

Endotoxins and Allergy: Lessons from the Murine Model

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Key Words

Asthma · Cytokines · Lipopolysaccharides · Mice · Ovalbumin

Abstract

Exposure early in life to organic dusts containing immunomodulatory components such as endotoxins and immunizing components such as aeroallergens may greatly influence whether subsequent encounters with allergens lead rather to sensitization or unresponsiveness. We investigated the effects of endotoxin in the context of allergen-mediated immune responses in a murine model of allergen sensitization. Systemic sensitization with ovalbumin induced high serum levels of allergen-specific IgE, predominant Th2-type cytokine production, eosinophilic airway inflammation and in vivo airway hyperactivity. Endotoxins were either applied systemically prior to sensitization, or via the airways prior to airway challenges, or by repeated inhalation during the first weeks of life prior to subsequent sensitization. Different effects of endotoxins on allergen-induced immune responses may be attributed to differences in dosing, route of application, time relationship with allergen sensitization and the concurrent exposure to endotoxin and allergen. The results of these studies may help to define the effects of endotoxin on allergen-mediated immune reactions and to further delineate the important interrelationships between environment and disease development. Finally, this may lead to new strategies in the prevention and treatment of allergic diseases.

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Introduction

The prevalence and incidence rates of atopic diseases such as bronchial asthma or atopic dermatitis have increased over the last two decades [1, 2]. Although the knowledge of the genetic background associated with the development of allergic diseases has greatly improved [3], this may not explain the fast and persistent increase over rather a short period of time. It is now well established that allergic diseases are the consequence of T helper cell (Th)-type 2 (Th2)-biased immune reactions to common environmental allergens [4], but the question remains why this type of immune response is steadily increasing. More recently, the ‘hygiene hypothesis’ tried to answer this question by stating that the lack of stimulating signals from the microbial environment early in life due to improved public health and hygiene may prevent the maturation of the immune system, thus favoring inappropriate immune reactions to common environmental and food antigens. This theory is supported by several epidemiological findings demonstrating that (1) the number of siblings [5], (2) the attendance of day-care facilities [6], especially in the first year of life [7], and (3) frequent episodes of common upper airway infections in the first year of life [8] are associated with a reduced risk of developing allergic diseases later in life. Moreover, a protective effect of exposure to high concentrations of microbial components, like lipopolysaccharides (LPS), fungi, organic dusts and animal proteins [9, 10], was noticed in children from farming families in several countries, demonstrating reduced risk of atopy, hay fever and asthma [10–18]. Con-

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1015–2008/03/0705–0255\$19.50/0

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clusively, these data suggest that stimulation of the innate immune system by one or more of these microbial factors, such as LPS, may initiate an inflammatory response that enhances Th1-type immune reactions, thus counterbalancing or inhibiting the development of 'pro-allergic' Th2-type immune responses [16–20].

LPS, a cell wall component of gram-negative bacteria, is a prominent pathogen-associated molecular pattern that is recognized by the innate immune system through members of the highly conserved family of toll-like receptors (TLRs) [21, 22]. Binding of LPS by TLRs causes activation of intracellular signal transducing molecules, including myeloid differentiation antigen 88 and nuclear transcription factor (NF)- κ B [reviewed in ref. 23], resulting in increased production of the proinflammatory cytokines TNF- α , IL-6 and IL-8 by antigen-presenting cells (APCs) such as macrophages and dendritic cells. In this way, direct airway contact with LPS results in intense activation of alveolar macrophages and a vast recruitment of neutrophils, both in humans and in animals [24, 25]. This clearly explains why endotoxin exposure leads to exacerbation of asthma severity and enhances allergic airway inflammation both in humans [26, 27] and in animal models [28, 29].

On the other hand, activation of dendritic cells by LPS leads to increased production of IL-12, the main regulatory cytokine initiating Th1- and antagonizing Th2-type immune responses [30–32]. First clinical evidence for this kind of a modulatory role of LPS in allergic immune reactions came from a study by Gereda et al. [20] showing that exposure to high levels of endotoxin in 61 infants with a high risk of asthma correlated with increased numbers of Th1-type IFN- γ -producing T cells, possibly explaining the reduced allergen sensitization rates in these children. This observation was partly challenged by a very recent analysis of the ALEX study demonstrating that high endotoxin exposure of farmers' children in Germany and Switzerland was not only associated with low prevalence rates of atopy and asthma, but also with reduced secretion of TNF- α , IL-12, IL-10 and IFN- γ by PBMC after stimulation with LPS or mitogen [17]. The authors concluded that environmental exposure to endotoxins thus may favor the induction of immune tolerance against ubiquitous allergens.

