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Lipopolysaccharide binding protein-deficient mice have a normal defense against pulmonary mycobacterial infection

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Abstract

Lipopolysaccharide (LPS) binding protein (LBP) facilitates the transfer of LPS of Gram-negative bacteria to the pattern recognition receptor CD14, resulting in activation of immunocompetent cells. LBP can also facilitate the binding of lipoarabinomannan, a major cell wall component of mycobacteria, to immune cells. To determine the role of LBP in the immune response to pulmonary *Mycobacterium tuberculosis* infection, LBP gene-deficient (−/−) and normal wild-type (WT) mice were intranasally infected with *M. tuberculosis*. LBP−/− mice displayed a similar survival and mycobacterial outgrowth in lungs and liver, although they demonstrated a reduced lymphocyte recruitment and activation during the early stages of infection. The clearance of pulmonary infection with the non-pathogenic *M. smegmatis* was also unaltered in LBP−/− mice. These data suggest that LBP does not contribute to an effective host response in *M. tuberculosis* infection.
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Keywords: Tuberculosis; Lipopolysaccharide binding protein; Inflammation; Innate host defense; Murine model

Introduction

Lipopolysaccharide binding protein (LBP) is an acute phase reactant predominantly derived from the liver [1]. LBP plays an important role in the activation of immunocompetent cells by lipopolysaccharide (LPS) from Gram-negative bacteria. LBP binds and disaggregates LPS, strongly promoting delivery to the pattern recognition receptor CD14 [2]. Subsequent LPS-induced intracellular activation proceeds via Toll-like receptor (TLR) 4 in the presence of an additional extracellular protein, MD-2 [3–5]. Elimination or inhibition of LBP largely prevents LPS-induced toxicity in experimental animals, indicating that LBP is required for the transfer of LPS to its receptor complex in vivo [6–9]. Interestingly, LBP gene-deficient (−/−) mice were unable to mount an effective early inflammatory response to the Gram-negative bacteria *Salmonella typhimurium* [10–13], *Klebsiella pneumoniae* [14,15], and *Escherichia coli* [16], which resulted in an enhanced bacterial outgrowth and an increased lethality when compared to normal wild-type (WT) mice. Together, these data suggest that LBP is important in the chain of events that leads to an adequate innate immune response to at least some Gram-negative infections by virtue of its capacity to present LPS to the CD14/TLR4 receptor complex.

Mycobacterium tuberculosis is responsible for approximately two million deaths per year worldwide [17,18].

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The organism is primarily transmitted via the respiratory route and pulmonary tuberculosis is the most common disease manifestation. The induction of an appropriate innate immune response is essential for the control of infection [19]. Lipoarabinomannan (LAM) is a cell wall component of mycobacteria that shares many physicochemical properties with LPS [20]. LAM isolated from *M. tuberculosis* strains is capped with mannose residues at the nonreducing arabinofurasonyl termini (ManLAM) [21], whereas LAM derived from rapidly growing nonpathogenic mycobacteria lacks mannose caps (AraLAM) [22]. AraLAM is much more potent in eliciting inflammatory responses by isolated mononuclear cells than ManLAM [23–25], although in whole blood in vitro and in mouse lungs in vivo ManLAM is able to produce inflammatory responses that resemble LPS effects [26–29]. In cell lines transfected with CD14 and/or TLRs, the effects of AraLAM are mediated via LBP and CD14, after which signal transduction occurs via TLR2 [30–33]. Although ManLAM induces virtually no effect in this in vitro system [31,34], various effects of ManLAM on monocytic THP-1 cells and macrophages can be inhibited by anti-CD14 antibodies, suggesting that CD14 may play a role in at least some ManLAM-induced intracellular responses [25,35].

