Update on Toll-like Receptor Ligands and Allergy: Implications for Immunotherapy

Anthony A. Horner, MD

Corresponding author
Anthony A. Horner, MD
Departments of Medicine and Pediatrics and The Sam and Rose Stein Institute for Aging, University of California, San Diego, 9500 Gilman Drive, Mail code 0663, La Jolla, CA 92093-0663, USA.
E-mail: ahorner@ucsd.edu

Current Allergy and Asthma Reports 2006, **6:**395–401
Current Science Inc. ISSN 1529-7322

Innate responses to microbes are mediated in large part via toll-like receptors (TLRs) that recognize a diverse family of ligands produced by viruses, bacteria, and fungi. Great effort has been directed toward translating this knowledge into the development of therapies for the prevention and treatment of diseases, including those fueled by allergic (Th2-biased) hypersensitivities. In this review, we consider the ways in which ligands for different TLRs influence the allergic phenotype. In addition, an update on safety and efficacy data from clinical trials of allergic patients treated with TLR9 ligand-based interventions is provided. Finally, recent experimental results that help elucidate how ambient TLR ligand exposures influence allergic risk and their relevance to the development of TLR ligand-based therapeutics are discussed. Investigations presented within this opinion paper suggest that several TLR ligands could have clinical utility in the treatment of allergic diseases, whereas other TLR ligands appear less attractive, as they facilitate development of Th2-biased hypersensitivities in murine studies.

Introduction

Understanding of the molecular basis for innate immune recognition of microbial pathogens has increased dramatically in the past decade. Epithelial cells, mononuclear and polymorphonuclear immunocytes, and many other cell types have been found to express pre-formed pattern recognition receptors that identify potential pathogens[1,2••,3]. These receptors interact with microbial components and are often referred to as pathogen-associated molecular patterns (PAMPs). As originally defined, PAMPs were thought to meet certain criteria including 1) their expression by microbes but not mammalian cells, 2) conservation of structure across a wide range of pathogens, and 3) the capacity to stimulate innate immunity. However, although the term PAMP has been accepted into the vocabulary of immunology, it should be noted that PAMPs are not unique to pathogens, as they are also produced by microbes that do not cause disease. TLRs have been identified as having an important role in the detection of PAMPs and in host protection against microbial infections. As a caveat, TLR ligands are not readily produced by mammalian cells under physiologic conditions, but under conditions of tissue inflammation, oxidative stress, and/or necrosis, molecules with TLR stimulatory activities (heat shock proteins, modified RNA and DNA species, and potentially others) appear to be generated and released by dying cells [2••,4•].

At least 11 mammalian TLRs have been identified [2••,5], although not all are expressed in all species. TLR2 interacts with peptidoglycan (PGN), a constituent of cell walls that is associated with essentially all bacterial species, with the exception of *Chlamydia* and *Mycoplasma*. TLR2 also interacts with additional molecules associated with gram-positive bacteria, mycobacteria, and fungi. TLR3 responds to double-stranded RNA and may also respond to select species of single-stranded RNA. TLR4 recognizes most species of LPS. TLR5 is activated by bacterial flagellin. TLR7 (mouse and human) and TLR8 (human) respond to synthetic imidazoquinolones, as well as several single-stranded RNA sequences of viral origin. TLR9 is activated by DNA sequences that are rare in mammalian genomes but common in the genetic material of bacteria, fungi, and DNA viruses. TLR11 (mice) recognizes an undefined PAMP associated with uropathogenic bacteria and a profilin-like protein associated with *Toxoplasma gondii*. Certain TLRs (ie, TLR1, TLR2, and TLR6) are also recruited into the phagosome where they heterodimerize with other TLRs [2••]. Such interactions between different TLRs may serve to expand the variety of PAMPs that can be recognized by this family of proteins and, therefore, the number of microbes to which the innate immune system can respond.

