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MOLECULAR CYTOKINE REGULATION BY INTERFERON-α (IFN-α) IN BONE MARROW STROMAL CELLS: A POSSIBLE PARACRINE MODE OF ACTION IN CHRONIC MYELOID LEUKEMIA (CML).

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IFN-α has a myelosuppressive effect which correlates with a selective suppression of the malignant clone in a subset of patients with Ph¹ positive CML. In addition to a direct inhibitory action on progenitor cells, recent data demonstrated a differential regulation of cytokine cascades by IFN-α. We have shown that IFN-α inhibits expression of Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) in LTBMC stromal cells stimulated with Interleukin-1 (IL-1), Tumor Nekrosis Factor-α (TNF-α) or Endotoxin. The murine bone marrow stromal cell line +/+-1.LDA11 was used to further define regulatory mechanisms of IFN-α inhibition on GM-CSF expression. This cell line originated from a murine Dexter type culture and exhibits a preadiposite phenotype. As in human LTBMC, we could demonstrate a inhibitory effect of IFN-α coincubation on GM-CSF activity in serum free supernatants of +/+-1.LDA11 stromal cell cultures stimulated with IL-1 or TNF-α or the combination of IL-1 plus TNF-α. IFN-α inhibitory effect on GM-CSF expression was shown to be dose dependent. Northern blot analysis confirmed these data at the mRNA level. Reprobing of Northern blots for Interleukin-6 (IL-6) mRNA showed increased expression after INF-α incubation, demonstrating specific and differential regulatory effects of INF-α on cytokine production in bone marrow stromal cells. Inhibition of GM-CSF mRNA by INF-α was time dependent, starting at about 90 - 120 minutes post-treatment. Cycloheximide (CHX) incubation abolished the inhibitory effect of IFN-α on GM-CSF expression, suggesting the requirement of a labile protein. Reporter gene studies were used in order to evaluate the effect of IFN-α incubation on GM-CSF mRNA transcription. in stromal cells. For this purpose GM-CSF promoter fragments were subcloned into a luciferase expression vector. Neither constitutive nor TNF-α or IL-1 stimulated GM-CSF transcription was inhibited by IFN-α coincubation. On the other hand, Actinomycin-D chase experiments revealed a reduced GM-CSF mRNA stability after IFN-α incubation. These results show a regulatory role for IFN-α in the bone marrow microenvironment possibly involved in the myelosuppressive effect of IFN-α therapy or viral infections.

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HUMAN ALLOGENEIC NK-CELLS ACTIVATED BY IL-2 AND IL-12 EFFICIENTLY LYSE FRESH ACUTE LEUKEMIA BLASTS. B. Glass, M. Zeis, L. Uharek, T. Gaska, J. Steinmann, W. Gassmann, H. Löffler and W. Mueller-Ruchholtz. Department of Internal Medicine II, University of Kiel, Germany.

We have recently shown that the transfer of allogeneic natural killer cells in the context of cellular immunotherapy is able to cure mice from leukemia after allogeneic BMT. In humans, however, the high resistance of leukemia blasts to NK-cell-mediated lysis may be an obstacle to this type of therapy. In this study we investigated the role of IL-12 (known as natural killer stimulatory factor, NKSF) and IL-2 in the generation of potent activated allogeneic NK cells against fresh acute myeloid (n=10) and lymphatic leukemia (n=4) blasts. *Method*: PBMC from healthy donors were separated for removal of T cells by using anti CD3 MoAb coupled to immunomagnetic beads.This enriched NK-cell fraction (about 65% CD56+) was treated with various concentrations of IL-2 (100 U/ml to 1000 U/ml) and IL-12 (1 U/ml to 100U/ml) for 24 h at 37°C. Cytotoxicity was measured against acute leukemias in a conventional 4h ^{51}Cr-Release Assay. *Results*: Pretreatment of NK-cells with optimal doses of IL-2 (1000 U/ml) resulted in a more than two-fold enhancement of cytotoxicity against leukemia blasts whereas the response to optimal doses of IL-12 (100 U/ml) was slightly lower. The combination with IL-2 (500 U/ml) and Il-12 (100U/ml) most effectively activated the cytolytic capacity of NK-cells tested against myeloid and lymphatic leukemia blasts. NK-cells stimulated by the combination of these cytokines augmented cytotoxicity for more than twofold compared to optimal IL-2 pretreatment alone. *Conclusions*: Our results indicate that such IL-2 and IL-12 stimulated allogeneic NK-cells may have interesting therapeutic implications in the context of cellular immunotherapy against leukemia in humans. Experimental studies evaluating this phenomenon in a murine leukemia model are now underway.

