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Is Fas Ligand expression by glioma a major mechanism of inactivation of tumor infiltrating lymphocytes ?

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Gliomas appear to progress without significant impedance from the immune system, despite the presence of intratumoral T cell infiltration. To date, this has been thought to be the result of T cell immunosuppression induced by glioma derived cytokines. Here, we propose that cell contact mediated events also play a role, since we demonstrate the *in vivo* expression of Fas Ligand (FasL/CD95L) by human glioma and the efficient killing of Fas-bearing cells by glioma lines *in vitro*. This lytic activity was exhibited not only by established cell lines, but also by tumour cells tested *ex vivo* and early passage glioma lines. In the brain, glioma cells can potentially deliver a death signal to Fas^+ cells which include infiltrating leukocytes. Indeed, glioma cells are able to induce a Fas mediated apoptosis of autologous T cells derived from the tumour. In addition, there is the possibility of a direct effect on tumour growth since most glioma also express Fas. Data will be presented on the regulation of FasL expression since this may play a key role not only in tumour cell-immune system but also in tumour-tumour interactions.

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Apoptosis in tumor cells

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Genetically programmed cell death appears to be mechanistically similar to a type of cell death termed apoptosis, which is observed in many cell types following a variety of stimuli. Apoptosis is characterized by a dramatic morphological transformation of the cell, including shrinkage, cytoplasmic membrane blebbing, chromatin condensation, and disassembly into membrane-enclosed vesicles. Experiments with several cell-free extracts have revealed a link between mitochondria and apoptotic execution mediated by proteases of the caspase family and vital substrates. This link was originally suspected following the demonstration that Bcl-2 protein is concentrated in the outer mitochondrial membrane. Nuclear apoptosis, as assessed by morphology and DNA cleavage, appears to be triggered by opening of mitochondrial permeability-transition pores or "megachannels". Opening of these pores was previously shown to allow the free distribution of solutes of <1.5 kDa and of some proteins across the mitochondrial membranes. These studies suggest that the Bcl-2-regulated release of mitochondrial factors might lie upstream of caspase activation in many types of apoptotic execution.

Recently, Raf kinase has been linked to the function of Bcl-2. Raf family protein serine/threonine kinases are critical elements of signal transduction cascades controlling growth, differentiation and cell survival. The first cascade that was elucidated, the Ras-Raf-MEK-MAPK cascade, is involved in regulation of cell cycle progression as well as differentiation. We have analyzed the cell cycle connection of Raf. The data indicate that oncogenic activation of the c-Raf-1 protein can trigger the entry into the cell cycle without the action of an autocrine growth factor loop. The activation of the c-Raf-1 leads to an accumulation of high levels of cyclin D1 protein and a repression of the p27 Kipl cdk inhibitor under all tested culture conditions.

A critical event in linking this "classic" cascade to activated growth factor receptors in the plasma membrane is the transient membrane docking of Raf by Ras GTP. Recently, we have identified a novel cascade in which Raf functions to suppress apoptosis. In this case the shuttle protein is not Ras but Bcl-2 which targets Raf to the outer mitochondrial membrane where it phosphorylates a novel substrate, BAD (Wang, H.G., Rapp U.R. and Reed J.C., Cell: Vol. 87-120, 1996).