

# The role of innate immunity in occupational allergy: recent findings

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## Purpose of review

Discovery of a system of pattern recognition receptors has opened an entirely new field in inflammation research. This paper reviews studies from the last 18 months focusing on mechanisms of immune stimulation after exposure to microbial components and other exposures that induce or modulate innate immune responses.

## Recent findings

Perhaps the most studied pattern recognition receptor response pathway is endotoxin upregulation of cytokine production via lipopolysaccharide binding protein (LBP), soluble and membrane bound CD14, v-myb-dependent gene 2 (MD-2) and toll-like receptor (TLR) 4. This interaction, and that of synthetic TLR2-agonist (Pam3Cys4) with toll-like receptors TLR1 and TLR2, has recently been characterized at the structural level, and has been confirmed by a blunted response in knockout mice. Dectin-1 recognizes the hyphal form, but not the spores of *Aspergillus fumigatus*, giving the first known function of the dectin receptor. Martinez has hypothesized that inconsistencies relating to the protective effect of lipopolysaccharide exposure on allergy might be explained by a gene–environment interaction.

## Summary

We are on the brink of understanding the complex nature and interaction of the pattern recognition receptors and the cellular events following their binding of ligands. Further elucidation of gene–environment interactions will probably lead to important discoveries in the near future.

## Keywords

β-glucan, cytokines, endotoxin, innate immunity, toll-like receptors

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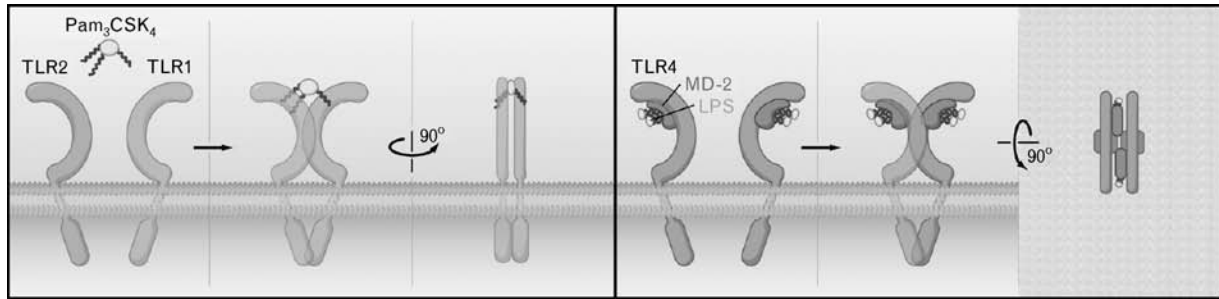
## Introduction

Phylogenetically, the innate immune response is the oldest part of the vertebrate immune system. It has developed through evolution in order to protect against attack from microorganisms. The organism's ability to detect and recognize microbial associated molecular patterns (MAMPs) is supported by highly conserved receptors in cell walls and in the interstitium, the so-called germ line encoded 'pattern recognition receptors' [1]. No prior exposure to these MAMPs is required in order to activate the immune system. The ligands are a host of different molecules from microorganisms, e.g. bacterial endotoxin (lipopolysaccharide; LPS), peptidoglycan, β-glucans, DNA from bacteria (CpG DNA) and RNA from viruses. MAMPs are recognized by the 10 different human toll-like receptors (TLRs) and others like the mannose receptors recognizing mannose [2], dectin receptors binding β-glucans and nucleotide binding proteins recognizing peptidoglycans from bacteria. Together with nucleotide-binding oligomerization domain (NOD)-like family and

the retinoic acid-inducible gene (RIG-I)-like helicases they comprise a comprehensive array of receptors enabling the innate immune system to respond to important microbial agents in the environment.

## The innate immune response

The innate response has become fashionable again; the ratio of reviews to original work is almost one to one. This is in part because of a paradigm shift; the discovery of TLR4 as the LPS receptor by Medzithov *et al.* [3] has led to description of three families of pattern recognition receptors: the TLR family, the NOD-like family and the RIG-I-like helicases. These are the receptors that sense the danger that Matzinger spoke of at the International Congress of Immunology in San Francisco in 1993 [4]. Study of these pathways may also tell us what other signals (most likely necrosis) are required to induce inflammation, and how the transition from inflammation back to homeostasis is regulated.

**Figure 1** Proposed mode of dimerization by toll-like receptors (TLR)

Reproduced with permission [15].

