Published in final edited form as:

J Allergy Clin Immunol. 2010 May ; 125(5): 955–962. doi:10.1016/j.jaci.2010.03.002.

Guilt by intimate association: What makes an allergen an allergen?

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Abstract

Why specific, ubiquitous, otherwise innocuous environmental proteins tend to provoke maladaptive, Th2-polarized immune responses in susceptible hosts is a fundamental mechanistic question for those interested in the pathogenesis, therapy and prevention of allergic disease. The current renaissance in the study of innate immunity has provided important insights into this question. The theme emerging from recent studies is that direct, (dys)functional interactions with pathways of innate immune activation that evolved to signal the presence of microbial infection are central to the molecular basis for allergenicity. This article reviews these data.

Keywords

allergy; allergenicity; Th2; asthma; innate immunity; pattern recognition receptor; Toll-like receptor; adjuvant; lipopolysaccharide; protease; carbohydrate

A central conundrum in allergic disease is why particular proteins have a propensity to act as allergens in susceptible hosts. In the case of aeroallergy, the proteins typically targeted by allergic responses represent but a tiny fraction of the airborne proteins routinely inhaled by humans. Furthermore, allergenicity is a very public phenomenon; the same subset of proteins drives allergenicity throughout the human population. Why are these specific, ubiquitous, apparently innocuous environmental proteins targeted by the immune system in this maladaptive way? The underlying mechanistic issues appear to devolve into two, likely interrelated, questions: (1) Why do such proteins tend to generate effector lymphocyte responses, as opposed to ignorance, tolerance or anergy? and (2) Why do the effector lymphocyte responses to such proteins tend to be Th2 polarized? Put this way, both questions suggest that answers are likely to be found through study of interactions between allergens and the innate immune system.

Innate and adaptive immunity: Evolution and fashion

The professional divide throughout much of the 20th century between immunologists studying innate immunity (initially known as “cellular immunity”) and those studying adaptive immunity (initially known as “humoral immunity”) was a holdover from the beginning days

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of immunology as a science. Throughout the latter half of 20th century, those studying adaptive immunity dominated the field. While this was in many ways a sociological/historical accident, it was fueled, at least in part, by the compelling nature of the problems under study (e.g., how almost limitless lymphocyte receptor diversity could be generated from a finite genome, what underlies tolerance to self, what the molecular substrates and biological functions of polarized effector and regulatory cell types are) and by the stunning, mechanistic progress made in addressing these problems. Recent molecular identification of innate immune pattern recognition receptors (PRRs) that play a central role in signaling the presence of infection—including Toll-like receptors (TRLs), nucleotide-binding domain and leucine repeat-containing receptors (NRLs), RIG-I-like helicases (RLRs) and C-type lectin receptors (CLRs)—has provoked a renaissance in the study of innate immunity^{1, 2}.

One lingering result of the previous adaptive immune-centric view of immunology is that the innate immune system is still presented in many immunology texts as a primitive, first-line defense against infection and injury that is employed until the more effective and sophisticated adaptive immune system can be brought to bear. There are, of course, numerous flaws in this formulation. At the most basic level, whereas the adaptive immune system can usefully be viewed as a single system—with its antigen receptors (T cell receptors, immunoglobulins) and cells (T and B cells) dependent on the evolution of RAG genes in jawed vertebrates—the innate immune system, present in all metazoans, cannot. “Innate immune systems” is a far better term for the diverse congeries of pathways and cells (including essentially all cells in vertebrates) that make up innate immunity. Further, the innate immune systems are no less sophisticated than the adaptive immune system (having been under evolutionary pressure for longer), nor are innate immune effector mechanisms in any way less effective than adaptive immune effector mechanisms. Even the standard textbook view of the kinetics of immune responses (the innate immune response handing off to the adaptive immune response) is misleading; activation of adaptive immune system is not associated with inactivation of the innate immune systems.

More generally, the distinction between innate and adaptive immunity is artificial in many ways. Innate and adaptive immunity are inextricably linked in vertebrates. For the purposes of the current discussion, it should be underscored that efficient activation of adaptive immunity is dependent on the innate immune systems. That is, while the adaptive immune system can generate responses to essentially any molecular structure, it relies on context provided by the innate immune system to discriminate which structures (under which conditions) should be responded to. Similarly, the innate immune system plays a central role in regulating the class, amplitude and resolution of adaptive immune responses thereby generated. The reverse is just as true: the adaptive immune system regulates inflammatory responses, in large part, via regulation of innate immune cell recruitment, activation, differentiation and counter-regulation.

These considerations suggest strongly that a general molecular basis of allergenicity is not likely to be found at the epitope level. Fundamental constraints on T cell activation, such as T cell receptor avidity and peptide density during antigen presentation, are certain to apply. However, despite much study of allergen B and T cell epitopes informed by an adaptive immune-centric view of the immunological universe, there do not appear to be common structural characteristics among allergen epitopes. Recent studies suggest, instead, that the widely diverse proteins that behave as allergens are linked by a common ability to drive innate immune activation.

