421

TRUNCATED α_1-ANTITRYPSIN IN LEUKEMIC BLASTS

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Truncated alpha$_1$-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 monoclonal 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 cytoreduction 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 alpha$_1$-antitrypsin in myeloid blasts by immunohistochemical analysis. Western blot analysis with immunostaining using monoclonal antibodies raised in our laboratory, revealed high levels of truncated alpha$_1$-antitrypsin in the granule fraction of leukemic cells. This finding suggests that the extent of GP-41 excreted reflects the degree ot 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 alpha$_1$-antitrypsin are under present investigation.

(1) Maubach et al., Blut 48: 243, 1984
(2) Meyer, Am. J. Clin. Pathol. 86: 461, 1986

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422

INFLUENCE OF THE CONTROL GROUP SIZE ON THE STANDARD DEVIATION OF CHEMOSENSITIVITY RESULTS IN THE DIFFERENTIAL STAINING CYTOTOXICITY (DISC) ASSAY

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We have previously shown, that the DiSC assay is well suited for pretherapeutic drug sensitivity testing of acute leukemia. A correct prediction of clinical response or resistance is achieved in more than 80% of all tested patients. The DiSC assay is based on the differential staining pattern of viable and dead malignant cells. The results are expressed as the mean percentage of surviving tumor cells in relation to untreated control groups (tumor cell survival).
Usually all experiments were performed at least in triplicate, but in order to define the optimal size of the control group in this study 18 different samples of untreated control cells were set up. The mean number of viable cells and the standard deviation were determined separately for 3, 6, 9, 12, 15 and 18 control samples. We found, that with 10 to 25% the variation coefficient for the averaged data of 6 control samples was quite low and could not be further reduced significantly through an increase of the sample size.
We conclude, that the utilization of 6 independent controls is sufficient to obtain reliable drug sensitivity information with the DiSC assay.

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423

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

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Results from in vitro studies and from allogeneic 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 1. 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 heparinized peripheral blood by Ficoll 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 6 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 immunotherapeutical approaches to AML.

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424

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

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In the murine system, bone marrow stroma cells play a key role in the regulation of B lymphocytopoiesis. 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 BLIN-1 were maintained in long term culture. The leukemic cells grew spontaneously only at high cell density (>10^6 cells/ml). Differentiation of the pre-B cells was analyzed on a FACSCAN by changes in antigen expression.
When BLIN-1 cells were cocultured with murine fibroblasts at cell densities of < 10^5 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, BLIN-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 BLIN-1. Long - term cultures with either murine fibroblasts or human stroma cells and BLIN-1 did not result in differentiation, i. e. changes in the expression of CD10, CD19, CD40 and sIgM.
Our data show a positive stimulatory effect of murine fibroblasts and human stroma cells on human pre-B cells. The coculture of BLIN-1 with stroma microenvironment provides a model system for the investigation of stroma - pre B cell interaction.

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