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In-situ cytokine therapy by immunokines: Induction of T-cell mediated antitumor immunity

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Tumor immunotherapy is aimed to induce tumor-specific T cell responses effective in eradicating disseminated tumors, as well as mounting a persistent tumor-protective immunity. We demonstrate here that a recombinant fusion protein consisting of an anti-ganglioside GD2 antibody and IL2 is able to induce eradication of established B78-D14 melanoma metastases in immunocompetent syngeneic C57BL/6J mice. This therapeutic effect is mediated by host immune cells, particularly CD8+ T cells. Analysis of the T cell receptor B variable gene repertoire of TIL demonstrated an overexpression of BV5 that was absent in PBL, skin or non-treated tumors. Following successful therapy animals displayed a long-lived antitumor immunity preventing melanoma growth in the majority of animals when challenged up to 4 month later. This effect was tumor-specific since no cross-protection against syngeneic EL-4 thymoma cells was observed. Furthermore, this tumor-specific protection can be transmitted horizontally to naive, syngeneic SCID mice by passive transfer of CD8+ T lymphocytes derived from immune animals. These results suggest that antibody-targeted delivery of cytokines provides means to elicit effective immune responses against established tumors in the immunotherapy of neoplastic disease.

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STIMULATION OF CD 40 UP-REGULATES COSTIMULATORY MOLECULES ON CHRONIC B-LYMPHOCYTIC LEUKEMIA CELLS AND INDUCES EFFICIENT KILLING OF STIMULATED AND NOT-STIMULATED B-CLL BY CYTOLYTIC EFFECTOR CELLS.

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Chronic B-lymphocytic leukemia cells (B-CLL) rarely induce clinically significant T cell mediated responses. Critical for the induction of tumor specific T-cell immunity is an adequate expression of tumor specific antigens and immunomodulatory molecules on the surface of the tumor cells. We therefore studied the regulation of costimulatory molecule on CLL cells by CD40 stimulation. Most chronic B-lymphocytic leukemia cells showed reduced or completely lost expression of adhesion or B7 costimulatory antigens. Interestingly it was possible to correct these defects. *In vitro* stimulation via CD 40 induced significant up-regulation of B7 costimulatory adhesion and MHC class II molecules. Subsequent stimulation of allogeneic T-cells with these pre-activated B-CLL led to an increase of CD8+ cells. Once primed these T cells showed B-CLL directed cytotoxicity in four out of five patients tested as assessed by a TNF-alpha release test. Taken together the experiments show that subsequent stimulation with pre-activated lymphoma cells induce a significant cellular anti-tumor immunity against B-CLL cells.

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IDIOTYPE VACCINATION FOR PATIENTS WITH NON-HODGKIN'S LYMPHOMA

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Idiotype (Id) vaccination takes advantage of the unique antigenic structure formed by the variable regions of the heavy and the light chain of the surface Ig expressed by lymphoma cells.

In one study, 41 patients with low-grade Non-Hodgkin's Lymphoma in remission after chemotherapy were immunized with Id-protein coupled to KLH and emulsified in an immunologic adjuvant. 20 patients generated a specific response against the Id of their tumor Ig. With a median follow-up of 5.3 years since the last chemotherapy, the median duration of Freedom from Tumor Progression (FFP) for the patients with immune response (IR) is 7.9 years compared to 1.3 years for the patients without anti-Id IR. Analysis of a more homogeneous subgroup of patients in first remission (n = 32) revealed a duration of FFP of 7.9 years for patients with IR vs. 1.3 years for patients without IR ($p = 0.0001$). The median time of survival after the last chemotherapy prior to vaccine has not been reached for the group with an anti-Id IR vs. 7 years for the patients without IR ($p = 0.04$). The only predictive parameter for the likelihood of developing an anti-Id IR was the absence of detectable tumor prior to the vaccine. Separate analysis of the subgroups with complete or incomplete remission confirmed the association of anti-tumor Id IR and duration of FFP. Two patients with residual disease experienced a complete regression of their tumor associated with the development of an anti-tumor Id IR.

An up-date of the current clinical trials will be given and the development of new vaccine strategies using dendritic cells or genetic vaccines will be given.

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CELLULAR THERAPY OF RENAL CELL CARCINOMA PAST, PRESENT, FUTURE

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Metastatic renal cell carcinoma (mRCC) is a disease with a mean survival of 6 to 10 months. Interleukin-2 (IL-2), the only approved therapy for mRCC, is associated with a 14% response rate and durable remissions in some patients with high performance status. This paper reports the clinical results from 62 patients enrolled in tumor infiltrating lymphocyte (TIL) cell therapy trials. Patients were eligible if they had mRCC with the primary tumor in place and an ECOG performance status of 0 or 1. Patients were treated with cytokine prior to nephrectomy and preparation of TIL. No significant toxicites were noted in these studies. Overall, 5 patients (9.1%) achieved a complete response and 14 patients (25.5%) achieved a partial response. The responses were durable with a medium duration of 14 months (range 0.8+ - 64+). The actuarial survival was 65% at one year and 43% at two years with an overall median survival for all patients of 22 months (range 2 - 70+ months). The median survival for responding patients has not yet been reached (range 2 - 63+). These results demonstrate that immunotherapy with TIL provide clinical benefit in the majority of patients.

Dendritic cells (DC) are potent antigen presenting cells. We isolated DC's from patients with RCC by culturing the adherent fraction of blood mononuclear cells for 7 days in GM-CSF, with or without IL-4. The mature DC's were loaded with the autologous tumor lysate and added to the TIL's once a week for two consecutive weeks. IL-4 was essential for obtaining low CD14 and a high B7-1, B7-2, and CD40 expression of the DC's, a phenotypic characteristic which appeared to be correlated with antigen presentation. DC stimulated TIL cultures showed an increase in growth expression, upregulation of CD3+ (CD8+ and CD4+) and TCR+ cell population, downregulation of the CD56+ natural killer cell population, and enhanced autologous tumor cell lysis. DC's when loaded with tumor antigens are potent immune cells and induce clonal expansion of CTL with specific anti tumor activity in RCC patients. Expanded DC's can be used in adoptive immunotherapy strategies alone or in combination with other biological agents.