

Miami *Nature Biotechnology* Short Reports  
*TheScientificWorld* (2001) 1(S3), 60SR  
ISSN 1532-2246; DOI 10.1100/tsw.2001.180

## MITOCHONDRIAL DEPOLARIZATION DURING TNF $\alpha$ -INDUCED APOPTOSIS IS CASPASE-DEPENDENT AND INDEPENDENT OF BCL-X<sub>L</sub> AND THE PERMEABILITY TRANSITION PORE

E. Cepero, B.W. Johnson, and L.H. Boise

Dept. of Microbiology and Immunology, University of Miami School of Medicine of Miami  
School of Medicine, Miami, FL 33101

**INTRODUCTION.** Mitochondria are intimately involved in apoptosis<sup>1</sup> and loss of the mitochondrial transmembrane potential ( $\Delta\Psi_m$ ) has been considered an early event in apoptosis. However, release of cytochrome *c* from the intermembrane space precedes  $\Delta\Psi_m$  and leads to caspase activation<sup>1</sup>. From this point of view mitochondria can be seen as an initiator of programmed cell death. However, the role of the mitochondrial transmembrane potential and whether it is the cause or the consequence of cell death remains controversial. Our laboratory has shown that TNF $\alpha$  signaling in the IL-3-dependent pro-B cell line FL5.12 can induce apoptosis by sending a type I and a type II signal. In FL5.12 cells caspase 8 can directly activate caspase 3 (type I) and this can be inhibited by zVAD-fmk. The type II signal involves the caspase-8 mediated cleavage of Bid, which is not completely inhibited by zVAD-fmk, proceeds through the mitochondria and is blocked by Bcl-x<sub>L</sub>. Thus, TNF $\alpha$ -induced apoptosis in FL5.12 cells can be blocked synergistically by Bcl-x<sub>L</sub> and zVAD-fmk. The goal of this project is to assess the ability of Bcl-x<sub>L</sub> to block mitochondrial dysfunction during TNF $\alpha$ -induced apoptosis.

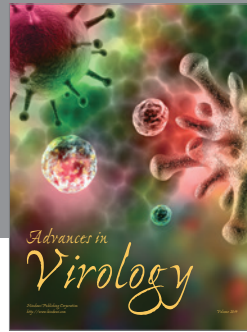
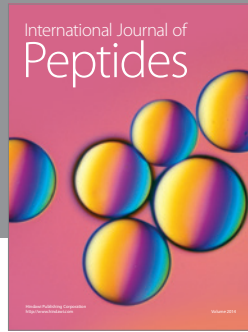
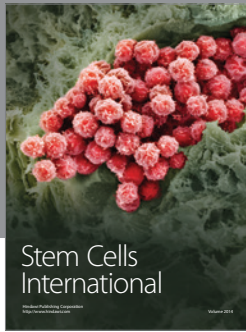
**MATERIALS AND METHODS.** AnnexinV-FITC, propidium iodide staining, and TMRE staining were performed as described<sup>5</sup>. For the cytochrome *c* release assay cells were mechanically lysed in Buffer A and 150  $\mu$ g of the mitochondrial fraction (10,000 x g pellet) and an equal volume of the cytosol (100,000 x g supernatant) were subjected to western blotting for cytochrome *c*<sup>5</sup>. For DNA isolation, cells lysed in RSB plus 0.5% NP-40, then resuspended in RSB + 2X SDS buffer<sup>5</sup>. Samples were incubated with 400  $\mu$ g/ml Proteinase K at 50°C overnight. 1  $\mu$ g of DNA was run on a 1.5% agarose gel.

**RESULTS.** FL5.12 Neo and Bcl-x<sub>L</sub> undergo apoptosis in response to TNF $\alpha$  to the same extent, however differences have been observed at the mitochondrial level. The caspase inhibitor zVAD-fmk has no effect on TNF $\alpha$ -induced cell death in Neo cells but is able to cooperate with Bcl-x<sub>L</sub> and block apoptosis. In order to determine whether Bcl-x<sub>L</sub> is blocking TNF $\alpha$ -induced mitochondrial dysfunction, determinants of mitochondrial physiology, i.e.  $\Delta\Psi_m$  and cytochrome *c* release, were examined. After six hours of TNF $\alpha$ -induced apoptosis, in the presence and absence of zVAD-fmk, FL5.12 Neo cells show a loss  $\Delta\Psi_m$  and release of cytochrome *c*, which is not affected by zVAD-fmk. FL5.12 Bcl-x<sub>L</sub> cells, on the other hand, retain their cytochrome *c* within the mitochondria but surprisingly show a loss of  $\Delta\Psi_m$ . This suggests that cytochrome *c* release and loss of  $\Delta\Psi_m$  are separate events. Additionally, since TNF $\alpha$  signaling results in loss of  $\Delta\Psi_m$ , the effects of the permeability transition pore (PTP)

inhibitor Cyclosporin A were assessed. Cyclosporin A alone was unable to prevent loss of  $\Delta\Psi_m$ , suggesting that the PTP is not involved in TNF $\alpha$ -induced cell death. Since the combination of zVAD-fmk and Bcl-x<sub>L</sub> are required to block  $\Delta\Psi_m$ , the data are most consistent with depolarization occurring at the level of downstream caspases. The mechanism of caspase-activated depolarization is not characterized, however, it is unlikely through opening of the permeability transition pore as Cyclosporin A was unable to prevent mitochondrial depolarization. Taken together, these data suggest that mitochondria can assume two positions in the death pathway. In a mitochondrial-dependent pathway release of cytochrome *c* is required as the initiating event and thus places the mitochondria upstream in the death pathway. In a mitochondrial-independent pathway effector caspases are directly activated and they can target the mitochondria for inactivation, just as they target the genome and the cytoskeleton, thus placing the mitochondria downstream in the death pathway.

#### **REFERENCES.**

1. Green, D.R. and Reed, J.C. (1998) *Science* 281,1309-1312
2. Bossy-Wetzell, E., Newmeyer, D.D., and Green, D.R. (1998) *EMBO J.* 17, 37-49
3. Johnson, B.W. and Boise, L.H. (1999) *J. Biol. Chem.* 274, 18552-18558
4. Johnson, B.W., Cepero, E., and Boise, L.H. (2000) *J. Biol. Chem.* 275, 31546-31553



**Hindawi**

Submit your manuscripts at  
<http://www.hindawi.com>

