

The role of innate immunity in the pathogenesis of asthma

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Purpose of review

The increase in prevalence of allergic diseases, in particular of asthma, poses great difficulties to healthcare institutions in industrialized countries. According to the hygiene hypothesis, a linkage exists between exposure towards microbes in early childhood and the development of allergies; however, the original view that stimulation of the host's immune system by microbes exclusively protects against the development of allergies and asthma has been challenged by recent studies, which are summarized in this review.

Recent findings

Recent studies in mice revealed that infection with a series of microbes in the context of allergen exposure enhances antigen sensitization. Furthermore, in studies using purified toll-like receptor ligands and live bacteria, innate immune activation via MyD88 has been shown to be a causative factor in sensitization. The view that innate immune activation, under circumstances yet to be elucidated, may be a causative factor for the development of allergies is backed by epidemiologic data showing a protective effect of genetic variants, which impair toll-like receptor signaling.

Conclusion

Recent studies in mice suggest that innate immune stimulation via microbes or their compounds, in a dose and time-dependent manner, can cause allergen sensitization, and this notion has lately been supported by epidemiologic data.

Keywords

asthma, dendritic cells, innate immunity, toll-like receptors

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Introduction

The assumption that immune responses elicited by microbial compounds protect against the development of allergic diseases, including asthma, has been raised with the hygiene hypothesis, introduced by Strachan in 1989. In fact, epidemiologic data showed that exposure to Lipopolysaccharide (LPS), a cell wall compound of Gram-negative bacteria acting as a ligand for Toll-like receptor (TLR)-4, protects against atopic diseases [1]. However, these results were challenged by another study [2], and there is increasing evidence that under certain circumstances, activation of the immune system by various pathogens and their partial structures may be a causative factor in the development of allergies and asthma.

Impact of viral respiratory infection on asthma: evidence from models in mice

The view that viral infections in early childhood protect from atopic diseases has been supported by epidemiologic studies [3]. However, other studies concluded that events of viral infections in early childhood are positively correlated with the development of asthma in later life [4]. On an experimental basis, the latter data were backed

by a study [5] published in the late 1990s showing that first-time inhalational exposure towards ovalbumin (OVA) in the context of respiratory infection with respiratory syncytial virus (RSV) enhances allergic airway inflammation in mice after reexposure to OVA, indicating that infection may act as an adjuvant on antigen sensitization. Recently, innate immune activation rather than adaptive immune responses, elicited by respiratory infection with Sendai virus, a mouse virus bearing similarities with RSV, was shown to promote airway inflammation [6•]. Furthermore, RSV-infected epithelial cells, in contrast to healthy epithelial cells, fail to inhibit antigen-specific T-cell responses [7•], providing a potential explanation for the enhancement of antigen airway inflammation described before. Regarding rhinoviruses, a recent study [8•] suggested an augmenting effect on allergic airway sensitization as well, but mechanisms involved still need to be defined.

A potential role of dendritic cells in allergic airway sensitization during viral infection was demonstrated by two independent studies [9,10] on the impact of infection with influenza virus in an asthma model. Here, mice sensitized and challenged with OVA and infected with influenza virus showed enhanced airway inflammation after OVA challenge as compared with

noninfected controls. However, Marsland *et al.* [10] infected mice after intraperitoneal sensitization to OVA, thus providing a model resembling exacerbation of preexisting asthma, whereas Dahl *et al.* [9] infected mice with influenza virus prior to intraperitoneal sensitization with OVA, leaving open the question of whether the effect observed was due to enhanced sensitization or increased responses to respiratory challenge. However, these studies further supported the view that the original hygiene hypothesis, stating that infections generally inhibit atopic diseases, may bear an oversimplification [11]. Furthermore, Marsland *et al.* [10,12] provided evidence that timing of infection related to allergen exposure is an important factor when deciding whether exacerbation of airway inflammation takes place or not, as they only observed an enhancing effect of infection on allergen-induced responses during early stages of infection, whereas at later time points, suppressive effects were observed [12]. These data also underlined the importance of dendritic cells in the process of allergen sensitization proposed earlier [13], as the effects observed were linked to upregulation of costimulatory molecules and trafficking of dendritic cells [9,10].

