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CD4+ and DC requirements for optimal CD8+ CTL induction against tumors
Molecular triggers of DC activation sufficient for induction of CD8+ CTL responses include agonistic CD40 antibody or LPS. In natural immune responses specific CD4 cells, reactive with peptide antigens presented by MHC class II molecules on DC, can also drive maturation of immature DC to the mature DC state required for CD8+ CTL response induction. CD4+ T helper cells to a large extent operate through upregulation of CD40L which then interacts with CD40 on DC to cause the required DC activation. Important cognate interactions for full CD8+ CTL induction by activated DC are CD80/CD86 on the DC, costimulating CD28 on the CD8 cells. For maintenance and full expansion of CD8+ T cells, interaction of 4-1 BBL (another member of the TNF(R) family) on DC with 4-1 BB on CD8+ CTL is also important. In the absence of CD80/CD86 costimulation, the 4-1 BBL -> 4-1 BB interaction appears to be inactive. Thus proper induction, expansion and maintenance of CD8+ CTL responses involves delicate interactions between CD4+ T-cells, DC and CD8+ T-cells involving several members of the TNF(R) family, including as signal transduction molecules CD40 on DC and 4-1 BB as well as CD27 on CD8+ CTL precursors. To prevent untoward destruction of antigen bearing DC by activated CD8+ CTL, DC protect themselves by upregulation during maturation of SPI-6, a member of the serpin family that specifically inactivates granzyme B and thereby blocks CTL-induced apoptosis. Interestingly, T helper 1 cells, which best induce CTL responses, cause SPI-6 expression and subsequent DC resistance. In contrast T helper 2 cells neither induce SPI-6 nor resistance to CTL mediated lysis of DC.

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rViscumin, a novel anticancer agent – preclinical and clinical development status

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rViscumin is a type II ribosome inactivating protein exhibiting cytotoxic and immunostimulatory properties.
In whole blood from healthy donors rViscumin lead to intracellular cytokine induction (IL-1β, IL-6, TNF-α) in predominantly monocytes and granulocytes which in consequence underwent apoptosis. Increased levels of the respective mRNAs were measured in PBMC. Peak cytokine induction was close to the IC_{50} measured as apoptosis or decreasing viability indicating that the immunomodulatory activity of rViscumin is linked to the induction of apoptosis. Strong growth inhibition was found in human tumor cell lines and xenografts: the mean IC_{50} value was 0.16 ng/ml (IC_{70} 0.4 ng/ml) for a set of 20 cell lines (Freiburg panel) and 0.9 ng/ml (IC_{70} 3 ng/ml) for 42 human xenografts. In the NCI 60 cell line panel the mean GI_{50} was 1 ng/ml (20 pM). Out of both panels prostate carcinoma, colon, ovarian, small and non small cell lung cancer lines were relatively sensitive as compared to the mean value. Pronounced antitumor activity was seen in vivo in B16 melanoma and xenograft models (CXF-280 colon cancer, LXFS 538 small cell lung cancer). In two different bladder cancer models and tumor colonisation models for metastasis inhibition rViscumin also lead to a prolongation of survival.
rViscumin is in phase I clinical development. In two ongoing EORTC phase I studies rViscumin is administered systemically (s.c. and i.v., respectively) to patients with refractory solid malignant tumors.
In patients with superficial bladder cancer six weekly intravesical instillations lead to a complete remission of the marker lesion in 3 of 12 patients examined in a dose finding phase I/II study so far. In another dose finding phase I/II trial intrapleural rViscumin therapy is being investigated in patients with malignant pleural effusion.

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Therapy of human tumors in NOD/SCID mice with patient derived re-activated memory T cells from bone marrow

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Our previous studies with animal tumors showed that bone marrow (BM) is a site where tumor cells are controlled in a dormant state by the immune system. Here we studied BM of breast cancer patients with respect to memory T cell content, their specificity and functional activity (Feuerer et al. Nature Med., 2001). We found that around 70 % of CD3 T cells in patients' BM were CD45RO positive memory T cells. The proportion of memory T cells among the CD4 and CD8 T lymphocytes was much higher in BM of cancer patients than in healthy donors ($P < 0.001$). Patients with disseminated tumor cells in their BM had more memory CD4 T cells than patients with tumor cell negative BM. Based on a functional analysis of 87 patients and 11 normal donors, we demonstrated that a majority of primary operated breast cancer patients contained in their BM functional memory T cells with the specificity for tumor associated antigens (TAA). Patients' BM and PB contained CD8 T cells which bound to tetramers consisting of HLA-A2 and peptides derived from the TAA MUC1 and Her-2/neu. BM but not PB memory T cells from patients could be re-stimulated in short-term culture to IFN-γ producing and cytotoxic effector cells by autologous dendritic cells pulsed with respective tumor lysate or with above mentioned TAAs. A single transfer of re-stimulated bone marrow T cells into NOD/SCID mice caused regression of autologous tumor xenotransplants involving their infiltration by human T cells, tumor cell apoptosis and necrosis. T cells from peripheral blood showed much lower anti-tumor reactivity. Our findings reveal a regular specific recognition of cancer TAAs by the human immune system and point to a novel cancer immunotherapy using memory T cells pre-existing in cancer patients.

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Effect of Immunostimulatory CpG-oligonucleotides in chronic lymphocytic leukemia B cells

Thomas Decker and Christian Peschel

Bacterial DNA and phosphothioate oligonucleotides containing a CpG motif (CpG-ODN) can activate cells of the immune system such as monocytes, dendritic cells and B cells. Protective immune responses against pathogens and tumor cells were observed in murine models when mice were treated with CpG-ODN. Recent results have demonstrated strong activation of human immune cells as well. Apart from stimulating cells of the immune system, CpG ODN have many direct effects on B-CLL cells such as cell cycle entry, cytokine production and upregulation of potential target antigens for antibody therapy. In our studies, we demonstrated that B-CLL cells activated with the CpG-ODN DSP30 upregulate important costimulatory molecules such as CD80 and CD86. This effect was further enhanced upon costimulation with CD40ligand or IL-2. Resting B-CLL cells are poor stimulators of autologous or allogeneic T cells but we demonstrated autologous and allogeneic immune recognition of B-CLL cells stimulated with CpG-ODN in mixed lymphocyte reactions.