Signaling pathways activated by TLR ligands lead to nuclear factor (NF)-κB and mitogen-activated protein kinase (MAPK) activation, cytokine gene transcription (eg, interleukin [IL]-6, IL-10, and IL-12), costimulatory molecule expression (eg, CD40 and B7), and other cellu-

lar responses [2••,6]. Interestingly, despite their common use of several signaling molecules, qualitative differences exist in the cellular responses elicited by ligands for different TLRs. It is believed that collateral signaling pathways are responsible for this diversity. For example, molecular studies have found that TIR domain-containing adapter protein (TIRAP), TRIF-related adapter molecule (TRAM), and TIR domain-containing adapter protein inducing interferon (IFN)-β (TRIF) participate in signaling through only a limited number of TLRs [2••]. Moreover, ligands for some TLRs (ie, TLR3, TLR4, TLR7, TLR9) induce type 1 IFN production in responsive cells by upregulating expression of IFN-regulatory factors, whereas ligands for TLR2 and potentially other TLRs do not [2••,7]. Furthermore, unlike all other TLRs investigated, MyD88 does not participate in signaling through TLR3 [2••,5]. Further discussion is beyond the scope of this paper; however, it is becoming increasingly clear that in addition to TLRs, which recognize PAMPs at the cell surface or within lysosomes and endosomes, additional cytoplasmic receptors exist for the detection of PAMPs, including members of the nucleotide-binding oligomerization domain (NOD), caspase activation and recruitment domain (CARD), and helicase families of proteins [2••,5].

TLR4 and Allergy

In the laboratory, lipopolysaccharide (LPS) has been found to have divergent effects on the allergic phenotype. LPS (TLR4) has long been known to promote murine immunoglobulin (Ig)E synthesis in vitro. In vivo, LPS has been found to inhibit the development of allergen-specific IgE and asthma if delivered within 6 days of allergen sensitization [8]. Paradoxically, in these same studies, LPS exposure after this period led to an exaggeration of the asthmatic phenotype. Eisenbarth et al. [9] have also reported on the seemingly schizoid immunologic activities of LPS. These investigators found that LPS induced either Th1- or Th2- biased responses to intranasally (IN) coadministered ovalbumin (OVA) when delivered at high or low doses, respectively; low-dose LPS vaccinations also primed mice for development of the asthmatic phenotype. In our own unpublished studies, we have confirmed that at low and high doses, LPS functions as a Th2- and Th1-biasing adjuvant, respectively.

The paradox of LPS's bipolar adjuvant activities has yet to be adequately explained, but experiments conducted by Kaisho et al. [10] with MyD88-deficient dendritic cells may be relevant to its understanding. These investigators demonstrated that MyD88-deficient dendritic cells activated with LPS induced Th2-cell differentiation, whereas LPS-activated, wild-type dendritic cells induced Th1-cell differentiation. Considered in context with the studies of Eisenbarth et al., these observations suggest that a MyD88-independent LPS signaling pathway induces Th2-cell development and is responsive to LPS at low concentrations, whereas the MyD88-dependent pathway dominates at high LPS concentrations and favors Th1-cell development.

TLR9 and Allergy

Viral and bacterial DNA have inherent immunostimulatory activities that are not shared with mammalian DNA [2••,5,11,12]. The molecular basis for this specificity lies in the high content of unmethylated CpG dideoxynucleotides found in microbial DNA, whereas these sequences are infrequent in mammalian genomes. In addition to microbial DNA, synthetic immunostimulatory sequence oligodeoxynucleotides (ISS-ODNs) activate TLR9, but murine and human homologs of the receptor appear to have unique flanking DNA sequence requirements [2••,5].

ISS-ODN has been found to induce an innate response dominated by cytokines (eg, IL-10, IL-12, type 1 IFNs, IFN-γ) that are known to inhibit the allergic phenotype [2••,5,12,13]. Moreover, mice injected with ISS-ODN have elevated serum cytokine levels for a week or longer [13]. As an allergen-independent therapeutic agent given to Th2-sensitized mice within hours of allergen challenge, ISS-ODN has proven effective in attenuating hypersensitivity responses associated with asthma, allergic conjunctivitis, and allergic rhinitis [11,12,14,15].

It is generally believed that the innate cytokine response to ISS-ODN plays a key role in its ability to rapidly attenuate allergic manifestations, but this may not be the only basis for its anti-allergic activities. We found that ISS-ODN inhibits leukotriene synthesis during the late-phase nasal hypersensitivity response and attenuates cellular production of leukotriene (LT)C_4 synthase, a key enzyme in the leukotrine synthesis pathway [14]. Additionally, we observed that ISS-ODN downregulates IL-4 receptor expression [16] and IL-4 responsiveness (STAT6 phosphorylation; Horner et al., Unpublished data). Hayashi et al. [17•] have further reported that ISS-ODN induces alveolar macrophages, pulmonary dendritic cells, and resident lung epithelial cells to produce high levels of indoleamine 2,3-dioxygenase (IDO), an enzyme that degrades tryptophan into immunoinhibitory metabolites. These investigators went on to demonstrate that ISS-ODN–mediated inhibition of Th2-biased airway hypersensitivity responses was at least partially IDO-dependent. In another published report, Hessel et al. [18] found that ISS-ODN inhibits effective antigen presentation to Th2 cells by lung antigen-presenting cells (APCs) and inhibits IgE-mediated Th2 cytokine release by lung-associated basophilic cells. These and other experimental results suggest that ISS-ODN attenuates the allergic phenotype by a variety of redundant and complementary mechanisms.