Toll-like receptor ligands and the development of asthma

Although the studies mentioned above provided a linkage between infection and development or exacerbation of asthma, the question of whether the effects observed were due to specific actions of the microbe, or due to immune responses directed against it, was largely left unanswered. On the basis of epidemiologic data suggesting a causative role for TLR-mediated activation in the development of asthma [2,14], Eisenbarth *et al.* [15] tested the potential adjuvant effect of LPS in an asthma model in mice. Using a model of asthma based on inhalation of OVA for sensitization and challenge, they found that OVA alone, after removal of contaminating LPS, was not able to induce airway sensitization. However, if LPS was added at 100 ng per mouse, there was Th2-biased antigen sensitization, resulting in production of OVA-specific IgE titers and eosinophilic airway inflammation after reexposure to OVA in the absence of LPS, which was absent in mice deficient in TLR4, the cellular receptor for LPS [15]. In a following study [16] performed in the same laboratory, the OVA sensitization induced by LPS was linked to activation of MyD88, a molecule crucially involved in signal transduction induced by TLRs. Furthermore, LPS-mediated activation of dendritic cells could be demonstrated as a hallmark event in sensitization using adoptive transfer experiments [16]. Of importance, particularly in the light of controversial epidemiologic data on LPS exposure and atopic diseases, in the original report as well as in a recent study on dose-dependent effects of LPS on asthma development,

higher doses of LPS did not induce allergic airway sensitization but caused Th1-biased immune responses with a predominance of anti-OVA IgG_{2a} antibodies instead [15,17]. Furthermore, a role for tumor necrosis factor (TNF) in Th2-biased sensitization towards OVA by low-dose LPS could be established, whereas Th1-biased responses induced by high levels of LPS were dependent on IFN γ [17]. According to a recent study [18^{••}], dose-dependent effects of TLR activation on allergen sensitization is not a feature specific for TLR4, as pulmonary TLR3 activation by double-stranded RNA (dsRNA), at low doses, initiated Th2-biased adaptive immune responses as compared with Th1-biased responses when high doses were applied.

Although these studies provided important insights on how the activation of the innate immune system may or may not cause allergen sensitization, a linkage of these findings with respiratory infection was lacking. Apart from viral infections, respiratory infections with certain bacteria, in particular *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, clinically resulting in atypical courses of pneumonia, have been repeatedly associated with development of asthma [19]. Using a model of respiratory infection with *C. pneumoniae* in mice, it was recently shown that mice exposed to human serum albumin, an LPS-free antigen, during infection with *C. pneumoniae*, were sensitized, resulting in eosinophilic airway inflammation after reexposure [20^{••}]. This sensitization was dependent on MyD88 signaling, providing a direct link-

Figure 1 Studies on dose-dependent effects of immune stimulation in mice

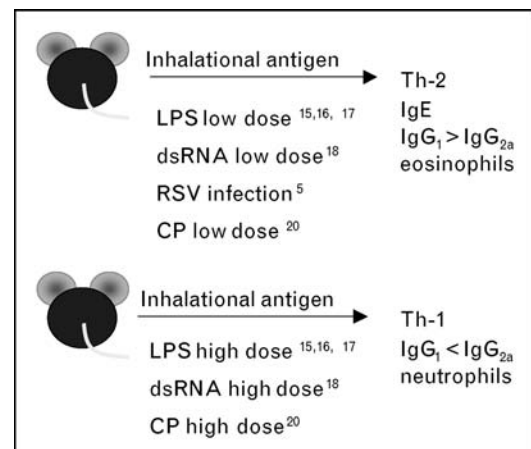


Fig. 1 summarizes data obtained so far with models based on simultaneous inhalational exposure of mice towards antigen and an inflammatory stimulus. In four independent studies, dose-dependent effects of immune stimulation were demonstrated, resulting in Th2-biased antigen-specific responses when mice were treated with low doses of LPS or infected with low doses of a pathogen. At higher doses, the same stimuli (with the exception of RSV) induce Th1-biased immune responses. Numbers refer to references of the corresponding studies in the text. LPS, lipopolysaccharide; RSV, respiratory syncytial virus.

