

The Innate Immune System and Its Role in Allergic Disorders

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Key Words

Innate immunity · Hygiene hypothesis · Toll-like receptors · Allergy

Abstract

Background: There has been an increasing prevalence of allergic diseases in the Western world over the last decades. The hygiene hypothesis has been proposed as a possible explanation for this epidemical trend in allergy. A key role in this theory is assigned to the reduced microbial stimulation of the Toll-like receptors (TLRs) in early life, which could lead to a weaker Th1 response and a stronger Th2 response to allergens. The individual immunological response is determined by the interplay between the dose and timing of exposure to endotoxins, other environmental factors and genetic predisposition. In the development and progression of allergic disorders, the innate immune system plays an important role.

Objective: In this review, we discuss the paradoxical effects that may appear when the innate immune components are triggered. We review the influence of changes in the gene sequence and TLR expression in relation to the overall pattern of commensals and pathogens. We explored the possibility of alternative stimulations of the immune system by CpG oligodeoxynucleotides and probiotics as therapeutic devices against this endemic disease in Western society. **Methods:** Selection of papers was based on the importance of their contribution to the understanding of innate immunity and its implications.

Results and Conclusion: The innate immune system

plays an important role in both the protection against and the enhancement of allergic disorders, but the mechanisms are still unclear. Nevertheless, gene polymorphisms and triggers of the innate immune system provide therapeutic targets for protection against and treatment of allergic disorders.

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Introduction

The innate immune system is an important first-line defense against potential pathogens [1]. It consists principally of physical, chemical and cellular elements [2]. To provide an immediate response in the setting of danger, rapid detection of pathogens is necessary [3]. This occurs by Toll-like receptors (TLRs), which are the sensors of innate immunity [4]. Activation of the system leads to effector functions through antibacterial peptides, such as defensins, that are considered as natural broad-spectrum antibiotics [5].

However, activation also leads to signaling to the adaptive immune system, modifying the shaping of the Th1/Th2 balance [3]. Although the allergic response is an example of a typical adaptive immune response, involving an abnormally strong Th2 response, experimental evidence demonstrated that innate immune components may play a role in priming the immune system at an early age and in the development of allergy.

In this review we discuss the impact of components of the innate immune system on the onset and progression

of allergic disease in order to attempt to reveal mechanisms that explain the environmental and genetic influences on the increase of allergy-related disease in Western society and to provide future therapeutic approaches to the prevention and treatment of this endemic trend.

How Triggering of the Innate Immune System Protects against Allergy

The hygiene hypothesis tries to explain the association between the increasing prevalence of allergy and the Western lifestyle [6]. It says that 'the apparent rise (in the prevalence of allergic diseases)... could be explained if allergic diseases were prevented by infection in early childhood, transmitted by unhygienic contact with older siblings, or acquired prenatally... Over the past century declining family size, improved household amenities and higher standards of personal cleanliness have reduced opportunities for cross-infection in young families. This may have resulted in more widespread clinical expression of atopic disease' [7].

In the search for the immunological basis of the hygiene hypothesis, and in suggesting that a reduced microbial stimulation of the TLRs on the innate immune cells in early life leads to stronger Th2 responses to allergens, the terms 'missing immune deviation' and 'reduced immune suppression' have been introduced [8].

The critical period for the establishment of the Th1/Th2 balance is the early life period [6]. Whereas the immune system of humans in early childhood is thought to have a Th2 bias [9], this Th2 skew gradually diminishes during the first 2 years of life in nonallergic individuals. In allergic infants, this does not occur [9]. The interaction of pathogen-associated molecular patterns (PAMPs) with TLRs results in the release of several Th1 cytokines, such as IFN- γ and interleukin (IL)-12, and Th2 cytokines, such as IL-4 and IL-5. These cytokines are mainly responsible for driving T cell polarization [10].

