



**THE MECHANISM OF ACTION
OF
TUMOUR NECROSIS FACTOR - α**

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ABSTRACT

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The *in vivo* administration of Tumour Necrosis Factor - alpha (TNF- α) as an antineoplastic agent has been severely restricted by dose-limiting side effects. Neutrophils, monocytes and endothelium are believed to be involved in the generation of these unwanted side effects. In the mouse, the administration of human TNF, which binds only to the murine p55 TNF receptor (TNFR55), is much less toxic than murine TNF, which binds to both murine TNF receptors. In view of this species specificity, human TNF mutants with selective binding to the human TNF receptors were employed to examine the role of these receptors in the mediation of TNF's cytotoxic and proinflammatory activities. The TNFR55-selective mutants stimulated proinflammatory activity which was markedly less than wild-type TNF. TNF- α 's priming of human neutrophils for superoxide production and antibody-dependent cell-mediated cytotoxicity, platelet-activating factor (PAF) synthesis and adhesion to endothelium were reduced by up to 170-fold. Activation of human endothelial functions represented by adhesiveness for neutrophils, E-selectin expression, neutrophil transmigration and IL-8 secretion were also reduced by up to 280-fold. The TNFR75-selective mutant did not stimulate any proinflammatory activity implying that TNFR75 facilitates the role of TNFR55 in mediating these activities. However, the TNFR55-selective mutants exhibited similar potency to wild-type TNF in causing cytotoxicity of a human laryngeal carcinoma-derived cell line and cytostasis in a human leukaemic cell line. Therefore, the *in vivo* use of TNFR55-selective mutants may result in reduced side effects whilst maintaining the antitumour activity of wild-type TNF.

The signal transduction mechanisms by which TNF- α elicits proinflammatory activity were examined in neutrophils and monocytes. Cytosolic phospholipase A₂ (cPLA₂), the rate-limiting enzyme in the production of eicosanoids and PAF, was selected for investigation. The rapid phosphorylation of cPLA₂ on serine residues by TNF- α was demonstrated and found to be coupled to the production of PAF in human monocytes. The TNFR55-selective mutants stimulated less cPLA₂ phosphorylation and PAF production than wild-type TNF and this is in keeping with the neutrophil and endothelial proinflammatory effects described.

The life span of the mature neutrophil *in vivo* is relatively brief (24 hours) and it is shown here that TNF- α shortens this time markedly. Apoptosis is induced by TNF- α in the majority of neutrophils within 3 hours as demonstrated by microscopy (light and fluorescent), DNA fragmentation gels and propidium iodide binding. The TNFR55-selective mutants induce significantly less apoptosis than wild-type TNF and implies similar TNF receptor biology for neutrophil proinflammation and apoptosis. Thus TNF- α , in stimulating neutrophil proinflammatory activities, induces an early noninflammatory death.

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