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The role of innate immunity in the pathogenesis of asthma: evidence for the involvement of Toll-like receptor signaling

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Infectious diseases have a major impact on both the development and the severity of asthma. The rise in incidence of asthma in industrialized countries over the last decades has been attributed to increased hygiene standards as well as the concomitant usage of antibiotics, which together lower the incidence of infections. Although this point of view is supported by both clinical studies and experimental approaches in mice, an increasing body of evidence suggests that certain infectious diseases may predispose for the development of asthma, thus challenging the 'hygiene hypothesis' in its classical form. Toll-like receptors (TLRs) are centrally involved in orchestrating immune responses towards various micro-organisms. Because of this, it is tempting to speculate that signaling through TLRs may be involved in mechanisms provoking Th1- or Th2-biased immune responses and may, therefore, be an important factor in either preventing or promoting allergic airway disease. This review summarizes clinical and experimental data from mouse models focused on the impact of TLR-signaling on allergic asthma.

Keywords: Allergy, asthma, endotoxin, mouse models, polymorphisms, Toll-like receptors

The hygiene hypothesis

During recent decades, asthma has become a major epidemic affecting up to 32% of the population in Western industrialized countries, and making a substantial impact on morbidity rates.^{1,2} Such a dramatic rise in incidence within a relatively short period of time cannot be attributed to genetic determinants alone. Hence, environmental factors are thought to act as major risk factors for the development of asthma. The inverse relationship between infectious and atopic diseases in Western countries has paralleled decreasing rates of serious infections due to

both, increased hygiene standards, and the expanded availability of antibiotics. This relationship between infection and allergic diseases has led to the formation of the hygiene hypothesis,^{3,4} and more recent studies attribute this relationship to a shift of balance between effector T-cell subtypes towards Th2-helper cells in the absence of early exposure towards microbes.⁵ Certain infectious agents (*e.g.* hepatitis A virus) can actively skew the balance between Th1- and Th2-helper T-cells towards the Th1 subtype.⁶ Furthermore, epidemiological studies on a potential association between exposure towards microbial compounds and the prevalence of atopic diseases indicate an inverse relationship between the presence of bacterial lipopolysaccharide (LPS) and the development of asthma in children.^{7,8} However, recent data indicate that certain pathogens (*e.g.* respiratory syncytial virus [RSV], influenza virus, *Mycoplasma pneumoniae* and *Chlamydomphila pneumoniae*) can either promote the development of asthma or exacerbate pre-existing asthma.^{9–14} The paradigm that microbial compounds inhibit allergic diseases has also been challenged

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by results from experimental and epidemiological studies that show a positive correlation between LPS exposure and allergy incidence and severity.^{15,16} These observations suggest that, while the increase in the prevalence of asthma may be generally linked to a decrease in infections, certain respiratory pathogens may actually enhance the development of asthma.

Evidence from studies on genetic variants and expression patterns of TLRs

The TLR family of pattern recognition receptors is a major component of the innate immune system.^{17,18} Ligation of all TLRs (except TLR3) leads to the activation of a MyD88-dependent signal transduction cascade.^{19–21} Interaction of LPS with its receptor TLR4 can also activate a distinct signaling pathway involving TRAM and TRIF (TIR domain-containing adaptor inducing interferon- β [IFN- β]), leading to the induction of IFN- α and IFN- β via activation of IRF-3 (interferon regulatory factor-3).^{22–24} Activation of TLR3 by double-stranded RNA activates only the TRIF-dependent pathway and does not involve MyD88.¹⁹ On the other hand, activation of TLR9 by CpG DNA and binding of single stranded RNA to TLR7 (in mice) or TLR8 (in humans) both induce IFN- α and IFN- β release via MyD88.^{21,25}

Genetic variants of Toll-like receptors and their impact on infectious, immunological and cardiovascular diseases have been extensively studied.²⁶ In particular, the Asp299Gly polymorphism of human TLR4 co-segregates with Thr399Ile and is present among about 8% of individuals in Caucasian populations.^{26,27} This variant was discovered in the course of a study to determine why some individuals do not develop symptoms of bronchoconstriction after inhalation of LPS.²⁷ The presence of this receptor variant was associated with decreased inflammation both *in vivo* and *in vitro*. This observation prompted a series of studies investigating the potential impact of this polymorphism on atopic diseases including asthma. An initial report found no evidence for any association of either the Asp299Gly or other variants of TLR4 with the incidence of atopic diseases;²⁸ however, subsequent studies indicated that TLR4-variants may indeed affect risk or severity of asthma. For example, in a population of British Caucasians, Asp299Gly was associated with severity, but not incidence, of asthma.²⁹ Similarly, a study of Swedish children found a 4-fold higher prevalence of asthma among Asp299Gly carriers, and in a Finnish population, the presence of Asp299Gly in combination with C/T –590 of the IL-4 gene also showed an association with asthma.³⁰ In contrast to these results, Asp299Gly of TLR4 was found most frequently in a group consisting of children with less severe asthma

in a Turkish population.³¹ Taken together, there is evidence that TLR4 variants may influence the pathogenesis of atopy and asthma. The sometimes contradictory results published so far may be due to the fact that endotoxin content in the patients' environment may not have been addressed.³² In addition to TLR4, the impact of polymorphisms of the TLR2 receptor on the pathogenesis of atopic diseases has been also been investigated. Here, a polymorphism within the promoter region of TLR2 (–16934) was identified as a major risk factor for the development of asthma among children living on farms.³⁰ Furthermore, the Ser249Pro polymorphism of TLR6, which forms heterodimers with TLR2 to recognize diacylated lipopeptides, was associated with a lower risk of asthma in African Americans.³³