Allergen independent ISS-ODN therapy potently inhibits allergic hypersensitivity responses, but the effect may only be temporary. In studies by Broide et al. [15], Th2-sensitized mice receiving ISS-ODN had attenu-

ated airway allergen-challenge responses (experimental asthma) for up to 4 weeks. However, 8 weeks after ISS-ODN treatment, airway hypersensitivity responses were similar to those of Th2-sensitized mice receiving a control oligodeoxynucleotide. In a murine model of allergic rhinitis, we confirmed that allergen-independent ISS-ODN delivery protects Th2-sensitized mice from end-organ hypersensitivity responses for 4 but not 8 weeks (Horner et al., Unpublished data). Taken together, experimental evidence currently available suggests that although allergen-independent ISS-ODN delivery rapidly inhibits the activities of effector Th2 cells and other cell types participating in the hypersensitivity response, this intervention does not necessarily translate into the efficient re-education of the memory B and T cells that maintain allergen-specific hypersensitivities.

Applying the vaccination paradigm to its study, ISS-ODN has been shown to be a potent systemic and mucosal adjuvant, promoting long-lived and Th1-polarized adaptive responses in both animals and humans [12,19,20]. Moreover, allergen/ISS-ODN vaccination is reported to be protective in murine models of asthma and anaphylaxis, with antigen-specific responses being found to both prevent and reverse the Th2 polarized responses associated with allergen/alum sensitization [12,19,21]. Unlike the rapid, allergen-independent, but relatively short-lived Th2 inhibition elicited directly by ISS-ODN, the adaptive responses induced by allergen/ISS-ODN vaccination are believed to mature over weeks, to be allergen specific, and to imprint on memory lymphocytes and their subsequent responses to allergen encounter.

Allergen ISS-ODN Conjugates (AIC) and Allergy

Development of AICs began approximately 10 years ago, and ragweed-specific AIC (Amb A I AIC) is the first TLR ligand–based allergic disease intervention to make it to clinical trials. These modified allergens were found to be far more immunogenic than similar doses of allergens mixed with ISS-ODN in murine vaccination studies [22,23]. However, of potentially greater importance, oligodeoxynucleotide conjugation was found to render allergens resistant to binding by allergen-specific IgE. As a consequence, AIC challenge of Th2-sensitized mice elicited far weaker hypersensitivity responses than challenge with native allergen in models of asthma and anaphylaxis [19,22]. Furthermore, Amb A 1 AIC was more than 100-fold less allergenic than native ragweed allergen in end point dilution skin testing studies of ragweed-allergic patients [24]. Another clinical trial report demonstrated that after six weekly injections of Amb A 1 AIC, ex vivo ragweed-specific cytokine responses by peripheral blood mononuclear cells obtained from treated allergic patients were shifted away from Th2 and toward Th1 cytokine production [25]. Additionally, in the only efficacy trial published to date, ragweed-allergic patients treated with the six Amb A 1 AIC injection schedule were found to have attenuated nasal-allergen challenge responses after the ragweed season and several months after the final AIC injection [26••]. Moreover, although clinical improvement was not noted during the ragweed season immediately following AIC treatment, fewer respiratory symptoms were reported by patients during the following ragweed season. As yet unpublished results from a second Amb A 1 AIC clinical trial were presented by William Busse et al. at the 2006 American Academy of Asthma, Allergy, and Immunology meeting. The study results confirmed the efficacy of the six AIC injection schedule and the one-season delay in significant clinical improvement. However, a subset of AIC-treated patients receiving additional AIC booster injections prior to ragweed season two did no better than the placebo group and far worse than the group only receiving AIC injections before the first season of the clinical trial. These results were unanticipated and suggest that we still have much to learn about AIC immune modulation in allergic patients. Nonetheless, although questions of safety, optimal dosing, and efficacy remain, AIC and other ISS-ODN–based interventions continue to hold promise as therapies for the reversal of allergic hypersensitivities.

