

LPS-binding protein as a target molecule in allergy and asthma

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Functionally and in the context of allergy and asthma, LPSs are JANUS-FACED molecules possessing favorable as well as undesirable traits. In low dose, they can induce a strong inflammatory and antimicrobial response in different mammalian tissues. At the same time, endotoxin tolerance of the innate immune system is observed either immediately with high dose or chronically with constant low dose LPS. Early and continuous LPS exposition of infants was associated with a protective effect for the development of allergy and asthma, whereas in existing bronchial asthma, it may augment allergic inflammation. Furthermore, chronic LPS application has been shown to induce strong pulmonary inflammation with irreversible transformation of bronchial tissue and signs of chronic obstructive pulmonary disease in an animal model. All of these double-headed effects are probably caused by the complex molecular and immunologic processes triggered by the encounter of LPS with soluble factors and cells on the mucosa or, in case of a septic shock, in the bloodstream.

LPS, its structure, and its biological function either for the gram-negative bacterial cell wall or in human diseases (eg, local inflammation or systemic diseases) have been extensively studied and reviewed over the past decades.¹ LPS has had its second spring in research since the innate immunity receptors of the Toll-like group were discovered and signal transduction induced by LPS has been understood better.

LPS may generally approach the mucosa in two ways: either as an integrated structure of the gram-negative bacterial cell wall or, probably more often, as an aggregate of monomeric LPS.² It was the membrane-anchoring lipid A, the phospholipid part of the molecule, that was shown

Abbreviations used

BAL: Bronchoalveolar lavage
LBP: LPS-binding protein

to induce the biological activities of LPS on macrophages, monocytes, or dendritic cells. According to current knowledge, LPS acts as a monomer. Soluble LPS-binding protein (LBP) binds to LPS aggregates and converts these or eventually a membrane-bound structure to monomeric units. Such complexed LBP-monomeric LPS is then transported to the receptor CD14, which is membrane-bound on the cell surface of, for example, a macrophage. Coaggregation of CD14-LBP-LPS with the Toll-like receptor 4–MD-2 complex initiates signal transduction, release of mediators from phagocytes, and maturation of cells such as dendritic cells. From this data it was expected that LBP played a central role in the regulation of this process.

LPS-binding protein was cloned in the 1990s. It is a 60-kd glycoprotein synthesized by hepatocytes, and was shown to possess high similarity to human bactericidal/permeability-increasing protein from granulocytes, which also binds LPS.^{3,4} Surprisingly, LBP knockout mice (LBP^{-/-} mice) were not protected consistently from septic shock after systemic administration of LPS, depending on the mice used for knockout.⁵ This was in contrast with *ex vivo* blood reactivity such as TNF- α releasability after LPS induction in these mice, which was 1000-fold reduced compared with wild-type mice. Therefore, it was concluded that blood is not the compartment primarily responsible for cytokinemia resulting from endotoxemia. The consequent question was whether there are compartments other than hepatocytes where LBP is explicitly produced. LBP was then shown to be elevated in the lung of allergic asthmatics as measured in bronchoalveolar lavage (BAL).⁶ Furthermore, in these experiments, LBP^{-/-} mice, in contrast with wild-type mice, did not respond to allergen challenge in an ovalbumin asthma model with airway hyperreactivity, demonstrating that LBP was the mediator of LPS activity in the lung and also that hyperreactivity in the asthma model depends on LPS adjuvance effects. Interestingly, LBP^{-/-} mice still showed

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the same pulmonary inflammation as wild-type mice, indicating that inflammation is partly independent from hyperreactivity. In summary, the lung is an important compartment for LBP because the protein is produced there in the context of inflammation, and LBP also seems to be a central regulator for LPS turnover on the environmentally exposed mucosa.

In this issue of the Journal, Brass et al present another milestone of LBP biology in the lung as the very important compartment of allergic, inflammatory, and occupational diseases. First, they demonstrated that LBP was specifically upregulated in the lung after LPS stimulus, because in LBP^{-/-} mice, this upregulation did not take place. Second, the number of polymorphonuclear leukocytes in the BAL was not different in both mice. Third, resulting activation of TNF- α , IL-1 β , and IL-6 release in BAL, submucosal cell proliferation, and thickening of airway submucosa was abrogated when LBP was missing. These data clearly confirm that LBP is a central regulator of inhaled LPS in the bronchial mucosa and that both the protective and the harmful effects of LPS may be strongly influenced by LBP. These observations lead to several interesting conclusions.

