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

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Thesis submitted for the degree of Doctor of Philosophy

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December 2000
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-α) 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-α with an increased production of proinflammatory cytokines. Neutrophils were primed by pretreatment with TNF-α 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-α and LPS. In a human alveolar type II respiratory epithelial cell line (A549) TNF-α upregulated the expression of the adhesion molecule ICAM-1, whereas LPS had no effect. However, in concert with IFN-γ or a cocktail of cytokines (IL-1β, TNF-α and IFN-γ) LPS had an enhancing effect on ICAM-1 upregulation.

The mechanisms of the synergistic effects of LPS and TNF-α were investigated. The LPS receptor CD14 on the surface of neutrophils was upregulated by TNF-α, which correlated with an increase in LPS-binding, possibly at least in part accounting for the priming effect by TNF-α. Interestingly, while it is believed that endothelial cells are CD14-negative our studies showed that these cell express CD14. Expression of CD14 on HUVEC could be modulated and was dependent on protein synthesis. The incorporation of radioactive amine 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-α may operate, the intracellular signalling pathways stimulated by these mediators were examined in detail in HUVEC. Synergy between the two pathways was found to be due to increased transcription. Enhanced activation of the transcription factor NF-κB 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.
Table of Contents

Abstract ...................................................... II
Declaration .................................................. III
Acknowledgements .......................................... IV
Publications, Presentations, Scholarships and Awards........ V
Table of Contents ........................................... VII
Abbreviations ................................................ XIV
Index of Figures ............................................. XVII
Index of Diagrams .......................................... XX
Index of Tables ............................................. XX
Index of Photographs ...................................... XXI

Chapter 1 Introduction ...................................... 1
1.1 GENERAL INTRODUCTION ................................ 3
1.2 CELLS INVOLVED IN THE INFLAMMATORY RESPONSE .... 7
  1.2.1 Inflammatory leukocytes .......................... 7
  1.2.1.1 Monocytes and Macrophages .................. 7
  1.2.1.2 Neutrophils .................................... 8
  1.2.2 Local tissue cells ................................ 11
  1.2.2.1 Epithelial cells .............................. 11
  1.2.2.2 Endothelial cells ............................ 12
1.3 MICROBIAL TOXINS (EXOGENOUS MEDIATORS) AND THEIR EFFECTS ON HOST CELLS 13
1.4 BACTERIAL LIPOPOLYSACCHARIDE (LPS) .................. 16
  1.4.1 History ............................................. 16
  1.4.2 Definitions ....................................... 16
  1.4.3 Structure of LPS ................................ 16
  1.4.4 Biological activity .............................. 18
  1.4.5 LPS serum levels ................................ 18
  1.4.6 Serum factors .................................... 18
  1.4.7 Cellular receptors for LPS ...................... 19
  1.4.8 Summary of the Current concept of LPS-induced cell activation 20
  1.4.9 Fate of LPS in the circulation .................. 20
1.5 ENDOGENOUS MEDIATORS OF INFLAMMATION AND INTRACELLULAR SIGNALLING

1.5.1 Introduction .................................................. 24

1.5.2 Tumour necrosis factor alpha (TNF-α) .................. 21
1.5.3 Interleukin-1 (IL-1) ......................................... 22
1.5.3.1 Signalling through the TNF-α receptor .......... 25
1.5.3.2 Signalling by IFN-γ ................................ 26
1.5.3.3 Signalling by IL-1 .................................. 28
1.5.3.4 Signalling by LPS .................................. 29
1.5.6 The nuclear factor-κB (NF-κB) pathway ............... 30
1.5.7 The mitogen-activated protein (MAP) kinases ......... 31
1.5.7.1 The ERK cascade .................................... 31
1.5.7.2 The JNK cascade .................................... 33
1.5.7.3 The p38 cascade .................................... 34
1.5.8 Special reference to individual cell types ............. 35
1.5.8.1 Monocytes and macrophages ....................... 35
1.5.8.2 Neutrophils ........................................ 36
1.5.8.3 Respiratory epithelial cells ....................... 36
1.5.8.4 Vascular endothelial cells ......................... 37
1.5.9 Ceramide and sphingomyelinase ......................... 37

1.6 INTRACELLULAR SIGNALLING.......................... 24

1.6.6 The nuclear factor-κB (NF-κB) pathway ............... 30

1.7 THE ENDOTOXIN RECEPTOR CD14 ......................... 39

1.7.1 Introduction .................................................. 38
1.7.2 The physiological role of CD14 ......................... 38
1.7.3 CD14 as a pattern recognition receptor for bacterial products 39
1.7.4 The role of CD14 in vascular disease ................. 40
1.7.5 Soluble CD14 .............................................. 40
1.7.6 Signalling and CD14 ...................................... 40
1.7.7 Signalling by other GPI-anchored proteins .......... 41
1.7.8 Is CD14 a raft protein? .................................. 42
1.7.9 Could CD14 be a co-receptor for other membrane proteins? 42
1.7.10 CD14 has a potential role in bacterial dissemination 43
1.7.11 Soluble CD14 as a potential therapeutic agent in gram-negative infection

