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Cytotoxic T-cells with grafted, tumor-specific recognition functions

Bernd Groner, Elfriede Klundt and Uwe Altenschmidt. Institute for Experimental Cancer Research, Tumor Biology Center, Breisacher Str. 117, D-79106 Freiburg, Germany

Adoptive immunotherapy of cancer utilizes the transfer of tumor reactive immune cells to achieve regression. The success of this procedure is dependent upon the specificity of the transferred immune cells, their number and their ability to reach their target cells. For this purpose we genetically modified T lymphocytes. These cells assume recognition specificity for particular tumor cells. Tumor cells overexpressing the ErbB-2 receptor have served as a model. The target cell recognition specificity is conferred to T lymphocytes by transduction of a chimeric gene encoding the ζ chain of the T cell receptor (TCR) and a single chain antibody (scFv) derivative of a monoclonal antibody (FRP5) directed against the human ErbB-2 receptor. The chimeric scFv(FRP5)-ζ gene was introduced into primary mouse T lymphocytes via retroviral gene transfer. Naive T lymphocytes were stimulated with IL-2 and PHA and infected by co-cultivation with a retrovirus-producing packaging cell line. The expression of the scFv(FRP5)-ζ fusion gene was detected in more than 75 % of the T cells. These T cells lysed ErbB-2 expressing target cells in vitro with high specificity. We tested the anti-tumor efficacy of scFv(FRP5)-ζ expressing T cells in a syngeneic animal model. ErbB-2 expressing tumor cells were transplanted into Balb/c mice and tumors established. The mice were treated with autologous, transduced T cells. The adoptively transferred scFv(FRP5)-ζ expressing T cells caused total tumor regression. The presence of the transduced T lymphocytes in the tumor tissue was monitored. No humoral response directed against the transduced T cells was observed. Antibodies directed against the human ErbB-2 receptor were detected upon tumor lysis.

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T CELL REGENERATION AFTER CHEMOTHERAPY: IMPLICATIONS FOR IMMUNOTHERAPY IN THE SETTING OF MINIMAL RESIDUAL DISEASE

Crystal L. Mackall, Frances T. Hakim, Thomas A. Fleisher, Margaret R. Brown, Dimiter S. Dimitrov, Ronald E. Gress, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Immune based therapies for cancer are most effective in the setting of low tumor burden, suggesting that it may be desirable to administer immunotherapy upon completion of antineoplastic regimens which contain cytotoxic chemotherapy. The success of such an approach however, is dependent upon host immunocompetence in this setting. Analysis of T cell regeneration in a series of patients after cytotoxic chemotherapy has shown that an age dependent decline in thymic regenerative capacity is a primary factor limiting immunocompetence. Recovery of total CD4+ T cell numbers is particularly thymic-dependent with profound, prolonged CD4+ depletion in patients greater than 12-15 years of age. While animal models suggest that peripheral expansion of small numbers of mature T cells could potentially also contribute to CD4+ regeneration, this process is limited by an abnormally high rate of activation induced apoptosis in CD4+ cells after chemotherapy. CD8+ T cell regeneration, although rapid and apparently not subject to age dependent declines in thymopoiesis, results in the accumulation of abnormal subsets (CD28-CD8+, CD57+CD8+) with a prolonged deficiency of the normally predominant CD28+CD8+ subset. Therefore, host immunocompetence after chemotherapy induced T cell depletion is limited by quantitative CD4 deficiency, increased rates of activation induced apoptosis, and CD8+ subset disruption. The development of specific approaches to rapidly restore immunocompetence after cytotoxic chemotherapy is important for the success of immune based therapies for minimal residual neoplastic disease.

