide (Cyclo) 750 mg/m^2 for OC every 4 wks without hematopoietic growth factor support. The desirable dose of rhIL-3 based on a phase I/II study in this setting was 5 or 10 μg/kg/day. A study was designed to determine whether rhIL-3 would allow CT administration every 3 wks with 17 pts treated to date. Cyclo was administered 750 mg/m^2 and CBDCA was dose adjusted to creatinine clearance: 60-80 mL/min: 257 mg/m^2, 80-120 : 300 mg/m^2, 120-140: 340 mg/m^2, >140: 385 mg/m^2. A total of 6 cycles (c) was administered. RhIL-3 (5 or 10 μg/kg/d) was given sc d2-11 in each c. At 5 and 10 μg doses are 10 (46c) and 7 (33c) pts evaluable for toxicity and 10 (43c) and 7 (27c) pts for efficacy. Side effects were fever and headache controllable with acetaminophen. At 5 μg rhIL-3 in three c (2 pts) and at 10 μg in six c (5 pts) urticaria occurred. In 4 episodes dyspnea and/or oedema was observed. This reaction only occurred during c3-6 and was controlled with antihistamine and prednisolone. CT could be administered every 3 wks in 43/70 c (25/43 c at 5 μg, and in 18/27 c at 10 μg (NS)), every 4 wks in 13/70 c and > 4 wks in 14/70 c. No platelet transfusions were required. Thus, in 61% of c it was possible to give a CT dose intensification of 33%. If full dose CT were to be given every 4 wks it would have been possible to administer in 80% of c in time. Conclusion: with rhIL-3 CT dose intensification of 33% is possible by reducing CT intervals, while no platelet transfusions were required with rhIL-3.

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RECOMBINANT HUMAN GRANULOCYTE COLONY STIMULATING FACTOR (R-MetHuG-CSF) IN PATIENTS WITH HODGKIN'S DISEASE AND NON-HODGKIN'S LYMPHOMA

B Mengelkoch, M R Nowrousian, A Martin, G Schwab S Seeber

For both Hodgkin's disease and non-Hodgkin's lymphoma the outcome of chemotherapy has been shown to correlate closely with the dose intensity of treatment. However, dose intensification is limited most often by severe myelosuppression with the subsequent risk of fever and infections.

We performed a clinical trial in 17 patients with Hodgkin's disease or non-Hodgkin's lymphoma to evaluate whether r-metHuG-CSF could facilitate the safe and timely administration of an intensive chemotherapy regimen. Patients who developed neutropenia ≤ 0.5 x 10^9/L for more than two days and / or fever ≥ 38.2°C and / or signs of infection after a cycle of chemotherapy (CEBOPP/VIM protocol administered at intervals of 21 days), as well as patients in which chemotherapy had to be delayed due to an ANC ≤ 1.5 x 10^9/L on day 1, were eligible for treatment with r-metHuG-CSF. In the subsequent cycles r-metHuG-CSF was given subcutaneously at a dose of 5 μg/kg/d from day 11 to 20.

16 of 17 patients were evaluable, one patient had received only 1 day of r-metHuG-CSF treatment. 15 of the 16 evaluable patients experienced neutropenia with an ANC of less than 0.5 x 10^9/L during the chemotherapy course preceeding r-metHuG-CSF treatment, whereas only 5 patients had ANCs ≤ 0.5 x 10^9/L after the subsequent therapy with r-metHuG-CSF ($p<0.01$).Overall analysis showed that the duration of ANC nadirs ≤ 0.5 x 10^9/L was on average 3.27 days in 41 cycles without r-metHuG-CSF compared to 1.78 days in 52 cycles with r-metHuG-CSF treatment. The administration of chemotherapy had to be delayed only for 2.47 days (mean value) during cycles with r-metHuG-CSF. Side effects probably related to r-metHuG-CSF, were moderate muscle and joint pain in 3 patients and chills in one patient. In general, r-metHuG-CSF was well tolerated. Under this treatment regimen 11 patients reached complete remission, 4 patients reached partial remission and one patient had stable disease. One patient was treated adjuvantly after gastrectomy.

In conclusion, r-metHuG-CSF allowed the safe and timely administration of this intensive chemotherapy regimen and reduced myelosuppression for patients with Hodgkin's disease and non-Hodgkin's lymphoma.

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A RANDOMIZED PHASE III STUDY OF RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR (rhG-CSF) IN CHILDHOOD HIGH RISK ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)

K. Mempel, A. Reiter, E. Yakisan, E. Odenwald, M. Pfetsch, G. Schwab, H. Riehm, K. Welte.

In the multicenter trial ALL-BFM 90, we have initiated a phase III study of rhG-CSF in children with High Risk ALL. High risk (HR) patients are characterized by at least one of three criteria: 1. Prednisone poor response (≥ 1000/mm^3 absolute blasts number in the blood at day 8 after 7 days' exposure to prednisone), 2. failure to achieve complete remission at day 33 of induction therapy, and 3. t(9;22). The primary objective was to test whether rhG-CSF reduces the incidence of febrile neutropenic episodes. The second objective was to examine whether rhG-CSF administration allows closer adherence to planned dosing schedule and to determine the overall response to chemotherapy. HR-ALL pts are randomized to receive either 9 cycles of chemotherapy (HRG I) or 9 cycles of chemotherapy (day 1-6) followed by rhG-CSF (day 7-20; HRG II). Up to date, 20 pts have been treated according to this protocol (HRG I: 10 pts, HRG II: 10 pts). In HRG II, rhG-CSF is well tolerated without G-CSF related adverse events. In each arm, one pt relapsed. The incidence of neutropenia was 37% in HRG I and 10% in HRG II. More importantly the incidence of febrile neutropenia was 14% in HRG I and 5% in HRG II. These data demonstrate that rhG-CSF allows for reduction of the incidence of febrile neutropenia in HR-ALL-patients.

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TREATMENT OF IDIOPATHIC NEUTROPENIA WITH LOW DOSES OF RECOMBINANT HUMAN GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF/NEUPOGEN)

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We describe the case of a 62-year-old woman affected by idiopathic neutropenia, at high risk of developing life-threatening infections. Clinical history, serial full blood counts, and bone marrow examinations indicated a diagnosis of chronic idiopathic neutropenia. Her diagnosis was established in 1978. She hat frequent, sometimes life-thretening problems with infections (furunculus, pneumonia, stomatitis ulcerosa, abscessus). She spent 310 days in hospital until we started to give the G-CSF.

The patient has been receiving continuous G-CSF treatment for 22 months. She needs very small amount of G-CSF (1 μg/kg/day). Her absolute neutrophil count have remained in the region of 3500-6000/μl. Since 23 of May 1991 the patient didn't spend any day in hospital.

The patient has experienced complete resolution of stomatitis, fever and malaise. The administration of G-CSF in patient with idiopathic neutropenia significantly increased the absolute neutrophil counts ($p < 0.001$).

G-CSF was effective in reducing the severity of neutropenia and infectious complications in our patient.

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G-CSF IN THERAPY OF CYCLIC NEUTROPENIA AND CHRONIC IDIOPATHIC NEUTROPENIA IN ADULT PATIENTS

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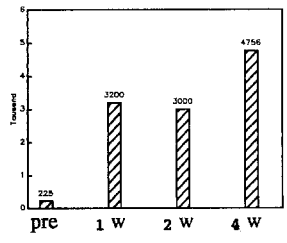
Twelve adult patients with chronic neutropenia, including 7 patients with *idiopathic sporadic neutropenia*, 2 with *idiopathic familial neutropenia* and 3 with *cyclic neutropenia* have been treated with rhu-met-G-CSF (Amgen, Thousand Oaks, USA). Treatment has been started in all patients with 3 μg/kg/d sc once daily. Doses have been modified according to WBC. All patients had a rapid increase of absolute neutrophil counts. Data are shown for idiopathic neutropenia (base line, after 1, 2 and 4 weeks). Doses required ranged from 0.1 to 9 μg/kg/d. Treatment has been continuously given up to three years in patients with severe preceeding infections.



The clinical efficacy of the treatment was excellent with abrogation of significant infections. One patient with *idiopathic sporadic neutropenia* recovered after 151 days of treatment with an ANC of >2000 /μl after stop of G-CSF. In a patient with *familial cyclic neutropenia* cycle length shortened from 21 to 14 days. In another patient with acquired *idiopathic cyclic neutropenia* the cycle length of 120 days remained constant but the nadir of ANC rose from 0 to 400 /μl.

This patient was taken off therapy because of urticaria related to G-CSF on day 850. There were no further significant adverse events. No loss of effectivity was observed during long-term treatment. We conclude that G-CSF is safe for long-term treatment of idiopathic neutropenie with severe preceeding infections. As response to treatment is quic, it may also be an effective interventional treatment in acute infections in these patients.

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REDUCTION BY GRANULOCYTE-MACROPHAG COLOGYSTIMULATING FACTOR (GM-CSF) OF HEMATOLOGIC TOXICITY INDUCED BY HIGH-DOSE CHEMOTHERAPY IN PATIENTS WITH METASTATIC BREAST CANCER. Hansen F., Stenbygaard L. and Skovsgaard T.

Twenty patients with recurrent metastatic breast cancer treated with high-dose myelosuppressive antineoplastic drugs (Cyclophosphamide 2,5 g/m^2 or Epirubicin 130 mg/m^2 both q 3. weeks) as first or second line chemotherapy were randomized in a prostective study to GM-CSF (n=11) 5 microg/kg/dag for ten days after cessation of chemotherapy or control (n=9). Compared to the control-group highly significant reduction in granulocyte nadir duration (two days (0-5) with GM-CSF vs. seven (2-11) days) and severity (WBC 0.4 x 10^9/l with GM-CSF vs. 0.2 x 10^9/l) was found. No difference in frequency of neutropen fever or antibiotic use could be observed.

Even though the patients treated with GM-CSF at random were more heavily pretreated with chemotherapy, there was a surprisingly higher responserate in these patients as compared to the control-group, namely 64% vs. 22%, resp. No severe side effects were seen, but presumably due to GM-CSF one patient developed an allergic type 1 reaction and one patient developed a possible pericardial exudation. Both were fully reversible after cessation of GM-CSF treatment.

Keywords: GM-SCF, chemotherapy, breast cancer.

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HUMAN G-CSF IMPROVES WOUND HEALING IN NEUTROPENIC MDS PATIENTS

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Cytokines and growth factors are widely used to promote growth and proliferation of hematologic cell populations. Improvement of wound healing by stimulation with G-CSF has been reported in patients suffering from Kostmann syndrome, Felty syndrome or from neutropenia due to chemotherapy.

We report on two patients with MDS/RA (HA, female, age 76; SH, male age 82); duration of disease was 3 months and 5 years, respectively. HA was admitted for neutropenia (neutrophils: 0.45-0.9 G/l), epistaxis and a growing ulcerous wound in the pubic area (diameter 60 mm) already pretreated with antibiotics for 10 days. Surgery was not possible due to poor heart condition and thrombocytopenia refractory to donor platelets. 30 MU G-CSF were administered subcutaneously daily for 5 days resulting in neutrophil counts of 3.15 G/l and effective wound granulation and epithelialisation. The patient died of cardiac failure on day 10.

SH was admitted for infected hematoma of the left thigh. Subcutaneous infection progressed due to severe neutropenia (neutrophils < 0.5 G/l). Incision and resection (ulcus diameter 30 mm with deep invasion into the fascia) was performed. 20 days later the defect measured 120 x 50 mm, reaching the knee joint, and the patient underwent a second surgical intervention. Enterococcus, staphylococcus epidermidis and bacterium xerosis could be cultured from direct swabs. Therapy with G-CSF at a dose of 30 MU s.c. was started on day 61. Neutrophils reached 19.5 G/l and G-CSF was reduced to every other day. Complete wound healing without any further surgical intervention was achieved by day 88 and SH was dismissed. After discontinuation of G-CSF the patient is well and has normal differential blood counts.

We conclude that G-CSF is successful in promotion of wound healing in MDS patients due to enhancement of neutrophil production. Leukemic conversion of MDS during G-CSF was not observed.

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EXTRAMEDULLARY HEMATOPOIESIS IN MYELODYSPLASTIC SYNDROM - INDUCED BY CYTOKINES?

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We report on a 44 year old male patient presenting in 5/88 with moderate thrombocytopenia, transfusion dependent macrocytic anemia and normal WBC. Trephine biopsy showed hypercellular marrow without fibrosis with trilineage dysplasia (MDS/RA). Cytogenetic analysis was 46,xy. In 10/88 vasculitis was diagnosed. From 10/88 to 12/88 three cycles of GM-CSF (250 $\mu g/m^2$ s.c. - 14 days) were administered, resulting in both transient leukocytosis and increased platelet counts. Bone marrow aspirations showed dysplasia but no blast proliferation. In 5/89 vasculitis progressed, splenomegaly and hemolytic anemia developed requiring prednisolon. In 3/90 high dose erythropoietin was started (400 IU/kg i.v. twice weekly) and continued till 6/90. There was no change clinically, bone marrow smears and cytogenetics. In 10/90 the patient complained of pain in the lumbosacral region and neuralgia in both legs developed; a CT scan was negative. Both pain and neurological symptoms (paraplegia and sensibility disorders) progressed. A CT scan and an MRT showed an intraspinal tumor (D2 - D11). Although severe thrombocytopenia refractory to high dose i.v. immunoglobulin and platelet support (HLA class I & lymphocytotoxic antibodies) developed, therapeutic laminectomy was performed in 6/91, but only a part of the tumor could be resected. Histologically the tumor consisted only of erythropoiesis with dysplasia without excess of blasts. Wound healing was without complications. After surgery gamma irradiation and therapy with IFNα (6 μg s.c. 3 times/week) were performed. The patient recovered totally from neurological disorders and is still alive but transfusion dependent because of severe cytopenia.

We conclude: intraspinal extramedullary hematopoiesis is a rare symptom in MDS. Although this infiltration was diagnosed months after GM-CSF and HD-EPO therapy, it could be induced by cytokine therapy.

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KINETICS OF HUMAN NEUTROPHIL SURFACE MARKER EXPRESSION AND CHEMOTAXIS DURING rhG-CSF TREATMENT

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G-CSF is a hematopoetic growth factor required for proliferation and differentiation of hematopoeitic progenitor cells. It is now being successfully used to overcome neutropenias of various etiologies. Recently, we demonstrated that rhG-CSF induced neutrophils from patients with severe congenital neutropenia showed altered surface marker expression (upregulation of $Fc_\gamma RI$ (CD64) and CD14 and downregulation of $Fc_\gamma RIII$(CD16)) as well as decreased chemotaxis towards a variety of chemoattractants including fMLP. To separate the effects of the underlying disease from those of the rhG-CSF therapy, we investigated neutrophils from patients receiving cytotoxic chemotherapy (n = 6) and healthy adults (n = 5) after application of rhG-CSF.

Results: Neutrophils from 6 patients receiving daily application of rhG-CSF (Neupogen®, 300μg sc.) were studied ex vivo one day before, three times during and 3-5 days after cessation of rhG-CSF treatment. Expression of $Fc_\gamma RI$, CD14 and CD54 (measured by flow cytometry) increased during therapy reaching a maximum at 3-5 days after initiation of rhG-CSF therapy, whereas expression of $Fc_\gamma RIII$ decreased to a minimum after 6-9 days. Chemotaxis of neutrophils under agarose towards fMLP was also reduced during therapy. Investigation of surface marker expression and chemotaxis 3-5 days after cessation of rhG-CSF revealed return to levels before therapy. To exclude the possibility that the observed alterations were caused by the underlying disease or chemotherapy, five healthy adults were treated with a single dose of rhG-CSF (Neupogen®, 300μg, sc.). A continuous upregulation of $Fc_\gamma RI$ and CD 14 starting 3h after application with a maximum after 48 hours ($Fc_\gamma RI$) and 18 hours (CD14) and a downmodulation of $Fc_\gamma RIII$ reaching a minimum at 48 hours was observed. Chemotaxis towards fMLP decreased 3 to 4 h after application and returned to normal after 6 h, whereas expression of $Fc_\gamma RI$, CD14 and $Fc_\gamma RIII$ showed baseline values after 96 hours.