TLR2 and Allergy

Our laboratory has compared how PGN (TLR2) and ISS-ODN (TLR9) influence antigen-specific responses in murine vaccination studies [6]. Mice that are IN immunized with antigen and PGN were found to develop Th2-biased adaptive responses and were primed for Th2-biased airway hypersensitivity responses upon subsequent IN antigen challenge. In contrast, IN antigen/ISS-ODN vaccination induced Th1-polarized adaptive responses but failed to prime mice for allergen-specific hypersensitivity responses. Confirming our results, another TLR2 ligand (lipopeptide Pam-3-Cys) has been found to promote Th2 responses in immunized mice [27] and human Th2 cell development in in vivo cultures [28]. However, in previously Th2-sensitized mice, treatment with lipopeptide Pam-3-Cys has also been reported to inhibit allergen-specific airway hypersensitivity and Th2-cytokine responses, whereas allergen-specific IFN-γ responses were induced [29]. These discordant experimental results will require further investigation to be reconciled.

Although understanding is limited, the quality of innate responses elicited by TLR2 ligands may help explain their Th2 adjuvant activities. For example, LPS has been shown to elicit a far stronger IL-12 response (Th1 promoting) from dendritic cells than did TLR2 ligands, whereas TLR2 ligands were found to induce greater mast cell degranulation and release of Th2-promoting cytokines [30–32]. Likewise, we have observed that bone marrow–derived dendritic cells (BMDDCs) activated with PGN expressed higher levels of OX40L and produced more IL-6 (Th2-promoting) but less IL-12 than BMDDCs activated with

ISS-ODN [6]. Mechanistic studies additionally suggest that TLR2 agonist activation of extracellular signal-regulated kinase 1/2 dependent pathways may contribute to their Th2-adjuvant activities [28].

From a clinical perspective, atopic dermatitis patients are generally heavily colonized by TLR2-expressing bacteria (*Staphylococcus aureus*) and display evidence of Th2-biased immune dysregulation [33]. Moreover, *S. aureus* colonization and infections can precipitate atopic dermatitis flairs for which antibiotic therapies are often effective. Additionally, investigators have found that application of *S. aureus*-derived lipoteichoic acid (TLR2) to murine skin induces an eczematoid inflammatory response [34]. Given these observations and our knowledge of the immunologic activities of TLR2 ligands, it is reasonable to speculate that microbial interactions with TLR2 could contribute to the genesis of eczema and other allergic diseases.

Other TLRs

It remains to be determined whether TLR3, TLR5, and TLR7/8 ligands can significantly influence the allergic phenotype, but their immunomodulatory influence on adaptive immunity has been investigated to some extent. TLR3 ligands have been found to serve primarily as Th1-polarizing adjuvants [35]. As with TLR2 and TLR4 ligands, conflicting reports exist as to the influence of flagellin (TLR5) on adaptive immunity. It was originally described as a Th1 adjuvant, but more recent investigations suggest that highly purified flagellin might actually favor Th2-biased adaptive responses [36]. Additionally, ligands for TLR7 and TLR8 (ie, R848 and its derivatives) have consistently been described as Th1 adjuvants. Furthermore, R848 has been reported to inhibit Th2 effector cell cytokine production while promoting production of IFN-γ by human Th2 cells [37]. Along with other studies reviewed in this perspectives article, these investigations attest to the wide spectrum of immune responses elicited by ligands for the different TLRs.

TLRs, Ambient Exposures, and Allergic Risk

Because of the ubiquitous distribution of microbes in our environment, it has been suggested that TLR ligand exposures via the intestines, inspired air, and skin provide an important molecular link between microbes, immune development, and atopic risk [11,38,39]. Unfortunately, although both intellectually attractive and popular, this hypothesis is far from proven. The experimental evidence is considered further in the following sections.

Aeroallergen exposure is a clear prerequisite for the development and persistence of respiratory allergic diseases [40,41]. Nonetheless, whereas for some allergens (ie, cockroach and mites) higher exposure levels are associated with an increased sensitization risk, for other allergens, including those associated with animals, increased levels of exposure have paradoxically been associated with decreased atopic risk in several studies [40,41]. These and other observations suggest that aside from allergens themselves, other immunomodulatory elements within living environments influence the balance between immune homeostasis and dysregulation. In further support of this view, endotoxin (TLR4) has been reported to be present at higher concentrations in homes with regular exposures to animals than in homes without animal exposures [39,42]. Moreover, infants raised in homes with high ambient endotoxin levels have been found to be at low relative risk for developing allergic hypersensitivities in many, although not all, published reports [39,43].

