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ACTIVE CELL DEATH IN OVARIAN CARCINOMA CELLS CAN BE INDUCED WITH BENZAMIDE RIBOSIDE, AN INHIBITOR OF THE GUANOSINE PATHWAY

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The C-glycoside Benzamide riboside (BR) specifically blocks the enzyme IMP dehydrogenase, which catalyses the conversion of inosine 5'-monophosphate (IMP) to xanthine 5'-monophosphate (XMP), the rate limiting step in the de novo synthesis of guanosine nucleotides. The resulting depletion in intracellular GTP and dGTP could be responsible for cell death in proliferating cells with high DNA synthesis rates.

Here we demonstrate that BR (applied in concentrations between 1 and 30 μM) acts upon the fast growing human carcinoma cell line N.1 in three successive but overlapping stages. In the first phase BR acts as a morphogen, leading to growth retardation, partial differentiation and occasional nuclear amplification. This phase was followed by a stage of cell death of an apoptotic phenotype, accompanied by DNA laddering, c-myc up-regulation, and cdc 25A down-regulation. The third stage of BR action is characterized by cell death of a non-apoptotic phenotype.

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APOPTOSIS AND CYTOKINE RELEASE ARE BIOLOGICAL RESPONSES MEDIATED BY RECOMBINANT MISTLETOE LECTIN IN VITRO

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Human blood cells, either primary cells or transformed cell lines, are frequently used to investigate the potency of mistletoe lectin (ML) to enhance the release of those cytokines which are relevant in terms of immunomodulation (e.g. IL1, IL6, IL12, IFNγ, TNFα).

Here we describe the effect of recombinant ML (rML) on human cells in vitro and compare its activity to plant derived ML I. Firstly, we tested cultures of human monocytic (THP-1) or leukemic (MOLT-4) cell lines for ML cytotoxicity. The IC_{50} of rML as determined for MOLT-4 cells was in the low pM range. Investigations on the cause of ML-induced cell death showed that concentrations near the IC_{50} quantitatively led to apoptotic morphology, whereas at higher ML-concentrations necrosis was predominant. FACS analysis revealed monocytes as likely primary target cell population for ML action. Therefore we investigated monocytic cells in the cytokine inducing concentration range of rML on the level of gene transcription (RT-PCR) and on the level of protein secretion (ELISA).

In the case of ML-treated THP-1 cells assayed for IL-1ß there was a correlation of the amount of cytokine in the cell culture supernatant and the increasing rate of apoptotic cells. The concentration of secreted IL-1ß was reduced when an inhibitor of the interleukin-1ß converting enzyme (ICE) was added. Obviously, production of the mature cytokine stimulated by ML is an active process which is likely to be linked to apoptosis via ICE. Apoptosis was not found in the tested concentration range when treating cells with an ML-holoprotein consisting of an inactivated A-chain coupled with a normal B-chain, showing the ribosome-inactivating activity is needed for biological response.

For other cytokines a characteristic bell-shaped concentration-effect curve was found, which is consistent with *in vivo* findings suggesting that the beneficial effect of ML I (or of the standardized aqueous extracts) is only observed at low doses in a relatively narrow range. The detection of the respective mRNAs by RT-PCR showed that the ML-induced cytokines are actively synthezised, which is likely to be the first step in the cascade of immune reactions leading to general immunostimulation and to an activation of the natural defense system against tumor cells.

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REDUCTION OF CD3-MEDIATED APOPTOSIS IN HUMAN T CELLS BY CD28-COSTIMULATION: POSSIBLE MECHANISMS

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We have previously observed, that stimulation of resting human T cells with high density anti-CD3 leads to proliferation (CD28minus-proliferation) and subsequent anergy and apoptosis. This negative signaling could be counteracted if cells were stimulated with low density submitogenic anti-CD3 together with CD28-antibody (CD28plus-proliferation). Here we further explore these two modes of stimulation as a model for "productive" vs. "abortive" T cell proliferation.

To examinate the mechanism of apoptosis induction via TCR/CD3 and its prevention by CD28-costimulation we looked for differences in T cell specific cytokine expression in ELISA or rPCR and apoptosis mediators p53, bcl-2 and bcl-x_L. Production of IL-2, IL-4, γIFN and TNFα varies considerably between T cells of different donors but is consistently higher during CD28plus proliferation. No consistent difference in the expression of p53 was observed. The expression of bcl-x_L and bcl-2 appears to be elevated during CD28plus-proliferation.

We also found that the apoptosis preventing effect of CD28-costimulation was lost at low cell concentrations.

This finding confirm the second signal model of T cell activation claiming that triggering of the TCR/CD3 complex constitues a negative signal which needs to be counteracted by second signal, e.g. via CD28 to induce an effective long lasting immune response. The reduction of apoptosis in the presence of CD28-costimulation is possibly due to increased lymphokine production and bcl-x_L expression.

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RETINOBLASTOMA PROTEIN-DERIVED PEPTIDES: FURTHER DEFINITION OF THEIR ANTINEOPLASTIC PROPERTIES

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In an initial study we have demonstrated by cell culture experiments the antineoplastic activity of peptides which were previously deduced from the tumor suppressor retinoblastoma protein (RB). We have now further dissected the structural determinants responsible for this activity which can be observed with breast cancer, osteosarcoma and leukemia cells. Previously, we had shown that an oligomeric RB-derived peptide blocks the stimulatory effect of fetal calf serum (FCS) on the cell cycle progression of the RB-defective human osteosarcoma cell line SAOS-2 at a concentration of 10 μM. We have now found that linear RB-like peptides are also able to reverse the FCS-induced growth stimulation of SAOS-2 cells at [50 μM]. This effect is specific since the vector sequence coupled to the active RB peptide portion and responsible for the latter's internalization into the cell does not have any effect on SAOS-2 cells when applied on its own at [50 μM]. Similarly, the vector-coupled RB peptides, but not the vector itself, inhibit both the insulin- and IGF-1-driven cell cycle progression of the RB-positive human breast cancer cell line MCF-7 at [10 μM]. Moreover, we also show that over 90% of the cells of the human leukemia cell line K562 are dead when incubated in the presence of each an all-D and an all-L oligomer of the active RB peptide sequence at [100 μM] for 48 hours. Interestingly, the all-D isomer is still active at [10 μM] leading to over 50 % of tumor cell-directed cytotoxicity, whereas the all-L isomer is inactive at [10 μM], implying that protease stability contributes to the activity of the RB peptide oligomer. Our present results should be instrumental for the further refinement and optimal minimization of antioncogene products such as RB into growth-inhibitory peptide therapeutics.