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Endotoxins, asthma, and allergic immune responses

J.R. Lapa e Silva [a], M.D. Possebon da Silva [b], J. Lefort [c], B.B. Vargaftig [c,*]

[a] *Hospital Universitario Clementino Fraga Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil*
[b] *Escola Paulista de Medicina, Federal University of São Paulo, São Paulo, Brazil*
[c] *Unité de Pharmacologie Cellulaire, Unité Associéé Institut Pasteur/INSERM no. 485, 25, rue du Docteur Roux 75724, Paris Cedex 15, France*

Abstract

Asthma severity depends to a great extent on the levels of endotoxin present in the microenvironment. Although favouring a Th1 cytokine response that could be beneficial to the asthmatic, lipopolysaccharide (LPS) aggravates bronchopulmonary inflammation by several mechanisms. These include neutrophil and eosinophil recruitment, and release by activated macrophages of pro-inflammatory cytokines and nitric oxide. LPS exerts its biological actions through its interaction with CD14. The genetic locus of CD14 is close to the genomic region controlling levels of IgE. A polymorphism in the CD14 promoter region seems to favour high serum IgE levels. Genetic influences may thus control circulating levels of sCD14 and by this mechanism modulate Th1/Th2 balance and IgE synthesis. LPS exposure, although hazardous to the asthmatic, seems to exert a role in the maturation of the immune system in children towards a Th1-skewed pattern. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

The understanding of the interactions between environmental changes and individual predisposition to respiratory diseases is increasing, and this includes the relationship between microorganisms and allergy. The demonstration that endotoxin, and specifically its main constituent lipopolysaccharide (LPS), is present in indoor dust and that the severity of asthma is related to the amount of endotoxin present in house dust in a dose-dependent manner (Michel et al., 1996), opened new horizons for the study of the role of LPS in allergic inflammation. Interactions between inhaled endotoxins and allergens in the bronchoalveolar compartment of the lungs of asthmatic patients could amplify the local inflammatory response, rendering the clinical manifestations more severe and difficult to control. In other words, endotoxins present in the indoor environment could act as an adjuvant to allergens.

The possible mechanisms involved in these phenomena will be discussed here, including a broader approach to endotoxins as adjuvants in relation to the Th1/Th1 paradigm, so important to the understanding of asthma pathogenesis.

* Corresponding author. Fax: +33-145-688703.
E-mail address: vargafti@pasteur.fr (B.B. Vargaftig).

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2. LPS as a pro-inflammatory agent

Endotoxins are a constituent of the outer layer of gram-negative bacteria and can be found in house dust, tap water, and in milk. LPS is the main component of endotoxin and it is formed by a phosphoglycolipid, called lipid A, that is covalently linked to a hydrophilic heteropolysaccharide (Rietschel et al., 1994). Lipid A connects the polysaccharide chains and it is responsible for the LPS toxicity. Five types of LPS receptors have been characterised. CD14 is a glycoprotein of 55 kD expressed in monocytes, macrophages, and, in smaller amounts, in neutrophils. There are two forms of CD14, mCD14, a membrane receptor that allows myeloid cell activation, and sCD14, a soluble form present in the extracellular compartment. The complex sCD14/LPS can attach to non-myeloid cell targets, such as endothelial and epithelial cells, leading to their activation. Recently, Striz et al. (1998) demonstrated induction of IL-6 and IL-8 release by bronchial epithelial cells through this mechanism. The nature of the signalling events of the CD14/LPS complex in mammalian cells has been elusive until recently, when TLR2, a member of the human Toll-like receptor family, was shown to confer LPS responsiveness, that also depends on LBP and CD14. Interestingly, this Toll receptor family of proteins is highly conserved and seems to have the same functions in the fruit fly, *Drosophila* (Ulevitch, 1999). CD14 is also a regulator of T cell activity and this can have great relevance to the pathogenesis of asthma.