LBP can be produced in the lung by alveolar epithelial cells [36], and patients with various inflammatory lung diseases display elevated LBP concentrations in their bronchoalveolar lavage fluid [37,38]. Recently, locally expressed LBP was found to be important for host defense against Gram-negative pneumonia [15]. Although to our knowledge LBP levels have not been reported in the pulmonary compartment of patients with lung tuberculosis, the serum concentrations of LBP are elevated in such patients [39]. In the present study, we sought to determine the role of endogenous LBP in host defense against lung tuberculosis. For this purpose, we intranasally inoculated LBP−/− and LBP+/+ WT mice with virulent *M. tuberculosis* and monitored survival, mycobacterial outgrowth, and host responses. In addition, the outgrowth of avirulent *M. smegmatis*, expressing AraLAM rather than ManLAM, was compared in LBP−/− and LBP+/+ WT mice.

Materials and methods

Mice

LBP−/− mice, backcrossed 11 times to a C57Bl/6 background, were generated as described previously [9]. Normal LBP+/+ C57Bl/6 WT mice were purchased from Harlan Sprague–Dawley Inc. (Horst, the Netherlands). Female mice were used at age 8–10 weeks and maintained in biosafety level 3 facilities. All experiments were approved by the Animal Care and Use Committee of the University of Amsterdam (Amsterdam, the Netherlands).

Experimental infection

Pulmonary tuberculosis was induced exactly as described previously [40–42]. In brief, a virulent laboratory strain of *M. tuberculosis* H37Rv was grown in liquid Dubos medium containing 0.01% Tween 80 for 4 days. A replicate culture was incubated at 37°C, harvested at mid-logarithmic phase, and stored in aliquots at −80°C. For each experiment, a vial was thawed and washed two times with sterile 0.9% NaCl. Mice were lightly anesthetized by inhalation with isoflurane (Upjohn, Ede, the Netherlands) and intranasally inoculated with 50 μl of mycobacterial suspension. The intranasal route of infection has been described previously by us and others, and results in a reproducible infection of the lung with subsequent dissemination to liver and spleen [40–44]. Exact inoculum strength was determined by plating 10-fold dilutions of the suspension on 7H11 Middlebrook agar plates immediately after inoculation. Mice were inoculated with 10^5 Colony Forming Units (CFU) *M. tuberculosis.* After 2 and 6 weeks, groups of 6–7 mice per time point were anesthetized by FFM (fentanyl citrate 0.079 mg/ml, fluanisone 2.5 mg/ml, midazolam 1.25 mg/ml in H_2O) and sacrificed by bleeding out the vena cava inferior. Lungs and one lobus of the liver were removed aseptically and homogenized with a tissue homogenizer (Biospec Products, Bartlesville, OK) in 5 volumes of sterile 0.9% NaCl, and 10-fold serial dilutions were plated on Middlebrook 7H11 agar plates to determine bacterial loads. Colonies were counted after 21 days of incubation at 37°C. CFUs are provided as total per gram lung or liver tissue. In an additional experiment, mice were intranasally inoculated with *M. smegmatis* (ATCC 14468, Rockville, MD). *M. smegmatis* was grown in the exact same way as *M. tuberculosis* and plated on Middlebrook 7H11 agar plates to determine inoculum strength. Mice were intranasally inoculated with 10^6 Colony Forming Units (CFU) *M. smegmatis* and were sacrificed 24 and 72 h after inoculation. Lungs were homogenized and plated. Lung bacterial colonies were counted after 4 days.

For cytokine measurements, lung homogenates were diluted 1:2 in lysis buffer (150 mM NaCl, 15 mM Tris, 1 mM $MgCl{\cdot}H_2O$, 1 mM $CaCl_2$, 1% Triton X-100, 100 μg/ml Pepstatin A, Leupeptin, and Aprotinin, pH 7.4) and incubated at 4°C for 30 min. Homogenates were centrifuged at 1500 × *g* for 15 min after which the supernatants were sterilized using a 0.22-μm filter (Corning, Corning, NY) and stored at −20°C until further use.

Histologic examination

Lungs for histologic examination were harvested at the designated time points, fixed in 10% formalin, and then embedded in paraffin. Four-micrometer-thick sections were stained with hematoxylin and eosin, and analyzed for inflammation and granuloma formation by a pathologist who was blinded for groups.

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