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IL-4 induced upregulation of BCL-2 in CLL patients in vivo and in vitro

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Interleukin-4 (IL-4) protects chronic lymphocytic leukemic B-cells (B-CLL) from programmed cell death (PCD). In experimental models, the decrease in apoptosis is accompanied by an upregulation of the bcl-2-protein, localized at the inner mitochondrial membrane. We investigated the in vivo and in vitro effect of IL-4 on the expression of bcl-2 and on apoptosis in CLL. bcl-2 mRNA was detected by RT-PCR using a set of primers annealing in the coding region of bcl-2. bcl-2 protein was analysed by flow-cytometry using a mouse anti-human monoclonal antibody (Dako-bcl-2, 124, Dako, Hamburg). CLL cells were cultured with 100 U/ml IL-4 for 7 days.

Fourteen of 23 (61%) tested CLL-patients expressed bcl-2 mRNA. In three patients studied by flow-cytometry bcl-2-protein was found in 100% of CLL-cells. Addition of IL-4 to cultured CLL cells led to an 1.7 fold increase of bcl-2 protein.

In an ongoing multicenter study the antiproliferative effect of IL-4 is studied in a phase II trial. One patient in partial remission, included in the study, was treated with subcutaneous injection of 2 µg/kg/3 x per week IL-4 (Essex, München). The cellular bcl-2 protein-level increased 1.8 fold within the first week. During the second week bcl-2 expression returned to baseline levels and did not change up to 5 weeks of treatment.

In conclusion, CLL cells express bcl-2 at the RNA and protein level. IL-4 increases bcl-2 protein expression in CLL-cells in vitro and in vivo. However, the latter effect is limited to the first week of treatment. Further investigation is necessary to explore the mechanism of cellular signal transduction of IL-4 resulting in upregulation of bcl-2.

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G-CSF AND IL-6 SERUM-LEVELS IN PATIENTS WITH MYELODYSPLASTIC SYNDROMES AND APLASTIC ANEMIA

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G-CSF serum levels in healthy persons show a negative correlation to neutrophil counts. Furthermore, IL-6 levels seem to play an important role in the recruitment of early progenitor cells. We therefore measured endogenous serum G-CSF and IL-6 levels in 16 patients with myelodysplastic syndromes (MDS) and five patients with aplastic anemia (AA). These disorders are characterized by decreased leucocyte, thrombocyte and erythrocyte counts. Cytokine serum levels were determined by using the MTT bioassay in two murine cytokine dependent cell lines (7-TDI and NFS-60). 4/16 patients (=25%) with MDS received myeloablative chemotherapy and the consecutive time course of cytokine-levels before, during and after therapy was also observed. In spite of low leukocyte counts (median 1700/µl), endogenous G-CSF serum levels were not increased in 14/16 patients with MDS (median G-CSF serum level in MDS 7,5 pg/ml; G-CSF in healthy volunteers 25 ± 20 pg/ml). This was also true for serum IL-6 levels (median IL-6 in MDS 2,5 pg/ml, in control 29 ± 39 pg/ml). However, in patients who received chemotherapy a rapid increase of G-CSF levels up to peak values of 10.000 pg/ml paralleled with decreasing neutrophil counts could be noticed.

In patients with AA serum G-CSF revealed normal (25 ± 29 pg/ml) but not increased levels in spite of decreased leukocyte count. For IL-6 reduced serum levels to a mean of 2 pg/ml were observed. From these results we conclude that in some patients exogenous support with G-CSF could be beneficial in e.g. episodes with infections to increase neutrophil counts. Furthermore, reduced IL-6 serum levels could reflect on an impaired recruitment of stem cells. This could explain for some part cytopenia in MDS and AA and therefore give insights in pathophysiological mechanisms in these malignant hematological diseases.

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DEVELOPMENT OF A BIOLOGICAL RESPONSE MODIFIERS SYSTEM BASED UPON STIMULATION OF BLOOD WITH OZONE EX VIVO AND REINFUSION.

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In the last few years we have evaluated a novel agent, namely ozone, as a cytokine inducer upon blood leukocytes ex vivo.[1] Human blood (about 300 ml) is collected in heparin and Ca^{2+} (~ 6mM) in ordinary transfusion bags, briefly treated with ozone at concentrations of about 70 µg/ml and reinfused in the donor within 20 minutes. The ordinary anticoagulant (CPD) has been eliminated because we have shown that Ca^{2+} enhances ozone action. The procedure has been standardized[2] and it is very simple, safe, rapid and inexpensive.

At the selected concentrations, ozone is not toxic for blood cells as shown by minimal hemolysis (1.4-2.7%), almost negligible decrease of intraerythrocytic GSH levels, no change of cellular viability and progressive production of cytokines such as IFNα, β and γ, IL-1, IL-2, IL-6, IL-8, TNFα, GM-CSF as well as TGFβ upon incubation.[3] Autohemotherapy carried out in normal volunteers has not revealed side effects and actually some subjects report a feeling of well being.[4] Interestingly, while the above cytokines do not become detectable in blood, there is an increase of Mx protein in circulating leukocytes 48-72 hours after reinfusion, suggesting that endogenous production of IFNs has caused the expression of Mx protein.[4]

At the moment autohemotherapy is carried out in patients with chronic hepatitis (B and C), recurrent papilloma virus infection and not otherwise treatable solid tumors. As far as the quality of life is concerned, the benefit/risk ratio is far superior than that after exogenous administration of IFNs and/or IL-2. Compliance of patients is excellent and the cost of treatment is negligible.

In conclusion, we have demonstrated that there is a rational basis for using autohemotherapy and we feel that, although this treatment is still considered unorthodox, it may become a very useful immunotherapeutic modality.[5]

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Immunomagnetic removal of tumor cells using monoclonal antibodies against B-cell epitopes or tumor associated breast cancer antigens

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High dose therapy regimens with autologous hematopoietic stem cell support has shown improved relapse free survival and increased complete remissions in several cancers. To overcome a risk of relapse due to retransfused graft contaminating tumor cells, monoclonal antibodies and polysterene paramagnetic microspheres have been used highly efficacious for removal (purging) of tumor cells from bone marrow or peripheral blood mononuclear cells. Among the most treated malignancies are lymphomas, acute leukemias, neuroblastomas and certain lung and breast cancers.

We have established a production of monoclonal antibodies for immuno-magnetic purging of B-cell malignancies (CD10, CD19, CD20, CD22, CD23, CD37) and breast cancer cells (c-erbB-2, Gp 55, Gp 42) to be used in a closed system magnetic cell separation device (MaxSep™).

The functionality of each individual antibody has been tested and validated in small scale assays. Purging of bone marrow from non-Hodgkin's lymphoma patients was performed on the MaxSep™ employing a cocktail of the B-cell antibodies (CD19, CD20, CD22, CD23, CD37). With two cycles of immunomagnetic removal, a complete elimination of target cells was achived. Randomized prospective clinical trials (CUP, GOELAM) comparing the efficacy of high dose chemotherapy with purged or unpurged hematopoetic stem cell support in poor risk follicular NHL are currently in progress.

By using the breast cancer antibody panel, the MaxSep™ immunobead procedure removed in one purging cycle more than 4 log tumor cells from MNC preparations spiked with breast cancer cell lines.

The results clearly indicate that the antibody panels can be safely used for effective elimination of tumor cells from B-lymphoma or breast cancer patients undergoing high dose treatment regimes with autologous hematopoietic stem cell support.

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