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THE FUNCTION OF LYTIC GRANULES IN TUMOR THERAPY INDUCED BY IL-2 ACTIVATED KILLER LYMPHOCYTES

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Immunotherapy of metastatic cancer by tumor-infiltrating lymphocytes (TIL) expanded in vitro in IL-2 or by IL-2 alone has been reported. The cellular and molecule mechanism of this new and promising mode of therapy and its side effects is not clearly understood. In murine tumor systems we have found a small population of highly cytocidal TIL at the tumor site (peritoneal cavity). The effectors are small-to-medium sized, non-dividing lymphocytes, exhibiting highly specific cytolytic activity in vitro, but interestingly, no lytic granules, perforin or BLT-esterase activity. When cultured with IL-2 the TILs rapidly differentiate into dividing CTL and possessing cytolytic granules and their constituents. Stimulated secretion of these lytic granules, upon lymphocyte-tumor cell interaction in vivo, may be responsible for the anti-tumor effects during IL-2/TIL therapy in vivo. Secreted granule constituents may play a key role in the extravasation of TIL. We also suggest that the secreted cytocidal granules of IL-2 activated TIL in vivo, rather than IL-2 in itself, are responsible for a number of undesirable side effects observed in the course of IL-2 immunotherapy.

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REGIONAL INFUSIONS AND PERFUSIONS WITH CYTOKINES

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Over a period of 5 years various protocols regarding the locoregional administration of cytokines have been carried out in mice, rats and humans. IL2 has been administered via the hepatic artery in patients (pts) with liver metastases with no success. IL2 was administered intrapleurally and intraperitoneally in phase I-II studies in mesothelioma and ovarian cancer pts. In ovarian cancer IL2 was given in combination with T-cells and bispecific antibodies. The response rate in mesothelioma pts was 23% and in ovarian cancer pts 44%. TNFα was administered by isolated limb perfusion in combination with gamma-Interferon and Melfalan in pts with stage III melanoma or irresectable soft tissue sarcomas of the limbs with remarkable success (90 % CRs; 90% limb salvage rate).

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INTERLEUKIN-2 AND INTERFERON IN ADVANCED RENAL AND OVARIAN CANCER. Selby P. Institute for Cancer Studies, St James's University Hospital, Leeds LS9 7TF, UK.

Interleukin-2 has been used by continuous intravenous infusion in five studies in Europe and in the United States to treat patients with advanced renal cancer. Three hundred and twenty seven patients treated have been compared to 390 control patients taken from the Eastern Collaborative Oncology Group database and matched to the Interleukin-2 treated patients. In this analysis interleukin-2 appeared to prolong survival and the benefits were greatest in a sub-group of patients with a better underlying prognosis as shown by good performance status, a single site of disease and a disease free interval of longer than one year from surgery to interleukin-2 treatment. A similar analysis with interferon alpha treatment also suggests prolonged survival compared to matched control patients. Studies with interferon in ovarian cancer have focused on the treatment of advanced disease. Our studies however have identified the role of interferon as a maintenance agent following chemotherapy for advanced ovarian cancer. Accrual and feasibility in this study can now be reported but the effect on disease free survival cannot yet be analysed.

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TUMOR NECROSIS FACTOR: MECHANISMS OF ACTION AND POTENTIAL ROLE IN CANCER TREATMENT.

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TNF, especially in combination with interferon, is cytotoxic for many transformed cell lines and has antitumor activity in vivo. The latter effect can be either host-mediated, or directly on the tumor cells. The fairly severe systemic toxicity of TNF, however, is a major hindrance towards clinical applications. Although hTNF and mTNF exert a similar cytotoxic μg/mouse for mTNF, and 500 μg/mouse for hTNF, a 50-fold difference. There are two types of receptor, a smaller, TNF-R55, and a larger, TNF-R75. As the latter in the mouse is not recognized by hTNF, we have concluded that the TNF-R75 plays a major role in the high systemic toxicity. Triggering of the TNF-R55 is sufficient to mediate direct antitumor activity. In the human system, however, there is no discrimination by the two species of TNF. However, we have now characterized hTNF mutants which still retained full activity on the TNF-R55, but no longer trigger hTNF-R75. Considering the results in mice, we would expect that these hTNF mutants would have reduced toxicity in primates, but would have maintained wild-type, direct antitumor activity. Such a mutant, R32W, indeed has retained the same antitumor activity as wild-type TNF, as assessed in vivo in nude mice carrying a human HT29 tumor.

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CYTOKINE GENE-TRANSDUCED TUMOR CELLS IN CANCER THERAPY: TUMOR INHIBITION BY NON-SPECIFIC AND SPECIFIC IMMUNE MECHANISMS. Parmiani G. and Colombo M.P. - Istituto Nazionale Tumori, 20133 Milan (Italy).

Rodent tumor cells expressing transduced cytokine genes like IL-2, IL-4, IL-6, IL-7 are growth inhibited thanks to the local recruitment of different immune cells and granulocytes. To see whether even genes coding for hemopoietic factors could affect tumor growth and to study the underlined mechanism, the mouse colon carcinoma C26 was transduced with the gene encoding human G-CSF. G-CSF producing C26 cells (C26/G-CSF) did not growth in syngeneic mice and such inhibition was due to an influx of polymorphic neutrophils (PMN) at the tumor site. Immunocytochemistry and *in situ* hybridization indicate that PMN were responsible for inhibition of tumor take and that they expressed mRNA for IL-1α, IL-1β and TNFα. In mice given C26/G-CSF after 600 R irradiation, the tumors grew to 1-2 cm and then regressed completely in 70% of mice. During the growing phase, tumors were infiltrated first by PMN (days 15-20), then by macrophages and lastly by T cells. Depletion of CD8+ T cells significantly reduced tumor regression. Depletion of PMN by anti-granulocyte mAb lowered the number of T cells infiltrating the tumor and prevented regression. These results indicate that inhibition of C26/G-CSF tumor take and regression occur through different mechanisms which involve PMN and PMN-T cell interactions, respectively, as well as release of different cytokines.

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RATIONAL DEVELOPMENT OF CYTOKINES FOR CLINICAL APPLICATION

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Biological therapy with growth factors and cytokines is currently finding its place as a new treatment modality for malignant disorders and provides preclinical and clinical scientists with a fascinating new challenge. Cytokines generally have a broad spectrum of in vivo effects like modulation of immune response, stimulation of hemopoiesis, direct regulation of cellular growth and differentiation, toxicity for tumor cells, action on tumor vascularisation, etc.. Therapy with cytokines follows totally different rules than for cytotoxic drugs. Test systems like the tumor stem cell assay or animal models tailored for the preclinical investigation of cytotoxic agents are of minor efficacy in the investigation of biological agents where predictions of likely antitumor properties are often inaccurate. Cytokines exert numerous primary effects and induce cascades of secondary effects. Thorough study of the various influences warrants the presence of the whole immune system and, as cytokines tend to be species-specific, optimal data can only be obtained from recombinant human material applied in humans.

The lack of reliable predictive in vitro- and animal models of human cancer implies that the therapeutic potential of a "biological" can only be assessed following careful study in man. Consequently, also for ethical reasons the effectiveness of clinical studies must be improved by closely relating them to basic research programs utilising material from cytokine-treated patients for "ex vivo" research. Thus, a "Task Force Biologicals" was established by the European Organisation for Research and Treatment of Cancer (EORTC) aiming to coordinate preclinical and clinical research on cytokines.