Conclusions: The results obtained from the healthy test subjects clearly demonstrate that neither the malignant disease nor chemotherapy, but rhG-CSF induced the profound alterations of Fc_γ receptor and CD14 expression and chemotaxis in neutrophils *in vivo*. The continuous character of the surface marker alterations without appearance of subpopulations and the increase in CD14 expression suggests that preactivation rather than immaturity of rhG-CSF induced neutrophils alone might be responsible for the observed phenomena.

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G-CSF, GM-CSF AND IL-8 POTENTIATE Fcγ RECEPTOR ACTIVATION OF GRANULOCYTES

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FcγRII and FcγRIII represent important activation structures for PMN using intracellular calcium mobilization. In the present studies we analyzed the effects of GM-CSF, G-CSF and IL-8 on Fcγ receptor activation. Freshly isolated PMN were activated by using $F(ab')_2$ fragments of FcγRII and FcγRIII monoclonal antibodies either with or without GaM crosslinking. In addition, we added the cytokines. The phenotypic change of expression of Fcγ receptors was measured. H_2O_2 production and calcium flux using the dihydrorhodamine (DHR) and Fluo-3 AM methods, resepectively. There were no changes in expression of Fcγ receptors, but a significant enhancement of PMN activation via Fc receptors by all three cytokines. We observed an increase of H_2O_2 production 4.5 fold by G-CSF, 2 fold by GM-CSF and 3.5 fold by IL-8. A FcγRIII-B specific monoclonal antibody for PMN (1D3), which was alone unable to mobilize Ca^{1+}, together with all three cytokines was a potent stimulator. The effects of GM-CSF and G-CSF were calcium independent, in contrast, IL-8 also enhanced calcium mobilization significantly. In summary, all three cytokines potentiate the Fcγ receptor activation of PMNs and therefore play a significant role in inflammatory granulocyte activation as in leukocytoclastic vasculitis.

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G-CSF RECEPTOR EXPRESSION ON NEUTROPHILS FROM PATIENTS WITH SEVERE CONGENITAL NEUTROPENIA

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Severe congenital neutropenia (SCN) is a disorder of myelopoiesis characterized by a maturation arrest on the level of promyelocytes with absolute neutrophil counts below 200/μl in the peripheral blood. In this study we investigated the expression of receptors for the granulocyte colony-stimulating factor (G-CSF) on neutrophils from patients with SCN during G-CSF therapy. The normal G-CSF receptor expression on neutrophils is in the range of 480-1200 receptors per cell. Neutrophils from SCN patients express increased numbers of receptors in the range of 2100-3900 receptors per cell. The dissociation constant of the binding of G-CSF to the G-CSF receptor is not altered as compared to healthy donors. In contrast neutrophils from patients suffering from cyclic neutropenia express normal G-CSF receptor numbers (400-900 receptors per cell). In addition, we have compared the G-CSF receptor cDNA of neutrophils from healthy donors and SCN patients using the polymerase-chain-reaction technique. We could not detect any major alterations in the G-CSF receptor cDNA in SCN patients. Preliminary cDNA sequencing data also did not reveal any point mutation. From this data we conclude that there is no defect in G-CSF receptor expression and no alteration in the sequence of the G-CSF receptor mRNA in SCN.

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LONG-TERM EFFECTS OF RHG-CSF TREATMENT IN PATIENTS WITH SEVERE CONGENITAL NEUTROPENIA

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Severe congenital neutropenia is a disorder of myelopoiesis characterized by severe neutropenia secondary to either a maturational arrest of myelopoiesis at the level of promyelocytes (Kostmann's-Syndrome; SCN) or regular cyclic fluctuations in the number of blood neutrophils with a median ANC below 500/µl (cyclic neutropenia). We have treated 32 patients with SCN and 4 patients with cyclic neutropenia. Thirty of 32 patients with SCN and all 4 patients with cyclic neutropenia responded to rhG-CSF treatment with an increase of the median ANC to above 1000/µl. The doses needed to achieve and maintain the response varied between 0.8 and 120 µg/kg/d. Long-term treatment did not exhaust the myelopoiesis: The mean ANC remained stable up to 5 years of treatment. The increase in ANC was associated with dramatic clinical responses: significant reduction of severe bacterial infections, reduction of intravenous antibiotic treatment episodes, and reduction of hospitalizations. No severe bacterial infections occured in any of the rhG-CSF responders during long-term treatment. Severe adverse events, most likely associated with the underlying disease, included the development of MDS/Leukemia in two patients, and osteopenia/osteoporosis in 12 patients. These results demonstrate the benefical effects of rhG-CSF treatment in severe congenital neutropenia patients.

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TREATMENT WITH rhG-CSF INCREASES THE CD62 -EXPRESSION ON THE SURFACE OF THROMBOCYTES

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The effect of rhG-CSF on platelets was studied in 20 healthy volunteers with the thrombometer, a specially developed device which is described in detail. Additionally, conventional aggregation tests were performed. Low doses of rhG-CSF enhance functional platelet activity, as shown by significant acceleration of the occlusion of the thrombometer channel. Similar results were found in conventional aggregation tests using collagen for induction. With G-CSF concentrations of 0,1 and 1,0 ng/ml the time of response was significantly accelerated and the maximum response was observed in a higher proportion of platelets. However, the second phase of aggregation induced by epinephrine was significantly inhibited by 1,0 ng/ml G-CSF.

The expression of CD41, CD42 and CD 62 on platelets' surface was determined in ten patients before and after administration of G-CSF (FACscan flow cytometer).Quality controls were done by calculating the events positive for CD41 and CD42, which were expressed in nearly 100 % of the platelets without being changed by the cytokine. The expression of CD62 in the platelets' surface however was significantly enhanced indicating a depletion of the α-granules. No platelet aggregation was observed.

CD62 expressed on thrombocytes' or damaged endothel cells' membrane is a receptor for macrophages. This property facilitates rapid adhesion of leucocytes to endothelium at regions of tissue injury as well as platelet-leucocyte interactions at areas of inflammation and hemorrhage.- In contrast CD62 can also have an antiinflammatory function because exposure of TNF-α-activated neutrophils to plasmatic CD62 inhibits their CD18-dependent adhesion to resting endothelium and superoxide production.- Our contribution to the pathophysiological involvement of CD62 is the demonstration of the increase of membrane-bound CD62 after admission of rhG-CSF. For discussion of the consequenzes of this observation we have to refer to papers of other authors, published in the last years.

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RECOMBINANT ALPHA INTERFERON (IFN) FOR NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA: A REPORT OF 52 PATIENTS. M. Montastruc, C. Fabères, G. Marit, C. Bilhou-Nabera, Ph. Bernard, P. Cony-Makhoul, M. Puntous, K, Bouabdallah M.J. Gharbi, A. Broustet, J. Reiffers.

Fifty-two patients (pts) (median age - 51 years) with Philadelphia chromosome positive (Ph^{1+}) chronic myeloid leukemia (CML) have been treated with IFN (5 x 10^6 units/m^2) within six months of diagnosis (median 1.5 months (mths), range 1 - 6 mths). We divided the pts into three groups according to Sokal's classification: low risk group (n = 24), intermediate risk group (n = 19) and high risk group (n = 9). Forty-three pts acheived a complete hematological response (CHR) as defined by the Houston criteria. The cytological response was evaluable in 40 pts: 23 pts (57.5 %) demonstrated a partial or major cytogenetical response (more than 65 % Ph^1 negative metaphases). The hematological and cytogenetical responses were influenced by the risk factors, as the percentage of CHR and cytogenetical responses was higher for the pts from the low or intermediate risk groups (88 % and 41 % respectively) than for the high risk group (55% and 22 %). Transformation occurred in four pts who did not demonstrate a cytogenetical response. The estimated chance of surviving at three years was 83% for the overall population. Toxicity was mild but IFN had to be interupted in four pts for cardiac (n = 2), liver (n = 1) or neurological (n = 1) tocxic effects. These results confirm that IFN is a very effective treatment for CML.

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COMBINATION IN CHRONIC MYELOGENOUS LEUKEMIA (CML) OF CYTOSINE ARABINOSIDE AND INTERFERON α-2b

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IFN α has a unique activity in CML leading to complete and partial remissions in 15-30% of the patients. To improve these results, we are currently treating patients with Ph'+ CML with a combination of cytosine-arabinoside at a maximum dose of 20 mg/m^2 SC on 5 days per week and IFN α-2b. IFN α-2b is started at a dose of 3 MU/m^2 SC daily and escalated to the maximum tolerated dose. 48 patients (25 male, 23 female, median age 44 years) have been entered into the trial. 15 patients have been pretreated with other regimen for a median time of 32 months. 33 patients are without pretreatment. The treatment has been well tolerated. Besides the IFN α related side-effects some patients experienced gastrointestinal toxicity with nausea and vomiting after prolonged Ara-C application. The median observation time in the study is now 8 months and patients are still entered. Up to now complete hematologic remissions have been achieved in 24 patients, and partial ones in 15 patients. The rate of complete hematologic remissions was higher in patients without pretreatment (55%) compared to patients who have been pretreated (40%). Five partial cytogenetic remissions have been observed and 3 minor reductions in the Ph'+ cell clone. All cytogenetic responses have been found in patients without pretreatment. We conclude that a combination of cytosine-arabinoside and IFN α-2b is well tolerated in patients with CML. Early results are encouraging. Longer follow up times are necessary to evaluate whether combination therpy will give superior results compared to a treatment with IFN α alone.

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SUBTYPE SPECIFICITY OF THERAPY-INDUCED AND AUTO-IMMUNE HUMAN IFN-α ANTIBODIES

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During rIFN-α2 therapy a minority of patients develops high-titered antibodies neutralizing the injected rIFN-α2. The rIFN-α-antibodies from six of such patients, who lost their clinical response to rIFN-α2 and showed a relapse of their leukemia (3 CML, 3 HCL) despite continuous rIFN-α2a-therapy, as well as IFN-α-specific antibodies from two patients with systemic lupus erythematosus (SLE) were characterized. The anti-IFN activity was purified by sequential Protein G - and rIFN-α2 affinity chromatography and was found to consist only of IgG-antibodies. These antibodies were further tested for their capacity to neutralize the antiviral and antiproliferative activity of various rIFNs-α-subtypes. All six sera tested showed a common pattern of neutralization (MDBK-VSV bioassay) distinct from the SLE-antibodies. All six neutralized rIFN-αA and rIFN-αK consistently with a higher titer against αA. Three of the six sera neutralized αA, αK, αC, αC/J1 and αI, but not αJ, αJ1 and some other subtypes. Therefore, from the structure of the C/J1-hybrid, it seems that one epitope recognized by these three sera is at the NH_2-terminal half of the molecule. In contrast, the SLE associated antibodies neutralized the antiproliferative and antiviral activity of every subtype tested. These data indicate that the therapy-induced antibodies against rIFN-α2 recognize very selected epitopes on the rIFN-α2-molecule suggesting that only a part of the rIFN-α2 molecule is immunogenic.

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RECOMBINANT ALFA-2A INTERFERON AFTER ABMT IN ACUTE LEUKAEMIA

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From November 1987 to October 1989 six patients with acute leukaemia, who achieved their first complete remission with standard chemotherapy followed by autologous bone marrow transplantation (ABMT), were consecutively treated with r.interferon alfa-2a (rA-IFN). Patients (4 ALL and 2 ANLL) were from 11 to 44 years old, 4 of them (2 ANLL and 2 ALL) were reinfused with autologous bone marrow purged with ASTA-Z 100 mcg/ml/ 2 x 10^7 cells and in the remaining 2 ALL patients immunomagnetic purging was employed. Conditioning regimens were BUCY in 4 patients and CY-TBI in the others. rA-IFN started at median time of 6 months (4-11) after ABMT when complete consolitated hemopoietic recovery occurred. The rA-IFN dose was 500.000 IU/sqm 3 times a week for 2 years. None of the 6 patients presented significant toxicity and only 3 short suspensions occurred for fever or ALT level increase. The incidence of infectious complications were particularly low compared with other autotransplanted groups of patients who received similar antinfectious prophilaxis. One case six months after ABMT and 20 days after acyclovir prophilaxis interruption presented a mild herpes-zoster complication which required new acyclovir therapy and resolved 10 days later. The amount of these patients is extremely low because the study was early interrupted to start a new protocol including IL-2; but the long duration of the good continous complete remission (3/5 years after ABMT) in all these unselected and consecutively treated patients is very interesting.

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TREATMENT OF HEPATOCELLULAR CARCINOMA (HCC) WITH A COMBINATION OF EPIRUBICIN (E) AND INTERFERON ALPHA 2B (IFN)

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In vitro experiments indicate higly synergistic effects of combining IFN with cytostatic drugs such as anthracylines (A). In a phase I/II-study 31 patients (pts) with progressive inoperable HCC were treated with E 20mg/m^2 weekly x 4 and IFN 3 Mio IU/m^2 s.c. 5 x weekly for 4 weeks, followed by one week off treatment. In case of at least no change (NC) and tolerable toxicity the therapy was continued. Escalation of E in steps of 5mg/m^2 per cycle was attempted.

PTS characteristics: Median age 56 years (21 - 69); male 20, female 11 PTS. Pretreated with A 3 pts.

Toxicity and treatment: Total number of cycles 92; median 3 (1-9) per PT. Worst toxicity per PT (WHO): WBC ^{o}III 38%, ^{o}IV 6%; platelets ^{o}III 9%, ^{o}IV 0%; diarrhoea ^{o}III 5%, ^{o}IV 0%; nausea/vomiting ^{o}III 5%; ^{o}IV 0%; IFN related fever (maximal ^{o}II) in 53%. IFN-related wasting syndrome 2 PTS, no severe organ toxicity.

Response: 1/31 MR (3%); 11/23 NC (35%; 3 A pretreated) for a duration of 2 - 9+ months; 19/31 P (61%). Median time to progression for all PTS 81 days; for PTS with MR/NC 7.8 months.

Conclusion: IFN only moderately increased E toxicity. The toxicity of treatment was graded as moderate by the PTS. About 40% of PTS will profit form E/IFN and achieve NC/MR. Activity in A pretreated PTS warrents further evaluation.

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PERI-OPERATIVE ADMINISTRATION OF INTERFERON ALPHA IN PATIENTS WITH ADVANCED MALIGNANT TUMORS : IMMUNOLOGICAL EFFECT AND CLINICAL TOLERANCE.

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Surgical procedures may be associated with an inceased risk of tumor spreading due to surgical mobilisation of the tumor and transient post - operative immunosuppression. Recurrences may result either from early growth of micrometastases already present at the time of surgery or from the seeding of malignant cells shed during operative manipulation of the tumor. Immunomodulators have been proposed to correct the immunological impairement induced by surgical procedures.

From 1/92 to 7/92, 23 patients with advanced stage cancer underwent surgical resection with peri-operative interferon-alpha administration. Patients received interferon alpha-2a (Roferon-A), by daily subcutaneous injection for fourteen days, starting on three days before surgery. Incremental doses were 2,3,5,7,9 and 12 x 10 6 IU for 4,4,3,3,3 and 6 patients respectively. Peripheral blood lymphocyte (Pbl) subset numbers were assessed using flow cytometric analysis the day before injection (D-3), before surgery (D-1), at the day 4 and 12. Absolute numbers of total T cell (CD 3+) and NK cell (CD 56+, CD 3-) were determined, as well as auxillary T cell (CD 4+), activated T cell (CD 3+, Dr+), and B cell (CD 19+) counts. Short-term cytotoxicity of PBMC against K 562 and DAUDI target cells in a 4-hour standard chromium release assay were determined.

