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COSTIMULATORY SIGNALS THROUGH INTERACTION OF B7.1 AND CD28 PREVENT "VETO" DEATH OF CYTOTOXIC T CELLS DURING TUMOR TARGET CELL LYSIS

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Expression of B7 on tumor cells can circumvent T cell tolerance and lead to the generation of tumor cell specific T cell immunity. Therefore, gene modified tumor cells with vectors for the expression of B7 have been extensively studied in experimental tumor cell vaccination. The effect of B7 expression on the generation of protective anti-tumor immunity has been attributed primarily to (1) activation of anergized tumor specific T cells and (2) more efficient generation of tumor specific killer T cells. We have thoroughly investigated the role of costimulation through B7.1 and its receptor, the CD28 molecule, in the generation of cytotoxic T cells (CTLs) against MCF-7 breast cancer cells. MCF-7 cells were transfected using a retroviral vector containing the human B7.1 gene. Cytotoxic T cells were generated by co-culture of allogeneic T cells with irradiated MCF-7 or MCF-7-B7.1 for time intervals up to 7 days in the presence of low dose IL-2. In this setting, we describe for the first time that activated, MCF-7-specific T cells undergo activation induced cell death after killing of the target cell. To describe this phenomenon we coined the the term "veto" kill. Instead of proliferation and clonal expansion, up to 90 percent of the CTLs underwent apoptotic cell death. The veto kill could be blocked by 50 % when binding of the Fas ligand to its receptor, the CD95 (APO-1/Fas) molecule, was prevented. Fas ligand was detected in the activated T cells but not in MCF-7 and a panel of other breast cancer cell lines. This excludes an active role of the target cell, at least in this setting, during veto kill and indicates that the veto kill is due to an autocrine production of the Fas ligand by CTLs. Costimulation of T cells with B7.1 expressed on MCF-7 drastically reduced the sensitivity of the CTLs to veto apoptosis. The same effect was seen when the T cells were stimulated with an agonistic anti-CD28 antibody. Costimulation of the CTLs did not alter expression of the pro-apoptotic Bax protein and the Fas ligand in Western blot analysis. These experiments show that costimulatory signals through B7.1 and its receptor CD28 prevent veto apoptosis of activated T cells during tumor cell killing. Thus, a major role of B7.1 in tumor cell vaccination might be the prevention of T cell death by altering T cell susceptibility for apoptosis.

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ACTIVATION-INDUCED CELL DEATH OF T CELLS IS PREVENTED BY COCULTURE WITH DENDRITIC CELLS

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T cell apoptosis or programmed cell death is crucial to maintain T cell homeostasis thereby preventing autoimmunity. Repeated stimulation renders T cells susceptible to activation induced cell death (AICD), which is mediated through autocrine production of the CD95 ligand (CD95L) and subsequent CD95 ligation. To date mechanisms for the prevention of AICD have not been described. Dendritic cells (DC) are professional antigen presenting cells able to prime naive T cells and initiate antigen-specific T cell responses. This suggest that antigen presentation by DC may activate T cells without inducing concomitant AICD. In this study we have evaluated whether DC can inhibit AICD induced by CD95 ligation. DCs were generated by culturing peripheral blood monocytes enriched by counterflow elutriation in the presence of GM-CSF and IL-4 for 7 days. T cells were activated with PHA/PMA. Apoptosis of T cells was assessed by flow cytometric DNA analysis. Spontaneous apoptosis of T cells amounted to 25%, addition of an agonistic anti-CD95 antibody antibody which mimics CD95 ligand increased cell death to 64%. Coculture of these T cells with increasing amounts of dendritic cells prevented apoptosis in a dose dependent fashion; the incubation of 10^5 DCs /ml with 10^6 T cells /ml in the presence of the apoptosis inducing anti-CD95 antibody resulted in a 33% apoptosis rate, which was only slighlty higher than the 25% spontaneous rate. This protective effect could be reduced by 50% by adding to the cell mixture an anti-CD58 antibody, further addition of an anti-CD80/B7.1 and an anti-CD86/B7.2 antibody led to an even more pronounced effect. Our findings suggest that dendritic cells can protect T cells from AICD, with CD58 ligation playing a key role. This would allow the dendritic cells to sustain T cell effector functions even if CD95 is crosslinked on activated T cells.