The ligation of LPS to CD14 depends on the presence of a transporter protein, the LPS binding protein or LBP. LBP is an acute phase protein that circulates in plasma and binds to lipid A forming high affinity complexes that enhance the capacity of low concentrations of LPS to bind and activate macrophages and neutrophils. Under normal conditions, little LBP is present in the bronchoalveolar compartment. After inhalation of antigen, there is extravasation of LBP and sCD14 to this compartment, due to rapid increase in bronchial microvascular permeability. This allows the LPS that was inhaled with the antigen to amplify the inflammatory response to the antigen. Dubin et al. (1996) elegantly demonstrated that a very high increment of LBP (158-fold) and sCD14 (31-fold) occurs in the bronchoalveolar compartment of asthmatics 24 h after allergen inhalation, compared with the levels before antigen provocation. These components were functionally active, as demonstrated by in vitro experiments. These findings were recently confirmed by Virchow et al. (1998) 18 h after segmental provocation, when they found a net increase of sCD14 in the bronchoalveolar lavage (BAL) fluid. Using an experimental model of allergy, we have recently demonstrated a synergistic effect of ovalbumin and LPS inhalation in allergic mice, increasing bronchial hyperresponsiveness (BHR) to methacholine and eosinophil influx to the bronchoalveolar compartment (manuscript in preparation). Interestingly, this synergy was only seen when ovalbumin inhalation preceded that of LPS, suggesting that the effect of ovalbumin on the microvascular permeability, with extravasation of LBP and sCD14, would allow an increased effect of LPS.

Once present in the inflamed airways of asthmatics, LPS would act to increase this inflammation and consequently aggravate asthma severity. We used another mouse model to demonstrate that intraperitoneal administration of LPS results in marked BHR to methacholine that is independent of TNF-α or neutrophil accumulation in the lungs (Lefort et al., 1998). However, evidence from different laboratories shows that LPS-induced macrophage activation results in increased production of cytokines such as IL-1β and TNF-α, as well as nitric oxide, through the up-regulation of inducible nitric oxide synthase or NOS2. In animal models of septic shock it has been demonstrated that IFN-γ, TNF-α, and IL-10 are involved in the regulation of LPS-induced NO release (ter Steege et al., 1998). It is well known that nitric oxide can combine with eosinophil-derived reactive oxygen intermediates, resulting in peroxinitrite, one of the most potent oxidant products that could harm bronchial epithelium. LPS also induces the recruitment of neutrophils to the bronchial lumen (Nightingale et al., 1998). Neutrophil accumulation in the bronchoalveolar compartment in the absence of infection has been

recently linked to severe forms of asthma (Jatakanon et al., 1999). It has also been demonstrated that LPS increases eosinophil survival through increased GM-CSF production by peripheral blood mononuclear cells (Saitou et al., 1997). Peden et al. (1999) demonstrated increased influx of eosinophils to the nose 4 h after LPS inhalation by atopic patients. They also found a correlation between nasal GM-CSF and eosinophil response to LPS in these subjects. These findings suggest that LPS will increase airways inflammation through different mechanisms, including aggravation of the eosinophilic inflammation.

3. LPS and the Th1/Th2 balance in asthmatics

It seems clear that LPS in general shifts the cytokine response towards a Th1 balance. Mattern et al. (1994) demonstrated that LPS/lipid A induces T cell proliferation and production of IFN-γ but not of IL-4, IL-5, or IL-10. This T cell proliferation depends on the presence of LPS-activated monocytes and cell-to-cell contact, suggesting a role for CD40L or other co-stimulatory molecules. Other evidence indicates that the ligation of CD14 to LPS can induce production of IL-12 by macrophages. This provides an important signal to a Th1 shift, through the production of IFN-γ by macrophages. Pugin et al. (1994) indicated that dendritic cells stimulated by LPS produce IL-12 in a dose-dependent manner according to the levels of sCD14. Moreover, Watanabe et al. (1999) demonstrated that lipid A can inhibit IL-4 production by CD4 + T cells without inhibiting the production of IFN-γ.