Additional evidence linking TLR-function with susceptibility towards the development of allergic diseases was presented by Lauener *et al.*³⁴ in 2002. Based on the observation that children raised on farms during the first year of life are at lower risk to develop allergic diseases,³⁵ expression levels of TLR2, TLR4 and CD14 in samples derived from these probands were determined using RT-PCR. Here, significantly higher levels of CD14 and TLR2, but not TLR4, were found among children living on farms compared to controls.³⁴ Interestingly, these data are consistent with *in vitro* studies showing that LPS up-regulates expression of CD14 and TLR2 by mouse macrophages but does not affect TLR4.³⁶ Although direct links between up-regulation of TLRs, exposure towards microbial products, and the pathogenesis of allergic diseases have yet to be established, these data support the notion that TLR signaling may be involved in the development of allergic sensitization and/or the severity of allergic symptoms. However, no final conclusion regarding the mechanism leading to disease in an individual with impaired sensing of TLR-ligands or increased expression of TLRs can be drawn as yet.

Experimental evidence for a linkage of TLR-signaling and antigen sensitization

Epidemiological studies suggest that the TLR4 ligand LPS can either promote or inhibit the development of asthma in humans.^{7,8,16} Experimental evidence supporting a role for TLR4 in promoting allergic airway sensitization and airway inflammation after antigen challenge was obtained in studies with C3H/HeJ mice, which lack functional TLR4.³⁷ Surprisingly, when sensitized with ovalbumin (OVA) in alum by intraperitoneal injection, these mice exhibited less pronounced airway inflammation after intranasal OVA challenge compared to control wild-type mice.³⁸ The authors concluded that the effect of TLR4 was caused by a disturbed maturation of the immune system due to diminished recognition of commensal microbes.³⁸

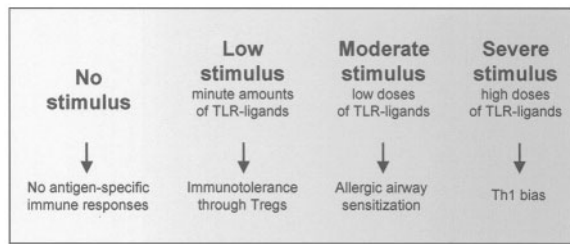


Fig. 2. Dose dependency of the effects of LPS on allergic sensitization. Published data suggest that moderate doses of LPS administered together with an antigen induce Th2-biased antigen sensitization, but high levels of LPS induce Th1 responses that counteract allergy development. However, some data suggest that LPS can also induce antigen-specific Tregs.

However, a subsequent study was able to establish a direct link between LPS exposure and allergic sensitization employing a model based on intranasal immunization of BALB/c mice with LPS-depleted OVA, with or without added LPS.³⁹ Here, mice only developed airway inflammation if LPS was added to OVA during the sensitization period.³⁹ The induction of pulmonary Th2-cell responses was dose dependent (Fig 1). LPS doses 1000-fold higher than those promoting Th2-cell responses induced a Th1-cell bias and prevented allergic airway sensitization.³⁹ Thus, these data provide evidence that LPS promotes development of asthma, and thus support

epidemiological evidence for a link between endotoxin exposure and asthma in children.¹⁶ This study also provided a potential explanation for seemingly conflicting results.^{7,8} A more recent study based on these observations showed that LPS effects on sensitization to OVA required MyD88.⁴⁰ The allergy-promoting effect of LPS in murine models of asthma was independently verified.⁴¹ The fact that LPS, a ligand that usually induces Th1-biased inflammatory responses,⁴² triggers Th2 responses is surprising, but not unprecedented. For example, *Leishmania* infection can cause Th2-biased inflammation in the lung but, in other body compartments, the pathogen causes Th1-biased immune responses.⁴³ Hence, modulation of TLR-dependent immune responses in the lungs may differ from those of other organs (Fig. 2A,B).

Tri-acylated lipopeptide is a ligand for heterodimers of TLR2 and TLR1. When applied in a murine model that involves subcutaneous injection of OVA, tri-acylated lipopeptide promoted antigen sensitization.⁴⁴ Mice sensitized with OVA in the presence of lipopeptide exhibited elevated OVA-specific IgE and IgG₁ levels and enhanced airway resistance after metacholine challenge compared to controls that received OVA alone. Thus, evidence indicates that signaling through TLR2 or TLR4 can promote the development of asthma. In contrast, subcutaneous application of a TLR9 ligand (CpG-DNA) during antigen