In consideration of the apparent association between ambient endotoxin exposure levels and allergic risk, it is important to note that endotoxin-rich environments also generally contain elevated levels of other immunostimulatory microbial products. These include ligands for TLR2 and TLR9 [44,45]. Furthermore, several man-made pollutants have been found to promote development of allergic hypersensitivities [46]. Although much has been learned in recent years, the complexity of daily environmental exposures has hampered efforts to develop a comprehensive understanding of their impact on allergic risk. In this regard, we reasoned that direct study of the immunologic activities of unpurified but clinically relevant environmental samples might prove enlightening. In our initial paper, we demonstrated that sterile house dust extracts (HDEs) stimulate BMDDCs to upregulate expression of costimulatory molecules and produce IL-6 and IL-12p40 at high levels [47•] but little IL-12p70 (Horner et al., Unpublished observations). Moreover, the relative bioactivities of HDEs correlated with their endotoxin content. Additionally, HDE immunostimulatory activities were found to be partially dependent on TLR2, TLR4, and TLR9, and almost completely dependent on MyD88. These findings provide direct and compelling evidence that TLRs play a dominant role in mediating innate proinflammatory responses to particulates present in environments of daily living.

In more recent investigations, we assessed how airway exposures to HDEs influence adaptive responses to a co-delivered antigen (ovalbumin [OVA]) [48••]. In initial experiments, mice were IN immunized with OVA and HDE at three weekly intervals. Surprisingly, while the adjuvant potential of these HDEs varied (collected from 10 consecutive San Diego suburban homes), all IN OVA/HDE–immunized mice developed Th2-polarized adaptive responses and evidence of experimental asthma upon subsequent IN OVA challenge. As in previously described studies of innate immunity, the adjuvant activities of HDEs were found to be MyD88-dependent.

The results of the experiments discussed might be construed to suggest that many, if not all, living envi-

ronments intrinsically promote the development of Th2-biased airway hypersensitivities. However, these studies fell short of modeling environmental airway exposures in several ways. For example, endotoxin and other immunostimulatory elements are likely to be ubiquitous in inspired air [47•,49,50], suggesting that physiologic airway exposures should be fairly continuous, whereas in our experiments, mice were exposed to HDEs only once a week [48••]. Moreover, healthy human airways have relatively few neutrophils [51], whereas the dose of HDE used for weekly vaccinations induced a neutrophil-rich airway inflammatory response that was readily detected 24 hours later [48••].

Given the issues just discussed, additional experiments were conducted in which mice received three weekly IN OVA vaccinations, as in the original investigations, whereas HDEs were IN delivered 1) daily, at 1/7th the weekly immunization dose (noninflammatory to airways), beginning 7 days before the first and finishing with the last OVA immunization; 2) weekly with OVA; or 3) both [48••]. Interestingly, despite receiving the same total dose as mice treated with HDE on a weekly basis, OVA-immunized mice receiving daily low-dose HDE did not develop Th2-biased airway hypersensitivities. Furthermore, daily airway HDE exposures protected mice from the development of Th2-biased airway hypersensitivities with concurrent weekly IN OVA/HDE immunizations.

Recognizing that immunostimulatory elements are ubiquitous in inspired air [49,50] but that levels vary widely, these results suggest a new paradigm by which ambient exposures impact on airway immunity and allergic risk. According to this model, basal exposures to endotoxin and other immunostimulatory materials present in environments of daily living are generally not sufficient to provide adjuvant activity in the airways, but rather serve to attenuate innate responsiveness. However, periodic exposures to ambient air with high levels of immunostimulatory elements may provide sufficient adjuvant activity to induce a breakdown in allergen tolerance if a priori immunologic dampening by basal exposures is inadequate. These results also provide a novel rationale for considering the prophylactic use of daily low-dose TLR ligand–based therapies to prevent development of allergic hypersensitivities.

Conclusions

Relatively recently it has become apparent that TLRs play a key role in immunologic detection and innate responsiveness to microbes. Therefore, it is logical to consider whether pharmacologic and environmental exposures to TLR ligands might also influence the genesis and duration of allergic hypersensitivities. Experimental evidence in support of this view has been discussed extensively in this review.

