

P012 What role do caspases play in TNF α stimulated neutrophil apoptosis?

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Tumour necrosis factor- α (TNF α) stimulated early killing of human neutrophils remains ill defined and poorly understood. While TNF α -induced apoptosis in most cell types is caspase-dependent, previous studies in neutrophils have shown that broad-spectrum caspase inhibitors have no effect on the rate of constitutive apoptosis, indeed, Z-VAD-FMK has been reported to increase TNF α -induced cell death. The aim of this study was to explain this effect of Z-VAD-FMK and define the role of mitochondria in TNF α killing. Here we describe data examining the role of broad-spectrum caspase inhibitors (Z-VAD-FMK, Boc-D-FMK), and specific caspase-8 and -9 inhibitors on TNF α stimulated early killing of neutrophils. Z-VAD-FMK (0.3-30 μ M) was shown to inhibit TNF α stimulated early killing, indicating the central role of caspases in this process. In contrast we observed an increase in apoptosis at concentrations of Z-VAD-FMK \geq 100 μ M (cytotoxic or alternative mechanism?). Boc-D-FMK (0.3-300 μ M) produced a concentration dependent inhibition of TNF α -stimulated killing. The caspase-8 specific inhibitor (Z-IETD-FMK) produced similar results as Boc-D-FMK. In contrast, the inhibition of caspase-9 (Ac-LEHD-CMK) only marginally inhibited TNF α killing, suggesting a mitochondrial independent pathway.