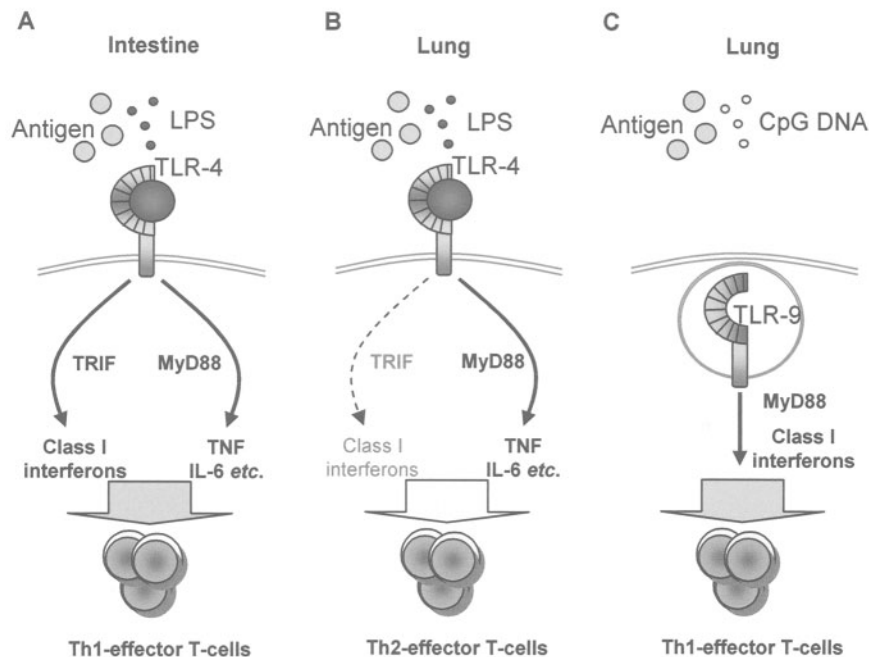


Fig. 1. Effects of TLR-activation on development and shaping of adaptive immune responses in the lung. In most body compartments (*i.e.* the intestine), LPS-mediated activation of TLR4 favors the generation of a Th1-biased immune response. However, in the lung LPS-mediated activation can cause a Th2-biased allergic sensitization. This may be explained by down-modulation of TRIF-dependent signal transduction and the lack of LPS-mediated interferon induction in the lung, by mechanisms that remain unclear. In such a setting, the diminished induction of class I interferons by LPS would promote Th2-biased adaptive immune responses. However, MyD88-dependent class I interferon induction by TLR9 ligands is still intact, which may explain why CpG-DNA can shape a Th1-biased immune response in the lung.

sensitization inhibited the development of allergic responses following antigen-challenge.⁴⁴ Furthermore, intraperitoneal administration of TLR3 and TLR7 ligands led to decreased OVA-sensitization.⁴⁵ Therefore, different TLRs affect antigen sensitization in divergent ways, possibly due to the fact that activation of TLR9, TLR3, and TLR7 strongly induces class I interferons, which may counteract Th2-biased immune responses.²¹

Effects of TLR-signaling in the previously sensitized host

Studies done on the impact of isolated TLR-ligands, in particular LPS, on the severity of asthma in already sensitized individuals point towards a positive impact of endotoxin exposure on the severity of clinical features of asthma.^{46,47} In particular, it was observed that endotoxin concentration in bio-aerosols present in the environment exhibited a much stronger positive correlation with asthma severity compared to the concentration of common allergens.^{46,47} However, studies on the effect of LPS and other TLR ligands during antigen challenge in murine models have yielded conflicting results: both intranasal and systemic administration of LPS to OVA-sensitized mice during antigen challenge was reported to inhibit airway inflammation.^{48,49} In line with these data, i.p. administration of a TLR2 ligand during intranasal antigen challenge also inhibited allergic responses,⁴⁴ and intranasal administration of a combined TLR2/4 ligand had similar effects.⁵⁰ In contrast, a recent study addressing the issue of endotoxin contamination of commercially available OVA-preparations found that LPS can enhance airway inflammation in a murine model that employed TLR4-deficient mice.⁵¹ Furthermore, intranasal administration of peptidoglycan, in contrast to CpG, also enhanced allergic airway inflammation.⁵² In sum, the evidence from animal studies does not conclusively answer the question whether, or how, TLR ligands may enhance or ameliorate allergic asthma. During sensitization, LPS can induce diametrically opposed immune responses depending on the dose used, with low levels promoting Th2 responses and high levels promoting Th1 responses.³⁹ Therefore, it is tempting to speculate that comparable mechanisms apply for immune reactions in previously sensitized individuals as well, and may also account for the discrepancies observed in animal studies.

In contrast to the controversy on the effect of LPS on asthma, clinical data unequivocally demonstrate a connection between both viral and bacterial respiratory infections and exacerbations of asthma, but the mechanisms leading to deterioration of disease are not completely understood.^{53,54} Recent studies investigating the role of influenza virus infection on asthma in murine models have provided experimental proof-of-principle

that influenza enhances airway inflammation in mice sensitized and challenged with OVA.^{55–57} Activation of antigen-presenting cells, in particular airway dendritic cells (DCs), appears to be a hallmark event in the mechanisms involved in allergic sensitization.^{55–57} TLR signaling critically impacts DC maturation and activation,^{58,59} but whether DC activation during allergic sensitization is mediated by TLRs responding to viral antigens^{60,61} or endogenous ligands released during infection remains to be clarified.