No W.H.O. grade IV toxicity were observed. A significant post-operative fall of the total Pbl count, of CD3+; CD4+; CD19+; CD3-56+ occured from D-3 to D4. The decrease were not significant for CD3+Dr+. Values were not significantly different, between D-3 and D12, only for CD3Dr+ and CD3-56+. Cytotoxicity against K 562 and Daudi target cells increased significantly from D-3 to D-1, and from D-1 to D1, with a significant fall of cytotoxicity against Daudi from D-3 to D4.

Peri-operative interferon alpha administration is well tolerated even at the 12.10 6 IU doses. In spite of treatment and increasing cytotoxicity activity, we observed a post-operative fall for the majority of the imunological parameters. Further studies are necessary to compared with a control group, with more patients treated with 12.10 6 IU daily.

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PHASE II STUDY OF IFN α-2B IN REFRACTORY ACUTE LEUKEMIA.

P. Heußner, H.-D. Kleine, E. Petershofen, H.-J. Tischler and M. Freund.

IFN α has an antiproliferative effect on normal and abnormal hematopoietic stem cells. Besides this effect it may modulate the stem-cell stroma interrelationship. It may stimulate cytotoxic T-cells by enhancement of MHC class II expression. The incorporation of IFN α into maintenance immunotherapy together with IL-2 is currently under discussion. On this background we report on a trial with IFN α-2b in patients with advanced and refractory acute leukemia.

Treatment schedule: Treatment was started with 5 MU daily SC and was modified according to the clinical course.

Patients: Six patients have been treated (med. age 29 yrs.; 24-53 yrs.) Four patients had acute myeloid leukemia (AML M2: 2, M4: 1, M4Eo: 1). Two patients had acute lymphocytic leukemia (both T-ALL). All patients had manifest disease with more than 50% blasts in the bone marrow. Patients were selected not to have rapidly progressive diesease allowing the application of the cytokine.

Treatment and toxicities: IFN α was given for a median of 31 (14-114) days at a dose of 5-10 MU SC daily. The following toxicities $\geq$grade 3 WHO were observed: fever 5, GPT 2, pulmonal 1, infection 4 (1 pneumonia), pains 1. The patients with AML ware transsusion dependent for platelets and erythrocytes. No significant additional bleeding was observed.

Results: One AML patients had stable disease and three had disease progression. Of the ALL patients one had disease stabilization and one progression.

Conclusions: IFN α is well tolerated in patients with refractory AML and ALL if they are in a relatively stable condition. The effectivity of IFN α-2b as a single agent is poor in patients with refractory AML or ALL.

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INITIAL RESULTS, EXPERIENCES WITH INF ALPHA 2b IN THE TREATMENT OF HAEMATOLOGICAL MALIGNANCIES

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Authors have used INF alpha 2b in cases of haematological malignancies for three years.

The number of cases not too high, and non fo CML cases was so called "early" CML.

Our cases are: 5 CML, 3 Non-Hodgkin Malignant Lymphoma, 4 Myeloma multiplex, 3 Essential thrombocythemia.

The TNF alpha 2b was used as monotherapy in the cases of low grade Non-Hodgkin Malignant Lymphoma, and Essential thrombocythemia, and was combined with chemotherapy in the other cases.

On the basis of our initial results, we recommend the INF alpha 2b in the treatment of haematological MALIGNANCIES in suitably selected cases.

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INTERFERON ALPHA-2B THERAPY COMBINED WITH STANDARD CHEMOTHERAPY IN 102 ADVANCED CANCER PATIENTS

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Precious published works (M. Freund et al. Eds, Springer-Verlag, 1992) have confirmed the wide range of clinical usefulness of IFN alpha - based therapy in cancer patients (pts).

We present in this paper a retrospective analyses of 102 cases with advanced neoplasias treated between 1986-1992 by a sequential administration of IFN alpha and standard chemotherapy. There were 71 W, 31 M, aged 18-76 y, with solid cancers 99 pats and lymphomas 3 pts. The treatment consisted of a sequential association of IFN alpha (also with RA all-trans) and CHT plus Tamoxifen (for appropriate cancers). The IFN schedule was of monthly series, one series consisting of 3 million IU/d for 3 consecutive days. CHT, appropriate for each primary cancer also administered in monthly series. The results are grouped according to the status of the disease (subsets of pts) at the onset of IFN-based therapy, and refer especially to long term survival (1-5 y +). For minimal residual disease (MRD) from 39 cases there were 37 CR (24/24 breast ca, 6/6 CR melanomas, each STS and RCC 2/2 RC, 2/4 gastric and head and neck cancers together, 1/1 lymphoma. For progressive disease, pre-IFN-based therapy there were 63 pts, and post IFN-CHT treatment there were 58 SD and 5 PD. In 3 of failure pts association of IFN alpha and bropirimine (IFN-inducer) appeared an unusual good response. Conclusions: 1) IFN alpha-based therapy is a very useful one especially in MRD. 2) The therapy must be individualized for selected subsets of pts and for each patient day to day.

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The influence of low oral doses of human leukocyte derived interferon alpha on the immune system of chronically HBV infected patients with depressed immunological response.

B. Ratajczak

Low oral doses of the interferon were given to a group of seven children with limphoblastic acute leucaemia in the state of remission, chronically HBV infected. Interferon alpha was produced by Hayashibara Biochemical Laboratories Inc. Okayama, Japan, in tablets of 50 IU and 100 IU respectively. Immunological response was checked by measuring population and subpopulations of limphocytes, level of immunoglobulin and complement C_3 fraction. A distinct stimulation of cellular immune response was observed: the fraction of activated limphocytes T increased significantly, index CD_4/CD_8 became normal and population of limphocytes B increased gradually. There was no influence of interferon treatment on immunoglobulin and complement C_3 fraction serum level. The interferon treatment improved the patients' general condition and shortened the period of intoxication after cytostatic treatment. No side effects were observed. None of the children eliminated the HB virus during the six months treatment.

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RHIL-4 INHIBITS GROWTH OF HUMAN LUNG TUMOR CELL LINES IN VITRO AND HAS THERAPEUTIC ACTIVITY ON XENOGRAFTS IN VIVO.

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Cytokines play an important role in activating the immune system against malignant cells. One of these cytokines, IL-4 has entered clinical phase I trials because of its immunoregulatory potency. In the present study we report that rhIL-4 has direct antiproliferative effects on some human lung cancer cell lines in vitro as measured by a human tumor cloning assay (HTCA). This activity could be abolished by neutralizing antibody against rhIL-4. The biological response of the tumor cells to the cytokine is correlated with expression of receptors for hIL-4 on both the mRNA level and the protein level. The most responsive cell line CCL 185 secretes IL-6 after being incubated with rhIL-4. On the other hand, neutralizing antibodies against IL-6 showed no influence on the growth modulatory efficacy of rhIL-4 in this cell line. Furthermore, CCL 185 does not show detectable production of IL-1, TNF-α or IFN-γ after incubation with rhIL-4. Thus, the response to rhIL-4 is not mediated through autocrine production of these cytokines triggered by rhIL-4. In a next series of experiments the cell lines were xenotransplanted to BALB/c nu/nu mice. Subsequently, the mice were treated for ≥12 days with twice 0,5 mg/m^2 rhIL-4 (rhIL-4 was a kind gift from Dr. Urdal, Immunex, Seattle, USA) or control vehicle subcutaneously per day. Treatment with rhIL-4 yielded a significant inhibition of tumor growth versus control in the responsive cell lines CCL 185 and HTB 56, but no therapeutic effect in the non-responsive cell lines. Plasma levels of rhIL-4 were sufficient for in vitro growth-inhibition in the responsive lines. Histology of the tumors in both groups showed no marked infiltration of the tumors with murine hematopoietic and lymphocytic cells consistent with the species specificity of IL-4. We conclude that rhIL-4 has direct antiproliferative effects on the growth of some human lung tumor cell lines in vitro and in vivo which together with its regulatory effects on various effector cell populations makes this cytokine an interesting candidate for further investigation in experimental cancer treatment.
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INTERLEUKIN-4 SWITCHES THE PATTERN OF INTEGRIN EXPRESSION ON HUMAN TUMOR CELL LINES AND CAUSES A SELECTIVE INCREASE OF ADHESION TO MATRIX PROTEINS

E.D. Kreuser, F. Herzberg, P. Lang, M. Schöning, C. Mücke, and E. Thiel

Integrin receptors play a crucial role in cell-cell and cell-matrix adhesive function, and thus are supposed to influence invasion and metastasis. Very little is known about the impact of interleukins on integrin regulation in tumor cell lines. Therefore, we investigated the expression of 7α and 4ß integrin subunits on well (HT29) and poorly differentiated (SW620) human colon cancer cell lines using a panel of specific monoclonal antibodies and cDNA probes. HT29 and SW620 expressed similarly high levels of α_1, α_2, α_3, β_1, and ß4 subunits on the cell surface. No α_4, β_2, and β_3 was detected on either cell line. While α_5 was not expressed on HT29, SW620 showed higher levels of the laminin receptor $\alpha_6\beta_4$. The poorly differentiated cell line SW620 was resistant to IL-4, whereas HT29 was sensitive. Treatment with IL-4 induced a decrease in α_2, α_3, α_6, α_v, β_1, and β_4 integrin expression. However, α_1 subunit was markedly upregulated. In contrast to IL-4, there was no evidence that IL-1ß could modulate integrin expression on these cell lines. The function of integrin receptors was assessed by measuring adhesion to collagen, laminin, vitronectin, and fibronectin. IL-4 significantly increased the adhesion of HT29 to fibronectin, while attachment to collagen, laminin, and vitronectin remained unchanged. These results suggest differential integrin expression pattern on well and poorly differentiated tumor cell lines. We provide evidence that integrin expression may be selectively regulated by IL-4, but not by IL-1ß. Furthermore, IL-4 can alter adhesive behavior of tumor cells. Since IL-4 is currently studied in clinical trials, the metastatic potential of malignant tumors should be monitored thoroughly.

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BIOCHEMOTHERAPY OF ADVANCED MALIGNANT MELANOMA

J. Atzpodien, H. Kirchner, M. Volkenandt, M. Deckert, E. Lopez Hänninen, and H. Poliwoda

The combination of systemic chemotherapy and immunotherapy comprising interleukin-2 and alpha-interferon leads to significant tumor regressions in patients with advanced malignant melanoma. In contrast to chemotherapy by itself, the combination produces a significantly extended remission duration in the majority of treatment responders. We conducted 2 phase II studies to assess the potentially additive or synergistic effects of chemotherapy and immunotherapy in metastatic malignant melanoma patients:
The first study comprised two cycles of carboplatin (400mg/m²) and dacarbazine (750mg/m²); the sesond study included up to four cycles of cisplatin (25mg/m² x3 days), dacarbazine (220mg/m² x3 days), BCNU (150mg/m², cycle 1+3) and tamoxifen (20mg daily). Chemotherapy was followed by up to 2 cycles of a 6-week immunotherapy comprising interleukin-2 (5-20 million IU/m² sc 3x weekly) and alpha-interferon (3-6 million U/m² sc 3x weekly).
Among 25 evaluable patients in study I, there were 9 (12%CR, 24%PR) objective responders; median remission duration was 18+ months for complete, and 10+ months for partial responders. Chemotherapy intensification in study II lead to an increased response rate of 50% (9 out of 18 patients). In both studies, the progression free interval was significantly extended when compared to patients who received chemotherapy, only (historic controls).
The role of immunotherapy as maintenance in patients with advanced metastatic malignant melanoma is currently being evaluated in a prospective randomized trial.

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PROGNOSTIC FACTORS OF RESPONSE TO IMMUNOTHERAPY IN METASTATIC MELANOMA.

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Immunotherapy with IFNα and IL-2 is an active regimen in malignant melanoma and has shown response rates of 20 - 30%. In previous studies no prognostic parameters for response could be identified. 72 patients with progressive metastatic melanoma have been enrolled in various immunotherapy trials including IFNα and high dose IL-2 since 1987 with an overall response rate of 30%. 59 patients with MM treated in our phase II trials could be analysed to identify possible prognostic parameters for response. Patients were divided into three groups: responder (3 CR/14 PR), stable disease (16 SD/2MR), and non-responder (24 PD). All patients had measurable tumor, a Karnofsky index of > 70%, no CNS metastasis, and no severe cardiorespiratory or renal disease.
We examined the following pretreatment parameters for prognostic relevance of response: age, sex, performance status, time from diagnosis to onset of first metastases/ to begin of immunotherapy, tumor load, number of metastatic sites, organ sites of metastases, LDH, AP, ESR, and HLA-type. Of these several variables were found to significantly correlate with response: tumor load (p=0.023), number of metastatic sites (p=0.045), serum LDH (p=0.005) and AP (p=0.013). Tumor load, LDH and AP are no independent parameters. While time from diagnosis to onset of first metastasis is of no prognostic significance for response, the time between first diagnosis and begin of immunotherapy, usually reflecting metastatic disease necessetating systemic treatment, significantly correlates with probability of response (p=0.018). Since several HLA class I alleles have been shown to function as restriction elements for recognition of melanoma cells by specififc T cells in vitro, namely A1, A2, B44, and Cw7, we compared the frequency of these HLA antigens between responder and non-responder. We found A1, B44 and Cw7 to be increased in responder vs. non-responder. Our results indicate that in patients with MM tumor load, number of metastatic sites, LDH, and time from diagnosis to begin of immunotherapy are prognostic parameters for response to immunotherapy. These parameters may be useful to determine patients with good and poor risk for response to immunotherapy and are of relevance for stratification in randomized clinical trials.

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Surgery of metastatic melanoma following successful IL-2 based immunotherapy.

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Surgery of advanced metastatic melanoma is of limited value and usually not recommended. Immunotherapy using high dose IL-2 is effective in a substantial proportion of patients, however, the duration of responses is limited, and benefits in survival are not yet proven. This evaluation was done to determine the value of resection of residual tumor lesions following successful immunotherapy. 72 patients with progressive metastatic melanoma have been enrolled in various immunotherapy trials including IFNa and high dose IL-2 since 1987. 37 patients showed evidence of antitumor response (4 CR, 15 PR, 18 MR/SD). In patients responding to immunotherapy, residual lesions were resected, whenever technically possible and patients agreed to surgery (13 patients). 22 of the 24 responding patients without surgery relapsed, the median time to progression was 5 months (range 2-14), almost all initial relapses occured locally, 8 patients died so far.

11 of 13 patients who underwent surgery (9 PR, 4 MR/SD) were converted into CR by surgery. 4 of the 11 patients disease-free after surgery relapsed, 3 locally (2, 5, and 9 months after surgery), and one CNS 9 months after surgery. 7 patients are still free of recurrence (3+,3+,6+,12+,16+,18+,28+ months after surgery) and 9 of 11 are still alive. In the patient with CNS relapse complete resection of this lesion was again possible, and there was no evidence of recurrence for 9 months after this second surgery.

Histology revealed vital tumor cells in almost all resected specimens, however in 11 of 13 patients profound necrosis of the tumor tissue was observed. Of special importance is the observation that 3 patients with minor response or SD according to imaging procedures were found to have an almost complete response histologically. Interestingly, almost all metastases resected after immunotherapy had developed a fibrous capsule.

Surgical reevaluation and resection of residual lesions should be considered in patients with partial response after immunotherapy, and in selected cases also with stable disease. This approach offers the chance for extended disease free survival, and may be curative in certain patients.

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Tumor cells in peripheral blood of patients with malignant melanoma after successful immunotherapy.