Studies with ISS-ODN–based interventions have established their effectiveness in the prevention and reversal of Th2-polarized hypersensitivity states in mice. Additional results from placebo-controlled clinical trials suggest that Amb A 1 AIC may prove a safer and more effective immunotherapy reagent than unmodified ragweed allergens. However, until a large-scale clinical trial directly compares these reagents, this view will remain speculative. Nonetheless, given the limited efficacy of traditional immunotherapy reagents, if Amb A 1 AIC stands the test of time, this technology would be a medical breakthrough, as any aeroallergen or food allergen could be rendered more immunogenic and less allergenic by ISS-ODN conjugation.

Other studies discussed herein demonstrate that not all TLR ligands influence adaptive immunity in an analogous manner. Like ISS-ODN (TLR9), polyI:C (TLR3), and R848 (TLR7/8) have been found to be Th1 adjuvants. However, although conflicting reports exist, several TLR2 ligands and flagellin (TLR5) have been described as Th2 adjuvants, whereas LPS has been found to have both Th2- and Th1- adjuvant potential, depending on the dose used. Therefore, in addition to ISS-ODN, other nucleic acid derivatives that serve as TLR3 and TLR7/8 ligands could prove useful in developing novel immunotherapy strategies to treat allergic hypersensitivities, as they steer adaptive immunity away from Th2 polarization. In contrast, TLR2, TLR4, and TLR5 ligands appear far less promising as allergic disease therapeutics, due to their Th2-adjuvant potential.

Studies with HDEs demonstrated that much of their allergen-independent immunostimulatory activity is TLR-dependent. More surprising was the observation that HDEs have Th2 adjuvant activities. However, with low-dose daily delivery, HDEs also appear to have the capacity to desensitize mice to their adjuvant activities. Clearly, more study will be required to develop a complete understanding of how environmental exposures to the immunostimulatory contents of HDEs influence immune maturation and allergic risk. Nonetheless, from the perspective of drug development, these experiments and previously described experimental observations with LPS and Amb A 1 AIC, highlight the importance of considering both dose and dosing schedule in the development of TLR ligand–based therapeutics for allergic diseases.

Immunotherapy was shown to reverse allergic hypersensitivities almost 100 years ago. However, efficacy has always limited clinical utility of this therapeutic modality. During the past decade, select TLR ligands and ISS-ODN conjugated allergens have been found to have robust anti-allergic activities. It is hoped that during the next decade, our growing understanding of TLR ligands will lead to the development of a new generation of immunomodulatory therapeutics for the prevention and treatment of allergic hypersensitivities and their associated diseases.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance

1. Medzhitov R, Janeway CA Jr: **Decoding the patterns of self and nonself by the innate immune system.** *Science* 2002, **296:**298–300.

2.•• Akira S, Uematsu S, Takeuchi O: **Pathogen recognition and innate immunity.** *Cell* 2006, **124:**783–801.

This review provides an up-to-date and comprehensive review of the molecular pathways by which TLRs activate host immunity. In addition, Akira et al. discuss current understanding of TLR independent pathways by which PAMPS activate host immunity.

3. Hoebe K, Janssen E, Beutler B: **The interface between innate and adaptive immunity.** *Nat Immunol* 2004, **5:**971–974.

4.• Rifkin IR, Leadbetter EA, Busconi L, et al.: **Toll-like receptors, endogenous ligands, and systemic autoimmune disease.** *Immunol Rev* 2005, **204:**27–42.

A comprehensive review of endogenous TLR ligand production and its potential role in the pathogenesis of autoimmune diseases.

5. Ishii KJ, Akira S: **Innate immune recognition of nucleic acids: beyond toll-like receptors.** *Int J Cancer* 2005, **117:**517–523.

6. Chisholm D, Libet L, Hayashi T, Horner AA: **Airway peptidoglycan and immunostimulatory DNA exposures have divergent effects on the development of airway allergen hypersensitivities.** *J Allergy Clin Immunol* 2004, **113:**448–454.

7. Moynagh PN: **TLR signalling and activation of IRFs: revisiting old friends from the NF-kappaB pathway.** *Trends Immunol* 2005, **26:**469–476.

8. Tulic MK, Wale JL, Holt PG, Sly PD: **Modification of the inflammatory response to allergen challenge after exposure to bacterial lipopolysaccharide.** *Am J Respir Cell Mol Biol* 2000, **22:**604–612.

9. Eisenbarth SC, Piggott DA, Huleatt JW, et al.: **Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen.** *J Exp Med* 2002, **196:**1645–1651.

10. Kaisho T, Hoshino K, Iwabe T, et al.: **Endotoxin can induce MyD88-deficient dendritic cells to support T(h)2 cell differentiation.** *Int Immunol* 2002, **14:**695–700.