Evidence for an impact of TLR activation prior to antigen contact on subsequent antigen-specific responses

In addition to data presented above showing effects of immune activation simultaneously with antigen exposure on allergic responses, there is also evidence that exposure to microbes **prior** to the encounter with an antigen may influence risk for development of allergies towards those same antigens upon subsequent encounter. Based on the inverse relationship between endotoxin exposure and allergies,^{7,8} studies in mice were performed to assess the effect of LPS on the development of Th2-biased responses towards OVA. Here, LPS administration to mice prior to antigen sensitization significantly suppressed allergic responses, including IgE levels and eosinophilia.^{62,63} This was associated with general bias towards Th1 responses, and could be blocked by antibodies against interleukin-12 (IL-12).⁶³ The inhibitory effect of LPS exposure on subsequent antigen sensitization was also demonstrated in newborn mice⁶⁴ and in a model of prenatal exposure.⁶⁵ Thus, there is evidence that TLR4-dependent signaling **prior** to the first encounter with an antigen prevents subsequent allergic sensitization in a non-antigen specific manner, possibly through the induction of a Th1-immune phenotype.

A potential role for TLR signaling in immunotolerance induction

According to the hygiene hypothesis, infections in early life inhibit development of atopic diseases by shaping the immune system towards a Th1-biased phenotype. However, there is also an increasing body of evidence that tolerization against antigens present in the environment is an important step in preventing allergic responses. Recent studies on Foxp3-deficient mice connect these observations with the development of CD25⁺Foxp3⁺ regulatory T cells (Tregs).^{66,67} Tregs have been extensively studied in relation to autoimmune diseases and organ transplant rejection, and several lines of evidence suggest that Tregs also play a major role in the regulation of allergic diseases. One study reported that

bee workers who are regularly stung possess antigen-specific Tregs that secrete IL-10 upon antigen stimulation *in vitro*.⁶⁸ Also, there is a strong negative correlation between the presence of IL-10 secreting T-cells and clinical symptoms of atopy in probands sensitized towards a variety of allergens.⁶⁷ Although these data have not yet been independently confirmed, they provide the first link between the presence of antigen-specific Tregs and protection from allergic responses. Data from mouse studies indicate that naturally occurring, non antigen-specific Tregs that develop in the thymus also inhibit the development of asthma.⁶⁹ Interestingly, development and function of these Tregs have been linked to TLR2 signaling. Total numbers of Tregs are lower in TLR2-deficient mice than in wild-type controls, and activation of Tregs by TLR2 ligands attenuates inhibitory effects in models of infectious disease.^{70,71} Whether these interactions of TLR-ligands with Tregs play a role in allergic disease as well remains to be studied.

The exact mechanism by which antigen-specific Tregs that secrete IL-10 are generated remains unknown. Induction of tolerance by intranasal exposure of mice towards OVA prior to systemic sensitization generates antigen-specific Tregs, but this depends on induction of IL-10 by DCs exposed to antigen in the absence of pro-inflammatory stimuli.^{72,73} However, these studies did not address potential endotoxin contamination of the antigen preparations used. Later studies found that OVA preparations free of any endotoxin contamination are more effective in sensitizing naïve mice compared to pure preparations.⁷⁴ These findings indicate that LPS at trace concentrations possesses inhibitory properties. It is not yet entirely clear whether these inhibitory effects are due to LPS or possibly other contaminants. However, a recent study reported that intranasal treatment of mice with OVA in the presence of heat-killed *Listeria monocytogenes* led to the induction of antigen-specific Tregs,⁷⁵ which suggests that low-level TLR stimulation may exert tolerizing effects. Interestingly, a recent report indicates LPS can induce IL-10 producing Tregs,⁷⁶ but the potential impact on allergic diseases has not yet been studied.

TLR-ligands as a therapeutic approach in asthma patients

Protective effects of TLR ligands in the context of asthma and allergy were first demonstrated for microbial DNA in 1999,⁷⁷ shortly after the initial report on immunoreactive properties of non-methylated CpG-DNA in a murine sepsis model.⁷⁸ Vaccination of mice with plasmid-delivered antigen (in contrast to expressed and purified antigen) led to a Th1-biased, antigen-specific immune response that was induced by plasmid DNA.⁷⁷ Later studies in genetic knock-out mice indi-

cated that TLR9 is the receptor for non-methylated CpG-DNA. The strong induction of Th1 responses by CpG-DNA, mediated by TLR9-dependent release of class I interferons, was also demonstrated to inhibit allergic inflammation in murine asthma models.^{79,80} The fact that TLR9 ligands can redirect allergic responses is most likely related to the strong induction of class I interferons, in particular by plasmacytoid dendritic cells (pDCs) that are involved in immune defense against viral and intracellular pathogens.^{81,82} However, it should also be noted that there are differences in TLR9 expression patterns between mice and primates. Primate TLR9 is exclusively expressed on B cells and pDCs, but mice also express TLR9 on myeloid dendritic cells and monocytes.⁸¹ Consistent with this concept, a recent study showed relatively weak immune responses towards vaccines in the presence of CpG-motifs in rhesus macaques.⁸³ However, repeated injection of CpG ligands causes severe immunopathology in mice.⁸⁴ Thus, it may not be appropriate to extrapolate murine results on the safety and effectiveness of TLR9-directed therapies to humans. However, initial clinical trials on an allergy vaccine based on coupling of a CpG-DNA motif to ragweed demonstrated anti-allergic effects of treatment in patients, which indicates a redirection of established antigen-specific Th2 responses to Th1 responses.^{85,86} Furthermore, TLR9 ligands are not only effective as adjuvants in treating allergies, but also exert effects on allergic responses when administered alone without an antigen.⁸¹ In fact, inhaled CpG monotherapy can prevent allergic reactions in experimental models in both mice⁸⁷ and primates.⁸⁸

