Z405 NO-induced apoptosis in mouse macrophage cell line RAW 264.7

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LPS and IFN-γ are known to induce nitric oxide synthase in macrophages and the resultant NO causes apoptotic cells death in the activated cells. To study the role of BCL-2 proteins, RAW264.7 cells were transfected with bcl-X_L, which inhibits apoptosis, and bcl-X_S, which accelerates apoptosis, respectively. Stable transfectants were selected and confirmed by RT-PCR. NO production in response to LPS and IFN-γ caused apoptosis in RAW 264.7 cells and vector transfected control within 24 hr. Both NO production and apoptosis were inhibited by N^G-monomethyl L-arginine (NMMA), a competitive inhibitor of NO synthase. In contrast, bcl-X_S transfectant appeared slightly susceptible and bcl-X_L transfectant appeared slightly resistant, although NO production was similar to control cells. These results indicate that both BCL-X_L and BCL-X_S are involved in apoptotic pathway in activated macrophage cell death, and NO plays a major causative role.

Z406 IL-4 gene therapy: tumor cells expressing membrane-bound form of IL-4 induce antitumor immunity

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Cytokine gene transferred tumor cells activate lots of bystander cells. To circumvent such problem, membrane-bound forms of IL-4 (IL-4mb) were expressed on MethA tumor cells. IL-4mb on tumor cells was able to support cell growth of the IL-4 dependent cell line, CT.4S. The IL-4/TNFa tumor clone stimulated the proliferation of spleen cells from 2C TCR transgenic mouse and Th2 D10 cells. In addition, the IL-4/TNFa tumor clone lost tumorigenicity, and the mice injected with the tumor cells acquired antitumor immunity. These results suggest that IL-4/TNFa tumor clone provide both signal 1 (TAA/MHC) and a signal 2 (IL-4mb) for effective lymphocytes activation. The proposed tumor vaccine may serve as an effective gene therapy method to avoid the toxicity of recombinant cytokines and bulk bystander leukocyte stimulation encountered in conventional cytokine gene therapy.