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THE USE OF ANTI-CD3/CD28-DYNABEADS TO RESTIMULATE HUMAN ANTIGEN SPECIFIC T CELLS OR EXPAND NAÏVE T CELLS

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The development of improved culture methods for efficient generation and expansion of antigen-specific T cell clones are important for the potential therapeutic use of these cells in adoptive immunotherapy of human malignant diseases. Techniques for cloning human T cells have used initial stimulation with antigen, APC and IL2 in limiting dilution cultures, followed by restimulation to maintain growth and antigen specificity. The requirement for repetitive stimulation with autologous/MHC-matched APC presenting exogenously added antigen can be a significant problem in long-term culture systems because the amount of available APC may be limited. In order to obtain a more general method that can be used for restimulation of established T cell clones, we have developed a method where we use Dynabeads coated with anti-CD3 and anti-CD28. Our results demonstrate that both antigen specific CD4+ and CD8+ T cell clones can be expanded in vitro by stimulation with anti-CD3/ CD28 Dynabeads. The method limits the quantity of antigen required and circumvents the need for APC. Furthermore, T cells remain antigen specific after several restimulations with anti-CD3/CD28 beads. The method is also applicable for expansion of naïve T cells from blood that can be used in adoptive immunotherapy of human diseases.

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BIOLOGICAL TUMOUR REGRESSION AND DORMANCY: A new therapetic approach from tumour stability analysis.

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BACKGROUND: The episodic phenomenology of prolonged arrest and biological remission has incidence of 6% and 0.7% respectively. This para-doxical natural phenomenon stresses the homeostatic self-reparative poten-tiality of the organism. Past studies of biological arrest of other diseases often provided valuable modalities, as by Wagner-Jauregg.
OBJECTIVE: The aim is to delineate the causative factors of the phenomenon, and explore the possibility of a new therapeutic approach.
METHODS: The statistical technique of Causation Analysis is applied to time-series pathophysiological data of patients displaying the phenomenon, and a significant causative factor turns out to be high perturbation of tumour temperature, oxygenation, glucose level, T-cell immune status or cytokine level. This shows that such perturbation can enable the tumour system instability, indicating arrest or regression. We use the recent advances in immunodynamics to show the basis of biological regression.
RESULTS: We delineate the threshold condition of high perturbation of the above pathophysiological parameters that will induce tumour instability or arrest. For this to occur, sigma value of perurbations >1.41. Empirical corroboration is furnished from Ewing sarcoma, clear cell sarcoma, lung carcinoma, fibrosarcoma, melanoma etc., which are regressed using temperature, oxygenation and other perturbations with sigma > 1.41. Thus the biological regression phenomenon offers a new anti-cancer approach.

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Hsp70-peptide activated autologous NK cells in the immunotherpay of cancer – a clinical pilot study

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As previously shown an incubation of NK cells with a 14-mer Hsp70-peptide stimulates both the proliferation and cytolytic activity against Hsp70 positive tumors, *in vitro*. An immunoreconstitution of tumor-bearing mice with Hsp70-peptide activated NK cells results in tumor regression. Before starting the clinical trial about 500 different tumor biopsies and bone marrow aspirates of leukemic patients have been screened for Hsp70 membrane expression. Especially lung, colorectal, pancreas cancer and leukemic blasts have been defined as Hsp70 positive. Therefore, patients with these tumors were included in our first clinical trial. Peripheral blood mononuclear cells (PBMC) of Hsp70 positive cancer patients were isolated by leukapheresis followed by Ficoll separation. Then PBMC were transfered into 250ml tissue culture bags (Cellgenix) and incubated for 4 days with Hsp70-peptide (cGMP-grade) plus low dose IL-2 (100 IU/ml) in serumfree X-Vivo 20 medium (GMP-grade). Following two washing steps the activated cells were reinfused into the patient on day 4. So far 6 patients suffering from solid tumors and 4 leukemic paptients have been treated. So far, none of the patients showed any negative side effects. In *in vitro* assays we demonstrate for all patients an activation of NK cells after treatment by the determination of cell surface markers (FACS) and in functional assays (standard Cr-51 release assays).

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OUR FINDING THAT THE GvM AND GvH EFFECT ARE CAUSED -AT LEAST PARTLY- BY DIFFERENT T CELL CLONES ALLOWS FOR THE FIRST TIME A SELECTIVE GvM POTENTIATION WITHOUT A PARALLEL GvHD INDUCTION

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Our animal experiments led to the following conclusions: (1) GvLR and GvHR are caused by different subsets. (2) Whereas GvHD-establishing T cell subset is of donor origin, GvL-responsible subset can be split into two T cell subpopulations, one (GvL/I-effect) being identical with the GvHD-causing subset (of donor origin) and the other (GvL/II-effect) being of recipient origin and representing a mixture of preexisting deblocked and newly recruited tumor-specific Tc cells. (3) The main difference between the GvL and GvT-effect is that the GvT/I-effect is, in contrast to the GvL/I- effect, a rather negligible component of the GvT reaction. (4) The GvL/I and the GvT/I effect are based on the activity of alloreactive, recipient-specific T subclones of the donor, the targets are all non-malignant and malignant, MHC I expressing, i.e. nucleated cells of the recipient (patient). There is no preference for transformed vs. non-transformed cells; favoured targets are rather cells which express both, the MHC II and MHC I antigen, due to the costimulation of Tc and Th cells. (5) The GvM/II-effect is based on the discrimination of the fine structural differences between malignant and non-malignant cells which can be recognized solely by autologous or syngeneic T cells. The GvL/II and GvT/II- reaction is expected to be slow in comparison to the GvL/I and GvT/I-effect but appears to be essential for the elimination of residual tumor cells and metastases, a capital problem in all tumor-therapeutical approaches. (6) An uncontrolled GvL/I or GvT/I-reaction can prevent the beneficial GvL/II or GvT/II-effect, as tumor-specific Tc of the patient which are the only effectors able to recognize and kill residual tumor cells, represent an important target of the GvM/I-effectors. It's why host immunocytes must be preserved (non-ablative instead of myeloablative regimen).