Missing immune deviation from Th2 to Th1 is thought to be caused by a decreased production of Th1 cytokines, as a result of a reduced stimulation of TLRs in a Western lifestyle [8].

Whereas experimental evidence, both in vitro and in vivo, supports the role of reduced shifting from Th2 to Th1 responses as a possible explanation for the increased prevalence of allergy, also other immune cell types can intervene to block either Th1 or Th2 activity or both, namely the regulatory T (Treg) cells, leading to the concept of 'immune suppression' [10].

Treg cells selectively express several TLRs, and are able to suppress both Th1 and Th2 responses by producing suppressive cytokines, such as IL-10 and TGF- β [3, 10, 11]. Some evidence suggests that exposure to lipopolysaccharide (LPS) enhances the suppressive functions of Treg cells [11]. However, the exact relationship between microbial stimulation of the TLR pathway and Treg cells is still unclear and the exact role of Treg cells in regulating allergen-specific Th2 responses is controversial [3, 8].

Numerous environmental factors associated with rural and urban living have been studied in an attempt to give an explanation for the increasing prevalence of allergic disorders in the developed world [6]. TLRs are transmembrane receptors with an extracellular leucine-rich repeat (LRR) domain and an intracellular Toll/IL-1 receptor (TIR) domain. The LRR mediates ligand-binding specificity, while the TIR domain is responsible for initiating signaling pathways through protein-protein interactions. The bacterial endotoxin or LPS, a component of the outer membrane of gram-negative bacteria, is recognized by TLR4.

In general, LPS-TLR4 binding induces downstream nuclear transcription factor $\kappa\beta$ (NF $\kappa\beta$) activation with the ultimate upregulation of different sets of genes that regulate inflammatory responses leading to the release of cytokines, chemokines and cells and thus also to the activation of the adaptive immune system [12].

Exposure to LPS in early life seems to stimulate Th1 activity directly or indirectly, which could lead to a possible protective effect against the development of allergy [8, 13, 14]. In a farming environment, the endotoxin levels are found to be higher than in a nonfarming environment [11, 14]. Children growing up on a farm or those having regular contact with farm animals are less susceptible to develop allergic disorders [15, 16]. In the blood samples of farmer's children, elevated levels of CD14, another primary receptor for LPS which has no cytoplasmic domain and is thus linked to other pattern recognition receptors (PRRs), were found [17]. An increased level of TLR2, a PRR mainly specific for gram-positive bacteria, was also present [8, 15]. In a recent study, in vivo administration of a TLR4 or TLR2 agonist (lipid A and peptidoglycan, respectively) on allergen sensitization or challenge induced a decrease in the allergic response [18]. The house dust endotoxin level in farm homes is found to be higher than in urban homes [19]. Exposure to endotoxin in early life was also found to be protective against asthma [20]. LPS induces the production of defensins and IL-10, which are important protective Th1 mediators in