CONCLUSIONS

Clinical, epidemiological, and experimental studies have elucidated the relationship between exposure of an individual towards TLR ligands and the development of atopic disease. The original hygiene hypothesis predicts that exposure towards microbial stimuli should protect against asthma and other diseases related to a Th2-immune phenotype. Since first reports on LPS and atopy supported this notion, it was concluded that TLR ligands generally act against the development of Th2-biased immune reactions. However, recent data indicate that this notion should be re-evaluated. Results obtained from experimental mouse models are still conflicting, perhaps due to differences in protocols. The same is true for studies investigating the potential impact of TLR variants on development and course of allergic diseases, which suggest a role of TLRs in asthma. Still, some aspects of the interaction of TLR ligands with the immune system to induce, shape or inhibit atopic disease are strongly supported by published data.

Studies performed in mice clearly show that LPS promotes the development of antigen-specific IgE and Th2-biased T-cells.^{39–41} One study also found a dose-dependent effect of LPS, with higher doses inhibiting development of asthma.³⁹ These results may help reconcile conflicting results obtained from epidemiological studies on the association of endotoxin with atopy,^{8,16} which could be due to differing levels of LPS exposure to subjects participating in these studies. On the other hand, data obtained from mouse studies on the effect of TLR9 signaling are consistent with clinical studies. These mouse studies clearly show that CpG-DNA induces type I interferons, can prevent the pathogenesis of asthma and may, therefore, be potentially useful as a therapeutic agent in established disease.⁸¹ These findings appear to be contradictory since LPS, like CpG-DNA, also strongly induces type I interferons. However, activation of TLR4 induces interferon via a pathway involving TRIF and IRF3, but TLR9 induces interferons via MyD88 and the transcription factor IRF-7.²⁵ Induction of interferons in the lung by LPS, in contrast to CpG DNA or single-stranded RNA, may be impaired, probably due to inhibition of TRIF-dependent signal transduction by unknown mechanisms (Fig. 2A–C). Thus, the opposing effects of TLR4 and TLR9/TLR7 ligands may be due to different cytokine patterns induced by these ligands. This could indicate that activation of the MyD88-dependent signal transduction pathway could favor the development of Th2-biased immune responses.⁴⁴

Currently available data on the effect of TLR stimulation during allergic sensitization and/or exacerbation of allergic disease are still controversial. Therefore, this issue will await further investigation. Both the intensity of TLR stimulation and the cytokine pattern induced after activation may be important contributors. In contrast to the divergent effects of TLR-activation observed during sensitization/exacerbation, studies investigating the effect of TLR activation prior to antigen exposure unequivocally showed an inhibition of subsequent symptoms of allergies. These observations suggest that the same stimulus can exert divergent effects on severity of atopic diseases depending on the timing of exposure. This may explain why the same viral infection can protect against development of allergic disease when encountered during early life, yet still exacerbate established allergic disease.^{89,90} Another question is whether LPS or other TLR ligands can also induce immunotolerance. This issue has not yet been directly addressed in murine models of asthma and allergy, but published data suggest that trace contaminants of LPS may prevent antigen sensitization,⁷⁴ and that LPS can also induce Tregs.⁷⁶ Further studies will be needed to provide clear answers to these important questions.

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REFERENCES

1. Eder W, Ege MJ, von Mutius E. The asthma epidemic. *N Engl J Med* 2006; **355**: 2226–2235.
2. Beasley R, Crane J, Lai CK, Pearce N. Prevalence and etiology of asthma. *J Allergy Clin Immunol* 2000; **105**: S466–S472.
3. Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; **299**: 1259–1260.
4. Strachan DP. Family size, infection and atopy: the first decade of the 'hygiene hypothesis'. *Thorax* 2000; **55** (Suppl 1): S2–S10.
5. von Mutius E, Pearce N, Beasley R *et al.* International patterns of tuberculosis and the prevalence of symptoms of asthma, rhinitis, and eczema. *Thorax* 2000; **55**: 449–453.
6. McIntire JJ, Umetsu SE, Akbari O *et al.* Identification of Tapr (an airway hyperreactivity regulatory locus) and the linked Tim gene family. *Nat Immunol* 2001; **2**: 1109–1116.
7. Gereda JE, Leung DY, Thatayatikom A *et al.* Relation between house-dust endotoxin exposure, type 1 T-cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; **355**: 1680–1683.
8. Braun-Fahrlander C, Riedler J, Herz U *et al.* Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; **347**: 869–877.
9. Johnston SL, Martin RJ. *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*: a role in asthma pathogenesis? *Am J Respir Crit Care Med* 2005; **172**: 1078–1089.
10. Murray CS, Simpson A, Custovic A. Allergens, viruses, and asthma exacerbations. *Proc Am Thorac Soc* 2004; **1**: 99–104.
11. Germ JE, Busse WW. Relationship of viral infections to wheezing illnesses and asthma. *Nat Rev Immunol* 2002; **2**: 132–138.
12. Hahn DL, Dodge RW, Golubjatnikov R. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. *JAMA* 1991; **266**: 225–230.
13. Sirmatel F, Ustunsoy H, Sirmatel O, Akdemir I, Dikensoy O. The relationship between *Chlamydia pneumoniae* seropositivity and peripheral vascular diseases, acute myocardial infarction and late-onset asthma. *Infection* 2003; **31**: 367–368.
14. Normann E, Gnarp J, Wettergren B, Janson C, Wickman M, Nordvall L. The association between *Chlamydia pneumoniae* antibodies and wheezing in young children and the influence of sex. *Thorax* 2006; **61**: 1054–1058.
15. Eldridge MW, Peden DB. Allergen provocation augments endotoxin-induced nasal inflammation in subjects with atopic asthma. *J Allergy Clin Immunol* 2000; **105**: 475–481.
16. Park JH, Gold DR, Spiegelman DL, Burge HA, Milton DK. House dust endotoxin and wheeze in the first year of life. *Am J Respir Crit Care Med* 2001; **163**: 322–328.
17. Medzhitov R. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001; **1**: 135–145.
18. Uematsu S, Akira S. Toll-like receptors and innate immunity. *J Mol Med* 2006; **84**: 712–725.
19. Hoebe K, Janssen EM, Kim SO *et al.* Upregulation of costimulatory molecules induced by lipopolysaccharide and double-stranded RNA occurs by Trif-dependent and Trif-