Therefore, endotoxin/LPS may affect allergen-induced immune reactions, such as sensitization, airway inflammation and development of bronchial asthma, by several distinct and divergent mechanisms: (1) by augmentation of inflammatory responses in already affected airways; (2) by modulating the Th1/Th2 balance, or (3) by induc-

tion of immune tolerance. To better delineate the kinetics and mechanisms of LPS in the context of allergen-mediated immune responses, we studied the effects of LPS in a murine model of allergen sensitization and airway inflammation [33]. In this model, BALB/c mice, genetically determined to high IgE responses [34], are systemically sensitized with ovalbumin (OVA) and develop high allergen-specific IgE serum levels and a predominant Th2-type cytokine profile in splenic mononuclear cells cultured with OVA in vitro. Repeated airway allergen challenges of mice sensitized in this way result in the development of airway inflammation, characterized by a vast influx of eosinophils into the small and middle-sized regions of the airways and increased in vivo airway reactivity to inhaled methacholine, measured by whole-body plethysmography.

Effects of Systemic LPS on Subsequent Allergen Sensitization

To investigate the effects of LPS on subsequent allergen sensitization, systemic administration of LPS derived from *Escherichia coli* was performed three times prior to OVA sensitization; controls received sham treatment with PBS prior to sensitization. This kind of systemic LPS exposure inhibited development of subsequent allergen sensitization in several ways: (1) OVA-specific IgE serum levels were markedly reduced, and (2) in vitro production of IL-4, IL-5 and IL-10 by splenic mononuclear as well as peribronchial lymph node cells was significantly suppressed [35]. Rather surprisingly, systemic application of LPS prior to sensitization did not directly enhance Th1 immune responses. Further, following systemic LPS treatment of sensitized mice, the numbers of eosinophils in bronchoalveolar lavage fluid following allergen airway challenges were significantly reduced compared to sham-treated sensitized and challenged mice. However, systemic LPS exposure before sensitization did not inhibit development of increased in vivo airway reactivity (table 1). To delineate a possible mechanism for the observed LPS effects, we injected mice with anti-IL-12 monoclonal antibody prior to LPS treatment. In vivo antagonization of IL-12 in this model reversed the inhibitory effects of systemic LPS treatment and led to increased serum levels of OVA-specific IgE and enhanced Th2 cytokine production, even compared to OVA-sensitized, challenged mice.

In summary, in this model, systemic LPS application prior to sensitization with allergen suppressed both aller-

Table 1. Effects of LPS on allergen-induced immune responses

Systemic treatment	PBS	LPS	PBS
Sensitization	OVA	OVA	OVA
Local treatment	PBS	PBS	LPS
<i>Systemic immune responses</i>			
OVA-specific IgE levels	↑	↓	↔
Th2 cytokines by splenic MNC	↑	↓	↔
<i>Local immune responses</i>			
Th2 cytokines by PBLN cells	↑	↓	↓
Th1 cytokines by PBLN cells	↓	↔	↑
Airway eosinophilia	↑	↓	↓
Airway hyperreactivity	↑	↔	↔

BALB/c mice were systemically sensitized with OVA followed by repeated airway allergen challenges. To investigate effects of endotoxins on allergen sensitization, LPS were administered systemically three times prior to OVA sensitization. To study effects of LPS on allergen-induced airway inflammation, LPS were administered three times locally after sensitization but prior to airway allergen challenges. Results of OVA sensitization are related to nonsensitized animals; results of LPS-treated animals are related to OVA-sensitized mice.

MNC = Mononuclear cells; PBLN = peribronchial lymph node.

gen-induced Th2 responses and development of airway inflammation in an IL-12-dependent manner, without enhancing overall Th2 immune responses.

This may be the consequence of insufficient activation and decreased antigen-presenting capacities of APCs following systemic LPS treatment, first demonstrated in vitro on human APCs [36–38]. Alternatively, repeated exposure to (low doses of) LPS may have induced LPS tolerance [39], characterized by downregulation of TLR expression, impaired activation of NF-κB and decreased production of proinflammatory cytokines [40–43]. In contrast to both assumptions, we did not observe decreases of the in vitro responsiveness of splenic MNC following activation with allergen, even after repeated systemic treatment with LPS. Therefore, the results with LPS in our model are best explained by its modulatory effects on the Th1/Th2 balance, supported by the outcome of the blocking experiment with anti-IL-12.

Effects of Local LPS on Allergen-Induced Airway Inflammation

To examine the effects of local application of LPS on allergic airway inflammation, LPS were administered intranasally three times after allergen sensitization, but

prior to airway allergen challenges. Not surprisingly, local LPS application after sensitization did not alter OVA-induced IgE synthesis or in vitro Th2 cytokine production by splenic mononuclear cells, but it did lead to reduced levels of IL-4 and IL-5 and increased IFN-γ production by peribronchial lymph node cells cultured with OVA in vitro [35] (table 1). Total cell counts in BAL fluids were slightly reduced, and the majority of infiltrating cells were now lymphocytes, whereas eosinophils were significantly reduced, compared to sensitized, challenged mice. In vivo antagonization with anti-IL-12 monoclonal antibody injected prior to local LPS administration again neutralized the effects of LPS on in vitro Th1 and Th2 cytokine production and inflammatory airway responses. Taken together, the data showed that local application of LPS prior to airway allergen challenges induced an IL-12-dependent shift from a predominant Th2 towards a predominant Th1 cytokine pattern in the local compartment, resulting in impaired local allergen-mediated immune and inflammatory responses.

