8

Clinical Aspects of Cytokine Control of B-Lymphopoiesis

W. Hiddemann, Ch. Buske, B. Wörmann, Department of Hematology and Oncology, University of Göttingen, Germany

Proliferation and differentiation in the various maturational stages of B lymphopoiesis are regulated through contact with stromal cells, specific cytokines and antigenic stimuli. Ex vivo B cells rapidly die by apoptosis. Research in humans has long been hampered by lack of reproducible *in vitro* systems. More recently this gap has been filled by the establishment of bone marrow stroma or murine fibroblast cocultures for growth of B cell precursors, and of a complex system using $F_{c\gamma}$ RII transfected L cells, CD40 ligand and IL-4 for maintenance and growth of mature B cells. Lymphoma and leukemic cells from B cell neoplasias at different stages of maturation closely resemble their putative physiologic counterparts, as on the genetic as on the functional level. Leukemic B cell precursors respond to IL-3, IL-7 and have functional IL-1 receptors. Cells from patients with B chronic lymphocytic leukemia respond to IL-4, while this cytokine induces apoptosis in follicular lymphoma. Negative growth regulators are also IL-10 and TGF β in B-CLL, IFN-α induces apoptosis in hairy cell leukemia. The data show that the *in vitro* systems can be successfully used for studies on the positive and negative growth regulation and the therapeutic manipulation of B lineage acute lymhoblastic leukemia and B non Hodgkin lymphomas.

9

Immunotherapy in Acute Leukemias - A new therapeutic approach?

L. Bergmann

Despite advantages by intensification of chemotherapeutic strategies and/or bone marrow transplantation (BMT), the disease-free survival (DFS) in patients with AML and ALL with high risk factors is still unsatisfactory. Immunotherapeutic approaches in AL have been supported by BMT data indicating a correlation between graft versus host (GvH) reaction and DFS. The GvH reaction is supposed to be associated with a graft versus leukemia (GVL) reaction, which may be responsible for the elimination of minimal residual leukemic blast populations. Therefore, therapeutic approaches inducing a GvL-like reaction in AML may be are desirable. For this, interleukin-2 (IL-2) administration may be a promising tool for eradication of minimal residual blast populations.

Acute leucemic blast cells of myelocytic and lymphoytic origin have been shown to be susceptible to activated cytotoxic cells (LAK-cells). Autologous cytotoxic T-cell lines/clones ($CD4^+$, $CD8^-$, $\gamma\delta^-$ T–cells) could be established reacting with autologous and allogeneic blast cells.

AML blasts themselves express IL-2 receptors, especially ß- and γ–chains, in a high proportion. Despite this, no proliferative effect of IL-2 on leukemic blast cells could be found in vitro. In our hand, we could demonstrate that the IL-2 recptors seem not to be functional for stimulation of blast proliferation, so that IL-2 administration may not induce disease progression.

So far, clinical trials with IL-2 have demonstrated that complete remissions (CR) can be achieved in patients with AML in partial remission (PR) with limited tumor burden (<20% blasts in BM). Various clinical trials with IL-2 in AML with or without autologous BMT and ALL with autologous BMT have been dealing with the feasibility of this approach. In a own phase II study in 2nd remission of AML with bolus IL-2, a possible benefit for DFS is suggested but has still to be confirmed by prospective randomized trials.

Medical Clinic III, J. W. Goethe University, Frankfurt, FRG

10

Apoptosis and the treatment of chronic lymphoid malignancies: the B-Chronic Lymphocytic Leukemia (B-CLL) model.

F. Caligaris-Cappio, D. Gottardi, A. Alfarano, A.M. De Leo, A. Stacchini, L. Bergui.

The central problem of B-CLL is why malignant cells accumulate in the G0 phase of the cell cycle. We have investigated whether B-cell chronic lymphocytic leukemia (B-CLL) and B-CLL-mimicking mantle cell lymphoma (MCL) in leukemic phase have a different pattern of expression of apoptosis/cell cycle-related genes. To this end, we have analyzed by Northern Blot and RT-PCR analysis the expression of Bcl-1, Bcl-2 and Bax genes in 27 patients presenting with B-chronic lymphoid leukemia. The CD5+, CD23+, sIg weakly positive B-CLL cells had the following gene pattern: Bcl-1 negative or weakly positive, Bcl-2 strongly positive, Bax positive. On the contrary, CD5 usually positive (7/12), CD23 negative and sIg strongly positive MCL malignant cells were Bcl-1 positive, Bcl-2 and Bax occasionally and weakly positive. Next, we have investigated whether Fludarabine, a drug with a marked activity in some B-CLL cases refractory to conventional treatment, might act by inducing apoptosis of malignant cells and whether apoptosis might be related to Bcl-2 downregulation. *In vitro* studies (morphology, FACS analysis, DNA electrophoresis on 2% agarose gel, RT-PCR and Northern Blot) were compared to the clinical consequences of the *in vivo* administration of the drug. When a significant downregulation of Bcl-2 mRNA was evident *in vitro* at 8 - 24h, it was followed by a downregulation of Bcl-2 protein and, subsequently, by a significant apoptosis: the *in vitro* data correlated with the *in vivo* outcome with a dramatic clinical response. On the contrary, when no Bcl-2 downregulation was observed, the cells also showed either low or absent evidence of apoptosis *in vitro* and the clinical response was slow and lagged behind, or the patients did not show any *in vivo* clinical response. It is plausible to conclude that Bcl-2 overexpression by inhibiting apoptosis leads to the relentless accumulation in G0 of malignant B-CLL cells and to suggest that an approach based upon the downregulation of Bcl-2 gene may be applied to the treatment of the disease.

Cattedra di Immunologia Clinica, Dipartimento di Scienze Biomediche e Oncologia Umana, Università di Torino, ITALY