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Cytotoxicity of mistletoe extracts and mistletoe lectins towards tumor cells due to the induction of programmed cell death (apoptosis)

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Investigation of in vitro effects of therapeutically administered mistletoe extracts (ABNOBAviscum®) and pure mistletoe lectins (mainly mistletoe lectin-1) on a variety of human and murine cell lines revealed a dosedependent growth inhibition of most cell lines. Thus, proliferation of murine P815, EL-4 cells or murine B-cell hybridomas and human cell lines such as MOLT-4, U937 and K562 was markely blocked by extracts and purified lectins.

The mechansims of growth arrest was shown to be due to the induction of programmed cell death (apoptosis). Thus, fragmentation of genomic DNA into oligonucleosomal bands 200 base pairs in length was observed within 20 h when tumor cells were incubated with mistletoe extracts or mistletoe lectins. Resting lymphocytes but not preactivated peripheral blood cells were fairly resistant to the induction of apoptosis with mistletoe extracts and purified ML-1. Also EBV-transformed cell lines proved to be resistant to lectin induced apoptosis. These data pointed to a rational basis for the direct cytotoxic effects of mistletoe extracts and lectins apart from the postulated immunostimulatory properties of these agents.

This direct cytotoxicity mediated by mistletoe lectins could be usefull in tumor therapy. For instance, mistletoe extracts injected directly into human xenografts transplantated into nude mice or perifumoral treatment of solitary oral squamous cell carcinoma led to a macro- and microscopially complete remission of the tumor in one patient. Experiments with whole mistletoe extracts have shown that the cytotoxic potential is not only due to the mistletoe lectin content and that different cancer xenografts react differentially to mistletoe treatment.

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Apoptosis of Ovarian carcinoma cells (N1) by TNF and it's possible Targets

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Ovarian cancer cell's were shown to synthesize Tumor Necrosis Factors (TNF's), as is the case for the N.1 cell line. Therefore, we investigate the effects of TNF's on N1 cells in order to unravel potential feedback mechanisms by autostimulation.

Further attention was payed to the expression level of the transcription factor c-myc, as it is known, that overexpression of c-myc often occurs in carcinomas.

When serum was withdrawn from cell cultures TNFα -application elicited active cell death (ACD) in N1 cultures more rapidly than TNFß, exhibiting morphological changes 4 and 5 days after stimulation, respectively.
Both Receptor types, TNF-R1 and TNF-R2 were expressed by N1 cells as evidenced by RT-PCR. Following TNFα-- exposure, c-myc expression is up-regulated, showing maximal transcript levels 6 hours after induction and maintained elevated even after 2 days and thereafter. Antiparallel to c-myc induction, mRNA levels of phosphatase cdc25 A were suppressed to undectable levels within 24 hours after TNFα treatment. CDC 25 A is an activator of the cdk4-cyclinD1 complex, which, once activated, permits progression through the cell-cycle. Cyclin D1/pradI mRNA itself remained unaffected when N1 cells were treated with TNFα.

Genistein, a phytoestrogen acting as a protein kinase inhibitor, arrested cell proliferation and prevented TNFα-triggered ACD.

C-myc and cdc25A mRNA-levels were downregulated by genistein application. After additional treatment with TNFα , there is no c-myc induction, both levels remained suppressed, indicating that tyrosine kinase activity is required for the mediation of TNF-initiated signals.

In the presence of 10 % FCS, application of TNFα reversed c-myc expression and resulted in slight downregulation of transcripts. Also cdc25A mRNA became repressed under these conditions.
The data obtained in this investigation suggest, that initiation and subsequent interruption of cell cycle-progression at crucial check points, which are still unidentified, might generate a stress condition triggering an emergency programm such as ACD.