Whenever the role of LPS in allergy, asthma, and inflammatory airway diseases is to be evaluated, analysis of the lung as a compartment must be performed directly and locally in the lung tissue. LBP is probably produced by respiratory type II epithelial cells.⁷ Still, there is no final explanation yet why LPS upregulates LBP in the bronchial mucosa. For biological regulation of a balanced response toward LPS, the induction of LBP by LPS would make sense. Second, the major effects of LPS in allergy and asthma as mediated by LBP must be viewed rather differentially. Once again, the results of Brass et al indicate that airway hyperresponsiveness, systemic and tissue inflammation, and immunization/sensitization are probably different processes. The biological systems involved in allergy and asthma pathogenesis are influenced rather differently by LPS stimulation, and LBP is thereby 1 of its key regulators. This is confirmed by observations, especially in mice but also in epidemiologic studies, demonstrating that LPS and other pathogen-associated molecules may induce different modulatory effects in allergy and asthma. When given intraperitoneally in mice, LPS may well modulate T_H cell response toward T_H1 induction and a suppression of specific IgE-antibody production.⁸ On the other hand, airway hyperresponsiveness in an asthma model of ovalbumin-sensitized mice is not consistently abrogated by LPS when applied intraperitoneally or intranasally. Furthermore, exposure of children to high levels of LPS in bed mattresses is associated with protection from allergic asthma and hay fever⁹; in contrast, protection from nonallergic wheezing is not correlated with LPS concentration but with the level of muramic acid as a marker for peptidoglycans, which are constituents of all cell walls of the bacteria and not only of gram-negative microbes like LPS.¹⁰

Their results probably led Brass et al to argue only very carefully in favor of the hygiene hypothesis because, on the

basis of a mouse model, they found more arguments for the harmful than the protective effects of LPS. This point is well taken, especially because in recent times several authors declared LPS to be the relevant marker for microbial load in the environment and could associate this load with an allergy and asthma preventing effect. Unfortunately, no reliable markers for microbial load and exposition other than LPS and muramic acids are available at the moment. However, one must take into account that several other microbial molecules such as CpGs, dsRNA, ssRNA, and cell wall components like peptidoglycans, lipoteichoic acids, or lipoarabinomannans may also modulate the innate immune response and thus could influence the genesis and course of allergy and asthma. The fact that the discussion about the hygiene hypothesis is probably too focused on LPS may be misleading and draws the attention away from the search for other relevant environmental factors.

Like other authors, Brass et al identified submucosal thickening after LPS inhalation in mice.¹¹ Whether LPS represents 1 of the responsible molecules for remodeling and fibrosis in chronic inflammatory diseases in human beings is still under debate. In human beings, sufficient data for LPS effects in the lung are missing, and the comparison of animal models showing airway remodeling with respective changes in human beings is still a problem. On the other hand, in occupational medicine, LPS seems to play a crucial role. Therefore, an interesting conclusion that can be drawn from the results of Brass et al is that LBP could evolve as the relevant target molecule for therapeutic interaction in LPS-induced lung diseases. Because it is very difficult to escape LPS and because there are no reliable targets to block exposition to all of the different species of LPSs themselves, LBP downregulation, for example by anti-LBP antibodies, could be 1 future strategy to influence chronic occupational lung diseases induced or augmented by LPS. This is especially important because there are still no relevant therapies available for the irreversible effects of chronic inflammatory diseases. Downregulation of LBP has not yet been proven to be an effective interventional strategy in such disease. Interestingly, antibodies against CD14 may protect primates from endotoxin-induced shock, demonstrating that intervention in another LPS-dependent process could be effective.¹² Surfactant protein A, produced in the alveolar compartment of the lung, is another candidate because it prevents interaction of LPS with LBP.¹³

Taken together, the elucidation of the role of LBP in the lung has become a central question for researchers who want to understand the effects of LPS in allergy, asthma, and chronic airway diseases. Also, LBP may become a therapeutic target in this context.

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