These important observations would suggest that LPS should theoretically be beneficial to asthmatics, re-equilibrating the cytokine profile skewed towards a Th2 response. However, LPS induces exactly the opposite effect in asthma, possibly by the pro-inflammatory mechanisms discussed above. Another possible explanation to this puzzle arises from the work by Blease et al. (1999), showing that IL-4, a cytokine present in high amounts in the airways of asthmatics, synergise with LPS to induce VCAM-1 expression in endothelial cells. We have demonstrated that VCAM-1/VLA-4 interaction is essential for BHR to methacholine and to the recruitment of eosinophils to the airways of allergic animals (Pretolani et al., 1994).

4. Role of CD14 in T cell biology and allergy

The biological effects of LPS depend on its interaction with CD14, either soluble or fixed in cell membranes. CD14 itself has an important role in lymphocyte biology, as elucidated in recent work by Nores et al. (1999). They tested a possible direct effect of CD14 on the regulation of T cell activation and function by showing that sCD14 induces inhibition of T cell proliferation through a marked inhibition of IL-2 production by these cells and also inhibits the production of IFN-γ and IL-4. This effect was considered secondary to the strong inhibition of IL-2 production.

Other evidence suggests that CD14 levels are genetically determined and influence the production of immunoglobulin-E (IgE), a major pathogenic component of allergy. There is mounting evidence that asthma develops predominantly in children that fail to mature their immune system towards a Th1 pattern (Holt, 1995). Newborns have naturally a Th2 skewed response, and a failure to mature IFN-γ responses to inhaled antigens may prolong the period during which Th2 responses are predominant (Martinez, 1999). This is the basic reasoning derived from the observations by Shirakawa et al. (1997) that, in Japan, children vaccinated with BCG were less prone to atopy and allergy. LPS, as we discussed previously, is a potent stimulator of dendritic cell maturation and can induce IL-12 production by these cells, which in turn will induce IFN-γ production by macrophages. LPS also induces upregulation of CD14 expression by macrophages. The CD14 gene is located in the chromosome 5q31.1, a region where probably the IgE regulation locus is present. Interesting work by Baldini et al. (1999) suggests that polymorphisms in the CD14 gene promoter region could influence the differentiation of T cells and the levels of serum

IgE. They worked with a large cohort of allergic and non-allergic children and found two homozygotic alleles. Children exhibiting the TT allele presented higher levels of circulating soluble CD14, lower levels of IgE, and lower levels of IL-4, whereas those presenting the CC allele had lower sCD14, higher IgE, and higher IL-4. TT homozygous children appear to benefit from the protective effect of higher levels of sCD14, which would allow stronger stimulation of Th1 responses during bacterial infections and LPS exposure in early life. As many epidemiological studies have firmly established the correlation between higher serum levels of IgE and allergy, the authors concluded that children with low circulating sCD14 had higher chances to present allergy. The authors suggest that genetic influences may thus control circulating levels of sCD14 and by this mechanism modulate Th1/Th2 balance and IgE synthesis. As the levels of CD14 are strongly up-regulated by LPS exposure, this may be a molecular model of gene/environment interaction in the development of IgE responses in children (Martinez, 1999).

We demonstrated that intranasal instillation of BCG to 10-day-old BP2 mice (a selection of hyper-IgE animals) markedly reduces their ability to mount an airways allergic response upon sensitisation 12 weeks later. This is expressed by a reduction in eosinophil recruitment to the lungs and a reduced BHR (manuscript in preparation).

5. Conclusion

LPS is an important adjuvant related to asthma severity through aggravation of bronchial inflammation but may re-equilibrate the Th1/Th2 balance to the Th1 side. Asthma and allergy are on the rise throughout the world, especially in the developed world, where cleaner, relatively aseptic microenvironments are frequently offered to affluent children, as part of the so-called Western style of life. The lack of exposure to LPS or to naturally occurring infections in children may delay maturation of the immune system towards a Th1-skewed response, thus facilitating the occurrence of allergy and asthma.

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