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Frequency and function of IL-2 receptor α, β and γ cains on blast cells from acute myelocytic leukemia.

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Preliminary clinical studies combining immunotherapy with interleukin 2 (IL-2) and chemotherapeutic strategies for treatment of acute myeloblastic leukemia (AML) suggest that IL-2 may improve the disease free survival of the patients. Since AML blasts have been shown to express IL-2 receptors, it is important to know, whether IL-2 may directly influence these leukemic cells. In initial studies, using flow cytometry to analyze surface expression of interleukin 2 receptors (IL-2R), we found expression of the IL-2R α chain on blast cells in 26% and of the IL-2R β-chain in 81% of the patients. To confirm these results at the transcriptional level, we studied the expression of RNA of the IL-2R α, β, and γ chains by RT PCR in the bone marrow of 38 newly diagnosed patients with AML and in three AML derived cell lines. RNA of the α chain was detectable in 11/38 patients, of the β chain in 10/38 patients and the of γ chain in 30/38 patients with AML. Blast cells obtained from colonies growing in semisolid media expressed mRNA for IL-2R. RNA for all three IL-2R chains was expressed in lines KG1, HEL 92.1.7 and K562. In comparison with unstimulated cell lines incubation of the three lines with various amounts of IL-2 over 3 and 14 days did not increase their growth or change message expression of IL-2R, IL-10, and TGF-β and surface expression of the adhesion molecules CD 11, CD 18, CD 29 and CD 54.

In conclusion, despite expression of IL-2 receptors AML blasts do not respond to IL-2 by proliferation, message expression for various cytokine genes and surface expression of cellular adhesion molecules.

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A PROLIFERATION ASSAY TO DETERMINE SPECIFIC T-CELL IMMUNITY USING HLA I-BINDING PEPTIDES. C Scheibenbogen, S Mayer, W Keilholz*, S Stevanovic*, M Anders, HG Rammensee*, U Keilholz, Med. Klinik V, Univ. Heidelberg, Hospitalstr. 3, 69115 Heidelberg and *DKFZ, Abt. für Tumorvirusimmunologie, Heidelberg, Germany

Reliable assays to asses the presence of specific memory T cells are of crucial importance for the development of tumor vaccines. Our aim was to develop a simple and sensitive assay to monitor the specific T cell response against HLA I-binding peptides. To establish specificity and sensitivity of the assay we used an HLA-A2 binding cytomegalovirus peptide (**FIAGNSAYEYV**, Parker et al., JI, 1992) and buffy coat preparations from HLA-A2 positive blood donors with known CMV-serostatus (IgG). In vitro lymphocyte proliferation was measured following cultivation of mononuclear cells (MNC) in 96 well-microtiter plates with or without addition of the HLA-A2 specific CMV peptide by determining BrdU-uptake using a colorimetric assay (Boehringer, MA). A variety of different culture conditions were compared (cell numbers, peptide concentrations, ± IL-2, ± CD4 cells, time in culture). The following conditions yielded highest sensitivity and specificity for discriminating between CMV+ and CMV- donors: Cultivation of $1x10^5$ MNCs/well in RPMI 1640 + 10% human AB serum for 12 days, stimulation with 10^4 pM peptide at days 0 and 7, addition of 30 IU/ml of IL-2 every other day from day 2 on. Under these conditions in 6/7 CMV+ donors specific proliferation (≥ 2 SD of mean of wells without peptide) was detected, while 5/6 CMV- donors showed no specific proliferation. Further donors are currently being tested. This assay offers the possibility to monitor specific T-cell responses against known HLA class I binding peptides following vaccination with viral or tumor antigens.