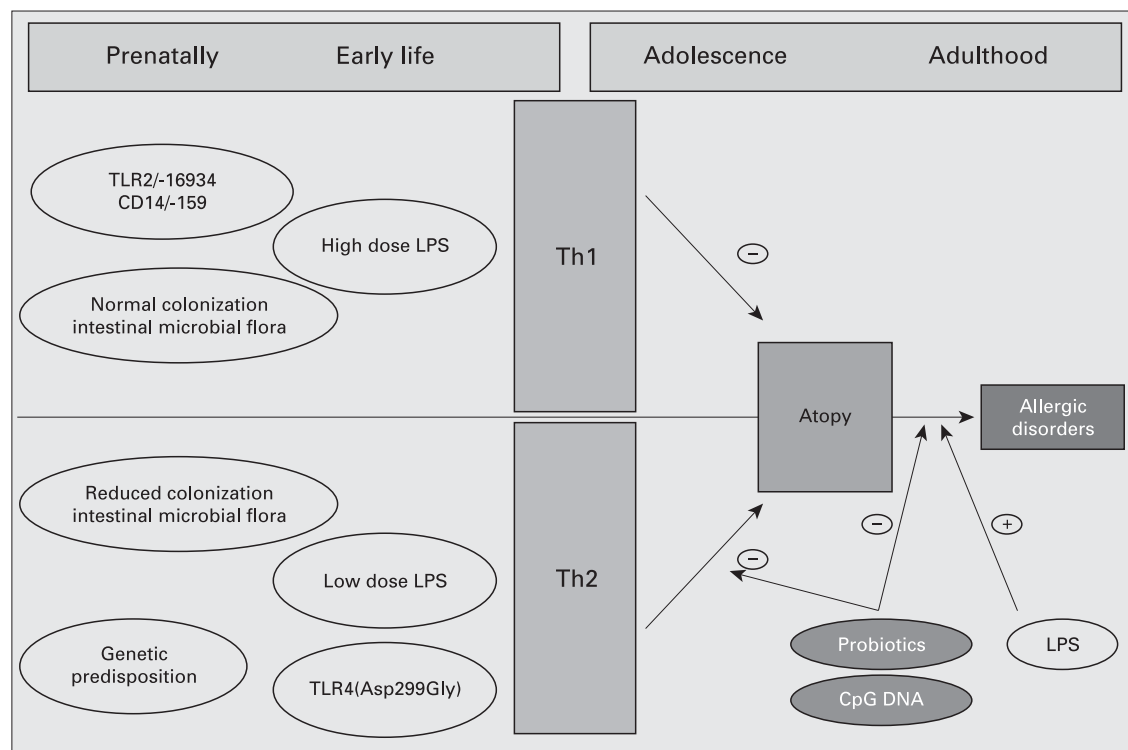


Fig. 1. The innate immune system intervenes in both the onset and later manifestation of allergic diseases. Depending on the time, type and intensity of innate triggering and the underlying genetic susceptibility of an individual, the innate response can alter the Th1/Th2 balance and can both suppress and enhance allergic inflammation and clinical expression. Gene polymorphisms of innate receptors as well as triggers of the innate immune system (LPS, CpG DNA and probiotics) may provide key therapeutic targets for protection against and treatment of allergic disorders.

the pathophysiology of asthma [20–22], but also of IL-12, which is important in the orchestration of cell-mediated immune responses and in the protection against bacterial pathogens [23, 24]. The farming environment is associated with a lower prevalence of both atopic and nonatopic asthma [16], but exposure to indoor endotoxin was found to be protective only against the development of atopic asthma [25]. These findings suggest that other contributing factors besides endotoxin may also play a role in the upregulation of innate receptors [15] and in the association with asthma [11, 20]. The immune responsiveness of an individual to environmental stress is determined by his or her genetic background.

Allergic diseases tend to be familial and have a genetic basis but the recent increase in the prevalence of allergy cannot be explained by genetic factors alone. Several environmental and lifestyle factors (e.g. prenatal maternal influences, allergen exposure, early life microbial exposure, air pollution, farming environment, dietary

factors) have been identified as influential factors. The interactions between our genetic system and these environmental factors eventually determine the individual immunological response (fig. 1).

Further assessment of the complex interactions between genetic and environmental determinants is required.

Stimulation of the innate immune system by LPS seems to be important in the ontogeny of the normal individual immune system [26]. However, although farmer's children seem less likely to have allergic disorders, and mutations and polymorphisms in genes encoding for innate components seem to modify the risk for the development of asthma and allergy, the question remains whether this is mediated by the innate immune system. To obtain a clear answer, additional well-performed studies are needed. Because asthma and other allergic disorders are not occurring in early life alone, these studies should cover a longer lifespan than only early childhood.

Although the hygiene hypothesis is promoted as the most plausible explanation for the increasing prevalence of allergy, it cannot be denied that there are lots of confounding factors in the current study designs, which need to be taken into account [27].

How Can Triggering of the Innate Immune System Enhance Allergic Disorders?