In the lung, the innate immune response is likely to be extremely dynamic, as the lung epithelium comes in contact with airborne environmental agents to a greater degree than occurs in the digestive or reproductive tracts. We will continue a tale we began in 2005 [5] which emphasizes TLR4 interactions with LPS in the recognition of bacteria, the manner in which the dectin receptor interacts with signals from fungi, and the emerging understanding of the way very different classes of organisms are recognized by the immune system (e.g. *Aspergillus fumigatus* is recognized through Dectin-1, TLR2 and TLR4 [6•] but is in a different phylogenetic kingdom than the prototype LPS sources, *Escherichia coli* and *Salmonella abortus*).

#### Toll-like receptors

LPS binds to v-myb-dependent gene 2 (MD-2) [7], and MD-2 induces clustering of TLR4 to initiate the signal cascade into the cell. LPS binding and clustering of TLR4 are differentially regulated by MD-2 and its interaction with LPS [8], and the result of the interaction may vary more than is appreciated now. The surface translocation of MD-2 and TLR4 are independently regulated [9] with TLR4 translocation being regulated by protein associated with TLR4 (PRAT4A) [10].

The tertiary structure of TLR4 with MD-2 and an antagonistic ligand, eritoran [11•], as well as that of TLR1, TLR2 and their ligand, synthetic TLR2-agonist Pam3Cys-Ser-Lys4-OH lipopeptide (Pam3CSK4) [12•] has recently been elucidated. All three TLRs have a structure dominated by leucine-rich repeats, shaped much like a horseshoe. TLR4 does not make direct contact with eritoran itself; the LPS antagonist with four acyl chains covers only the hydrophobic pocket in MD-2, as was predicted [13•]. The MD-2 eritoran complex binds at the concave face of the N-terminal and central domains of TLR4. It will be interesting to see how penta-acyl and hexa-acyl analogs of eritoran like *E. coli* LPS change the shape of MD-2, and hence activate TLR4. Activation through TLR4 is proposed to involve allosteric changes

of interactions of MD-2 with a second TLR4–MD-2–eritoran complex. Surprisingly, the interactions do not follow a single rule. Two of the three acyl chains of the synthetic TLR2 agonist Pam3CSK4 bind in a lipid binding pocket of TLR2 and the third acyl chain binds in a similar pocket in TLR1, both also at the junctions of the N-terminal and central domains of the TLRs. The r-form of LPS, which is the backbone of the molecule, is capable of stimulating TLR4 in the absence of CD14, which is required if there is O-antigen linked to the core LPS molecule [14] (Fig. 1) [15].

In a whole blood assay, IgE to the allergen Der p was required to upregulate eotaxin 2 in monocytes of nonsymptomatic atopic individuals, where it correlated with the level of specific IgE. Asthmatics with specific IgE to Der p responded to Der p with increased levels of eotaxin-2 and IL-5 from monocytes. LPS (and LPS-induced IL-10) abrogated eotaxin and IL-5 secretion [16], showing that this phenomenon is central in the downregulation of the type-2 immune response.

Upregulation of FcεRI on inflammatory monocytes (CD14<sup>dim</sup> CD16<sup>bright</sup> HLA DR<sup>+</sup>) of atopic dermatitis patients reduces their ability to respond to stimuli through TLR2 to synthesize TNFα [17]. The TLR2 responsiveness was restored when FcεRI expression was reduced by culturing without IgE. This represents a difference at the functional level, as patients with allergic atopic dermatitis express similar levels of TLR2 and TLR4 on monocytes and TLR2 and CD14 on peripheral granulocytes as healthy controls [18]. This also appears to be a monocyte-specific response, as there is no difference in the mRNA for surface-expressed TLRs and their functional response profile on myeloid dendritic cells of allergic atopic patients with over 1000 kIU IgE and controls [19]. Pattern recognition receptors can also regulate the inflammatory response, as cord blood-derived dendritic cells could be cultivated with *Bifidum* spp. bacteria to induce less IL-4 and more IL-10 and IFNγ in T cells through TLR2, but not through TLR4 [20].

Thymic stromal lymphopoietin (TSLP) is thought to trigger dendritic cells to promote a Th2 phenotype. Respiratory viral infections enhance Th2 inflammation in the asthmatic lung through expression of TSLP, which is enhanced by IL-13 and synergistically by IL-4 and through TLR3 by double stranded RNA [21].

### Short palate lung nasal clone

A large fraction of chronic stable asthmatics may be persistently infected with *Mycoplasma pneumoniae*. Short palate lung nasal clone (SPLUNC) is the palate lung nasal clone (PLUNC) isoform that is found in large airway epithelia of mice and humans [22]. SPLUNC inhibited *M. pneumoniae* concentrations in murine and human studies. With exposure to environmental allergens producing an excess of IL-13, SPLUNC expression may be downregulated and consequently unable to limit *M. pneumoniae* growth producing persistent airway inflammation [23].

