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Alveolar recruitment of ficolin-3 in response to acute pulmonary inflammation in humans

Ronni R. Plovsing[a,*], Ronan M.G. Berg[b,f], Lea Munthe-Fog[c], Lars Konge[g], Martin Iversen[d], Kirsten Møller[e,f], Peter Garred[c]

[a] Department of Intensive Care, University Hospital Rigshospitalet, Copenhagen Ø, Denmark
[b] Department of Clinical Physiology, Nuclear Medicine & PET, University Hospital Rigshospitalet, Copenhagen Ø, Denmark
[c] Department of Clinical Immunology, Laboratory of Molecular Medicine, University Hospital Rigshospitalet, Copenhagen Ø, Denmark
[d] Department of Lung Transplantation, University Hospital Rigshospitalet, Copenhagen Ø, Denmark
[e] Department of Neuroanaesthesiology, Neurointensive Care Unit, University Hospital Rigshospitalet, Copenhagen Ø, Denmark
[f] Centre of Inflammation and Metabolism, University Hospital Rigshospitalet, Copenhagen Ø, Denmark
[g] Centre for Clinical Education, University of Copenhagen and the Capital Region of Denmark, Copenhagen, Denmark

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ABSTRACT

Background: Ficolins serve as soluble recognition molecules in the lectin pathway of complement. They are known to participate in the systemic host-response to infection but their role in local pulmonary defence is still incompletely understood. The purpose of this study was to clarify whether acute lung and systemic inflammation induce recruitment of lectins in humans.
Methods: Fifteen healthy volunteers received LPS intravenously (IV) or in a lung subsegment on two different occasions. Volunteers were evaluated by consecutive blood samples and by bronchoalveolar lavage 2, 4, 6, 8, or 24 h after LPS ($n = 3$ in all groups), and gene expression patterns and protein levels of mannose-binding lectin (MBL) and ficolins were determined.
Results: Endobronchial LPS was associated with an increase in alveolar ficolin-3 and MBL levels ($p < 0.04$ and $p < 0.001$, respectively). IV LPS elicited a pronounced acute phase response with an increase in CRP ($p < 0.001$) and plasma ficolin-1 protein levels ($p < 0.001$), whereas no changes were observed in ficolin-1 gene expression patterns ($p = 0.11$) or plasma protein levels of MBL, ficolin-2, or ficolin-3.
Conclusions: LPS induces a tissue-specific recruitment of ficolin-3 and ficolin-1 in the lung and systemic compartment, respectively, suggesting an important role of distinct lectin complement pathway initiators in the local pulmonary and systemic host defence.

1. Introduction

The innate immune system constitutes the first line of defence against invading microorganisms. Pattern-recognition receptors (PRRs) detect pathogen-associated molecular patterns (PAMPs), which rapidly leads to activation of complement and opsonisation of pathogens for phagocytosis by macrophages and neutrophils (Medzhitov and Janeway, 2000; Medzhitov, 2007). In humans, there are at least five soluble recognition molecules that initiate the lectin pathway of complement (Basset et al., 2003; Endo et al., 2006; Garred et al., 2009; Holmskov et al., 2003; Ma et al., 2013; Matsushita and Fujita, 2001); mannose-binding lectin (MBL), collectin-11, and the ficolin protein family (ficolin-1/M-ficolin; ficolin-2/L-ficolin; ficolin-3/H-ficolin). MBL is produced in the liver and extravasates during inflammatory processes as part of the acute-phase response (Holmskov et al., 2003; Medzhitov and Janeway, 2000; Medzhitov, 2007). Ficolin-1 is expressed in peripheral blood monocytes and neutrophils and in type II alveolar epithelial cells in the lung (Endo et al., 1996; Liu et al., 2005; Munthe-Fog et al., 2012). Ficolin-2 is expressed in hepatocytes and ficolin-3 is expressed in bile duct epithelial cells, the liver and the lung (Akaiwa et al., 1999; Hummelshoj et al., 2005; Matsushita et al., 1996; Munthe-Fog et al., 2008; Sugimoto et al., 1998) with the highest expression pattern of ficolin-3 in human lung tissue (Hummelshoj et al., 2008). Hitherto, ficolin-3 protein has evaded quantification in the lungs, notwithstanding that it has previously been detected with antibodies in lavage fluid obtained from human

* Corresponding author at: Dept. of Intensive Care, University Hospital Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen Ø, Denmark. Fax: +45 35 45 27 37.
E-mail address: drplovsing@gmail.com (R.R. Plovsing).