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IMMUNO-GENE THERAPY OF HUMAN MELANOMA PHASE I/II CLINICAL TRIAL

Andrzei Mackiewicz , Stefan Rose-John
University School of Medical Sciences, Poznan,J. Guttenberg University, Mainz, Germany

In preclinical studies we demonstrated that ectopic expression of the murine IL-6 and IL-6 soluble receptor (sIL-6R) genes in murine melanoma cells inhibited thier *in vivo* growth potential and metastatic capacity due to the stimulation of a specific antitumour immune response. These results provided the basis for a phase I/II clinical trial of immunization of patients with autologous tumor cells admixed with allogeneic melanoma cells retrovirally (MSCV based double-copy bicistronic vector) transduced with IL-6 and sIL-6R genes. Nine patients with advanced malignant melanoma were enrolled in the study. Meleanoma metastatses were surgically excised single cell suspensions prepared and admixed with an equal number of allogeneic melanoma cells modified to secrete recombinant IL-6 and sIL-6R (5x 10^7 cells total). The mixtures were irradiated and then injected to patients on day 0, 14, 28, 32, then once a month for three months and subsequently at two months intervals. Patients receiving vaccine immunizations displayed delayed type hypersensitivity, activation of peripheral NK and T cells, as well as infiltration of inoculation sites and distant metastases by activated T cells mostly CD8-positive. Complete regression in one patient partial regression in two patients stable disease in one patient and mixed responses in two patients were observed. One patient with grade III melanoma has had no recurrence of disease during 9 months of treatment. There are three major conclusions of the study (i) this novel melanoma vaccine strategy is not toxic; (ii) the vaccine induced cellular (antitumor) immune responses associated with significant T-cell infiltration of distant metastatic nodules; and (iii) the feasibility of this approach as potential treatment of metastatic melanoma was demonstrated. Phase II clinical studies in which patient inclusion criteria will be expanded to include more patients with stage III disease will be carried out.

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GENETICALLY-ENGINEERED ALLOGENEIC RENAL CELL CARCINOMA VACCINES

D.J. Schendel[1], G. Willimsky[2], B. Maget[1], S. Cayeux[3,4], W. Uckert[2], Z. Qin[2], R. Oberneder[5], A. Hofstetter[5], T. Pezzutto[3,4], B. Dörken[3,4] and Th. Blankenstein[2].
Inst. of Immunology[1] and Dept. of Urology[5], Ludwig-Maximilians-Universität München, Max-Delbrück Center[2] and Robert Rössle Clinic[3], Berlin-Buch and Virchow-Clinic, Humboldt University, Berlin[4]

In light of the technical difficulties to prepare individual patient vaccines, allogeneic vaccination of patients with metastatic RCC is now considered. A number of immunological criteria should be considered in the selection of tumor lines for use in allogeneic vaccines. 1) RCC lines should have good levels of MHC class I and adhesion molecules. 2) In vitro functional studies should demonstrate that they express tumor-associated antigens that can be seen by T cells. 3) These T cell ligands should be common to RCC of other patients and be presented by prevalent class I molecules. MHC serological matching may not be suitable; for example, HLA-A2 as defined serologically exists as 21 variants that differ in their peptide binding regions. Therefore, vaccine and patient selection may require molecular MHC matching. Multiple genetic alterations may be necessary to obtain optimal immune stimulatory capacity with a tumor cell line: costimulatory molecules, like B7.1 may synergize with cytokines to improve immune responses. All genetic modifications need to be judged extensively for their impact on naive and memory T cell responses. Inclusion of suicide genes may allow non-irradiated allogeneic vaccines to be tested in patients who show strong alloresponses to the vaccine cells in vitro. While considerable effort is needed to select and modify vaccines according to these criteria the resultant vaccines can be better assessed in a standardized manner. The ability to assess whether tumor cell vaccines stimulate T cell responses in vivo will require a combination of cellular and molecular monitoring strategies. We have developed a potent allogeneic RCC vaccine, as judged by in vitro immunological studies. Two phase I trials comparing tumor cells altered to express IL-2/B7.1 or IL-7/B7.1, along with two suicide genes, are now being planned for RCC patients with advanced disease