### Dectin-1 receptor

The dectin-1 receptor was described in 2002 as the  $\beta$ 2-glucan receptor [24]. Dectin-1 has recently been shown to recognize the hyphal form of *Aspergillus fumigatus*, in combination with TLR2 and TLR4, but not the spores that are more commonly inhaled, allowing recognition of the potentially invasive form of the organism by alveolar macrophages [6•]. A similar complex of receptor–ligand interactions, that also includes the mannan receptor, is used by alveolar macrophages to recognize *Candida albicans* [25]. Dectin-1 promotes the fungicidal activity of neutrophils as demonstrated by inhibition of antifungal activity of neutrophils treated with antibodies against dectin-1, [26]. This is the first documented fungicidal activity of this molecule.

## Exposure studies

Exposure studies are a valuable tool to understand the mechanisms and the dose–response relationships. Recent studies have been performed in animals as well as in humans, elucidating the mechanisms and exploring the beneficial effects of CpG DNA as an adjuvant.

### Exposure to endotoxin in humans

Endotoxin responsiveness requires at least four extracellular and cell surface host proteins [LPS-binding protein (LBP), CD-14, MD-2, and TLR4]. These proteins interact with endotoxin in a series of protein–endotoxin and protein–protein interactions [27]. LBP catalyzes the transfer of endotoxin from bacterial membranes [28] or aggregates of endotoxin to soluble or membrane-bound CD-14 via a GPI-linked membrane anchor. Individual molecular complexes of endotoxin and CD-14 are released and serve as the substrate form of endotoxin for MD-2,

presented either in extracellular form [29] or membrane bound via association with TLR4 [30••]. Engagement of MD-2 with endotoxin and TLR4 results in cell activation and leads to the proinflammatory mediator release [31].

Levan *et al.* [32•] measured circulating soluble CD14 (sCD14) in 88 human subjects before and after exposure to 20  $\mu$ g inhaled endotoxin. Subjects who were homozygotic for CD14/-1619, CD14/-1359, and CD14/-159 demonstrated higher sCD14 than subjects with CD14 polymorphisms at baseline but were no different 24 h after endotoxin exposure. Further, these polymorphisms were not associated with LBP, C-reactive protein, or white blood cell counts before or after exposure.

Maris and colleagues [33] exposed 16 subjects to 100  $\mu$ g inhaled endotoxin and 6 h later recovered macrophages from bronchoalveolar lavage. Expression of mRNAs encoding for TLRs in the macrophages demonstrated TLRs 1, 2, 7, 8 and CD14, and reduced expression of mRNAs encoding TLR4. The authors concluded that endotoxin exposure reduces the levels of mRNAs regulating the expression of proteins involved in endotoxin signaling, especially TLR4 and MD-2, but not those regulating the expression of TLRs involved in the recognition of other MAMPs.

### Exposure to endotoxin in mice

A number of studies have evaluated endotoxin-induced inflammation in the lung to elucidate the function of the innate immune system in MAMP recognition. A study by Hadina *et al.* [34•] evaluated the dose-dependent pulmonary inflammatory potency of two types of endotoxin from *Neisseria meningitidis*, a lipooligosaccharide (LOS). Wild type hexaacylated endotoxin (LOS<sup>wt</sup>) and mutant pentaacylated endotoxin (LOS<sup>msbB</sup>) were administered by intranasal inhalation to mice. Hexaacylated LOS resulted in robust inflammatory responses while pentaacylated LOS was far less potent in recruiting neutrophils to the lungs and in increasing TNF- $\alpha$ , MIP-1 $\alpha$ , IL-6, G-CSF, and IL-1 $\beta$  concentration in bronchoalveolar lavage. A similar time course of inflammatory responses in lungs was found in both types of endotoxin exposures with peak levels of cytokines appearing at 4 h after exposure and neutrophils significantly elevated at 4–24 h.

Hadina *et al.* [34•] then evaluated responsiveness to MD-2 null mice versus MD-2 competent mice. Inhalation of hexaacylated LOS in MD-2<sup>-/-</sup> mice failed to induce neutrophil recruitment or cytokine upregulation demonstrating the importance of MD-2 in endotoxin responsiveness *in vivo*. They further reported that MD-2<sup>-/-</sup> mice mobilize airway inflammatory responses to purified

monomeric LOS:MD-2, confirming that the loss of responsiveness of MD-2<sup>-/-</sup> mice to endotoxin is due to the absence of MD-2.