As mentioned above, exposure to endotoxin may provide protection against allergic disorders. There is, however, evidence that endotoxins may have a dual opposing effect. Triggering of the innate immune system through endotoxin exposure can be both protective and harmful in allergy and asthma [21]. This depends on the dose of endotoxin exposure [6]. Whereas high doses in early life promote Th1 responses, low doses in later life favor Th2 responses [8, 28, 29].

In asthmatic patients, higher house dust endotoxin levels are associated with more pronounced symptoms, increased use of medication, a more important degree of airway obstruction and more wheezing in the first year of life [21, 30–33]. Exposure to LPS after sensitization aggravates the allergic inflammatory response [34]: e.g. endotoxin exposure in later life adversely affects patients with asthma by increasing the severity of the airway inflammation and the susceptibility to rhinovirus-induced colds and causing irreversible airway obstruction after chronic exposure [35]. This effect may be explained by LPS inducing the production of cytokines, chemokines (IL-8, activating neutrophils), adhesion molecules (ICAM-1), and other products, causing inflammation [35–37]. Thus, innate immunity may play a deleterious role in asthma [35].

Nevertheless, whether the immune response and modulation will result in allergic sensitization not only depends on the exposure to endotoxin per se, but also on the timing of the exposure, the dosage of endotoxin, environmental factors, and genetic predisposition [21] (fig. 1).

The Genetic Background and Implications of Innate Immunity

As mentioned above, gene-environment interactions will eventually determine the individual immunological response. Changes in the gene sequence can alter the immune responsiveness of an individual to environmental stress either negatively or positively. There is much vari-

ability among individuals in the response to inhaled toxins, suggesting that different polymorphisms act in concert with each other [38]. A genetic polymorphism encoding TLR2 (TLR2/-16934) was found to be associated with reduced susceptibility to asthma and allergy in farmer's children [11]. Only those farmer's children with this TLR2 polymorphism were significantly less likely to have asthma and current asthma symptoms [11].

Mutations in the extracellular domain of the TLR4 receptor (Asp299Gly and Thr399Ile) were found to be associated with a significant hyporesponsiveness to inhaled LPS in humans [38]. The TLR4 (Asp299Gly) polymorphism was found to be associated with a higher prevalence of asthma in school-age children, especially of atopic asthma, but no correlation was found with allergic rhinoconjunctivitis or skin prick test positivity [39]. The reduced capacity to produce IL-12 and IL-10 in response to LPS in children with the TLR4 polymorphism (Asp299Gly) suggests an aberrant function of the innate immune system [39]. However, the exact mechanism through which this happens and the physiologic and biologic implications are still unclear or have not yet been investigated [38, 40].

A CD14 polymorphism (CD14/-159) was found to be associated with low levels of total serum IgE and a decreased prevalence of a positive skin prick tests and self-reported hay fever and allergic rhinitis in adults [41]. Positive skin prick test results in children have been associated with increased soluble CD14 levels and low levels of total serum IgE [42]. Other studies found no association between the CD14/-159 genotype and asthma [39, 43], allergic rhinoconjunctivitis or skin prick test positivity [39].

Thus, the TLR4 polymorphism may be associated with the susceptibility of atopic children to develop asthma, but not with the development of atopic reactivity and IgE production. The CD14 polymorphism seems to have a more modest clinical importance. Nevertheless, these polymorphisms can provide key therapeutic targets to modulate LPS signaling in humans, and can perhaps modify protection against allergic disorders.