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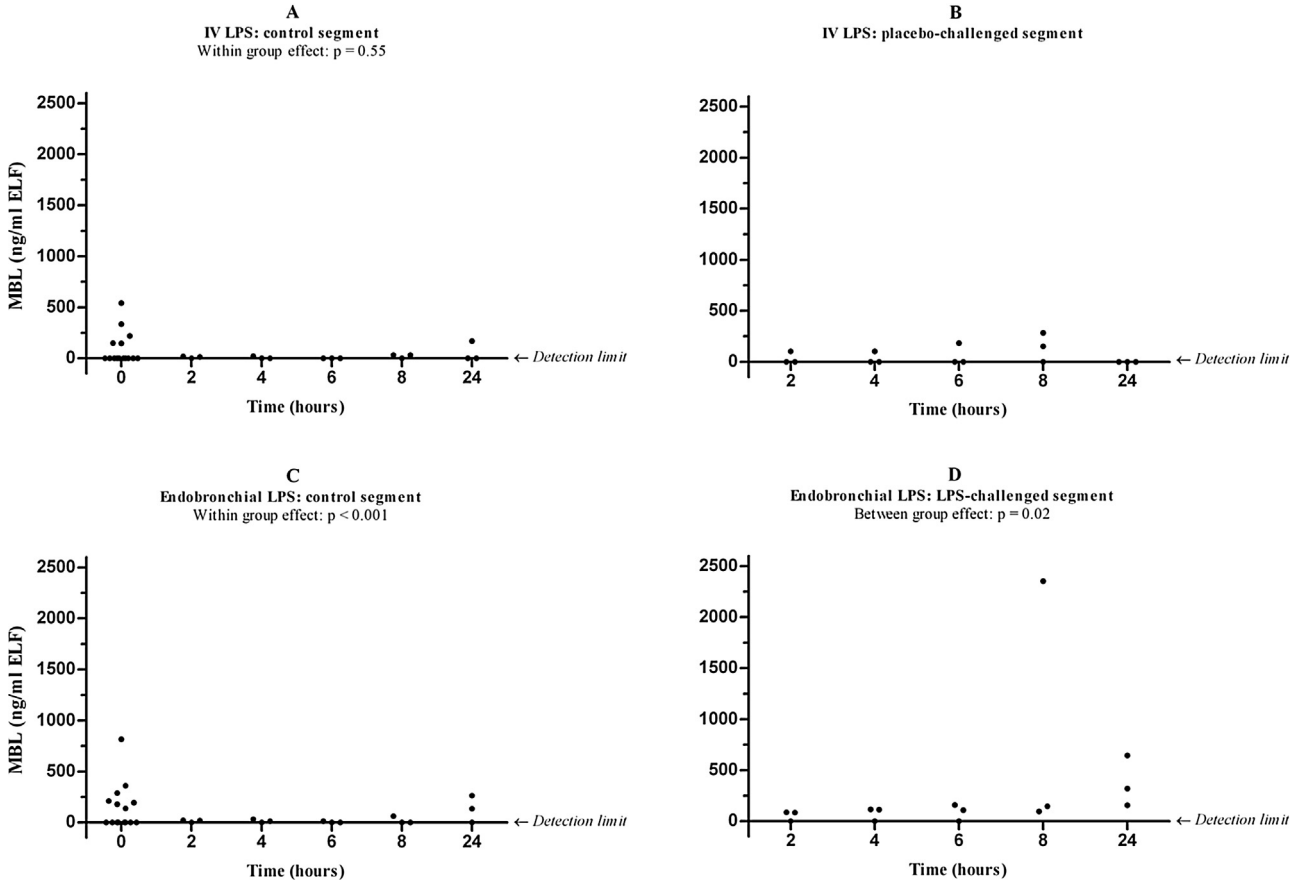