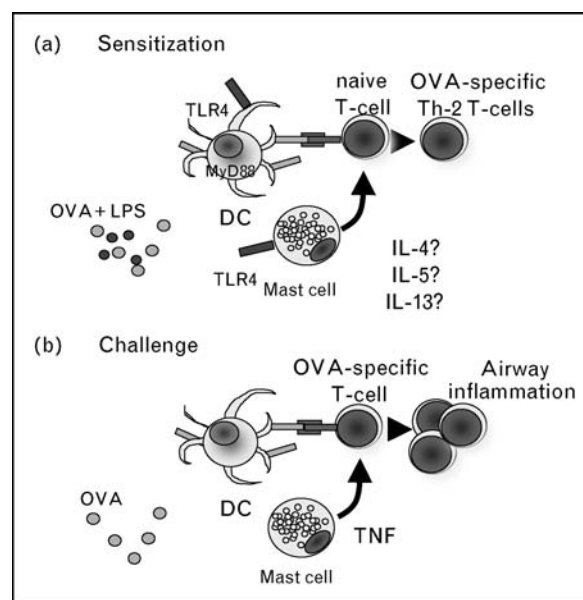
age to studies [16,20**] performed with purified TLR ligands. Furthermore, sensitization could be induced by adoptive transfer of dendritic cells infected *in vivo* or *in vitro*, thus being in line with two previous studies [20**,21*] showing that bone marrow-derived dendritic cells infected with *C. muridarum* exhibit a Th2-related phenotype. Most importantly, sensitization towards albumin was dependent on timing of infection [20**], as shown by Marsland *et al.* [12] in the context of influenza infection, as well as on dose of bacteria, as demonstrated for LPS and dsRNA ([15,17], Fig. 1).

Induction of airway sensitization by toll-like receptors: a role for mast cells?

As described above, dendritic cells have been repeatedly shown to mediate allergic airway sensitization in the lung [9,10,16,20**]. One important issue in this context is why activation of dendritic cells in the lung predisposes towards the generation of Th2-biased rather than Th1-biased immune responses. Although this question can be answered in part by the dose and time-dependent effects discussed above, data are also available that infection with the same pathogen, in that case *Leishmania*, causes Th2-biased adaptive immune responses in the lung, but Th1-biased immunity after subcutaneous injection [22]. Mast cells are mostly known as effector cells causing allergic reactions through IgE-triggered release of mediators, including histamine. However, more recent data show that they are also important regulators of adaptive immune responses by releasing a wide variety of cytokines, including IL-4, IL-5 and interferons (IFNs) [23]. Mast cells have been shown to be crucial for the development of asthma after inhalation of OVA [24], and recently the release of thymic stromal lymphopoietin by lung epithelial cells during injury was demonstrated to promote allergen sensitization through activation of mast cells [25**]. Mast cells have been shown to express TLRs [26], raising the question whether mast cell-derived cytokines, released after TLR ligation, may act on dendritic cells to shape immune responses and may cause allergic airway sensitization in the lung after simultaneous exposure towards antigen and LPS (Fig. 2).

This hypothesis was tested in a mouse model employing mast cell-deficient Kit^{W/Kit^{Wv}} mice [27]. Here, intranasal exposure of wild type, but not mast cell-deficient mice, towards OVA and LPS caused airway sensitization, and this could be restored by transferring wild type, but not TLR4-deficient mast cells [27], thus supporting the notion that TLR4-mediated activation of mast cells creates a Th2 bias (Fig. 2). However, the mechanism proposed by Nigo *et al.* [27], with LPS-induced release of eotaxin by mast cells promoting eosinophil influx, does not sufficiently explain these findings, as this would not explain how mast cell-dependent sensitization occurs. In

Figure 2 Proposed role of mast cells in allergic airway inflammation



(a) During the first encounter of an allergen in the presence of an inflammatory stimulus, activation of TLRs on mast cells induces the secretion of cytokines, including IL-4, IL-5 and IL-13. This may cause an environment favoring Th2-biased immune responses. Note that an interaction of mast cells with dendritic cells has not been demonstrated yet. (b) During antigen challenge, mast cell-derived TNF promotes airway inflammation. Note that the stimulus inducing TNF release by mast cells has not been elucidated yet. TLR, toll-like receptor; TNF, tumor necrosis factor.