1.7.12 In vivo trials with anti-CD14 antibody

1.8 THE NORMAL LUNG AND THE LUNG IN INFLAMMATION

1.8.1 Functions of the lung

1.8.2 The lung and infection

1.8.3 Pneumonia

1.8.3.1 Introduction

1.8.3.2 The immune system in lung infection

1.8.3.3 Gram-negative sepsis

1.8.4 Overview of current approaches to the treatment of sepsis

1.8.4.1 Conventional therapies

1.8.4.2 Novel therapies that target inflammatory mediators

1.9 HYPOTHESIS, AIMS AND SIGNIFICANCE

1.9.1 Hypothesis

1.9.2 Specific aims

1.9.3 Significance

Chapter 2 MATERIALS and Methods

2.1 MATERIALS

2.1.1 Lipopolysaccharide and cytokines

2.1.2 Antibodies

2.1.3 Other reagents

2.1.4 Culture dishes

2.1.5 Serum, culture media and buffers

2.2 METHODS

2.2.1 Preparation of mononuclear leukocytes and neutrophils

2.2.2 Plate purification of peripheral blood monocytes

by adherence to plastic

2.2.3 Differentiation of monocytes into macrophages

2.2.4 Culture of Human Umbilical Vein Endothelial Cells (HUVEC)

2.2.5 The human endothelial cell line HUVEC-C

2.2.6 Culture of the epithelial cell line A549

2.2.7 Human respiratory epithelial cell line 16HBE14o

2.2.8 Measurement of cytokines monocyte/macrophage
Chapter 3 Responses of Mononuclear Phagocytes to LPS and Pyrogenic Cytokines

3.1 INTRODUCTION

3.2 THE PRODUCTION OF TNF-α, IL-1β AND IL-6 BY LPS-STIMULATED MONOCYTES

3.3 THE EFFECT OF TNF-α ON THE LPS-INDUCED IL-1β PRODUCTION

3.4 THE EFFECT OF TNF-α, IL-1β AND LPS ON MONOCYTE IL-6 PRODUCTION

3.5 EFFECT OF TNF-α ON THE LPS-INDUCED PRODUCTION OF TNF-α BY MACROPHAGES

3.6 EFFECT OF TNF-α ON LPS-INDUCED UPREGULATION OF
Chapter 6  Effects on ICAM-1 Expression in Respiratory Epithelial Cells  98
   6.1 INTRODUCTION ................................................................. 99
   6.2 STIMULATION OF ICAM-1 ON RESPIRATORY EPITHELIAL.
   CELLS BY TNF-α, IL-1β, AND IFN-γ ........................................ 100
   6.3 SYNERGISM BETWEEN CYTOKINES ..................................... 102
   6.4 ENHANCEMENT OF THE EFFECT OF CYTOKINES BY LPS ............ 102
   6.5 SUMMARY AND CONCLUSIONS ............................................ 105

Chapter 7  The Effect of TNF-α and LPS on Cell signaling in
   Human Umbilical Vein Endothelial Cells .................................. 106
   7.1 INTRODUCTION ................................................................. 107
   7.2 SYNERGISM AT THE LEVEL OF mRNA ................................... 107
   7.3 EFFECTS ON TRANSCRIPTION FACTOR NF-xB ......................... 108
   7.4 EFFECTS OF TNF-α AND LPS ON MAP KINASES ....................... 109
      7.4.1 ERK ........................................................................... 109
      7.4.2 JNK ........................................................................... 110
      7.4.3 p38 ............................................................................ 111
   7.5 THE EFFECT OF CERAMIDE ON TNF-α AND LPS-INDUCED
      E-SELECTIN EXPRESSION IN HUVEC .................................... 112
   7.6 SUMMARY AND CONCLUSIONS ............................................ 113

Chapter 8  The LPS Receptor CD14 on Leukocytes, Endothelial and
   Respiratory Epithelial Cells ..................................................... 114
   8.1 INTRODUCTION ................................................................. 115
   8.2 RESULTS ........................................................................... 116
      8.2.1 Effects of TNF-α on monocyte CD14 expression ............... 116
      8.2.2 Effects of TNF-α on the surface expression of CD14 by
            human neutrophils ......................................................... 117
      8.2.3 Human Umbilical Vein Endothelial Cells ......................... 118
         8.2.3.1 Expression of CD14 on HUVEC ............................... 118
         8.2.3.2 Specificity of MY4 ............................................... 122
         8.2.3.3 Assessment of HUVEC cultures for monocyte contamination 123
         8.2.3.4 Loss of cell surface expression of CD14 during subculturing 124
         8.2.3.5 LPS-binding to endothelial cells .............................. 125
8.2.3.6 Estimation of the number of CD14 expressed on HUVEC

8.2.3.7 Modulation of CD14 expression on HUVEC and
dependence on protein synthesis

8.2.3.8 CD14 is synthesized by endothelial cells

8.2.3.9 Role of serum in the activation of endothelial cells by LPS

8.2.3.10 Anti-CD14 antibody blocks the LPS-induced endothelial cell
activation in the absence of serum

8.2.3.11 Endothelial cell-associated CD14 has a functional role

8.2.4 Respiratory Epithelial Cells

8.2.4.1 CD14 expression

8.2.4.2 Estimation of the number of CD14 on respiratory epithelial cells

8.2.4.3 Modulation of CD14 surface expression

8.2.4.4 LPS-binding to respiratory epithelial cells

8.2.4.5 Search for other binding sites for LPS

8.3 SUMMARY AND CONCLUSIONS

Chapter 9 Discussion

9.1 REGULATION OF RESPIRATORY EPITHELIAL CELL ADHESION

MOL E C U L E EXPRESSION BY MEDIATORS OF INFLAMMATION

9.2 EFFECTS OF TNF-α AND LPS ON VASCULAR ENDOTHELIAL

CELL FUNCTION

9.3 THE INFLUENCE OF CYTOKINES ON LPS-INDUCED RESPONSES

OF MONOCYTES AND MACROPHAGES

9.4 THE EFFECT OF TNF-α ON NEUTROPHIL RESPONSES TO LPS

9.5 MECHANISMS OF SYNERGISM

9.5.1 Changes in the expression of CD14

9.5.2 Intracellular signalling

9.6 THE ROLE OF THE LPS RECEPTOR CD14 IN IMMUNITY

AND DISEASE

9.7 CONCLUDING REMARKS

Bibliography