- independent pathways. *Nat Immunol* 2003; **4**: 1223–1229.
20. Beutler B. The Toll-like receptors: analysis by forward genetic methods. *Immunogenetics* 2005; **57**: 385–392.
21. Kawai T, Akira S. TLR signaling. *Semin Immunol* 2007; **19**: 24–32.
22. Yamamoto M, Sato S, Hemmi H *et al*. TRAM is specifically involved in the Toll-like receptor 4-mediated MyD88-independent signaling pathway. *Nat Immunol* 2003; **4**: 1144–1150.
23. Yamamoto M, Sato S, Hemmi H *et al*. Role of adaptor TRIF in the MyD88-independent Toll-like receptor signaling pathway. *Science* 2003; **301**: 640–643.
24. Hoebe K, Du X, Georgel P *et al*. Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. *Nature* 2003; **424**: 743–748.
25. Kawai T, Sato S, Ishii KJ *et al*. Interferon- α induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. *Nat Immunol* 2004; **5**: 1061–1068.
26. Schroder NW, Schumann RR. Single nucleotide polymorphisms of Toll-like receptors and susceptibility to infectious disease. *Lancet Infect Dis* 2005; **5**: 156–164.
27. Arbour NC, Lorenz E, Schutte BC *et al*. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000; **25**: 187–191.
28. Raby BA, Klimecki WT, Laprise C *et al*. Polymorphisms in Toll-like receptor 4 are not associated with asthma or atopy-related phenotypes. *Am J Respir Crit Care Med* 2002; **166**: 1449–1456.
29. Yang IA, Barton SJ, Rorke S *et al*. Toll-like receptor 4 polymorphism and severity of atopy in asthmatics. *Genes Immun* 2004; **5**: 41–45.
30. Adjers K, Karjalainen J, Pessi T, Eklund C, Hurme M. Epistatic effect of TLR4 and IL4 genes on the risk of asthma in females. *Int Arch Allergy Immunol* 2005; **138**: 251–256.
31. Sackesen C, Karaaslan C, Keskin O *et al*. The effect of polymorphisms at the CD14 promoter and the TLR4 gene on asthma phenotypes in Turkish children with asthma. *Allergy* 2005; **60**: 1485–1492.
32. Eder W, von Mutius E. Genetics in asthma: the solution to a lasting conundrum? *Allergy* 2005; **60**: 1482–1484.
33. Tantisira K, Klimecki WT, Lazarus R *et al*. Toll-like receptor 6 gene (TLR6): single-nucleotide polymorphism frequencies and preliminary association with the diagnosis of asthma. *Genes Immun* 2004; **5**: 343–346.
34. Lauener RP, Birchler T, Adamski J *et al*. Expression of CD14 and Toll-like receptor 2 in farmers' and non-farmers' children. *Lancet* 2002; **360**: 465–466.
35. Riedler J, Braun-Fahrlander C, Eder W *et al*. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; **358**: 1129–1133.
36. Matsuguchi T, Musikacharoen T, Ogawa T, Yoshikai Y. Gene expressions of Toll-like receptor 2, but not Toll-like receptor 4, is induced by LPS and inflammatory cytokines in mouse macrophages. *J Immunol* 2000; **165**: 5767–5772.
37. Poltorak A, He X, Smirnova I *et al*. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* 1998; **282**: 2085–2088.
38. Dabbagh K, Dahl ME, Stepick-Biek P, Lewis DB. Toll-like receptor 4 is required for optimal development of Th2 immune responses: role of dendritic cells. *J Immunol* 2002; **168**: 4524–4530.
39. Eisenbarth SC, Piggott DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, Toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen. *J Exp Med* 2002; **196**: 1645–1651.
40. Piggott DA, Eisenbarth SC, Xu L *et al*. MyD88-dependent induction of allergic Th2 responses to intranasal antigen. *J Clin Invest* 2005; **115**: 459–467.