Brass and coworkers [35–37] explored the responses of three different knockout mice to endotoxin inhalation exposure as compared to control mice. Unlike controls, IL-1 receptor null mice (IL-1RI<sup>-/-</sup>) did not develop submucosal thickening with subchronic exposure. The IL-1RI<sup>-/-</sup> also demonstrated diminished recruitment of inflammatory cells to the airways acutely and with repeated exposure. Similar experiments in CD14<sup>-/-</sup> mice revealed diminished inflammatory response to endotoxin challenge. Neutrophilia and increased lavage fluid TNF $\alpha$  could be restored in the acutely exposed CD14<sup>-/-</sup> mice by treatment with exogenous sCD14 via intratracheal instillation. CD44 is the major hyaluronate receptor on cell surfaces. Endotoxin inhalation in CD44-deficient mice revealed blunted secretion of TNF $\alpha$  and reduced macrophage motility *in vitro*. These studies coupled with those of Hadina *et al.* [34<sup>•</sup>] demonstrate the importance of MD-2, CD14, IL1RI, and CD44 in mounting an appropriate response to endotoxin and, by inference, Gram-negative bacteria.

#### Exposure to $\beta$ -glucan containing dusts

In a study of 36 volunteers the combined effect of dust and  $\beta$ -glucan inhalation was studied in a climate chamber. Bonlokke *et al.* [38<sup>•</sup>] found decreased nasal volume and increased IL-8 indicating that  $\beta$ -glucan enhanced the inflammatory effect of the dust on the upper airways. The administered doses of dust and  $\beta$ -glucan in this study were comparable to what can be found in offices in temperate climates. A repeat of this setup was performed on guinea pigs without a marked effect on the upper airways [39].

#### Exposure to CpG DNA as an adjuvant

A clinical trial was performed for a vaccine consisting of a ragweed-pollen antigen conjugated to CpG DNA in 25 adults allergic to ragweed. This trial showed a Th2  $\rightarrow$  Th1 shift in patients with increased amounts of IgG antibodies towards ragweed. Saline was used as the placebo control, however, so it is not clear if the adding of the CpG DNA was increasing the efficiency of the vaccine compared to standard protocols [40]. This finding is interesting, however, since veterinary vaccination protocols have used LPS as an adjuvant for decades, with a clear benefit.

#### Susceptibility factors

CD14 remains one of the most studied receptors for MAMPs, and polymorphisms have been linked to changes in the risk of allergy and asthma [41]. Two

recent studies of young adult populations have shown the CD14 159 T allele to be protective of allergy, with a stronger effect in people with a farm childhood. A study from France [42] showed that the CD14 159TT genotype was associated with lowered risks of atopy and nasal allergy in persons with exposure to farm residence early in life. In a study from Denmark [43,44] previously finding lower rates of atopy and allergy in farmers, it was shown that the CD14 159 T allele was associated with lower prevalence of allergy and this tendency was even stronger in persons with a farm childhood. These findings underline the effect of LPS, and stress the notion that in order for the effects of polymorphisms to unfold, the right exposure level has to be present. From earlier studies, however, we know that TLR2 is also important in order to achieve the beneficial effects of farm exposure. Interestingly, Martinez [45<sup>••</sup>] has hypothesized on the basis of four recent studies of CD14 polymorphisms, that the inconsistencies relating to the protective effect of LPS exposure on allergy in different populations might be explained by a gene–environment interaction with the 159CC being protective at higher domestic endotoxin exposures and the TT at lower exposure levels.

#### CD14 polymorphism and chemical exposures

As the polymorphisms have been associated with asthma in population studies, it becomes attractive to study the effects of these polymorphisms in workers with occupational asthma, in order to investigate if the TLRs might be important for other ligands, or if they are modulating the response of other stimuli. Bernstein *et al.* [46] showed in one such study of diisocyanate workers that combinations of CD14 C159T with IL4RA I50V and IL13 R110Q polymorphisms were significantly associated with occupational asthma due to hexamethylene diisocyanate (HDI), whereas only IL4RA showed an increased odds ratio for occupational asthma due to HDI (OR 4.1; 95% CI 1.4–12.7). For CD14 and IL4RA, the odds ratio was 5.2 (1.8–14.9) and the combination of the former with IL-13 yielded an odds ratio of 6.4 (1.6–26.2) indicating that HDI may induce the CD14-related pathway.

#### Conclusion

As the basis of the interaction between multicellular hosts and microbes is being elucidated at the molecular and cellular level, we will begin to understand how the innate immune system determines whether a microbe is a commensal partner that is to be tolerated or even supported, or a pathogen that warrants an inflammatory response and clearance. The importance of this interaction is documented by clinical and epidemiological surveys that show associations between microbially derived signaling and

health as well as the burden of disease of a given population.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 191).

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