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Recently a highly sensitive assay combining reverse transcription and polymerase chain reaction (RT/PCR) to assess for melanoma cells in peripheral blood has been developed. The detection of tyrosinase mRNA, a tissue spezific enzyme in melanocytes and melanoma cells in peripheral blood indicates the presence of melanoma cells.

We used RT/PCR assay to determine malignant melanoma cells in peripheral blood of 43 patients with malignant melanoma in different stages of disease. In none of 8 patients with stage I (localised tumor) but in 5 of 14 patients in stage II (regional lymph node metastases) tyrosinase transcripts were detected. Tyrosinase mRNA was found in all 21 patients with distant metastases (stage III). This method may be helpful to define a group of patients at high risk for development of hematogenous metastases, that would be a possible target group to explore adjuvant treatment strategies.

We then examined blood samples and bone marrow aspirates of 28 patients with metastatic malignant melanoma for presence of melanoma cells prior to and after therapy with IFN-a and IL-2. 10 patients showed antitumor response to immunotherapy: 3 complete remissions (CR) and 7 partial remissions (PR). 4 patients (3 PR, 1 stable disease) underwent subsequent resection of residual tumor lessions and had no clinical evidence of disease after the surgery. Tyrosinase mRNA was detected in blood and bone marrow samples from all patients with malignant melanoma prior to and after immunotherapy, including the patients without any clinical evidence of disease (median disease free survival 21 month, range 19-28 month). Tyrosinase transcripts were also detected in all patients with amelanotic melanoma. In contrast, no tyrosinase mRNA was determined in any of 30 healthy persons or 6 patients with other malignancies. The presence of residual melanoma cells in patients without clinical evidence of disease after successful immunotherapyover a prolonged time periods may confirm the assumption of sustained immunosurvaillence.

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T-cell-receptor (TCR) Vαβ usage of tumorinfiltrating T-cells in primary, regressing and progressing melanoma metastases following immunotherapy with IFNα and IL-2: evidence for a specific T-cell response.

Möhler, T., Willhauck, M., Scheibenbogen, C., Pawlita, M.#, Bludau, H.#, Brossart, P., Keilholz, U.

The identification and characterization of immunological effector cells mediating tumor regression in immunotherapy with IL2 is of great interest for understanding and further development of this therapeutic approach. Tumor infiltrating lymphocytes specific for autologous tumor cells can be expanded from certain melanoma tissues. T cells recognizing the same antigen use a limited TCR repertoire with a certain Vα and β variable region, determining the specifity of their receptor.

We therefore analyzed T-cell receptor V-Region distribution in tumor tissue from melanoma patients prior to and following immunotherapy with IL-2. We used a highly sensitive RNA-PCR method. After RNA-extraction from tissue and subsequent cDNA-synthesis semiquantitative PCR with different primers for all known Vα- and Vβ-T-cell receptor gene families (18 Vα and 20 Vβ) was performed.

12 tumor tissue samples were analysed including 6 samples of primary malignant melanoma and tumor samples of three patients after immunotherapy. The results were compared to control tissues (peripheral blood, unaffected skin, and liver tissue). The analysis of primary malignant melanoma tissue showed a weak overexpression of different Vβ-families. Preferential usage of different TCR-Vβ-genes was more obvious in tumor tissue of patients after immunotherapy.

Of special interest is a patient with a mixed response to immunotherapy with progressing and regressing skin metastases. In the regressing lesion we could demonstrate a predominant usage of TCR-Vβ11-Gene almost lacking in the progressing lesion. This suggests a role of Vβ11-expressing T-cells in mediating tumor regression in this patient. Cloning and sequenzing analysis are currently performed to assess wether this represents a true clonal T-cell proliferation.

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IN VIVO KINETICS OF CYTOKINE mRNA-TRANSCRIPTS IN PERIPHERAL BLOOD MONONUCLEAR CELLS (PBMC) FOLLOWING SUBCUTANEOUS IL-2 ADMINISTRATION: THE USE OF SEMI-QUANTITATIVE PCR.

R.Hilse, M.Meffert, J.Grosse, H.Kirchner, H.Poliwoda, and J.Atzpodien

We investigated the use of PCR for a semiquantitative estimation of cytokine expression patterns in PBMC before and after administration of IL-2 to patients with advanced renal cell carcinoma or malignant melanoma. mRNA of 9 cytokines was measured using a modified polymerase chain reaction protocol, which could detect 10-fold differences in mRNA-contents of stimulated PBMC *in vitro*.

Weekly RNA-samples of 7 patients receiving a total of 11 treatment cycles were examined for long term changes, in 2 patients frequent samples were taken immediately after IL-2-administration for transcript-kinetics. mRNA-expression for IL-4, IL-5, IL-6, IFN-γ, TNF-α, GM-CSF, TGF-β and IL-2-receptor-α was clearly detectable in most of the samples, including four healthy donors. However, our method could not detect significant changes in transcript-levels of PBMC during 3 days following injection of (A) 36 Mio.IU or (B) 2×18 Mio.IU rIL-2 daily.

This was in marked contrast to cytokine secretion assayed by ELISA. Thus, serum IL-2 peaked 2-4 hours after administration followed by secondary cytokines with a peak 2-16 hours later. Increases for TNF-α, IFN-γ, IL-6 and IL-2R serum levels were significant ($p<0,05$) with the highest response found for IL-6, increasing 35- (A) and 32-fold (B) at day 1, or 8- / 14-fold at day 2.

Comparing normal individuals to patients, only small differences in constitutive cytokine expression were seen (<10-fold) with no distinct pattern. During therapy, changes could be seen for all cytokines except for IL-2 and TGF-β. In one patient, a 100-fold increase for IL-6, TNF-α and IFN-γ transcripts was observed during week 4 of the second treatment-cycle, other changes were approximately 10-fold.

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REGIONAL IMMUNOTHERAPY: PERFUSION OF LIVER METASTASES WITH LAK CELLS

U. Keilholz, C. Scheibenbogen, M. Brado, W. Tilgen, and W. Hunstein

A regional approach of adoptive immunotherapy with interleukin-2 and lymphokine activated killer cells for the treatment of liver metastases is reported. The treatment consists of continuous infusion of interleukin-2 i.v. or into the splenic artery, and transfer of ex vivo generated lymphokine activated killer cells into the portal vein or the hepatic artery. 15 patients with malign ant melanoma, 2 with renal cell carcinoma, and 1 with thyroid carcinoma have been treated. All had progressive liver metastases. Trafficking studies using indium-oxine labelled cells revealed that >80 % of the LAK cells remained in the liver after regional adoptive transfer.

In 9 patients with liver metastases of cutaneous melanoma, 2 CR (24 and 23+ months), 1 PR (9+ months, converted to CR by surgery), 2 SD (10 and 11 months), ad 4 PD were observed. The lesion in the patient with PR was resectable after two cycles of treatment, and histology revealed almost completely necrotic tumor tissue surrounded by a dense fibrous capsule. No responses were observed in 6 patients with liver metastases of ocular melanoma, suggesting an immunologic difference between these two melanoma subtypes. 1 PR (6 months) and 1 SD (10 months) were achieved in 2 patients with renal cell carcinoma, and 1 SD (6 months) in the patient with thyroid carcinoma. Evidence for the crucial role regional cell transfer is provided by the observation in a patient with an anatomic variation of hepatic blood supply in whom we achieved complete and durable tumor regression. In this case anti-tumor responses were only observed in anatomic areas of the liver which were perfused with LAK cells.

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VIRUS MODIFIED CANCER VACCINES FOR THE ADJUVANT TREATMENT OF LOCALLY ADVANCED RENAL CELL CANCER

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Patients with locally advanced renal carcinoma are at high risk of relapse after initial radical surgery. We initiated a clinical phase II trial using autologous tumor vaccines for the surgical adjuvant therapy of renal cancer patients.

Seventy-two patients (pts) (25 female, 47 male; median age, 56 yrs; range, 28-77 yrs) with locally advanced renal carcinoma (pT3b-4pN0 or pTxN1-2M0) received autologous Newcastle Disease Virus modified and lethally irradiated tumor vaccines in combination with 1.8 million IU of IL-2 and 1.0 million U of IFN-α2, once weekly over 10 consecutive weeks. Toxicity was very mild with transient flu-like symptoms. Among 55 evaluable patients, there were 5 relapses (2pts, pT3aN1-2; 3pts, pT3bN0); the median relapse-free survival was 22+ months with a range from 6 to 41+ months; survival probability in this vaccine treated cohort was significantly better than in all historic controls.

Using Western blot analyses, we could demonstrate a vaccine specific in-vivo B-cell response in all patients receiving NDV tumor vaccine.

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RANDOMIZED PHASE II STUDY OF IL-2/IFN-ALPHA 2b VS IFN-GAMMA THERAPY IN METASTATIC RENAL CELL CANCER

S. Möllhoff, M. Goepel, T. Otto and H. Rübben

In a randomized phase II study we evaluated the response and side effects of a combined administration of Interleukin-2 (IL-2) and Interferon-alpha 2b (IFN-alpha 2b) versus Interferon-gamma (IFN-gamma) in patients with metastatic renal cell cancer.

Patients in group A received subcutaneous (sc.) 200 mcg IFN-gamma once a week. In group B patients were treated with a (sc.) combination therapy of IL-2 (36×10^6 IU/m^2 in week 1 and 4, 18×10^6 IU/m^2 in week 2,3, 5 and 6, twice a day for 5 days) and IFN-alpha 2b (5×10^6 IU/m^2) over 6 weeks once a day 3 times a week.

Up to now 60 patients were treated, 30 patients in each group. Toxicity of IFN-gamma treatment was absent. The therapy with IL-2 and IFN-alpha 2b led to sideeffects grade 2 (WHO): Fever, chivering, fatigue and weight-loss. Treatment were withheld in 20 %.

Follow up after 11 months (3-22 months) showed stable disease in 12 patients and progression in18 patients in group A. In group B there were 4 complete remissions, 2 partial remission and 24 patients with progressive disease.

Although the combination therapy showed 20 % objective response ($p<0.05$, Fisher-Test) no significant improvement on survival was seen ($p = 0.342$ logrank test).

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PRETREATMENT NATURAL KILLER ANTIGEN DENSITY CORRELATES TO CLINICAL RESPONSE IN TUMOR PATIENTS RECEIVING LONG-TERM SUBCUTANEOUS rIL-2 and rIFN-α

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A subset of peripheral blood natural killer (NK) cells has been found to exhibit high density surface expression of the NK associated CD56 antigen; it has been suggested that these NK cells respond to lower concentrations of IL-2 when compared to the majority of NK cells expressing cell surface CD56 at low density.

We evaluated density of the CD56 antigen on circulating NK cells of 47 patients with advanced renal cell carcinoma by flow cytometry. Patients received a combination of low-dose subcutaneous recombinant interleukin-2 (rIL-2) at 18 million IU/m²/day on days 1 and 2, followed by 3.6 million IU/m²/day, 5 days per week, over 6 consecutive weeks, in combination with recombinant α-interferon (rIFN-α) at 5 million IU/m², three times weekly. Antigen density of CD56 before therapy was found 2.2-fold higher ($p<0.005$) in patients who subsequently achieved a complete or partial tumor remission (n=10) when compared with patients who presented with progressive disease on therapy (n=11). After a 6-week treatment cycle, NK cells of treatment responders expressed significantly (2.1-fold; $p<0.005$) more CD56 antigens than NK cells in nonresponding patients.

These results suggested a potential role of both pre- and posttreatment NK antigen density levels as a biologic correlate to treatment response in tumor patients receiving low-dose rIL-2 and rIFN-α.

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Low-dose subcutaneous IL-2 following autologous bone marrow transplantation in cancer patients.

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28 patients (pts) entered an outpatient protocol designed to test the tolerance, and the clinical and biological effects of extended administration of SC low-dose IL-2 following high dose chemotherapy with autologous bone marrow rescue (BMT).

Two IL-2 regimens were used. Protocol A consisted of a once daily dose administered in 5-day cycles of 3 millions IU/m^2 SC rIL-2 (RU49637 kindly provided by Roussel-Uclaf) every other week. The pts were treated at home and the treatment was scheduled for 6 months (i.e. 12 cycles for 6 months) in the absence of major disease progression or side-effects. Protocol B consisted of a once daily dose of 6 millions IU/m^2 SC IL2 in 5 days/cycles for 6 consecutive weeks (i.e. 6 cycles for 6 weeks). IL-2 was started 60 to 90 days following BMT. Blood lymphocyte subsets and NK/LAK cytotoxic activity were determined monthly.

17 pts received regimen A and 11 pts were given regimen B. In both regimens, inflammatory skin reactions were the main side effect, leading to interruption of treatment in 1 case. No capillary leak was observed. Flue like syndrom was occasionny observed in protocol B. Hematological parameters were not adversely affected by IL-2. At the lowest dose levels (Regimen A), long term administration of IL-2 did not produce any changes of blood lymphocyte subsets. On the other hand, the administration of 6 Millions IU/m^2 IL-2 resulted in a significant increase of CD3+, CD25+ cells and CD3- CD56+ cells, and NK/LAK cytotoxic activity of fresh Pbls. This study confirms the feasability of long-term administration of SC lowdose IL2 following autologous ABMT. A dose of 6 Millions IU/m^2/d resulted in detectable activation of circulating lymphocytes. Further studies are needed to assess the clinical impact of prolonged low-dose iL-2 in this clinical setting.

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High activity of glycosylated r-IL2 in vivo : Results of a phase I study.

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SR 29009 (Sanofi Recherche) is a CHO-derived r-IL2, glycosylated on the threonine in position 3. Its specific activity is identical to native IL2. Dose escalation studies were performed using SR29009 either as IV bolus or IV continuous infusion in advanced-stage cancer patients. Treatment schedule consisted in three 5-day cycles with 9-day rest in between. 22 pts received IV bolus Q8-12 hrs at 4 dose levels (1;3;6;9 Millions IU/m^2/bolus) and 19 pts received IV continuous infusion at 5 dose levels (0.3;0.5;1;2;3 Millions IU/m^2/day). MTD were found to be 6 Millions IU/m^2 Q8 hrs/day and 2 Millions IU/m^2/day for bolus and continuous IV infusion respectively. Toxicities were similar in nature to those described under treatment with non-glycosylated rIL-2. DLT were mainly related to capillary leak. 1PR (NHL) was obtained. A significant rise of T-cell and NK cell subsets as well as NK/LAK cytotoxicity in blood was observed at day 8. Stimulation was already maximum for the lowest dose levels. At 0.5 Millions IU/m^2/day, the mean number of CD3+ CD25+ cells increased from 113 to 486/mm^3 on day 8. Similarly, CD56+ cells increased from 461 to 1345/mm^3, and cytotoxicity of fresh Pbl against K562 increased from 10% to 40% (E/T ratio = 25 : 1). In vivo, SR29009 appears to exhibit effects similar to those observed with higher doses (x 4-6 fold) of non glycosylated rIL2.

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INFECTIOUS COMPLICATIONS DURING IL-2 THERAPY IN AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMT) PATIENTS.

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AIM: To study the incidence of infectious episodes (IE) in a single centre series of 7 autologous BMT patients who received IL-2 in an attempt to prevent relapse.

PATIENTS. METHODS: IL-2 was given as a continuous i.v. infusion through a Hickman catheter with a portable pump: 200.000 IU/m^2/d for the first week and 400.000 IU/m^2/day thereafter during 12 weeks. IL-2 was started when full neutrophil engraftment was achieved and platelet counts remained stable over 50.000 platelets/mm^3 (median: day +64. Range: 38-118).

Median age was 39 years (5-46). 6 were male. BMT was performed for Acute Lymphoblastic Leukemia (3), Lymphoma (2), and Myeloma (2).

