CONVERSION FROM A TYPE I TO A TYPE II DEATH RECEPTOR-MEDIATED PATHWAY THROUGH A PARTIAL INHIBITION OF CASPASE-8

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INTRODUCTION. Bcl-2 is the prototypical member of a family of proteins which, when overexpressed in cells, are capable of either inhibiting or inducing apoptosis\(^1,2\). We have previously reported that Bcl-2 can cooperate with inhibition of caspase activation by zVAD-fmk to block TNF\(\alpha\)-induced cell death in a Bcl-2 cleavage-independent fashion\(^3\). We now propose a multiple pathway model of TNF\(\alpha\)-induced cell death that diverges at least at the level of caspase-8 activation. In this model, one mitochondria-independent (Type I) pathway is blocked by zVAD-fmk while the other mitochondria-dependent (Type II) pathway is blocked by Bcl-2\(^4\).

METHODS. The murine pro-B cell line FL5.12 was cultured and transfections were performed as described\(^3\). For TNF\(\alpha\)-induced cell death experiments, transfectants were incubated 6 hrs. at 37\(^\circ\)C in the presence of recombinant human TNF\(\alpha\) (TNF; 2 ng/ml) and cycloheximide (CHX; 10 \(\mu\)g/ml). Where indicated, the caspase inhibitor zVAD-fmk (Enzyme Systems Products) was added at the indicated concentrations. For anti-Fas cell death experiments, Fas-transfected FL5.12 cells were incubated with 1 \(\mu\)g/ml Jo2 (Pharmingen) and the indicated concentrations of zVAD-fmk. Viability was determined by flow cytometry double staining with Propidium Iodide (PI) and AnnexinV-FITC. Western blotting was carried out as described\(^4\). DEVDase activity was determined by incubating 5x10\(^5\) cells with 10 \(\mu\)M DEVD-G\(_1\)D\(_2\) (Oncoimmunin, Inc.) at 37\(^\circ\)C for 1 hour and analyzing samples by flow cytometry. The mean fluorescence intensity of the DEVD-G\(_1\)D\(_2\)-positive cells was calculated relative to the mean fluorescence intensity of DEVD-G\(_1\)D\(_2\)-positive Neo cells cultured in medium alone.

RESULTS AND DISCUSSION. Our data are most consistent with a model in which zVAD-fmk converts the death signal from a Type I to a Type II pathway through a partial inhibition of caspase-8, thereby allowing for protection by Bcl-2 or Bcl-x\(_L\) at the level of the mitochondria\(^4\). We are currently in the process of determining whether these results represent a receptor-specific or cell-specific phenomenon. We have found the same cooperativity between Bcl-2 expression and zVAD-fmk in another pro-B cell line, Baf3, in response to TNF\(\alpha\) treatment. Also, Bcl-2 lowers the amount of zVAD-fmk required to inhibit anti-Fas induced cell death in Fas-transfected FL5.12 cells. Interestingly, lower doses of zVAD-fmk were required to inhibit anti-Fas-induced cell death in vector control-transfected cells than that which was required to inhibit TNF\(\alpha\)-induced cell death in the same cells. Differences in the way TNFR1 and Fas receptor complexes are formed may determine the sensitivity to caspase inhibition. Conversely, the difference may be due to our comparison of a physiologic ligand (TNF\(\alpha\)) to an antibody-
mediated death stimulus (anti-Fas). Experiments to distinguish between these possibilities will be presented.

REFERENCES.