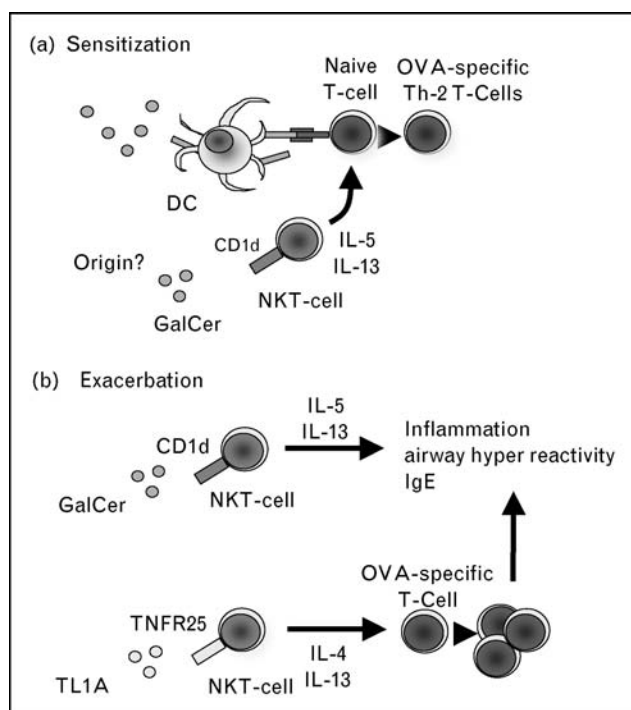
the context of allergen exposure in previously sensitized mice, however, recent reports linked mast cell-derived TNF to enhanced airway inflammation in OVA sensitization and challenge models ([28,29*,30*,31**], Fig. 2), and these effects were attributed to TNF acting during the antigen challenge ([29*,31**], Fig. 2). However, whether engagement of TLRs plays a causative role here remains to be elucidated. At this point, it has to be noted that a recent study [32**] provided evidence that c-Kit signaling in dendritic cells induced by allergens contributes to the induction of antigen-specific Th2-biased immune responses, being the first report on the impact of c-Kit on allergies in cells other than mast cells. Therefore, results obtained from c-Kit-deficient mice should be critically evaluated in the future.

Toll-like receptor-independent innate immune responses in allergen sensitization

Natural killer T (NKT) cells are population-expressing receptors found on natural killer (NK) cells as well as on T cells. They recognize glycolipid antigens via a receptor complex consisting of cluster of differentiation 1 (CD1d) and an invariant T-cell antigen receptor (TCR). A connection of glycolipid-mediated activation of NKT cells

and asthma was shown in a hallmark study [33] published in 2003. Here, NKT cell-deficient mice did not develop allergic airway inflammation after sensitization and challenge [33], and this was attributed to the release of IL-4 and IL-13 and subsequent induction of antigen-specific Th2 T cells (Fig. 3). The involvement of NKT cells in allergic inflammation was supported by studies in asthmatic patients showing an increased frequency of NKT cells within the sinus mucosa [34], and a subpopulation of NKT cells strongly secreting IL-5 after glycolipid stimulation was recently identified in human probands ([35], Fig. 3). Furthermore, a recent study [36] suggests that the activation of the newly described TNF receptor superfamily 25 (TNFRSF25) on NKT cells is a crucial event in the activation of Th2-biased antigen-specific CD4⁺ T cells, resulting in airway inflammation (Fig. 3). However, apart from the ability to influence T-cell responses, activation of NKT cells by glycolipid ligands may directly promote allergic airway inflammation in the absence of allergen independent of T cells ([37], Fig. 3).

Figure 3 Proposed effect of glycolipid-mediated activation of natural killer T cells in allergic airway inflammation



(a) During antigen sensitization, activation of NKT cells by glycolipids causes the release of cytokines, in particular IL-5 and IL-13, resulting in Th2-biased antigen-specific immune responses. Note that the physiological counterpart of synthetic glycolipids used in experimental settings has not been clearly elucidated yet. (b) Independently of T cells and antigen, glycolipid-mediated activation of NKT cells, probably through the release of IL-5 and IL-13, induces airway inflammation and an increase in total IgE. Another mechanism by which NKT cells may contribute to inflammation is the TNFRSF25-mediated release of IL-5 and IL-13, which promotes Th2 T cell-mediated inflammation. NKT cell, natural killer T cell; TNFRSF25, TNF receptor superfamily 25.

Thus, there is evidence that NKT cells may be a contributing factor towards allergen sensitization shaping T-cell responses, but may also be involved in exacerbation of asthma in previously sensitized individuals. So far, the identity of naturally occurring glycolipid antigens interacting with NKT cells has not been clarified. However, recent studies [38,39] showed that glycolipid antigens isolated from *Borrelia burgdorferi* [38] activate NKT cells [39], raising the question of whether microbes may influence immune responses through interaction of yet undefined ligands with NKT cells, providing a further link between infection and allergic airway inflammation.