RESULTS: IL-2 planned therapy was completed in 5 patients (1 early relapse, 1 refusal) in a median of 103 days (90-154). Median intensity dose of IL-2 was 2.8 x 10^5 IU/m^2/day (1.2 x 10^5-3.82 x 10^5).

10 infectious episodes who precised admission to the hospital were observed in 5 patients. 7 of these infections were microbiologically documented: 56% of isolations corresponded to gram negative and 44% to gram positive. First IE was detected at a median of 11 days (4-56) after the starting of IL-2. Median cumulative IL-2 dose until first infectious episode was 3 x 10^6 IU/m^2 (0.8 x 10^6 - 15 x 10^6). Catheter were removed in 3 of them.

CONCLUSIONS: In our experience, low dose continuous i.v. administration of IL-2 is associated with a high incidence of infectious episodes.

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INDUCTION OF LYMPHOKINE- (LAK) AND BACILLUS CALMETTE-GUÉRIN-ACTIVATED KILLER (BAK) CELLS AGAINST BLADDER TUMOR CELL LINES *IN VITRO*

A.Thanhäuser, A.J.Ulmer, A.Böhle, T.Mattern, M.Ernst, and H.-D.Flad.

Although intravesical therapy with Bacillus Calmette-Guérin (BCG) against superficial bladder cancer recurrences and carcinoma in situ is highly effective, its mode of action is still unclear. The bladder tumor cell lines BT-A, BT-B (grade 3 transitional cell carcinoma), SBC2, and SBC7 (grade 1 transitional cell carcinoma) are nearly resistant to natural killer cell activity *in vitro*. We could demonstrate that these cell lines are susceptible to lymphokine-activated killer cells generated by interleukin (IL)-2 or interferon (IFN)-γ. We have now investigated whether BCG itself is able to activate a LAK cell-like reaction against bladder tumor cells. Our results show that activation of PBMC with viable BCG is a potent way of generating cytotoxic effector cells against bladder tumor cells. In contrast, killing of the NK and LAK cell-sensitive K562 cell line was not enhanced by BCG-induced PBMC. Because of their different target cell pattern and their, compared to LAK cells, distinct way of activation these cytotoxic effector cells were termed "BCG-activated killer (BAK) cells". Antibodies neutralizing IFN-γ activity blocked the induction of BAK cell cytotoxicity, indicating that this cytokine is playing a crucial role during this process. With respect to the phenotype of BAK cells, our data show that the effector cells belong to the $CD8^+/CD56^+$ lymphocyte subset. Depletion of either $CD8^+$ or $CD56^+$ cells from BCG-induced PBMC led to a decrease of cytotoxicity against bladder tumor cells. Furthermore, positively selected $CD8^+$ cells could maintain the level of cytotoxicity exerted by BCG-induced PBMC whereas the depletion of $CD56^+$ cells from this population also eliminated the cytotoxic effect. A direct involvement of $CD4^+$ cells or macrophages in the killing of bladder tumor cells was not observed. In patients with superficial bladder cancer LAK and BAK cells might play an important role in the maintenance of the relapse free state. *(Supported in part by a grant of Institut Mérieux, Leimen)*

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LOCAL IMMUNE ACTIVATION BY INTRAVESICAL BACILLUS CALMETTE-GUÉRIN (BCG) AGAINST SUPERFICIAL BLADDER CANCER A.Böhle, A.J.Ulmer, J.Gerdes, E.Busemann, K.Dietrich, D.Jocham, and H.-D.Flad.

Intravesical immunotherapy against superficial bladder tumor recurrences and carcinoma in situ is a recognized and highly effective regimen in urology. To further clarify the mode of action of this approach, the local immune response of patients was investigated: The cytokines IL-1, IL-2, and TNF were determined in the urine before and after intravesical instillation by ELISAs and biological assays. Furthermore, bladder biopsies taken before and after the treatment course were analysed by means of immunohistology for the presence of mononuclear cell subsets. The results show a significant increase of urinary cytokines with a maximum 4-8 hours after the instillation of BCG which returned to baseline values within 24 hours. This intense local immune activation was further reflected by the accumulation of activated mononuclear cells, predominated by T cells as demonstrated with bladder biopsies. The local T-helper/T-suppressor cell ratio shifted towards the T-helper subset. These changes persisted for more than 1 year after the initial treatment course. In conclusion, this local immune response may be associated with the therapeutic success of BCG. Further analyses will dissect the role of each factor with regard to antitumor cytotoxicity against bladder carcinoma.
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ENHANCEMENT OF THERAPEUTIC EFFECTS OF INTERLEUKIN-2 ON TWO TRANSPLANTABLE MURINE TUMORS BY ASSOCIATION WITH CYCLOPHOSPHAMIDE

S.Dima, E.Badea, I.Corneci, A.Iosub, G.Mota and I.Pană

Enhancement of therapeutic effect of Interleukin-2 (IL-2) by association with Cyclophosphamide (Cy) was studied on EL-4 lymphoma maintained in ascitic form in syngeneic C57BL/6 (H-2^b) mice and lymphoreticulosarcoma (spontaneous origin) maintained in solid form in syngeneic CBA (H-2^k) mice. Immunotherapy with IL-2 (obtained by in vitro stimulation of EL-4 lymphoma cells with phorbol myristate-acetate) was applied 24 hours after transplantation of EL-4 lymphoma and 10 days after transplantation of lymphoreticulosarcoma by administration i.p. (intratumoral) in EL-4 bearing mice and s.c.(peritumoral) in lymphoreticulosarcoma bearing mice for three consecutive days. Cyclophosphamide was administered at a dose of 180 mg/kg i.p. six hours before the immune treatment with IL-2. The results obtained demonstrated that the prolongation of the survival rate expressed by the median survival time (MST) and the percentage of increasing life span (ILS) of the groups treated with IL-2 associated with Cy was significantly higher than that of the groups which receveid a single treatment with IL-2 or Cy. Enhancement of therapeutic effects of IL-2 in association with Cy on lymphoreticulosarcoma was revealed by inhibition of tumor growth with a marked regression in the volume of the established tumors and even resorption in some cases. We conclude that the antitumoral effect of IL-2 treatment was enhancemented by association with Cy a well known cytoreductive drug which selectively removed T-suppressor lymphocytes from the tumor bearers. This could be considered as an alternative to immuno- or chemotherapy in cancer.

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CELLULAR EXPRESSION OF P-GLYCOPROTEIN IS ASSOCIATED WITH TUMOR PROGRESSION IN RENAL CELL CARCINOMA PATIENTS

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The phenomenon of multi drug resistance (MDR) in human tumors has been attributed to the expression of a 170 kD membrane glycoprotein (P-glycoprotein). Immunophenotyping of untreated renal cell carcinomas showed P-glycoprotein expression in 48-100%. It has been suggested that P-glycoprotein contributes at least partially to the high degree of intrinsic chemoresistance of renal cell carcinomas. Furthermore, in vitro experiments demonstrated a possible relationship between high levels of P-glycoprotein expression and increased resistance to NK mediated cytotoxicity.
We evaluated the constitutive i.e., pretreatment P-glycoprotein expression of tumor cells in 25 patients with advanced renal cell carcinoma employing immunocytochemistry. Positive staining results were found in twenty tumor specimens (80%) using the monoclonal antibody C219. P-glycoprotein expression of tumor cells below 1% was associated with an extended progression-free survival when compared to patients with 1% or more positive tumor cells (Kaplan-Meier analysis, $p<0.005$).
These results suggested a potential role for P-glycoprotein as a correlate to tumor progression in vivo.

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AEROSOLIZED NATURAL INTERLEUKIN 2 FOR TREATMENT OF ADVANCED MALIGNANCY: RESULTS OF A PHASE I TRIAL.

W.E. Aulitzky. M. Kessler, M. Wilhelm, E. Huland, A. Thews, C. Peschel, C. Huber, J. Lorenz

To investigate the toxicity and clinical efficacy of aerosolized nIL-2 (Biotest) 15 patients presenting with advanced malignancy were entered into a phase I trial. 13 patients suffered from metastasizing renal cell carcinoma, 2 patients from advanced bronchial carcinoma. At start the patients received either 50000, 150000 or 300000 U nIL2 applied as a single dose. If no adverse events were observed, treatment was continued with the same dose 5 times daily for six weeks. In addition to standard invetigations detailed evaluation of the respiratory function was performed once weekly. Soluble interleukin 2 receptor serum levels and the effect on numbers and/or phenotype of lymphocytes in the bronchoalveolar lavage fluid were measured for assessment of biological response to inhalative nIL-2 treatment.
Treatment with aerosolized nIL-2 was well tolerated. Most prominent toxicity appeared to be resistant cough in all patients treated with 5x300000 U/d. No febrile reactions or other constitutional side effects were observed. A dose-dependent increase of the numbers of memory T lymphocytes, macrophages and eosinophil granulocytes could be demonstrated in BAL fluid. In addition, the treatment resulted an increased expression of adhesion molecules on lymphocytes. 1 patient suffering from renal cell carcinoma achieved a partial remission after 6 weeks of treatment with 5x50000 U/d. We conclude that treatment with aerosolized nIL-2 is biologically active and well tolerated and should be further tested in clinical phase II trials.

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SOLUBLE INTERLEUKIN-2 RECEPTORS ABROGATE IL-2 INDUCED CELLULAR ACTIVATION IN MICE AND MEN

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Soluble interleukin-2 receptors (sIL-2R) exert a potential role in immunoregulation. We investigated the ex vivo effects of sIL-2R on several interleukin-2 (IL-2)-dependent activation events. Proliferation of the IL-2-dependent mouse cell line CTLL-2 and isolated human PBMC stimulated with recombinant IL-2 (rIL-2) was suppressed by sIL-2R added to the culture medium in a dose-dependent way. Preincubation of sIL-2R with rIL-2 did not enhance this suppression. Cytotoxicity of rIL-2-stimulated human PBMC against the human cell lines K562 and Daudi was correlated inversely to the concentration of sIL-2R in the culture medium during rIL-2 stimulation. sIL-2R concentrations higher than 4.0 pM produced a significant decrease in cytotoxicity ($p<0.01$). Light microscopy of IL-2-stimulated PBMC revealed no signs of cellular activation when high dosages of sIL-2R had been added. The effect of different sIL-2R concentrations added to cultured human PBMC on secondary IL-2 and sIL-2R production was tested by ELISA. Initial supply with high sIL-2R dosages yielded weak increase and subsequent slow reduction of IL-2 levels. In contrast, strong secondary IL-2 production followed by rapid clearance was observed when low sIL-2R concentrations had been added. Endogenous shedding of sIL-2R in response to rIL-2 was abrogated by the initial exogenous addition of high amounts of sIL-2R whereas low exogenous addition of sIL-2R was followed by a continuing endogenous production of sIL-2R after five days of culture. Our studies may lead to a better understanding of IL-2-related immunoregulation in the preclinical and clinical settings.

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IMMUNOLOGICAL CHANGES IN CANCER PATIENTS RECEIVING RECOMBINANT HUMAN INTERLEUKIN-6

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In a phase I clinical trial of recombinant human Interleukin-6 (IL-6), 21 patients were entered to receive daily subcutaneous injections of IL-6 over 7 days followed by a two week observation period and another 4 weeks of daily IL-6 injections. Doses varied between 0.5 and 20 µg/kg body weight. 18 patients were evaluable for studying immune functions. At all dose levels IL-6 administration led to a marked increase in serum levels of C reactive protein and complement factor C3. Natural killer (NK) cell activity was reduced at doses exceeding 5 µg/kg. Similarly lymphokine activated killer (LAK) cell activity induced by in vitro culture over 4 days in the presence of 200 U/ml Interleukin-2 (IL-2) was suppressed at 10 and 20 µg/kg, as was the proliferative response to IL-2 in vitro. However no changes were observed in the proliferation induced by phytohaemagglutinin, pokeweed mitogen or fixed staphylococcus aureus. There were no changes in peripheral blood lymphocyte subpopulations as measured by CD4 and CD8, nor in the expression of HLA DR. Serum levels of immunoglobulins IgA, IgM and IgG remained unaffected by IL-6 treatment. In contrast we found consistent elevations in levels of IgE all over the dose range. We conclude that IL-6 inhibits NK and LAK activity in vivo which may be of interest in future studies with cytokine combinations and that the role of IL-6 in IgE related diseases might be more important than previously thought.

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ANTIINFLAMMATORY ACTIONS OF TYPE I INTERFERONS.

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We investigated the effect of interferons (IFN) on expression of IL-8 and other cytokines regulating inflammatory responses in various cellular models in vitro and in vivo. In peripheral blood mononuclear cells (PBMNC) of healthy individuals IL-8 gene expression which was upregulated in vitro was significantly reduced in presence of IFN-α. In dose titration experiments a reduction of the IL-8 protein was detected at IFN concentrations as low as 30U/ml. In CML patients with constitutive expression of IL-8 a reduction of IL-8 mRNA expression was seen after therapeutic administration of IFN-α. By contrast, in LPS stimulated granulocytes IFN failed to inhibit IL-8 expression in vitro. We investigated the mechanism of IL-8 inhibition in the THP-1 cell line more in detail. Nuclear run on assays and RNA decay analysis in presence of acinomycin D suggested that the effect was regulated predominantly at a posttranscriptional level. De novo protein synthesis was not required since the inhibitory effect was also detected in presence of cycloheximide. In addition to IL-8 expression we studied the effect of IFN-α on the synthesis of IL-1, TNF, IL-6 and IL-1RA in PBMNC and bone marrow stromal cell cultures. These experiments revealed an antagonistic effect of IL-1 action by IFN-α at two levels which was most striking in bone marrow stromal cells. Expression of IL-1 mRNA was downregulated whereas the production of IL-1RA was enhanced by IFN-α. In contrast expression of IL-6 and TNF was enhanced by IFN.

We conclude that IFN-α differentially regulates proinflammatory cytokines. The inhibition of IL-8 and IL-1 action suggest an antiinflammatory role of type I IFNs.

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CYTOKINE RESPONSE OF HUMAN MACROPHAGES AFTER STIMULATION WITH LPS AND A LIPID A ANALOG, TWO DISTINCT PATHWAYS

H.-P. Knopf, F. Herrmann and R.R. Schumann

One of the most potent stimulatory agents for the induction of cytokines in myeloid cells is the bacterial cellwallproduct LPS (Liopopolysaccharide or Endotoxin). In the bloodstream it forms a complex with LBP (LPS Binding Protein) and is recognized by effector cells via the CD14 receptor. Here we report on studies performed with human peritoneal macrophages that were stimulated in vitro with LPS and a synthetic LPS homologue in the presence and absence of serum. As revealed by ELISA-based analysis of the cellsupernatants, strong, serum-dependent resonses were seen for TNF-, IL-6, IL-8 and G-CSF production, while unstimulated cells produced basically only IL-8. Repeated stimulation of the cells with LPS resulted in adaptation that was different for certain groups of cytokines. The "adapted" cells produced much less TNF and IL-6 while IL-1 and G-CSF was superinduced. Stimulation of the cells with the Lipid A anolog MRL 593 showed a similar picture, given that MRL had to be used in higher concentration. "Adapatation" of the cells with MRL also resulted in an "adapted" response to a challenge with a high dose of LPS so that it might be useful as a therapeutic agent for preventing the septic shock syndrome, e.g. Northern blot analysis showed that the differentiated response of the cells towards a low dose LPS stimulation regarding cytokine production after an LPS challenge occured on transcriptional level, as mRNA levels were regulated accordingly. These results give evidence that two different pathways for LPS dependent stimulation of myeloid cells for cytokineproduction exist and experiments to further elucidate this phenomenon and possibly discover CD14 independent and dependent pathways are underway.

