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# **Bacterial Lipopolysaccharide and Tumour Necrosis Factor- alpha Synergism in Inflammation.**

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## Abstract

During infection with bacteria exogenous and endogenous mediators combine to form a complex network. Although the biological activities of individual pro-inflammatory agonists are well characterized, there is an incomplete understanding of the interactions between different mediators.

In order to expand the knowledge of mediator-interactions this thesis examines different aspects of 'cross-talk' between bacterial lipopolysaccharide (LPS) and tumour necrosis factor-alpha (TNF- $\alpha$ ) *in vitro* in relation to the respiratory epithelium, vascular endothelium, monocytes/macrophages and neutrophils.

Monocytes and macrophages responded to co-stimulation with LPS and TNF- $\alpha$  with an increased production of proinflammatory cytokines. Neutrophils were primed by pretreatment with TNF- $\alpha$  for an enhanced LPS-induced respiratory burst, and also showed a synergistic increase in their adhesive properties. Human umbilical vein vascular endothelial cells (HUVEC) responded with the synergistic upregulation of the adhesion molecules E-selectin, ICAM-1 and VCAM-1 when treated with TNF- $\alpha$  and LPS. In a human alveolar type II respiratory epithelial cell line (A549) TNF- $\alpha$  upregulated the expression of the adhesion molecule ICAM-1, whereas LPS had no effect. However, in concert with IFN- $\gamma$  or a cocktail of cytokines (IL-1 $\beta$ , TNF- $\alpha$  and IFN- $\gamma$ ) LPS had an enhancing effect on ICAM-1 upregulation.

The mechanisms of the synergistic effects of LPS and TNF- $\alpha$  were investigated. The LPS receptor CD14 on the surface of neutrophils was upregulated by TNF- $\alpha$ , which correlated with an increase in LPS-binding, possibly at least in part accounting for the priming effect by TNF- $\alpha$ . Interestingly, while it is believed that endothelial cells are CD14-negative our studies showed that these cells express CD14. Expression of CD14 on HUVEC could be modulated and was dependent on protein synthesis. The incorporation of radioactive amino acid into CD14 confirmed it to be of endothelial origin. Further work was carried out to determine why CD14 had not been detected previously. Functional studies revealed that cell-associated CD14 is required for LPS-induced endothelial cell activation, while serum factors act as enhancers. CD14 was detected on the A549 and 16HBE14o- respiratory epithelial cell lines, which also had not been previously described. However, because these cells are relatively insensitive to LPS, and the binding of LPS to these cells is CD14-independent, the relevance of this finding is not yet clear.

To understand how the synergism between LPS and TNF- $\alpha$  may operate, the intracellular signalling pathways stimulated by these mediators were examined in detail in HUVEC. Synergism between the two pathways was found to be due to increased transcription. Enhanced activation of the transcription factor NF- $\kappa$ B and, to a lesser extent, the MAP-kinases p38 and JNK were demonstrated.

This thesis has contributed to the knowledge of how the bacteriokine-cytokine network operates by demonstrating how two major proinflammatory mediators interact in modulating the inflammatory response. Furthermore the discovery of CD14 expression on endothelial cells not only provides greater insight in the pathogenesis of bacterial infection, sepsis and perhaps atherosclerosis, it is also likely to influence the future development of new treatment strategies for those conditions.

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