A role for Nalp3 in the induction of adaptive immune responses

In 2003, crystalline uric acid was identified as a danger signal other than TLR ligands, causing innate immune responses [40]. Later on, Nalp3, a member of the Nod-like receptor family, which interacts with apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and caspase-1 to form a complex referred to as inflammasome [41], was identified as the receptor interacting with uric acid [42]. On the basis of these findings, three laboratories recently showed that aluminium adjuvants, which are widely used in mouse models to induce Th2-biased immune responses, induce T-cell responses through activating Nalp3 as well, via a pathway independent of MyD88 signaling [43–45]. This may be, at least in part, due to release of uric acid through the interaction of alum with dendritic cells [46]. However, data published so far did not identify a Th1 bias in Nalp3-deficient mice after stimulation with alum adjuvants, thus requiring further studies to elucidate whether Nalp3 is specifically involved in the generation of Th2-biased immune responses.

Evidence from epidemiologic studies on genetic variants

As indicated above, epidemiologic data on the effect of innate immune activation on the incidence of asthma published so far yield conflicting results. On the basis of the initial findings on an association of endotoxin exposure and asthma [1,2], a series of studies investigating the potential effect of genetic variants of molecules involved in innate immunity was performed. Initial reports on the association of the Asp299Gly polymorphism of TLR4, which is found at a frequency of approximately 10% among whites and impairs cytokine induction by LPS [47], failed to detect any association with asthma [48,49] or found a positive correlation with the prevalence of asthma among children [50]. However, more recent studies [51,52], including a study focusing on severity of disease, found an association of these variants with milder forms of asthma. Regarding

TLR2, a receptor interacting with lipoproteins and lipopeptides, an initial study [53] correlated the presence of a polymorphism within the promoter sequence, TLR2/–16934, with decreased frequency of asthma in children living in rural areas. However, conclusions on the molecular mechanisms involved could not be drawn due to lack of information on the effect of this variant, in particular whether it impairs or enhances TLR2-mediated cellular activation. Recent studies [54,55*] on polymorphisms within the amino acid sequence of two coreceptors of TLR2, TLR1 and TLR6, which impair TLR-mediated signal transduction, supported the view that activation of TLR2 may be causative factor for the development of asthma by indicating a protective effect for these variants. Finally, inactivating mutations within IL-1 receptor-associated kinase M (IRAK-M), a negative regulator of TLR signaling, were recently identified in individuals diagnosed for early asthma [56**]. Thus, there is evidence that a state of hyperactivation of the innate immune system predisposes for development of asthma, which is in line with reports showing that an impaired immune response, as caused by TLR variants, has protective effects.

Conclusion

As summarized here, there is ample evidence in particular derived from studies in mice that innate immune activation can act as a causative factor in the development of asthma. Meanwhile, it has been convincingly shown that TLR activation in the context of initial antigen encounter can cause allergic airway sensitization. These data are available for LPS and dsRNA as well as *C. pneumoniae* as a bacterial pathogen, and there is evidence that comparable mechanisms may apply for RSV and influenza virus as well. In most of these studies, dendritic cells were shown to play a crucial part in the sensitization process. Whether microbe-mediated activation of dendritic cells alone is sufficient, or whether a third player is involved to create a bias towards a Th2 type immune response favoring allergic inflammation, still needs to be clarified. As shown in this study, NKT cells as well as mast cells may play an important role, in the latter case potentially through TLR-mediated activation, but this notion needs further investigation.

It has also to be noted that data on these issues are still controversial, regarding both experiments in mice as well epidemiological studies. This may be at least in part due to the strong dose and time-dependent effects of innate immune stimulation on allergic inflammation. However, recent epidemiologic data support the hypothesis that activation of the innate immune system may predispose for the development of asthma and other allergic diseases. In general, conflicting data may arise because of the strong dose and time dependency of effects of innate

immune stimulation on antigen sensitization, which have lately been demonstrated by several independent studies. Thus, the overall interpretation that innate immunity enhances allergic sensitization would bear an oversimplification as well as the original hygiene hypothesis supporting the opposite view. In fact, evidence is increasing that innate immunity has an impact on allergies, with the outcome depending on timing and extent of activation.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 82–83).

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