11. Horner AA, Raz E: **Do microbes influence the pathogenesis of allergic diseases? Building the case for Toll-like receptor ligands.** *Curr Opin Immunol* 2003, **15:**614–619.

12. Horner AA, Raz E: **Immunostimulatory sequence oligodeoxynucleotide-based vaccination and immunomodulation: two unique but complementary strategies for the treatment of allergic diseases.** *J Allergy Clin Immunol* 2002, **110:**706–712.

13. Kobayashi H, Horner AA, Takabayashi K, et al.: **Immunostimulatory DNA pre-priming: a novel approach for prolonged Th1-biased immunity.** *Cell Immunol* 1999, **198:**69–75.

14. Rhee CS, Libet L, Chisholm D, et al.: **Allergen-independent immunostimulatory sequence oligodeoxynucleotide therapy attenuates experimental allergic rhinitis.** *Immunology* 2004, **113:**106–113.

15. Broide DH, Stachnick G, Castaneda D, et al.: **Systemic administration of immunostimulatory DNA sequences mediates reversible inhibition of Th2 responses in a mouse model of asthma.** *J Clin Immunol* 2001, **21:**175–182.

16. Horner AA, Widhopf GF, Burger JA, et al.: **Immunostimulatory DNA inhibits IL-4-dependent IgE synthesis by human B cells.** *J Allergy Clin Immunol* 2001, **108:**417–423.

17.• Hayashi T, Beck L, Rossetto C, et al.: **Inhibition of experimental asthma by indoleamine 2,3-dioxygenase.** *J Clin Invest* 2004, **114:**270–279.

A novel and potentially important pathway by which ISS-ODN and other TLR ligands might impact on the allergic phenotype is described.

18. Hessel EM, Chu M, Lizcano JO, et al.: **Immunostimulatory oligonucleotides block allergic airway inflammation by inhibiting Th2 cell activation and IgE-mediated cytokine induction.** *J Exp Med* 2005, **202:**1563–1573.

19. Takabayashi K, Libet L, Chisholm D, et al.: **Intranasal immunotherapy is more effective than intradermal immunotherapy for the induction of airway allergen tolerance in th2-sensitized mice.** *J Immunol* 2003, **170:**3898–3905.

20. Halperin SA, Van Nest G, Smith B, et al.: **A phase I study of the safety and immunogenicity of recombinant hepatitis B surface antigen co-administered with an immunostimulatory phosphorothioate oligonucleotide adjuvant.** *Vaccine* 2003, **21:**2461–2467.

21. Racila DM, Kline JN: **Perspectives in asthma: molecular use of microbial products in asthma prevention and treatment.** *J Allergy Clin Immunol* 2005, **116:**1202–1205.

22. Horner AA, Takabayashi K, Beck L, et al.: **Optimized conjugation ratios lead to allergen immunostimulatory oligodeoxynucleotide conjugates with retained immunogenicity and minimal anaphylactogenicity.** *J Allergy Clin Immunol* 2002, **110:**413–420.

23. Tighe H, Takabayashi K, Schwartz D, et al.: **Conjugation of protein to immunostimulatory DNA results in a rapid, long-lasting and potent induction of cell-mediated and humoral immunity.** *Eur J Immunol* 2000, **30:**1939–1947.

24. Creticos PS, Eiden JJ, Balcer SL, et al.: **Immunostimulatory oligodeoxynucleotides conjugated to Amb a 1: safety, skin test reactivity, and basophil histamine release [abstract].** *J Allergy Clin Immunol* 2000, **105:**S70.

25. Simons FE, Shikishima Y, Van Nest G, et al.: **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.

26.•• Tulic MK, Fiset PO, Christodoulopoulos P, et al.: **Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response.** *J Allergy Clin Immunol* 2004, **113:**235–241.

This paper provides the first published evidence that ISS-ODN conjugated allergens have clinical efficacy in the treatment of allergic rhinitis.

27. Redecke V, Hacker H, Datta SK, et al.: **Cutting edge: activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma.** *J Immunol* 2004, **172:**2739–2743.

28. Agrawal S, Agrawal A, Doughty B, et al.: **Cutting edge: 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.

29. Patel M, Xu D, Kewin P, et al.: **TLR2 agonist ameliorates established allergic airway inflammation by promoting Th1 response and not via regulatory T cells.** *J Immunol* 2005, **174:**7558–7563.