41. Nigo YI, Yamashita M, Hirahara K *et al*. Regulation of allergic airway inflammation through Toll-like receptor 4-mediated modification of mast cell function. *Proc Natl Acad Sci USA* 2006; **103**: 2286–2291.
42. Agrawal S, Agrawal A, Doughty B *et al*. Different Toll-like receptor agonists instruct dendritic cells to induce distinct Th responses via differential modulation of extracellular signal-regulated kinase-mitogen-activated protein kinase and c-Fos. *J Immunol* 2003; **171**: 4984–4989.
43. Constant SL, Lee KS, Bottomly K. Site of antigen delivery can influence T cell priming: pulmonary environment promotes preferential Th2-type differentiation. *Eur J Immunol* 2000; **30**: 840–847.
44. Redecke V, Hacker H, Datta SK *et al*. Activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. *J Immunol* 2004; **172**: 2739–2743.
45. Sel S, Wegmann M, Sel S *et al*. Immunomodulatory effects of viral TLR ligands on experimental asthma depend on the additive effects of IL-12 and IL-10. *J Immunol* 2007; **178**: 7805–7813.
46. Michel O, Kips J, Duchateau J *et al*. Severity of asthma is related to endotoxin in house dust. *Am J Respir Crit Care Med* 1996; **154**: 1641–1646.
47. Schwartz DA, Thorne PS, Yagla SJ *et al*. The role of endotoxin in grain dust-induced lung disease. *Am J Respir Crit Care Med* 1995; **152**: 603–608.
48. Rodriguez D, Keller AC, Faquim-Mauro EL *et al*. Bacterial lipopolysaccharide signaling through Toll-like receptor 4 suppresses asthma-like responses via nitric oxide synthase 2 activity. *J Immunol* 2003; **171**: 1001–1008.
49. Hollingsworth JW, Whitehead GS, Lin KL *et al*. TLR4 signaling attenuates ongoing allergic inflammation. *J Immunol* 2006; **176**: 5856–5862.
50. Revets H, Pynaert G, Grooten J, De Baetselier P. Lipoprotein I, a TLR2/4 ligand modulates Th2-driven allergic immune responses. *J Immunol* 2005; **174**: 1097–1103.
51. Jung YW, Schoeb TR, Weaver CT, Chaplin DD. Antigen and lipopolysaccharide play synergistic roles in the effector phase of airway inflammation in mice. *Am J Pathol* 2006; **168**: 1425–1434.
52. Yamamoto K, Kawamura I, Ito J, Mitsuyama M. Modification of allergic inflammation in murine model of rhinitis by different bacterial ligands: involvement of mast cells and dendritic cells. *Clin Exp Allergy* 2006; **36**: 760–769.
53. Mallia P, Johnston SL. How viral infections cause exacerbation of airway diseases. *Chest* 2006; **130**: 1203–1210.
54. Martin RJ. Infections and asthma. *Clin Chest Med* 2006; **27**: 87–98, vi.
55. Dahl ME, Dabbagh K, Liggitt D, Kim S, Lewis DB. Viral-induced T helper type 1 responses enhance allergic disease by effects on lung dendritic cells. *Nat Immunol* 2004; **5**: 337–343.
56. Marsland BJ, Kopf M, Le Gros G. Viral infection and allergy. *Nat Immunol* 2004; **5**: 865; author reply 865–866.
57. Marsland BJ, Scanga CB, Kopf M, Le Gros G. Allergic airway inflammation is exacerbated during acute influenza infection and correlates with increased allergen presentation and recruitment of allergen-specific T-helper type 2 cells. *Clin Exp Allergy* 2004; **34**: 1299–1306.
58. Michelsen KS, Aicher A, Mohaupt M *et al*. The role of Toll-like receptors (TLRs) in bacteria-induced maturation of murine dendritic cells (DCs). Peptidoglycan and lipoteichoic acid are inducers of DC maturation and require TLR2. *J Biol Chem* 2001; **276**: 25680–25686.
59. Schnare M, Barton GM, Holt AC, Takeda K, Akira S, Medzhitov R. Toll-like receptors control activation of adaptive immune responses. *Nat Immunol* 2001; **2**: 947–950.

