TRUNCATED a1-ANTITRYPSIN IN LEUKEMIC BLASTS


Truncated α1-antitrypsin of MW 41 kDa has been found in high quantities in the urine of leukemic patients undergoing remission induction chemotherapy. This was established by monocolonal antibodies and N-terminal sequence analysis. Identity with the urinary glycoprotein GP-41 described by Maubach et al. (1) was proven. Excretion of GP-41 in the urine follows cyto-reduction of malignant blasts in acute leukemia. Peripheral blood leukocytes and cells from bone marrow of patients with acute myeloid leukemias of M2 and M3 morphology were investigated as a possible source of GP-41 since Meyer et al. (2) have described high amounts of α1-antitrypsin in myeloid blasts by immunohistochemical analysis. Western blot analysis with immuno-staining using monocolonal antibodies raised in our laboratory, revealed high levels of truncated α1-antitrypsin in the granule fraction of leukemic cells. This finding suggests that the extent of GP-41 excreted reflects the degree of tumor cell reduction during polychemotherapy. Thus, determination of GP-41 in the urine may be a simple way to early evaluate response in acute leukemia. The biochemical mechanisms leading to the accumulation of truncated α1-antitrypsin are under present investigation.

(1) Maubach et al., Blut 48: 243, 1984

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INHIBITION OF COLONY FORMATION OF LEUKEMIC BLASTS IN ACUTE MYELOID LEUKEMIA BY INTERLEUKIN-2 STIMULATED AUTOLOGOUS LYMPHOCYTE SUBSETS


Results from in vitro studies and from autologous bone marrow transplantation suggest a role for cytotoxic lymphocytes in the control of acute myeloid leukemia (AML). The present study was undertaken in order to identify the specific effector cells of the antileukemic effect. We analyzed peripheral blood samples and bone marrow aspirates from 10 AML patients in complete remission (CR). The target cells were autologous leukemic blasts isolated at diagnosis prior to chemotherapy and 2. cells from regenerating bone marrow in CR. Leukemic cells had been stored in liquid nitrogen for 2 to 3 months and were thawed just prior to the experiments. Mononuclear effector cells were separated from hepatoproliferative peripheral blood by Ficol Hypaque gradient centrifugation. They were stimulated with recombinant interleukin 2 (IL-2) for 2 to 3 days. Under sterile conditions they were incubated with fluorescence labeled antibodies against CD3, CD16 and the gamma/delta T cell receptor complex. Cells were isolated on an argon - equipped flow cytometer. Isolated effector cells and target cells were mixed at a ratio of 1 : 5 and incubated in a colony assay supplemented with recombinant GM-CSF for 10 - 14 days. Successful colony formation was obtained in 4 samples prior to chemotherapy and in 2 samples in complete remission. Leukemic blast colony formation was inhibited by the CD16 positive cells in 4 of the 4 samples and by the gamma/delta T cells in 3 of 4 samples. IL-2 stimulated unsorted peripheral blood cells had a stimulatory effect in 3 of 4 patients, while the sorted CD3 positive cells increased colony formation in 1 of 4 samples. Inhibition of colony formation of normal myeloid progenitors was observed in none of the 6 bone marrow aspirates in complete remission. We conclude that CD16 positive NK cells and gamma/delta T cells are able to exert an autologous antileukemic effect. This may of great value in immunosuppressive approaches to AML.

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PROLIFERATION OF HUMAN LEUKEMIC PRE-B CELLS INDUCED BY MURINE FIBROBLASTS AND HUMAN STROMA CELLS: ESTABLISHMENT OF A MODEL SYSTEM

C. Buske, B. Wörmann, Th. Büchner and W. Hiddemann

In the murine system, bone marrow stroma cells play a key role in the regulation of B lymphopoiesis. The aim of this study was the establishment of an in vitro culture system for analysis of interactions between stroma cells and human pre-B lymphocytes. Stroma cells from seven patients with hematological and non - hematological diseases, murine fibroblasts and the leukemic pre-B cell line BLN-1 were maintained in long term culture. The leukemic cells grew spontaneously only at high cell density (>10⁶ cells/ml). Differentiation of the pre-B cells was analyzed on a FACSCAN by changes in antigen expression. When BLN-1 cells were cocultured with murine fibroblasts at cell densities of <10⁵ cells/ml over 10 days, the absolute cell numbers increased 8 - 10 fold in 3 independent experiments. When cocultured in a diffusion chamber with semipermeable membrane, BLN-1 cells maintained a higher viability compared to the controls, but no real cell growth was observed. Identical results were obtained in coculture assays of human stroma cells from different donors with BLN-1. In another experiment, murine fibroblasts or human stroma cells and BLN-1 did not result in differentiation, i.e. changes in the expression of CD10, CD19, CD40 and IgM.

Our data show a positive stimulatory effect of murine fibroblasts and human stroma cells on human pre-B cells. The coculture of BLN-1 with stroma microenvironment provides a model system for the investigation of stroma - pre-B cell interaction.

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Proliferation of human leukemic pre-B cells induced by murine fibroblasts and human stroma cells: establishment of a model system.

C. Buske, B. Wörmann, Th. Büchner and W. Hiddemann.

In the murine system, bone marrow stroma cells play a key role in the regulation of B lymphopoiesis. The aim of this study was the establishment of an in vitro culture system for analysis of interactions between stroma cells and human pre-B lymphocytes. Stroma cells from several patients with hematological and non-hematological diseases, murine fibroblasts and the leukemic pre-B cell line BLN-1 were maintained in long term culture. The leukemic cells grew spontaneously only at high cell density (>10⁶ cells/ml). Differentiation of the pre-B cells was analyzed by FACSCAN by changes in antigen expression. When BLN-1 cells were cocultured with murine fibroblasts at cell densities of <10⁵ cells/ml over 10 days, the absolute cell numbers increased 8-10 fold in 3 independent experiments. When cocultured in a diffusion chamber with semipermeable membrane, BLN-1 cells maintained a higher viability compared to the controls, but no real cell growth was observed. Identical results were obtained in coculture assays of human stroma cells from different donors with BLN-1. In another experiment, murine fibroblasts or human stroma cells and BLN-1 did not result in differentiation, i.e. changes in the expression of CD10, CD19, CD40 and IgM.

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