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A C-TERMINALLY TRUNCATED MOLECULAR VARIANT OF THE NEUTROPHIL-ACTIVATING POLYPEPTIDE NAP-2 EXHIBITS ENHANCED BIOLOGICAL ACTIVITY

E.Brandt, F.Petersen, and H.-D.Flad

The neutrophil-activating peptide 2 (NAP-2), a member of the "intercrine"-family of chemotactic and reparative host defense cytokines, represents one of several N-terminally truncated cleavage products that originate from platelet-derived ß-thromboglobulin through proteolytic processing. Here we present evidence that there exists also a naturally occurring C-terminally truncated form of NAP-2 that is about four times more potent in eliciting neutrophil degranulation than the original cytokine. The novel molecule was detected in concentrates of culture supernatants from peripheral blood mononuclear cells and could be separated from authentic NAP-2 by several steps of column chromatography. According to amino acid sequence analysis it had a N-terminus identical to NAP-2, whereas electrophoretic analyses indicated a lower molecular weight as well as a higher isoelectric point. Immunochemical analyses performed with epitope-characterized antibodies raised against NAP-2 C-terminal synthetic peptides identified limited truncation at the C-terminus of the variant molecule. Comparison of reactivity patterns of these antibodies in Western blots as well as in a NAP-2 biologic assay (PMN degranulation assay) confirmed that the variant NAP-2 was truncated by at least one and by maximally three residues. Thus, there is for the first time evidence that proteolytic processing at the N-terminus is not necessarily the only mechanism regulating the formation of neutrophil-activating peptides, but that modification at the C-terminus may assist in the fine-adjustment of biological activity.

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THE NEUTROPHIL-ACTIVATING PEPTIDES NAP-2 AND IL-8 SHARE THE SAME RECEPTORS ON NEUTROPHILS: INHIBITION OF PRIMARY GRANULE EXOCYTOSIS FOLLOWING DOWN-MODULATION OF NAP-2 HIGH AFFINITY BINDING-SITES.

F.Petersen, H.-D.Flad, and E.Brandt

Interleukin 8 (IL-8) and the neutrophil-activating peptide 2 (NAP-2) are two closely related members of the "intercrine" family of host defense cytokines. The expression of at least two different receptor classes for IL-8 on human neutrophils (PMN) exhibiting similar affinities has been demonstrated recently. Using iodinated ligands we could directly demonstrate that ^{125}I-NAP-2 specifically bound to PMN with two different affinities, characterized by K_d-values of about 1.4 nM and 9.6 nM, respectively. Cold 72-residue IL-8 competed with iodinated NAP-2 for binding to the high affinity site(s) with practically equivalent efficacy, while it was significantly more effective in displacing ^{125}I-NAP-2 from its low affinity site(s) than was cold NAP-2 itself. As new findings, unlabeled IL-8 could completely displace ^{125}I-NAP-2 and vice versa, indicating that there are no distinct binding sites for either cytokine on PMN. In contrast to IL-8, NAP-2 did not induce PMN degranulation at concentrations (2 nM), engaging solely its high affinity site. However, short-term priming of PMN with the same amount of NAP-2 dramatically down-regulated degranulation inducible by higher concentrations of NAP-2 as a secondary stimulus. The IL-8-induced secondary response was also diminished, but to lower extents. These phenomena correlated with the rapid down-regulation and internalization of NAP-2 high affinity binding sites from the cell surface. Thus, our data provide direct evidence for a regulatory function of NAP-2 at very low concentrations, obviously occurring through the modulation of NAP-2 and IL-8 receptor expression on PMN.

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CIRCULATING CYTOKINES IN THE FEBRILE NEUTROPENIC PATIENT: CORRELATION OF INTERLEUKIN-6 AND INTERLEUKIN-8 WITH BODY TEMPERATURE

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Cytokines like Interleukin-1-beta (IL1), Interleukin-6 (IL6), Interleukin-8 (IL8) and Tumor-Necrosis-Factor-alpha (TNF) are involved in the pathogenesis of fever and infection. However, intra- and inter-individual values differ considerably and there is only limited data on early cytokine serum levels and their evolution in febrile neutropenic patients. Therefore, we measured cytokine levels in 14 adults with chemotherapy-induced aplasia and fever. Concentrations of IL1, IL6 and TNF were determined by IRMA, IL8 by ELISA in 11 specimens per patient.

IL1 and TNF were elevated in 5 of 10 patients with peak values of 113 pg/ml and 147 pg/ml, respectively. IL6 and IL8 were elevated in all patients with a maximum of 41.300 pg/ml (median 416 pg/ml, range 104 - 41 316 pg/ml) for IL6 and 25.6oo pg/ml (median 1100 pg/ml, range 150 - 26.000) for IL8. Both cytokines showed a high correlation (r=0.89). The individual IL6-concentration-time curve closely paralleled the temperature curve. In 10 of 14 patients IL6 was elevated before onset of fever and peaked at or one hour before the temperature maximum in seven cases. So also in the cytopenic patient lacking a main source of cytokine producing cells and cytokine target cells consistently high IL6 and IL8 serum levels can be detected very early in the course of fever and infection. The biological and clinical significance of this cytokine response and its regulation mechanisms remain to be determined.

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Sequential cytokine plasma levels in drug fever

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Determination of cytokine plasma levels possesses many promising features concerning monitoring and studying of an array of different diseases including febrile reactions. Detailed analysis of the role of these factors is of crucial importance for the understanding of the complex cytokine network. Investigation of cytokine blood levels however is complicated by their short half-life in circulation, the presence of soluble inhibitors and the ill-defined beginning of fever.

Looking for a suitable in-vivo-model allowing a sequential and well-defined analysis of cytokine plasma levels we chose the acute toxicity after intravenous amphotericin B (Am B) application consisting of fever, chills and hypotension. These side-effects were reported to be mediated by release of pro-inflammatory cytokines such as TNF α and Interleukin 1 (IL-1).

In order to compare mutual interations and different temporal patterns of liberation we determined a panel of cytokines including TNF α, s-TNF-receptor (s-TNF-r), Interleukin-1 β, Interleukin-1-receptor-antagonist (IL-1-RA), Interleukin-6 and Interleukin-8 from 15 patients suffering from acute leukemia and fungal infections. Serial EDTA-plasma samples were obtained before and up to 8 hours after start of Am B infusion. Samples were immediately centrifuged and stored at -40° C until analysis by ELISA (Medgenix and R&D Systems).

Patients experiencing adverse reactions showed TNF α peak plasma levels 90 -180 minutes after starting Am B infusion reaching maximum concentrations of 750 pg/ml. Concentrations declined to base levels within the following 2- 3 hours. IL-6 as well as IL-8 concentrations showed similar, but delayed changes of plasma concentration. Compared to TNF α s-TNF-r-levels peaked about 30 minutes later with a prolonged decrease to basal concentrations. In contrast to TNF α no circulating IL-1-β could be detected, while IL-1-RA demonstrated up to 5-fold increases in concentration.

We conclude that this model of a drug induced acute-phase-reaction offers wide possibilities for studying the behaviour of inflammatory cytokines and their inhibitors by means of plasma level determination.

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INTERLEUKIN-6 (IL-6) AND TRANSFORMING GROWTH FACTOR-ß (TGF-ß) IN ACUTE INFLAMMATION FOLLOWING TRAUMA: REGULATION OF NEUTROPHIL DEGRANULATION

U. Bank, D.Reinhold, D. Kunz and S. Ansorge

Inflammatory processes following severe trauma were found to be associated with an abnormal high secretion of inflammatory cytokines. These cytokines are discussed to be involved in neutrophil activation associated with the release of high amounts of destructive lysosomal proteases into the extracellular space. The task of our investigations was to evaluate the possible regulation of the degranulation of neutrophils by the immunostimulatory cytokine IL-6 and the immunosuppressive factor TGF-ß. We analysed the concentration of the α_1-antitrypsin-complex of the lysosomal protease elastase as markers for the degranulation of neutrophils as well as the levels of IL-6 and TGF-$ß_1$ in the plasma of patients with multiple trauma or after severe surgeries. The time courses of the plasma levels of IL-6 and the elastase-inhibitor-complex were found to be highly correlated, suggesting a possible regulatory role of this cytokine on the neutrophil degranulation. However the plasma concentrations of TGF-ß were not significantly altered in comparsion to the control group. In additional experiments, the effect of both cytokines on the degranulation of healthy donors was investigated in vitro. Pathological high concentrations of rhIL-6 up to 10^4 U/ml (as detected in several probes from the surgical area) were found to be capable to induce a significant degranulation of the azurophilic granules (56,3 ± 20,2% of the total cellular enzyme content) under serum free conditions as detected by measurement of elastase release by ELISA technique and enzymatic methods. In contrast to this, the degranulation of neutrophils was found to be uneffected by TGF-$ß_1$. In conclusion, these data suggest that the inflammatory cytokine IL-6 may contribute to the activation of neutrophil granulocytes in acute inflammatory processes following severe trauma, whereas the immunosupresive factor TGF-$ß_1$ seems to have no direct regulatory effects beside the described chemotactic effects on neutrophils.

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SPONTANEOUS PRODUCTION OF IL-1β AND IL-6 BY PERIPHERAL BLOOD MONOCYTES FROM PATIENTS WITH POLYMYALGIA RHEUMATICA AND GIANT CELL ARTERITIS A.D. Wagner, G.G. Hunder, J.J. Goronzy and C.M. Weyand

Polymyalgia rheumatica (PMR) and giant cell arteritis (GCA) are closely related syndromes. GCA is a vasculitic disorder of medium and large sized arteries. PMR represents a variant of the same systemic disease entity without evidence for vasculitic lesions. Both diseases are characterized by increased acute phase responses which have been correlated with elevated concentrations of IL-6 in the serum.

To answer the question whether IL-6 is of hematogenous origin, immunohistochemistry and in situ hybridization with IL-6 specific S^{35} labeled RNA probes was established to study the frequencies of IL-6 expressing peripheral blood mononuclear cells. A two color immunofluorescence assay with antibodies to CD68 and IL-6 was utilized to correlate IL-6 mRNA expression and IL-6 protein production in monocytes of patients and control individuals. 60 - 70% of circulating monocytes spontaneously expressed IL-6 mRNA compared to 15% in normal individuals. A strong correlation of IL-6 protein production and IL-6 mRNA was found in monocytes of patients and controls. Semiquantification of IL-6 mRNA and IL-1β mRNA by PCR demonstrated that about 10 to 60 fold higher amounts of mRNA were found in untreated patients compared to normal individuals. In contrast, we did not find evidence for excessive synthesis of TNFα mRNA. The overproduction of IL-6 and IL-1β in the absence of detectable TNFα mRNA establishes a specific cytokine pattern in circulating monocytes of PMR and GCA patients.

We analyzed the IL-6 mRNA expression in biopsy specimens from GCA patients applying in situ hybridization. Autoradiographs were analyzed by visual examination and by using an image-analysis system. Tissue infiltrating macrophages expressed IL-6 mRNA, however, the proportion of IL-6 $mRNA^+$ $CD68^+$ was lower than in the peripheral blood. Also, immunohistochemistry with an IL-6 specific antibody demonstrated that only a small fraction of macrophages produced this cytokine. These data suggest that in PMR and GCA, circulating monocytes are activated and produce IL-1 and IL-6. Tissue infiltration of macrophages is not accompanied by a local activation, in contrast, cytokine production is downregulated.

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SOLUBLE TUMOR NECROSIS FACTOR RECEPTORS INDICATE IMMUNE ACTIVATION IN HIV INFECTION

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We determined serum concentrations of soluble tumor necrosis factor receptor (sTNF-Rs) in 61 HIV infected individuals. Eighty-five percent of these had increased serum concentrations of sTNF-R type I (p55) (sTNF-R55) and 95% had increased sTNF-R type II (p75) (sTNF-R75). The extent of the increase of sTNF-R75 was greater in more advanced HIV infection (p=0.046) as it was measured by dividing the 61 individuals into two groups according to the median of the CD4+ T cell count. sTNF-R-55 did not differ between these two groups. A strong correlation was found between sTNF-R75 and the soluble immune activation markers ß2-microglobulin (rs=0.74, p<0.0001) and urinary neopterin rs=0.67, p<0.0001), and a less strong correlation with interferon gamma (rs=0.51, P=0.0001). The correlations observed for sTNF-R55 were also significant but were always weaker than that for sTNF-R75. A weak inverse correlation was found between the number of CD4+ T cells and sTNF-R75 (rs=-0.33, p=0.012), no such correlation was observed with sTNF-R55. Our findings suggest that increased concentrations of serum sTNF-Rs in HIV infection are linked to immune activation where synergistic action of interferon-gamma and the TNF-alpha system are likely to play an important role.

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21 kDa PROTEIN SERUM CONCENTRATION IN HIV-1 INFECTION DURING INTERFERON-(IFN ALPHA) THERAPY - CORRELATION TO CLINICAL STATUS

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Introduction: A 21 kDa protein (p21) is frequently found in HIV-1 infected patients and patients with proliferating urogenital or gastrointestinal tumors. Our objective was to evaluate the prognostic value of p21 protein levels in patients with advanced HIV-infection during cytokine therapy (IFN or IFN/combination). **Methods:** p21 serum levels were measured every two months by ELISA in 29 HIV-infected patients during a period of 2 to 15 months (median 8,6) and compared with clinical stage (WR), disease progression and response to therapy. 18 patients suffered from an infection or an inflammation disease during therapy and 6 from Kaposi sarcoma (KS). **Results:** In 15 patients with stable disease constant p21 levels (± 37 ng/ml) were observed. Changes of disease correlated with distinct increasing levels of p21 (in 16 patients with acute infection and/or inflammation disease, in 2 patients with progress of KS). After start of AZT-therapy, chemotherapy and antibiotic/ antimycotic therapy p21 level decreased. The highest amount of p21 (up to 165 ng/ml) was found in phases with opportunistic infections. The lowest amount of p21 (31 ng/ml) was observed in a clinically stable patient without an evident progress of disease.

Conclusions: Here, we detected increased levels of p21 in HIV-1 infected patients under IFN-therapy during acute opportunistic infection. Protein p21 may be a useful marker for the activation of the immunesystem and a prognostic marker during the course of IFN-therapy.

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THE EFFECTS OF THE SYNTHETIC PENTAPEPTIDE SPLENOPENTIN ON HUMAN HEMOPOIETIC PROGENITOR CELLS IN VITRO.

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Previous studies have shown that the diacetylated synthetic pentapeptide splenopentin (DAc-SP-5) accelerates hemopoietic recovery following sublethal irradiation and autologous bone marrow transplantation in mice, but the effects of the peptide on human bone marrow cells were still unknown. In this study, human granulocyte-macrophage (rhGM-CSF), macrophage (rhM-CSF), and granulocyte colony-stimulating factor (rhG-CSF) as well as interleukin-1α (rhIL-1α) and interleukin-3 (rhIL-3) were compared for their stimulatory activity on human granulocyte-macrophage colony-forming cells (CFC-GM) alone and in combination with DAc-SP-5. After depletion of accessory cells from bone marrow mononuclear cells (BMMNC) in semi-liquid cultures the combination of rhGM-CSF plus splenopentin stimulated the growth of CFC-GM/-M in dose-depended manner. Furthermore, similar effects were seen in combination of DAc-SP-5 plus rhIL-1α and and rhIL-3, but not in rhG-CSF and rhM-CSF plus splenopentin. In unseparated as well as in BMMNC enriched for CD34$^+$ cells comparable stimulatory effects of SP-5 alone were missed. Additionally, in BMMNC enriched for CD33$^+$/CD34$^+$ population, the preculture with DAc-SP-5 for 10 days enhanced the ability of rhGM-CSF, rhIL-1α and rhIL-3 to induce colony formation from this cell source. However, in all these combinations, mainly differentiation to macrophage lineage has been observed. Phenotypic analysis of precultured BMMNC with splenopentin leads to the suggestion that this compound may recruit a cell population being more sensitive to GM-CSF, IL-1α and IL-3, because the percentage of CD34$^+$ cells decreased rapidly, whereas the expression of HLA-DR$^+$ was enhanced. Therefore, this potentially interresting molecule might be a candidate as a therapeutic adjuvant with hemopoietic growth factors. Although, the examination of GM-CSF, IL-1α and IL-3 receptor expression in splenopentin-treated cells should be appropriate to learn more about it.