30. McCurdy JD, Olynych TJ, Maher LH, Marshall JS: **Cutting edge: distinct toll-like receptor 2 activators selectively induce different classes of mediator production from human mast cells.** *J Immunol* 2003, **170:**1625–1629.

31. Re F, Strominger JL: **Toll-like receptor 2 (TLR2) and TLR4 differentially activate human dendritic cells.** *J Biol Chem* 2001, **276:**37692–37699.

32. Supajatura V, Ushio H, Nakao A, et al.: **Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity.** *J Clin Invest* 2002, **109:**1351–1359.

33. Boguniewicz M, Leung DY: **Pathophysiologic mechanisms in atopic dermatitis.** *Semin Cutan Med Surg* 2001, **20:**217–225.

34. Matsui K, Nishikawa A: **Lipoteichoic acid from Staphylococcus aureus induces Th2-prone dermatitis in mice sensitized percutaneously with an allergen.** *Clin Exp Allergy* 2002, **32:**783–788.

35. Wang L, Smith D, Bot S, et al.: **Noncoding RNA danger motifs bridge innate and adaptive immunity and are potent adjuvants for vaccination.** *J Clin Invest* 2002, **110:**1175–1184.

36. Didierlaurent A, Ferrero I, Otten LA, et al.: **Flagellin promotes myeloid differentiation factor 88-dependent development of Th2-type response.** *J Immunol* 2004, **172:**6922–6930.
37. Brugnolo F, Sampognaro S, Liotta F, et al.: **The novel synthetic immune response modifier R-848 (Resiquimod) shifts human allergen-specific CD4+ TH2 lymphocytes into IFN-gamma-producing cells.** *J Allergy Clin Immunol* 2003, **111:**380–388.
38. Abreu MT, Fukata M, Arditi M: **TLR signaling in the gut in health and disease.** *J Immunol* 2005, **174:**4453–4460.
39. 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.
40. Frew AJ: **Advances in environmental and occupational diseases 2004.** *J Allergy Clin Immunol* 2005, **115:**1197–1202.
41. Platts-Mills TA, Woodfolk JA, Erwin EA, Aalberse R: **Mechanisms of tolerance to inhalant allergens: the relevance of a modified Th2 response to allergens from domestic animals.** *Springer Semin Immunopathol* 2004, **25:**271–279.
42. Gereda JE, Klinnert MD, Price MR, et al.: **Metropolitan home living conditions associated with indoor endotoxin levels.** *J Allergy Clin Immunol* 2001, **107:**790–796.
43. 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.
44. Roy SR, Schiltz AM, Marotta A, et al.: **Bacterial DNA in house and farm barn dust.** *J Allergy Clin Immunol* 2003, **112:**571–578.
45. van Strien RT, Engel R, Holst O, et al.: **Microbial exposure of rural school children, as assessed by levels of N-acetyl-muramic acid in mattress dust, and its association with respiratory health.** *J Allergy Clin Immunol* 2004, **113:**860–867.
46. Saxon A, Diaz-Sanchez D: **Air pollution and allergy: you are what you breathe.** *Nat Immunol* 2005, **6:**223–226.
47.• Boasen J, Chisholm D, Lebet L, et al.: **House dust extracts elicit Toll-like receptor-dependent dendritic cell responses.** *J Allergy Clin Immunol* 2005, **116:**185–191.
This paper provides the first direct evidence that sterile HDEs have TLR-dependent immunostimulatory activities.
48.•• Ng N, Lam D, Paulus P, et al.: **House dust extracts have both Th2 adjuvant and tolerogenic activities.** *J Allergy Clin Immunol* 2006, **117:**1074–1081.
In this follow-up paper to Boasen et al. [47•], we established that HDEs have both Th2-adjuvant and tolerogenic activities.
49. Becker S, Fenton MJ, Soukup JM: **Involvement of microbial components and toll-like receptors 2 and 4 in cytokine responses to air pollution particles.** *Am J Respir Cell Mol Biol* 2002, **27:**611–618.
50. Rabinovitch N, Liu AH, Zhang L, et al.: **Importance of the personal endotoxin cloud in school-age children with asthma.** *J Allergy Clin Immunol* 2005, **116:**1053–1057.
51. Alexis NE, Becker S, Bromberg PA, et al.: **Circulating CD11b expression correlates with the neutrophil response and airway mCD14 expression is enhanced following ozone exposure in humans.** *Clin Immunol* 2004, **111:**126–131.