60. Pauligk C, Nain M, Reiling N, Gerns D, Kaufmann A. CD14 is required for influenza A virus-induced cytokine and chemokine production. *Immunobiology* 2004; **209**: 3–10.
61. Marshall-Clarke S, Tasker L, Buchatska O *et al.* Influenza H2 haemagglutinin activates B cells via a MyD88-dependent pathway. *Eur J Immunol* 2006; **36**: 95–106.
62. Gerhold K, Blumchen K, Bock A *et al.* Endotoxins prevent murine IgE production, T(H)2 immune responses, and development of airway eosinophilia but not airway hyperreactivity. *J Allergy Clin Immunol* 2002; **110**: 110–116.
63. Gerhold K, Blumchen K, Franke A, Stock P, Hamelmann E. Exposure to endotoxin and allergen in early life and its effect on allergen sensitization in mice. *J Allergy Clin Immunol* 2003; **112**: 389–396.
64. Wang Y, McCusker C. Neonatal exposure with LPS and/or allergen prevents experimental allergic airways disease: development of tolerance using environmental antigens. *J Allergy Clin Immunol* 2006; **118**: 143–151.
65. Gerhold K, Avagyan A, Seib C *et al.* Prenatal initiation of endotoxin airway exposure prevents subsequent allergen-induced sensitization and airway inflammation in mice. *J Allergy Clin Immunol* 2006; **118**: 666–673.
66. Lin W, Truong N, Grossman WJ *et al.* Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *J Allergy Clin Immunol* 2005; **116**: 1106–1115.
67. Akdis M, Verhagen J, Taylor A *et al.* Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med* 2004; **199**: 1567–1575.
68. Akdis CA, Blesken T, Akdis M, Wuthrich B, Blaser K. Role of interleukin 10 in specific immunotherapy. *J Clin Invest* 1998; **102**: 98–106.
69. Lewkowich IP, Herman NS, Schleifer KW *et al.* CD4⁺CD25⁺ T cells protect against experimentally induced asthma and alter pulmonary dendritic cell phenotype and function. *J Exp Med* 2005; **202**: 1549–1561.
70. Suttmoller RP, den Broek MH, Kramer M *et al.* Toll-like receptor 2 controls expansion and function of regulatory T cells. *J Clin Invest* 2006; **116**: 485–494.
71. Liu H, Komai-Koma M, Xu D, Liew FY. Toll-like receptor 2 signaling modulates the functions of CD4⁺ CD25⁺ regulatory T cells. *Proc Natl Acad Sci USA* 2006; **103**: 7048–7053.
72. Tsitoura DC, Blumenthal RL, Berry G, Dekruyff RH, Umetsu DT. Mechanisms preventing allergen-induced airways hyperreactivity: role of tolerance and immune deviation. *J Allergy Clin Immunol* 2000; **106**: 239–246.
73. Akbari O, DeKruyff RH, Umetsu DT. Pulmonary dendritic cells producing IL-10 mediate tolerance induced by respiratory exposure to antigen. *Nat Immunol* 2001; **2**: 725–731.
74. Watanabe J, Miyazaki Y, Zimmerman GA, Albertine KH, McIntyre TM. Endotoxin contamination of ovalbumin suppresses murine immunologic responses and development of airway hyper-reactivity. *J Biol Chem* 2003; **278**: 42361–42368.
75. Stock P, Akbari O, Berry G, Freeman GJ, Dekruyff RH, Umetsu DT. Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. *Nat Immunol* 2004; **5**: 1149–1156.
76. den Haan JM, Kraal G, Bevan MJ. Lipopolysaccharide induces IL-10-producing regulatory CD4⁺ T cells that suppress the CD8⁺ T cell response. *J Immunol* 2007; **178**: 5429–5433.
77. Spiegelberg HL, Broide D, Tighe H, Roman M, Raz E. Inhibition of allergic inflammation in the lung by plasmid DNA allergen immunization. *Pediatr Pulmonol Suppl* 1999; **18**: 118–121.
78. Sparwasser T, Miethke T, Lipford G *et al.* Bacterial DNA causes septic shock. *Nature* 1997; **386**: 336–337.
79. Kline JN, Waldschmidt TJ, Businga TR *et al.* Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *J Immunol* 1998; **160**: 2555–2559.
80. Jain VV, Kitagaki K, Businga T *et al.* CpG-oligodeoxynucleotides inhibit airway remodeling in a murine model of chronic asthma. *J Allergy Clin Immunol* 2002; **110**: 867–872.
81. Krieg AM. Therapeutic potential of Toll-like receptor 9 activation. *Nat Rev Drug Discov* 2006; **5**: 471–484.
82. Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol* 2005; **23**: 275–306.
83. Hartmann G, Marschner A, Viveros PR *et al.* CpG oligonucleotides induce strong humoral but only weak CD4⁺ T cell responses to protein antigens in rhesus macaques *in vivo*. *Vaccine* 2005; **23**: 3310–3317.
84. Heikenwalder M, Polymenidou M, Junt T *et al.* Lymphoid follicle destruction and immunosuppression after repeated CpG oligodeoxynucleotide administration. *Nat Med* 2004; **10**: 187–192.
85. Creticos PS, Schroeder JT, Hamilton RG *et al.* Immunotherapy with a ragweed-toll-like receptor 9 agonist vaccine for allergic rhinitis. *N Engl J Med* 2006; **355**: 1445–1455.
86. Simons FE, Shikishima Y, Van Nest G, Eiden JJ, HayGlass KT. Selective immune redirection in humans with ragweed allergy by injecting Amb a 1 linked to immunostimulatory DNA. *J Allergy Clin Immunol* 2004; **113**: 1144–1151.
87. Jain VV, Businga TR, Kitagaki K, George CL, O'Shaughnessy PT, Kline JN. Mucosal immunotherapy with CpG oligodeoxynucleotides reverses a murine model of chronic asthma induced by repeated antigen exposure. *Am J Physiol* 2003; **285**: L1137–L1146.
88. Fanucchi MV, Schelegle ES, Baker GL *et al.* Immunostimulatory oligonucleotides attenuate airways remodeling in allergic monkeys. *Am J Respir Crit Care Med* 2004; **170**: 1153–1157.
89. Papadopoulos NG, Psarras S, Manoussakis E, Saxoni-Papageorgiou P. The role of respiratory viruses in the origin and exacerbations of asthma. *Curr Opin Allergy Clin Immunol* 2003; **3**: 39–44.
90. Papadopoulos NG, Kalobatsou A. Respiratory viruses in childhood asthma. *Curr Opin Allergy Clin Immunol* 2007; **7**: 91–95.