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LYMPHOCYTE PHENOTYPE IN THE PERIPHERAL BLOOD AND BONE MARROW OF PATIENTS WITH APLASTIC ANEMIA BEFORE AND DURING IMMUNSUPPRESSIVE THERAPY

U.Mentzel, R.G.Geissler, R.Rossol, A.Maurer, A.Ganser, and D.Hoelzer

The role of T-lymphocyte subsets in the development of aplastic anaemia (AA) remains poorly understood. Therefore we analysed by cytophometry the contribution and proportion of lymphocyte subpopulations in the peripheral blood (PB) and bone marrow (BM) of patients with AA before, after 6 weeks of treatment and additionally after 12 weeks of therapy with anti-lymphocyte globulin (ALG), methylprednisolon, and cyclosporine A (CSA). For double labeling immunofluorescence studies monoclonal antibodies directed against the following specifities were used: TCR$\alpha\beta$-, TCR$\gamma\delta$, δTCS1, CD2, CD3, CD4, CD8, CD16, CD56, CD38, and HLA-DR. In 13 patients with AA a significant decrease of CD3 positive T-cells was observed after 6 weeks of therapy (PB 35,9$\pm$12,0; BM 16,2$\pm$3,1) as compared with normal controls (PB 75,7$\pm$1,8; BM 39,8$\pm$3,1). Before therapy, the CD4/CD8 ratio in PB and BM did not differ from the ratio in the control population; however a reversed ratio (<1) was present in PB and BM after 6 and 12 weeks of therapy. The number of activated T-cells defined by the antigens CD38, HLA-DR and CD56 were low or in the normal range,and did not further decrease during therapy in contrast to the non-activated T-cells. $\gamma\delta$-T-cells were significantly decreased before and after 6 weeks of therapy as compared with healthy controls. However, the proportion of the $\gamma\delta$-subpopulation δTCS1 was markedly increased before (PB 39$\pm$3,5; BM 31$\pm$3,7) after 6 weeks (PB 58,7$\pm$2,9; BM 45,1$\pm$3,4) and esspecially after 12 weeks of therapy (PB 94,9$\pm$7,1) as compared with that in normal subjects (PB 17$\pm$2,0; BM 9,0$\pm$0,8). These data indicate that δ TCS1-T-cells are a T-cell population not affected by treatment with ALG, methylprednisolone and CSA . Further studies have to show whether this subpopulation has an effect on the hematopoiesis of patients with AA.

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Molecular and Pathobiological Role of the CD30-Ligand in CD30-Positive Malignant Lymphomas H.-J. Gruss, D. Williams, R. Armitage, C.A. Smith, and R.G. Goodwin

CD30, a cell surface antigen, has been identified on the neoplastic Hodgkin and Reed-Sternberg (H-RS) cells in Hodgkin's disease (HD), Ki-1 large cell anaplastic lymphoma (LCAL) cells, EBV-transformed Non-Hodgkin's lymphoma (NHL) and on activated lymphoid cells of either T or B cell origin. The CD30 molecule is a member of the TNF/NGF receptor superfamily. The corresponding CD30-ligand (CD30L) was identified on the membrane surface of a stimulated murine T cell clone (7B9) using a soluble, recombinant form of the extracellular portion of CD30. By direct expression cloning a 1,6 kB cDNA for the murine CD30L was isolated. A 1,7 kB human CD30L cDNA clone was isolated by cross hybridization of a stimulated peripheral blood T cell lambda library. The CD30L sequence revealed a 239(muCD30L)/ 234(hCD30L) amino acid type II membrane protein with approximately 75% homology. The C-terminal domain of the CD30L shows significant sequence homology to TNF-α, TNF-β, CD27L and CD40L. CD30L is another member of a growing ligand superfamily for the TNF/NGF receptor superfamily. CD30L mRNA is strongly expressed in activated T cells, monocytes/macrophages and granulocytes, but not in cultured H-RS cells. We are investigating the expression pattern of CD30L in primary HD tissue sections and in Ki-1 LCAL sections by using in situ hybridization. The CD30L induces proliferation of cultured T cells and H-RS cells and can induce cell death of the CD30-positive LCAL cell line Karpas 299. The CD30L enhances IgA secretion of EBV-transformed lymphoblastoid B cells. CD30L is a pleiotropic cytokine with a possible role as growth factor for the pathogenesis of Hodgkin's and Non-Hodgkin's lymphoma. Further studies for the role of CD30L in the pathobiology of CD30-positive malignant lymphomas are under investigation.

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ACTION OF DIPEPTIDYL PEPTIDASE IV (CD 26) AND AMINOPEPTIDASE N (CD 13) ON SELECTED CYTOKINES AND CYTOKINE PEPTIDES

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IL-1β, IL-2, TNF-β, G-CSF and other cytokines are characterised to have proline in the second position of the N-terminal peptide sequence. From this they could be potential substrates of the dipeptidyl peptidase IV (DP IV).

Using the method of capillary electrophoresis, here we show that purified soluble DP IV is capable of hydrolysing oligopeptides with sequences analogous to the N-terminal part of human IL-1b, IL-2, TNF-b and mouse IL-6 up to a length of 12 amino acids. Furthermore, it could be demonstrated that hydrolysis rates are negatively correlated the with chain length of the oligopeptides.

Glycosylation of threonine in the third position of the IL-2 hexapeptide sequence has no effect on the hydrolysis rate of this peptide by the DP IV.

In contrast to these results, no degradation was found in the case of rIL-1β, rIL-2, natural IL-2, and rG-CSF, using up to 1000-fold higher DP IV concentrations than in the experiments with oligopeptides. After incubation with both, DP IV and aminopeptidase N, also no cytokine degradation was found. Possible explanations for this results will be discussed.

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THE IMPACT OF HAEMATOPOIETIC SUPPORT IN THE TREATMENT OF RELAPSING AND RESISTANT AGGRESSIVE LYMPHOID MALIGNANCIES

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Thirteen patients (median age 39 years, range 22–58 years) have been treated with a high-dose chemotherapy protocol. Seven had a centroblastic NHL, one had a large cell mediastinal B-NHL, two high-grade pleomorphic or anaplastic T-NHL, one an immunoblastic and two more a high-grade not classifiable NHL. All patients had advanced disease and all have been extensively pretreated with chemotherapy and irradiation. Eight have been refractory to pretreatment, the others had relapsing disease. After a pre-phase of VCR 1.4mg/m² (maximum 2mg) i.v. days 1, 8 and prednisolone 60mg/m² p.o. days 1–10 the high-dose chemotherapy consisted of prednisolone 60mg/m² p.o. days 1–4, ifosfamide days 1–4, methotrexate 5,000 mg/m² day 1 as a 24 hour infusion, cytosine-arabinoside 1,000 mg/m² i.v. days 3 + 4, and etoposide i.v. days 3 + 4. Etoposide has been escalated from 170 mg/m² to 500 mg/m² at the present time. The dose of ifosfamide is currently escalated from 1,500 mg/m² to 2,500 mg/m² and finally 3,500 mg/m² as a continuous 24 hour infusion. The high-dose chemotherapy is repeated for a maximum of four times. During the pre-phase 12µg/kg filgrastim (recombinant G-CSF) are given twice daily. Apheresis of APBSC is done on days 5–7. APBSC are reinfused after the high-dose chemotherapy and filgrastim is given at a dose of 5µg/kg. With WBC rising to >1,000/µl aphereses are performed repeatedly. The pre-phase for induction of PBSC was excellently tolerated and a median of 10.4 x 10^4/kg (1.3–91.2) CFU-GM collected. After the first and second hd-chemotherapy course the stem cell yield was 20.6 x 10^4/kg (0.2–113.5) and 52.9 x 10^4/kg (0.2 – 95.8) respectively. The high-dose chemotherapy was associated with significant toxicities. In 36 courses WHO grade 3 and 4 side effects have occurred in the following number of courses: mucositis 14, diarrhoea 3, vomiting 5, sepsis 5, GOT/GPT 8. Granulopenia grade 4 and thrombopenia grade 4 lasted for a median of 7 and 4 days after course 1 and for 3 and 1 days respectively after course 2. Results: 3 CR, 7 PR, 2 PD, 1 patient is too early to evaluate. The high-dose regimen is tolerable and efficient when given with APBSC support. Sufficient numbers of peripheral stem cells could be collected after a pre-phase after preparation with filgrastim only and after chemotherapy. The protocol should be incorporated into the primary treatment of high-risk patients.

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ADVANCES IN METHODS TO MOBILISE PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) FOR AUTOLOGOUS OR ALLOGENEIC TRANSPLANTATION

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Many of the *in vivo* effects of the haemopoietic cell growth factors may well have been anticipated based on their known ability to stimulate proliferation and development of multipotent and lineage-restricted progenitor cells *in vitro*. What was not predicted from these *in vitro* studies, however, was the observed ability of at least some of these growth factors to mobilise large numbers of haemopoietic progenitor cells from the bone marrow into the peripheral blood. This was an added "bonus" effect of growth factors and one that is now being widely exploited in a variety of clinical situations.

Initially these mobilised PBPC were used, in combination with bone marrow cells, to facilitate the more rapid recovery of myeloid cells following transfer into patients receiving high dose cytotoxic therapy. But when experimental studies showed that the mobilised PBPC also contained primitive (repopulating) stem cells – this encouraged the use of PBPC alone as a source of cells for transplantation. In most of these studies however, collection of the PBPC was performed over several cycles of apheresis - and this was somewhat limiting to their widespread use.

Because of this, our approach has been to define the optimal growth factor regimen for mobilisation of PBPC such that apheresis is not required, i.e. to use whole blood and then to identify groups of patients who may benefit from receiving these cells either for autologous or for allogeneic marrow reconstitution.

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AUTOGRAFTING WITH RECOMBINANT G-CSF (FILGRASTIM) MOBILIZED BLOOD STEM CELLS IN PATIENTS WITH MALIGNANT LYMPHOMA

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In this report we summarize our experience with high-dose therapy and peripheral blood stem cell (PBSC) autografting in advanced malignant lymphoma. Since May 1991, 27 patients (19 male / 8 female) were included into this study. The median age was 37 years (range 19–58). 11 patients had Hodgkin's disease and 16 non-Hodgkin's lymphoma (9 low-grade / 7 high-grade NHL). Four patients received recombinant G-CSF (filgrastim) (5 µg/m²/day s.c.) during steady-state hematopoiesis, while in the remaining 23 patients recombinant G-CSF (filgrastim) was started 24 hours after conventional chemotherapy. For all patients, a target quantity of 0.4 x 10^9/kg total nucleated cells (TNC) was reached. A wide interindividual range with respect to the level of circulating hematopoietic progenitor cells (325-fold for CFU-GM/ml and 221-fold for CD34+ cells/µl) was observed. With a median number of 5 leukaphereses (range 2–11), a median of 12.7 x 10^4 CFU-GM/kg bw and 4.7 x 10^6 CD34+ cells/kg bw could be harvested, respectively. High-dose therapy consisted of either TBI (14.4 Gy, hyper-fractionated)/Cyclophosphamide (200 mg/kg) or the BEAM-protocol. With the exception of 1 patient who died of a respiratory distress syndrome 32 days following autografting, transplant-related toxicity was moderate. The low toxicity is reflected by the kinetics of hematological reconstitution: 15 (median) days for 1.0 x 10^9/l PMN and 11 days for 20 x 10^9/l platelets. Platelet reconstitution was closely related to the number of CD34+ cells reinfused. Twenty-six patients are evaluable for response: 5 patients (19%) relapsed after a median of 5 months (range 2–6) post-transplant. The remaining 21 patients are alive in remission with a median follow-up of 9 months (range 3–19). In summary, recombinant G-CSF (filgrastim) is highly efficient in mobilizing PBSC capable of restoring hematopoiesis after myeloablative conditioning therapy. The quantity of CD34+ cells harvested was inversely related to the amount of previous cytotoxic chemotherapy. Therefore, high-dose therapy with stem cell support should be considered as an upfront treatment modality in poor prognosis NHL patients.

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TRANSPLANTATION OF ENRICHED AUTOLOGOUS CD34+ HEMATOPOIETIC PROGENITOR CELLS INTO BREAST CANCER PATIENTS FOLLOWING HIGH-DOSE CHEMOTHERAPY.

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CD34+ progenitor cells were isolated from marrow and peripheral blood, for use as autologous hematopoietic progenitor cell support in 44 poor-prognosis breast cancer patients following high-dose chemotherapy. The study included 5 sequentially treated patient cohorts. All data are expressed as "median (range)": 20 x 10^9 marrow buffy coat cells (cohorts 1–4) and 63 x 10^9 filgrastim (recombinant G-CSF)-stimulated PBPCs from three leukaphereses (cohorts 4, 5) were incubated with biotinylated anti-CD34 antibody 12-8. The cells were then applied to a column of avidin coated beads (CEPRATE™ SC Stem Cell Concentrator) for purification. The positively selected fractions contained 166 (46–329) x 10^6 marrow cells that were 72 (50-85)% CD34+, and 324 (84–645) x 10^6 PBPCs/3 phereses that were 50 (21–73)% CD34+. The cohort description, as well as the number of days to granulocyte (ANC) count ≥ 500/µl and platelet transfusion independence (Plat Ind) are:

PATIENT COHORT	N	CD34+ MARROW	CD34+ PBPCS	GROWTH FACTOR	#DAYS TO ANC > 500	#DAYS TO PLAT IND
1	7	***		NONE	23	23
2	10	***		filgrastim	13	16
3	8	***		GM-CSF	18	18
4	12	***	***	filgrastim	9	9
5	7		***	filgrastim	9	13

Using a sensitive immunohistochemical technique, 1.3->5.0 logs of breast cancer cell depletion was documented in the positively-selected fractions of marrow (10 patients) and 2–>5 logs from the PBPC fractions (3 patients), in whom tumor was initially detected. To date no tumor has been detected in the CD34+ PBPC fractions. The engraftment rates for cohorts 4 and 5 were significantly faster than those of the other cohorts. The preliminary data suggest that since CD34+ PBPCs alone are capable of restoring hematopoiesis following high-dose therapy, a marrow fraction may no longer be needed for this purpose. Longer follow-up will be required to assess the ultimate therapeutic effect of the entire treatment program.

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LONG-TERM EFFECTS OF FILGRASTIM (RECOMBINANT G-CSF) IN SEVERE CHRONIC NEUTROPENIA

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In patients with severe chronic neutropenia, treatment with filgrastim (recombinant G-CSF) results in a rise in the absolute neutrophil counts and a significant reduction in infections (Bonilla *et al.*, N Engl J Med 320: 1574, 1989; Welte *et al.*, Blood 75: 1056, 1990). We report here the effects of long-term G-CSF subcutaneous administration in 48 patients (congenital n=32, cyclic n=4, idiopathic n=12) treated for 3-5 years. A sustained ANC response was seen in 30/32 congenital patients, in 4/4 cyclic patients, and in 10/12 idiopathic patients. The G-CSF doses needed to maintain these responses ranged between 1 and 60 μg/kg/d. The ANC responses were associated with a significant decrease in the incidence of severe infections and the need for intravenous antibiotics. G-CSF has been well tolerated in the majority of patients and resulted in a dramatic improvement in the quality of life. The adverse events noted included: osteopenia (n=12), vasculitis (n=2), mesangioproliferative glomerulonephritis (n=2), and the development of MDS/leukemia (n=2). These adverse events are most likely associated with the underlying disease and not caused by G-CSF treatment. These results demonstrate the feasibility of maintenance treatment with G-CSF without exhaustion of myelopoiesis and the beneficial effect of G-CSF in patients with severe chronic neutropenia.