Therapeutic Challenges Related to Innate Immunity

The gastrointestinal microflora provides a possible intervention site for therapeutic strategies in allergy prevention and treatment. It might be an important counter-regulator of the Th2-skewed immune system in early life

because of its potentially antiallergic processes like the promotion of Th1 immunity, the generation of TGF- β and the induction of oral tolerance and IgA production [44]. The gastrointestinal tract, which is sterile at birth, becomes colonized in the first months and years of life. This is determined by an interplay between genetic factors, environmental factors, diet and disease [45]. Moreover, the maturation of the immune system depends on the stimulus by the intestinal microbiota [45]. It was shown that the intestinal epithelial cells constitutively express TLRs [46, 47]. Changes in the overall pattern of commensals and pathogens in the intestinal microbial flora might be responsible for the increasing prevalence of allergic and inflammatory disorders [48, 49]. An altered bacterial colonization in young babies may lead to a persistent predominant Th2 immunity and later to the development of allergic diseases [50]. The development of atopic disorders is preceded by a reduced rate of intestinal colonization (in the neonatal intestinal microbiota of atopic patients the ratio of bifidobacteria to clostridia was reduced as compared to nonatopic patients) [44, 51]. The question arises whether manipulation of the intestinal microbiota can play a role in improving the health of the host or even in preventing atopic disorders.

Probiotics are live microbial feed supplements which beneficially affect the host by improving its intestinal microbial balance [52, 53]. They are able to stimulate our immune system and to augment the production of TGF- β and IL-10. They can also restore alterations in allergic individuals, such as an increased intestinal permeability, defective gut-specific IgA responses, and an insufficient normal gastrointestinal barrier function [44]. They have already proven to be safe at an early stage and to be effective in several nonallergic diseases [53, 54]. Some studies also point to a possible beneficial effect in allergic disorders [55, 56], e.g. in the treatment of atopic dermatitis in children [57]. Perinatal administration of the probiotic strain *Lactobacillus GG* is effective in the primary prevention of atopic eczema in at-risk children [44, 58]. These beneficial effects are strain-specific [45], and extend beyond infancy [59]. However, no conclusions can be drawn on the effect of probiotics in respiratory allergic diseases typically presenting at an older age. Furthermore, the long-term effects and the exact mechanisms by which probiotics work, remain unclear.

Another therapeutic target in the prevention and treatment of allergic disorders is the use of CpG oligonucleotides. Bacterial DNA differs from human DNA by its unmethylated CpG sequences and interacts in a highly specific way with TLR9 present in humans on B cells and

plasmacytoid dendritic cells [21, 60]. Activation of these cells by CpG leads to maturation, differentiation and proliferation of natural killer cells, T cells, monocytes and macrophages. This leads to the secretion of pro-inflammatory and Th1 cytokines and chemokines (IL-1, 6, 18, TNF, IFN- γ and IL-12) and contributes to the survival of humans [60]. CpG oligodeoxynucleotides (CpG ODN) resemble bacterial DNA and are capable of stimulating our innate immune system by eliciting IFN- γ and IL-12 and reducing the production of IL-4. Studies have shown that administration of CpG ODN during or even after the sensitization phase reduced or eliminated allergic asthma [61]. These effects were long-lasting and even more pronounced if CpG ODN was directly coupled to the allergen [60, 62]. While these findings point to a valuable use of CpG DNA in the understanding of the innate immune system and in the immunotherapy of allergy, many questions, such as the safety profile of CpG administration, remain unanswered.

Conclusion

The hygiene hypothesis has been postulated as a possible explanation for the increasing prevalence of allergic disorders in Western society. The interactions between our genetic system and several environmental factors determine our individual immunological response. A change of one or several of these factors seems to lead to a different immunological pattern and may consequently be responsible for the increase in allergic diseases.

The innate immune system has an impact on both the development and progression of allergic disorders. Depending on the time, type and intensity of innate triggering and the underlying genetic susceptibility of an individual, the innate response can both suppress and enhance allergic inflammation and clinical expression. However, the exact mechanisms remain unclear. Gene polymorphisms of innate receptors as well as triggers of the innate immune system (LPS, CpG DNA and probiotics) may provide key therapeutic targets, especially in the development of allergen-specific immunotherapy and vaccination regimens, for the prevention and treatment of allergic disorders.

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