Fig. 1. Mannose-binding lectin (MBL) protein levels in the epithelial lining fluid (ELF) of lavaged lung subsegments before (0 h) and after intravenous (IV) or endobronchial administration of LPS.
Data are presented as individual calculated values. 1A and 1C represents MBL levels from the control segment at baseline (0 h; $n = 15$) with follow-up measurements ($n = 3$ at each subsequent time point). 1B and 1D represents MBL levels from the placebo (1B) or LPS-challenged segment (1D) 2, 4, 6, 8, or 24 h after intervention ($n = 3$ at each time point). Between group effect (1B vs. 1D), $p = 0.02$; within group effect (1C vs. 1D), $p < 0.001$.

alveoli (Akaiwa et al., 1999), and its role in the local pulmonary defence in humans in vivo remains to be elucidated.

MBL is capable of recognizing all classes of pathogens, whereas ficolins have been shown to interact with many Gram-positive and -negative bacteria (Eisen, 2010; Endo et al., 2011; Matsushita, 2013). Ficolin-3 or MBL deficiency may predispose to severe pulmonary infection in humans (Eisen, 2010; Munthe-Fog et al., 2009), and the potential role of ficolins in the protection against *Streptococcus pneumonia* has recently been highlighted (Endo et al., 2012).

The human endotoxin model, in which *Escherichia coli* lipopolysaccharide (LPS) is administered to healthy volunteers, provides an opportunity for investigating acute inflammatory responses mediated through activation of Toll-like receptor (TLR)-4 (Lowry, 2005; Medzhitov and Janeway, 2000; Opitz et al., 2010). Furthermore, intravenous LPS (4 ng/kg) has previously been shown to cause complement activation in humans (Soop et al., 2004).

In the present study, we investigated whether administration of LPS in the lung and blood stream is associated with recruitment of lectin complement pathway initiators. We hypothesised that the acute pulmonary inflammatory response specifically involves a local recruitment of ficolin-3 to the alveolar compartment.

2. Materials and methods

2.1. Subjects

Fifteen healthy non-smoking males (age 23 ± 2 year; height 186 ± 5 cm; weight 80 ± 9 kg; all mean ± SD) were enrolled in the study after providing oral and written informed consent. All had an unremarkable medical history, with no signs of infection within 4 weeks ahead of the study day. The study was approved by the Scientific Ethical Committee of Copenhagen and Frederiksberg Municipalities, Denmark (protocol no. H-2-2009-131) and performed in accordance with the Declaration of Helsinki.

2.2. Study design

Subjects were challenged with *E. coli* LPS on two occasions separated by at least three weeks in a randomized, double-blind, cross-over study investigating LPS-induced inflammatory responses (Plovsing et al., 2014). They received either an intravenous (IV) or an endobronchial bolus dose of LPS (4 ng/kg in 10 ml saline) on the first visit, whereas the opposite intervention was performed on the second visit; simultaneously, placebo (10 ml of saline) was administered in the non-LPS challenged compartment. Data on inflammatory and cellular responses have been published elsewhere (Plovsing et al., 2014; Ronit et al., 2015).

2.3. Study day

Volunteers reported to the laboratory after an overnight fast. A peripheral venous catheter was inserted in the antecubital region for injection of LPS or placebo and an arterial line was placed in the left radial artery following local anaesthesia (lidocaine, 20 mg/ml). Afterwards, a bronchoalveolar lavage (BAL) was performed in the left lung (lingula; control segment) at baseline ($t = 0$ h), that is,