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INHIBITORY EFFECT OF PROSTAGLANDIN (PG) D_2, PGJ_2 AND Δ^{12}-PGJ_2 ON PROLIFERATION OF CELLS INVOLVED IN HOST DEFENSE

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PGD_2 and its metabolites PGJ_2 and Δ^{12}-PGJ_2 are known to exhibit potent antiproliferative effects. Although the exact mode of this action is not known, studies on antineoplastic therapy with Δ^{12}-PGJ_2 and other cyclopentenone prostaglandins have been initiated. Preliminary results suggest that this approach may be benificial.

We investigated the effects of these lipid mediators on cells involved in host defense.

Effects of PGD_2, PGJ_2 and Δ^{12}-PGJ_2 on monocytic, lymphocytic, myelocytic and mast cell cultures and on mixed lymphocyte cultures were tested using proliferation assays ([^{3}H]-thymidine uptake, MTT-test).

These Prostaglandins exhibited almost equipotent antiproliferative effects in the investigated cell systems. The IC_{50} was 2.5 to 20 µM for lymphoma cells, myelocytic cells and mast cells, whereas monocytic and histiocytic cells were more resistant (IC_{50} 30 to 70µM).

We conclude that PGD_2, PGJ_2 and Δ^{12}-PGJ_2 could suppress proliferation of cells involved in host defense. This may be relevant in situations of enhanced PGD_2 production and in treatment with cyclopentenone PGs in vivo.

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INTRACRANIAL MURINE GLIOMA 261 MODEL TO STUDY THE ANTITUMOR ACTIVITY OF AUTOLOGOUS CANCER VACCINES, PRODUCING VARIOUS CYTOKINES.

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We have established a permanent cell line from murine glioma 261 (Division of Cancer Treatment Repository, Frederick, Maryland, USA) which was maintained for years by serial subcutaneous transplantation in C57Bl/6 mice. We used these cells to study the effect of autologous cancer vaccines producing different cytokines. Glioma 261 cells are highly immunogenic, because pre vaccination with irradiated tumor cells will prevent or highly decrease mortality after intracerebral challenge with living cells. Vaccination with irradiated tumor cells 3 days after the intracerebral tumor challenge with $1x10^5$ cells will not effect survival. Glioma 261 cells can be transduced with cytokine producing adenoviral vectors nearly to 100%. The cytokine production of transduced cells will decrease 7 days after irradiation with either 10 or 20 Gy of Co^{60}-γ radiation. In C57Bl/6 mice the GM-CSF level peaks 24 h after subcutaneous injection of $1x10^6$ irradiated, GM-CSF producing cells and goes back to normal level by 72 h. Three days after intracerebral injection of $1x10^5$ glioma 261 cells C57Bl/6 mice were vaccinated with irradiated cells producing different levels of GM-CSF. Vaccines producing 280 and 140 pg/ml GM-CSF levels in blood 24 h after vaccination increased the survival. Experiments with vaccines producing other cytokines and various combinations of cytokines are in progress. We also study the combined effects of autologous, cytokine producing vaccines and intracranial irradiation.

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CLONING OF THE ACTIVE PRINCIPLE OF MISTLETOE: THE CONTRIBUTION OF MISTLETOE LECTIN SINGLE CHAINS TO BIOLOGICAL FUNCTIONS

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Mistletoe preparations are widely used in traditional medicine as a general immunomodulating agent and/or biological response modifier. The active principle of the preparations, mistletoe lectin (ML), is a heterodimeric highly active plant toxin closely related to the castor bean toxin ricin. The two chains termed A- and B-chain are linked by a disulfide bond.

In order to assess the contribution of a) the enzymatic activity of the A-chain and b) the lectin activity of the B-chain to the observed biological effects of ML a molecular cloning approach was applied to identify and characterize relevant structure/function relationships of ML.

A PCR-strategy was performed to clone a number of different fragments of the ML-gene from mistletoe genomic DNA. All fragments were identified to belong to one particular gene in the mistletoe genome. The full-length-sequences of both A- and B-chains were established by alignment of the fragments.

Expression vectors containing either the A-chain- or the B-chain coding region were constructed and the single chains were expressed in *E. coli* separately. After renaturation of the insoluble "inclusion bodies" functional A- and B-chains were obtained and subsequently associated in vitro yielding active ML-holoprotein.

The recombinant proteins were structurally characterized and their biological activities analysed (MOLT4-cytotoxicity, RIP-activity, induction of apoptosis, induction of cytokine secretion in PBMC).

The activities of recombinant ML are not altered compared to the glycosylated forms of plant ML. The single chains have no or little effect on MOLT-4 cells. We conclude, that both binding activity of the B-chain and the rRNA-glycosidase activity of the A-chain are prerequisites of the stimulatory action of ML on immunocompetent cells.

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DOES THE ACTIVITY OF THE Th2 AND THE Ts SUBSET, IN CONTRAST TO THAT OF THE Th1 AND THE Tc SUBSET CRITICALLY DEPEND ON THE INFLUX OF EXTRACELLULAR Ca^{++} THROUGH THE L-TYPE Ca^{++}-CHANNELS ?

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We propose for the first time that the Th2 and Ts cells, secreting the type 2, i.e. Th2-like cytokine profile, differ from Th1 and Tc cells, which synthetize type 1, i.e. the Th1-like profile of lymphokines, primarily in the activation of the PC (phosphatidyl choline) dependent PLC (phospholipase C), as opposed to the activation of the PI (phosphatidyl inositol) dependent PLC in the Th1 and Tc subset. Since only the PI dependent PLC mobilizes intracellular Ca^{++}, the activation of both , the Th2 and the Ts subset seems to depend in a critical way on the influx of the extracellular Ca^{++} which can be inhibited by Ca antagonists.

To test this hypothesis, we compared the lymphocyte activity in the presence and absence of different Ca-antagonists, using standard laboratory techniques (MLC,CML/LMC and PHA-proliferation-assay).

Our results show that all tested Ca-antagonists (nifedipine/nicardipine, verapamil and dilthiazem) significantly increased the activity of immunocompetent cells, the most efficient being nicardipine which showed even at the extremely low concentration of 1.10^{-12} M a 52% (!) overall PBL stimulation (in the PHA-proliferation assay).We interprete this observation as deblocking of the immunosuppressive T cell subsets, responsible for the generalized downregulation of the immunocyte activity.

To measure the T cell activity in vivo, we chose the highly sensitive AA(adjuvant arthritis)-model in rats (Lewis and WKY-strain). Whereas the classical Ca antagonists (nifedipine) led to a significantly increased reactivity of autoaggresive T cells in AA-induced animals, the Ca overload blocker cinnarizine inhibited the AA-establishment.