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Generation of Cytotoxic T Lymphocytes (CTL) in HLA Haplo- And Identical Individuals to Investigate Graft Versus Leukemia Effects

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It is well accepted that cytotoxic-T-lymphocytes (CTL) of the bone marrow donor are responsible for mediating graft-versus host disease (GvHD) and for graft versus leukemia (GvL) following bone marrow transplantation (BMT) between HLA-identical siblings. In order to analyse the GvL and the GvHD effect separately, we intend to generate specific CTLs directed only against Philadelphia-chromosome positive bone marrow mononuclear cells (BMMNC) from patients with chronic myelogenous leukemia. The Philadelphia-chromosome is a specific marker for chronic myelogenous leukemia. For our studies, we use colony-forming-units (CFU) inhibition-assays and the cell mediated lympholysis-assays (CML). We succeeded in generating CTLs directed against different allogenic HLA alleles in related individuals which act as possible restriction elements for a CTL-response. Furthermore, we generated a CTL-response between HLA-identical-siblings following a 4-time rechallenge in the mixed lymphocyte reaction (MLR). This finding could be explained either by differences in HLA-DP alleles or by a mismatch in the minor-antigens. Investigations are in progress to define CTL clones with specificity against Philadelphia-positive bone marrow blasts using the limiting dilution technique. In summary our preliminary data led us to the hypothesis that it is possible to generate a specific CTL-response between HLA-identical siblings without prior in vivo priming. This might have further clinical implications for HLA-identical BMT with respect to determine the CTL-precursor frequency of the donor with anti-leukemic specificity in advance.

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THE EFFECT OF TNF ALFA ON THE CYTOTOXIC ACTIVITY OF NATURAL KILLER CELLS IN VITRO

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Aside from being an effector molecule in antitumor cytotoxic reactions TNF also has immunoregulatory functions on different cells of the immune system. NK cells represent important effector cells of the innate immunity as they employ cytotoxic mechanisms against various tumor cells. They lead to tumor cell necrosis by perforin or tumor cell apoptosis by TNF. The aim of this study was to see whether in vitro pretreatment of peripheral blood lymphocytes (PBL) with recombinant human TNF alfa (rh TNF alfa) has an effect on NK cytotoxic function against the tumor cell line K 562. NK cell cytotoxicity was estimated by the 2 hr enzyme (LDH) cytotoxicity assay. PBL of healthy controls and patients with malignant lymphomas were pretreated for 30 min. in culture medium RPMI 1640 without phenol red and with 10% FCS and 100 U/ml of rh TNF alfa. The treated PBL were then used for the assessment of the change in NK cell activity compared with untreated PBL. The obtained results showed an inhibitory effect of TNF alfa on NK cell activity of controls and a lack of an effect on NK cell activity of investigated patients, whose NK cytotoxicity, with or without treatment, was very much below that for controls. These results point out a negative regulatory role of TNF alfa on NK cell activity of healthy controls, while this effect on NK activity of patients with malignant lymphomas was not obtained. As TNF acts only via its receptors type I or II it could be postulated that they were either not adequately expressed on NK cells of lymphoma patients or that there are defects in the transduction pathways, so that the effect observed for healthy controls could not be obtained in the investigated patients.

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TIL CULTURE AND EXPANSION FROM 42 HUMAN TUMORS. REINFUSION FOR IMMUNOADOPTIVE THERAPY IN 15 PATIENTS:PRELIMINARY DATA.

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Separation, in vitro culture and expansion of TIL from many types of solid tumors is possible in a medium with IL-2. Antitumor response has been observed in Literature after TIL reinfusion+IL-2 in melanoma and renal cancer patients. In the Medical Oncology Dept. of Forlì TIL have been grown in IL-2 from 42 types of human tumors over the past four years: 25 melanomas, 1 hepatocarcinoma, 1 breast, 2 gastric and 13 colorectal carcinomas. The tumor specimens were digested enzymatically to yield single cell suspensions which were then cultured in AIM-V and RPMI 1640 medium with 6000 IU/ml IL-2. TIL expanded from 1,4 x 10 fold to 3,2 x 10^6 fold over a culture period ranging from 9 to 71 days in 26 out of 42 cases (62%). From Feb.'93-Dec.'94, 15 patients were treated with TIL plus IL-2 in continuous infusion (12 million IU/m2 from day 1-5 and 14-18). The median number of infused cells was 4.2 x 10^{10} (range 0,043 x 10^{10}-20 x 10^{10}). 7 patients had colorectal cancer, 1 had gastric cancer and 7 had melanoma. Among these, 4 patients (1 melanoma and 3 colorectal cancer) with advanced disease progressed after 1,4,5,16 months. 11 patients were "disease free" when treated (the metastases were radically removed for TIL culture). The gastric cancer patient relapsed after 6 months, 2 out 4 colorectal cancer patients relapsed after.16,5 months, 2 are still disease free after 18 e 2 months. Only one disease free melanoma patient relapsed after 4 months and the other 5 patients are still disease free after 20,16,13,10,1 months.

The preliminary results reported here seem to indicate that TIL treatment should be used in patients with microscopic residual disease and a very high risk of relapse

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LYMPHOKINE INDUCED DIFFERENTIATION OF HUMAN NONLYPHOCYTIC LEUKEMIA CELL LINES

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Human leukemia cell lines ML-1, HL-60, THP-1, RC2A and PS-1 were treated by combinations of natural and/or recombinant tumor necrosis factor alfa (TNF-alpha), interferon-alfa (IFN-alpha) and interferon-gamma (IFN-gamma) to induce *in vitro* monocytic differentiation. Synergistic action of TNF-alpha, IFN-gamma and IFN-alpha was observed by morphological and immunological analysis. TNF-alfa alone induced monocytic differentiation of majority of myeloid and myelomonocytic cell lines. IFN-gamma alone induced strong expression of surface antigens HLA-ABC, HLA-DR and CD14. However, only limited morphological and functional differentiation and no adherence to surface of culture flask in any cell line was observed. Using both TNF-alpha and interferon-gamma in concentration 500 U/ml and we demonstrated strong induction of monocytic/macrophage differentiation. The most efficient differentiation was observed by the simultaneous induction by all three agents - TNF-alpha, IFN-alpha and IFN-gamma, as demonstrated by the adherence to plastic and glass surfaces and by the expression of the major monocytic antigens CD14, CD11b, and by monocytic morphology.