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GM-CSF AND G-CSF IN AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) FOR NON-HODGKIN'S LYMPHOMA (NHL). REPORT ON TRANSPLANTATION OF PURIFIED CD 34+ STEM CELLS

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Hemopoïetic growth factors are now being tested in several institutions, in an effort to reduce the duration of neutropenia after bone marrow trasnplantation (BMT). In the past 3 years, we have conducted or participated to several double blind multicentric studies using GM-CSF and G-CSF (Schering-Plough/Sandoz, Behring/Hoechst and Roche lab.) in patients (pts) with NHL. We wish to summarize these studies and also report on our own observations.

1- GM-CSF post-ABMT: double blind international studies and the French national trial that we conducted (Blood 1992, 180, 1149-1157) have clearly demonstrated the GM-CSF infusion post-ABMT significantly accelerates neutrophil recovery by 4 to 7 days, both in pts receiving unpurged marrow or marrow purged by Mafosfamide. The duration of hospitalization is reduced by 4 days with a possible cost benefit.

2- In pts with documented CMV infection requiring DHPG treatment, myelosuppression was not seen in those who received GM-CSF and DHPG concomitantly while in contrast a severe neutropenia developped in those who received DHPG after administration and discontinuation of GM-CSF (Lancet, 1989, 1273).

3- 13 pts. in our institution received GM-CSF in a compassionate use for delayed enraftment or engraftment failure after autologous (ABMT : 12) or allogeneic bone marrow transplantation (BMT : 1). The pretransplant regiment included total body irradiation (TBI) in 10 and consisted of high dose polychemotherapy only in 2. Interestingly 9 pts were transplanted for acute myelocytic leukemia (AML), a disease in which the presence of receptors to GM-CSF has been detected in vitro in about 50% of cases, 2 pts hat NHL, and 2 ALL. Of the 12 ABMT, 10 pts received marrow heavily treated in vitro by Mafosfamide and 1 marrow purged by long term marrow culture (LTC). In 7 (AML 4, ALL 2, NHL 1), granulopoietic recovery occured within 3 days (1-16). This included a pt with refractory ALL who received LTC marrow, developped cytomegalovirus (CMV) infection and had not engraftment by day 31: in this pt the absolute nucleated count (ANC) peaked to 2.7 10^9/l, 3 days later. An additional pt with AML had not engrafted by day 63 when GM-CSF was administered for 12 days with no efficacy. Infusion of back up marrow (which has never been effective in our past experience) combined with GM-CSF and Cyclosporine A was follwoed by sustained engraftment occuring 17 days later. 1 pt (AML) had a minor and transient response and 4 failed. 6 additional pts in the context of entgraftment failure have since received in various sequences GM-CSF and Cyclosporine A + back up marrow after no response to GM-CSF. 3 have recovered sufficient hemopoïesis strongly suggesting a role of Cycloporine A in engraftment failure (manuscript in prepration).

We conclude that GM-CSF is now the first line treatment for poor engraftment and/or engraftment failure. We do not freeze any longer back up marrow in pts autografted after pretransplant regimens not containing TBI. In pts with engraftment failure post TBI, who do not respond to first line GM-CSF, we suggest that the combination of back up marrow and Cyclosporine A on top of GM-CSF should be further evaluated.

4- We administered GM-CSF at a dose of 250 μg/m²/day to 5 pts with resistant NHL and bone marrow involvement after the BEAM myeloablative regimen; reconstitution occurred within the same delays than observed after ABMT in 3. We propose that GM-CSF may replace ABMT in highly selected cases of non-Hodgkin's lymphoma with progressive disease and bone marrow involvement (Lancet 1991, 338, 601).

5- We have transplanted since September 1992 8 NHL pts with CD 34 purified stem cells followed by infusion of GM-CSF. Successful engraftment was obtained in all with recovery to $0.5x10^9$ PMN/l by day 14 (11-25) and to $50x10^9$ plts/l by day 23 (13-32). The expansion of CD 34+ cells in short term liquid culture with 6 cytokines (including GM and G-CSF) is another approach toward transplantation with total abrogation of neutropenia. Overall the introduction of hemopoietic growth factors in transplant units has considerably changed the situation in several aspects. Further studies will combine GM-CSF and G-CSF to other cytokines.

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RANDOMIZED DOUBLE-BLIND PLACEBO-CONTROLLED PHASE III STUDY OF RECOMBINANT HUMAN GRANULOCYTE/MACROPHAGE COLONY STIMULATING FACTOR (rhGM-CSF) AS ADJUNCT TO INDUCTION-TREATMENT OF HIGH GRADE MALIGNANT NON-HODGKIN'S LYMPHOMAS

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We have evaluated recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) (Sandoz-Schering/Plough) as an adjunct for COP-BLAM in the primary treatment of high grade malignant non-Hodgkin's lymphomas (NHL). Patients (n = 182, stage II-IV, age 15-73 years), were randomized to rhGM-CSF (400 μg) or placebo for 7 days s.c. following chemotherapy. Efficacy was analyzed for patients receiving at least 70% of study medication (n = 125). The frequency of clinical relevant infection was reduced by rhGM-CSF (28 vs 69 infections, 16 vs 30 patients, P = .02) with a cumulative probability of remaining infection free in 70% vs 48% (P = .05, log rank test at 190 days). Periods of neutropenia (P ≤ .01 in 5/8 courses), days with fever (2.1 vs 4.0, P = .04) and days of hospitalization for infection (3.5 vs 8.0 days, P = .01) were significantly reduced. Complete response (CR) rates, assessed by prognostic risk, were 15/19 (79%) in treated vs 20/21 (95%) in controls in the low-risk group (P = .12). In the high-risk group 31/45 (69%) of treated patients achieved CR vs 25/52 (48%) of controls (P = .04). No difference in survival has been seen after 1 year. Only injection site reactions (45% treated vs 7% controls) and rash (26% vs 2%) occurred more frequently in treated patients (n = 179). These data show that rhGM-CSF ist well tollerated in most patients with NHL, significantly reduces infection and improves response.

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PERIPHERAL BLOOD PROGENITOR CELLS (PBPC) USED ALONE OR TO AUGMENT MARROW AS HEMATOLOGIC SUPPORT OF SINGLE OR MULTIPLE CYCLE HIGH-DOSE CHEMOTHERAPY

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Summary

The principal morbidity and mortality of high dose chemotherapy with autologous bone marrow support (ABMT) relates to the infectious complications which occurs during 3-4 week aplasia until the marrow autograft recovers. Progenitor cells can be mobilized into the peripheral blood compartment by hematopoietic growth factors, alone or used after chemotherapy. We describe four trials using cytokine-mobilized peripheral blood progenitor cells (PBPC). In the first trial, PBPC collected after GM-CSF administration are used to augment marrow. Reconstitution of trilineage marrow function occurred quickly, resulting in short hospital stays and fewer platelet transfusions. In a second study, GM-CSF/chemotherapy-mobilized PBPC were used as the sole hematopoietic support during high dose chemotherapy. Granulocyte and platelet reconstitution was rapid. Time to hematopoietic recovery, transfusion requirements and duration of hospital stay were all significantly improved for the patients receiving PBPC compared with similar patients receiving marrow alone. However, some patients had poor platelet engraftment. Two recent trials were designed to explore multiple high-dose therapy. In the third trial, PBPC with and without marrow made it feasible to deliver two sequential cycles of high dose therapy. The fourth trial utilizes PBPC in addition to cytokines to deliver four cycles of dose-intensive therapy utilizing doses of chemotherapy that could not be given with cytokine support alone. PBPC appears to make multiple course combination high dose therapy feasible, is particularly useful to support platelets, and may enhance the safety, tolerance and cost of high dose therapy.

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HEMATOPOIETIC GROWTH FACTORS IN AIDS THERAPY

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A number of clinical trials have indicated the utility of erythropoietin, GM-CSF, or G-CSF in improving hematologic parameters in HIV-1 infected patients. Other clinical benefits have been demonstrated in large placebo-controlled trials with erythropoietin and, in smaller controlled trials, with GM-CSF. Several aspects of HIV-1 disease may be important distinguishing features of the use of growth factors in this as opposed to other clinical conditions: a) the ability of certain cytokines to stimulate virus replication, and (b) the interaction of cytokines with the intracellular metabolism of certain drugs. Recently developed methods of quantitating circulating levels of HIV-1 have permitted the evaluation of these issues clinically. In a Phase I clinical trial treating cytopenic HIV-1 infected patients in cohorts of three with four different dose levels of recombinant human interleukin-3, we monitored virologic as well as hematologic parameters. No increase in circulating viral burden was detected by either quantitative competitive PCR or by quantitative culture. A trial testing the ability of GM-CSF to affect the antiviral properties of zidovudine is currently in progress and preliminary results will be discussed.

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USE DE GRANULOCYTE MACROPHAGE-COLONY STIMULATING FACTOR (GM-CSF) IN ADVANCED HIV INFECTED PATIENTS (P) WITH NEUTROPENIA

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Severe neutropenia and functional defects of neutrophils are an increasing problem in treating advanced HIV infected P. Neutropenia may result from bone marrow failure due to HIV by itself, or maybe.secondary to drug toxicity (mainly Zidovudine, ganciclovir, chemotherapy) or bone marrow infiltration (Mycobacterial infection, neoplasms). P. with severe neutropenia are at a higher risk of developing bacterial infections.

Since both GM-CSF and G-CSF have been successfully used in cancer to optimalize dose intensity and to prevent neutropenia and secondary infections in P. receiving myelotoxic agents, clinical trials have recently been conducted in ARC and AIDS P. with neutropenia to determine their potential benefits and toxicity profile. Our previous experience in treating P. with non Hodgkin's lymphoma (NHL) with a 6-cycle third generation regimen (ProMnCE-CYTABOM), with GM-CSF given at alternate cycles, clearly demonstrated that doses of chemotherapy following the adjunction of GM-CSF were significantly higher than doses given without prior GM-CSF administration. GM-CSF was largely well tolerated in those P., with no sign of active viral replication as measured by HIV-1 P24 Antigenemia. We have also performed an open clinical trial in P. with severe neutropenia to assess the efficacy and tolerance of GM-CSF with a three weekly subcutaneous administration in P. who initially responded to a daily dose. 24 out the 28 enrolled P. (median duration : 40 days) showed a rise of the absolute neutrophil count (ANC) above the target value of $1000/mm^3$ after a median period of 3 day at 3 mcg/kg/d. Only 5/28 P. were given antiretrovirals while other concomitant drugs included mainly ganciclovir (8) and pyrimethamine (10). Of the 21 P. who entered the maintenance phase at 3 weekly doses, 12 (57 %) showed a sustained increase of the ANC >1000, while in the remaining 9 P., a new episode of neutropenia was observed.

In contrast to P. with NHL, adverse reactions were commonly observed, mostly fever (68 %) and flu-like symptoms (50 %). Furthermore among the 22 P. without antiretroviral therapy who were P24 Ag negative at baseline, a detectable P24 antigenemia was found in 9 P. These preliminary data on this original schedule of GM-CSF administration was effective in preserving ANC levels at >1000 in 57 % of the responders. Such an approach could enhance P.'s compliance and have a direct impact on the cost/benefit evaluation of such therapy. In addition, antiretrovirals should always be associated with GM-CSF to reduce the increased risk of viral replication.

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GM-CSF IN ACUTE MYELOID LEUKEMIA

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Attempts to further improving the curative treatment for AML have to overcome the problem of residual disease represented by leukemic cells surviving in a therapy resistant state. A novel approach aims at recruiting those cells into a sensitive state by the use of GM-CSF before and together (priming) with chemotherapy. In vitro, the recruitment of leukemic blasts to colony forming cells in presence of GM-CSF (Blood 67:1448, 1986) is documented by numerous reports. Given to patients with newly diagnosed AML GM-CSF priming decreased the proportion of leukemic cells in G_0 and increased cells in sensitive cycle phases within 24-48 h (Blood 77:700, 1991). A similar priming with preinfusion of GM-CSF for a variable period of 0-8 days before chemotherapy started resulted in significantly inferior outcome and more persistent leukemias than in historical controls suggesting protective effects of GM-CSF (Blood 79:2246, 1992). In a first study we showed that GM-CSF following chemotherapy in high risk AML effectively reduced both neutrophil recovery and early mortality and had no adverse impact on leukemic regrowth and remission duration (Blood 78:1190, 1991). In current randomized study in patients with newly diagnosed AML we give GM-CSF from 24 h before chemotherapy and then on to neutrophil recovery which is repeated in each of the initial 5 treatment courses and is compared to chemotherapy alone. 2 1/2 years from study start and after entering 72 patients (median age 50, range 17-75 years) this update in the GM-CSF group and controls shows 78% and 81% CR, 2 and 5 persistent leukemias and a clearance of day 16 b.m. blasts to < 5% in 59% and in 38%, and remission duration shows a trend in favour of the GM-CSF group. In addition to recruitment, other effects of GM-CSF like enhancement of Ara-C cytotoxicity (Leukemia 3:789, 1989; Ch. Reuter et al. Leukemia in press) may contribute to this strategy. Thus, GM-CSF appears not to antagonize antileukemic chemotherapy. Whether GM-CSF priming and longterm administration ultimately improves the cure rate in AML should be shown some later from the multiple course strategy used in this study.

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GM-CSF IN A DOUBLE-BLIND RANDOMIZED, PLACEBO CONTROLLED TRIAL IN THERAPY OF ADULT PATIENTS WITH DE-NOVO ACUTE MYELOID LEUKEMIA (AML).

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In this multicenter trial was evaluated, whether rhu GM-CSF given concomitant and after chemotherapy (CT) can improve the outcome of AML patients by increasing the cytotoxic effect of CT and by reducing the rate of infectious complications. Induction and early consolidation therapy included cytarabine (Ara-C, 100 mg/m^2, day 1 -8 CIVI), daunorubicin (60 mg/m^2, IV, day 3 - 5,) and etoposide (100 mg/m^2, day 4 - 8, 2 h IV infusion) with reduced dosages in the second induction and early consolidation course. Late consolidation included one cycle with high-dose Ara-C (3g/m^2, 12 doses) and daunorubicin (30 mg/m^2, day 7 - 9) for patients aged 50 years and younger, whereas patients over 50 years received a reduced dose of Ara-C (0.6g/m^2, 12 doses). Patients were randomized after the first induction course to receive either rhu GM-CSF (E. coli, 250 μg/ m^2/ day, s.c.) or placebo starting 48 hours prior to the second induction and the subsequent courses and given throughout chemotherapy until absolute neutrophil count had recovered > 500/μl. Eighty out of 82 patients (median age = 50 years) included into the study could be randomized to receive either GM-CSF (n = 41, median age = 50 years) or placebo (n = 39, median age = 50 years). 64 out of 82 patients (78%) achieved a complete remission (CR). 15 patients (18.5%) were treatment failures. Two patients died and in one patient the response to therapy was not evaluable. There was no statistically significant difference in CR rate between patients of the GM-CSF (81%) and placebo group (79%) nor between patients aged 50 years or less and those over 50 years old. The proportion of relapse free survival at a median follow up of 12 months is 48 % in the GM-CSF versus 33% in the placebo group (p = 0.64). The proportion of survival of the GM-CSF group at 22 months is 36% versus 56% in the placebo group (p = 0.64). GM-CSF did not significantly shorten the period of critical neutropenia and prolonged the period of critical thrombocytopenia especially in patients aged over 50 years. The overall incidence of infectious complications as well as the non-hematological toxicity was similiar in boths groups. Thus GM-CSF therapy is feasible in AML therapy while its influence on remission quality and survival remains still open.

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