Effects of Continuous Exposure of Neonatal Mice to LPS

Life in a farming environment is characterized not only by exposure to high concentrations of microbial components, such as LPS, but also by broad contact with possibly sensitizing components, like environmental allergens. To delineate the effects of early exposure to one or the other or the combination of these two factors on allergic disorders, we investigated the consequences of continuous exposure of infant mice to aerosolized LPS and/or allergen for subsequent allergen-induced Th2 and inflammatory responses. Preliminary data showed that exposure of neonatal mice to LPS (three times a week, 10-min inhalation of LPS aerosol, total of 4 weeks) failed to prevent Th2-mediated immune responses upon later allergen sensitization, whereas pre-exposure to allergen or to the combination of LPS plus allergen completely suppressed allergen sensitization and inhibited the development of allergen-induced airway inflammation and in vivo airway hyperreactivity (table 2). This inhibition was due to allergen-specific tolerance, as indicated by absent allergen-specific Th2 and Th1 immune responses. Moreover, combined exposure to LPS plus allergen prior to allergen sensitization induced a general shift from a predominant Th2 towards an unspecific Th1 immune responsiveness. These data indicate that early and repeated inhalant exposure to allergen may induce specific unresponsiveness,

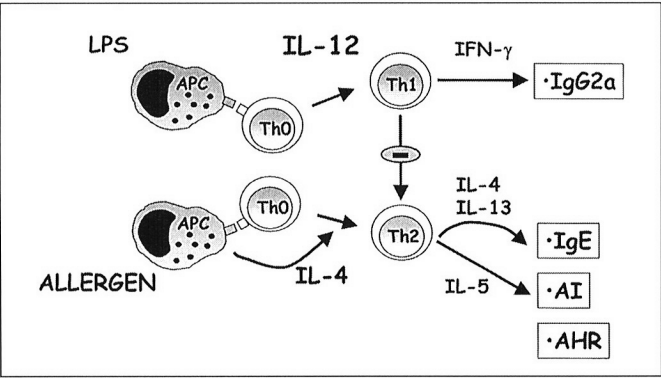


Fig. 1. Prevention of allergen sensitization and development of airway eosinophilia by LPS. In this murine model of allergen sensitization, LPS suppresses allergen sensitization and eosinophilic airway inflammation in a manner dependent on the presence of IL-12, which plays a pivotal role in the induction of Th1 immune response. AI = Airway inflammation; AHR = airway hyperreactivity.

Table 2. Effects of aerosolized LPS and/or OVA exposure on allergen sensitization and allergen-induced airway inflammation

Neonatal exposure	PBS	LPS	OVA	LPS + OVA
Sensitization	OVA	OVA	OVA	OVA
<i>Systemic immune responses</i>				
OVA-specific IgE	↑	↔	↓	↓
Th2 cytokines	↑	↔	↓	↓
Th1 cytokines	↓	↔	↔	↑
<i>Local immune responses</i>				
Airway eosinophilia	↑	↔	↓	↓
Airway hyperreactivity	↑	↔	↓	↓

BALB/c mice were exposed to aerosolized PBS, LPS, OVA or LPS plus OVA three times per week from the day of birth for 4 weeks. All mice were systemically sensitized with OVA. Controls were sham sensitized with PBS instead of OVA. Results of OVA-sensitized animals are related to sham-sensitized mice; results of pretreated animals are related to OVA-sensitized mice.

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and that additional, but not exclusive, exposure to endotoxin may further decrease the susceptibility for sensitization to a variety of common allergens.

Conclusion

In conclusion, LPS prevents systemic IgE and Th2 cytokine responses and development of airway eosinophilia in an IL-12-dependent manner when administered shortly prior to allergen sensitization (fig. 1). Further, airway administration of LPS prior to allergen airway challenges suppresses Th2 cytokine production and airway inflammatory responses even after systemic sensitization with allergen has been achieved. The potential benefit of LPS for treatment of *established* allergic diseases seems limited by the facts that (1) LPS exposure prior to airway allergen challenges was unable to prevent the development of increased AR, and (2) LPS – as demonstrated in various studies in humans and animals – enhances established asthmatic reactions. Still, treatment with LPS (or similarly acting immunomodulatory mediators) may provide a novel mode to *prevent* sensitization in children with a high risk of developing allergic diseases.

Exposure of infant mice to allergen and LPS suppressed subsequent Th2 immune responses, probably by induction of T cell tolerance. Moreover, the combination of allergen and LPS exposure may not only inhibit allergen sensitization to a particular allergen but, at the same time, decrease the susceptibility for sensitization to a variety of common allergens by inducing a general shift from a predominant Th2 towards a Th1 immune responsiveness. Therefore, this tactic may be beneficial for the preventive approach in individuals with a high risk of developing allergies or atopic diseases. Future studies have to prove if exposure to LPS or other immune modulators may be beneficial for the prevention of allergic sensitization and disease, and which form of application is most effective.

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