Allergenicity resulting from molecular mimicry of the LPS receptor: A mite too close for comfort

PRRs of the innate immune system, such as the TLRs, play a critical role in regulating the function of antigen presenting cells^{1, 2}. Exogenous antigen presentation by dendritic cells in the absence of direct PRR stimulation normally leads to tolerance³. Moreover, elegant reductive experimental systems have shown that the efficient generation of effector T cell responses by dendritic cells depends on the presence of TLR ligands in the same phagolysosome as the antigen being presented⁴. PRRs of the innate immune system also play a central role in regulating the class of adaptive immune response generated. This is particularly well understood for Th1 and Th17 responses. PRRs on dendritic cells play an essential role in driving dendritic cell production of Th1- and Th17-polarizing cytokines such as IL-12, IL-23 and IL-6^{1, 2, 5}. The mechanisms underlying Th2 polarization have been less well understood. However, it is clear, for example, that PRR-driven production of TSLP by airway epithelial cells and granulocytes can prime dendritic cells for Th2 polarization via OX40/OX40L interactions, something that is likely abetted by basophil production of IL-4^{6, 7}.

Several lines of evidence have linked exposure to lipopolysaccharide (LPS), the paradigmatic ligand for TLR4, with protection from the development of allergic asthma⁸. While allergic disorders clearly have a heritable component, the rapid rise in the prevalence of allergic (and autoimmune) disorders in westernized environments in the latter half of the 20th century suggests that the reasons for this epidemiological shift lie in the environment. The hygiene hypothesis posits that early childhood exposure to microbes or microbial products inhibits the propensity to develop allergic (and autoimmune) disease, likely through the development of robust counter-regulatory responses^{9, 10}. A variety of studies have shown that children raised on farms are at lower risk of atopy and allergic asthma¹¹⁻¹⁴. In the search for mechanism, several groups have shown that house dust LPS levels (in farming and non-farming households alike) are inversely correlated with atopy and allergic disease¹⁵⁻¹⁹. Recent study of gene (TLR complex gene polymorphisms) - environment (level of microbial product exposure) interactions has also provided compelling support for a link between LPS exposure and allergic disease^{20, 21}. It should be noted, however, that epidemiological and human challenge data also indicate that LPS exposure can also exacerbate established asthma, allergic or not, as well as induce non-atopic wheezing^{15, 22-26}.

In addition to providing confirmation of the ability of LPS to exacerbate established allergic asthma²⁷, studies in murine models have provided key mechanistic insights into the variable role of LPS exposure in both facilitating and inhibiting the development of allergic asthma. Consonant with the predictions of the hygiene hypothesis, a key variable appears to be the dose of LPS involved. Bottomly's group has shown that, whereas airway sensitization with the model antigen, ovalbumin (OVA), in the presence of "very low-dose" (< 1 ng) LPS leads to tolerance, sensitization with OVA in the presence of "low-dose" (100 ng) LPS leads to the TLR4-dependent generation of airway Th2 airway inflammatory responses^{28, 29}. On the other hand, airway sensitization with OVA in the presence of "high-dose" (100 µg) LPS drives Th1 inflammation, likely along with a strong regulatory response as well^{28, 29}. These data were initially surprising. The dogma at the time was that Th1, not Th2, differentiation was dependent on TLR signaling³⁰. However, while TLR-independent pathways *can* drive Th2 development, additional studies have also underscored an important role for TLR4 signaling in Th2 polarization and the robust development of experimental allergic asthma^{31, 32}, including in more physiological models of airway exposure to natural allergens³³.

Trompette, et al. recently discovered a more intimate link between TLR4 signaling and allergic sensitization to a specific allergen³⁴. House dust mites are major sources of aeroallergens for patients with allergic asthma³⁵. Secreted by mite gut epithelial cells and concentrated in house

dust mite fecal particles³⁶, the major group 2 allergens, Der p 2 and Der f 2, are highly allergenic. Indeed, among defined house dust antigens, these antigens have the highest rates of skin test positivity in atopic patients³⁷. Of note, these major allergens are homologs of MD-2, the secreted, LPS-binding member of the TLR4 signaling complex and the founding member of the MD-2-related lipid-recognition (ML) domain family of proteins^{38, 39}. Given the fact that MD-2 and Der p 2 are structurally homologous⁴⁰⁻⁴³, Trompette et al. searched for functional homology as well. They reported that: (a) Der p 2 drives TLR4 signaling through direct interactions with the TLR4 complex, reconstituting LPS-driven TLR4 signaling in the absence of MD-2 and facilitating it in the presence of MD-2; (b) Der p 2 facilitates LPS signaling in primary antigen presenting cells, with or without MD-2 being present; and (c) the *in vivo* allergenic activity of Der p 2 mirrors its *in vitro* functional and biochemical activity: Der p 2/LPS efficiently drives airway Th2 inflammation *in vivo* in a TLR4-dependent manner, retaining this ability in the absence of MD-2³⁴.

These data suggest that Der p 2 tends to generate effector lymphocyte responses because of its ability to activate the innate immune system through TLR4 signaling. Of course, natural exposure to allergens is not to purified, recombinant proteins, but to complex molecular mixtures; there is no *a priori* reason that the molecules driving innate immune activation be the same as the proteins recognized as allergens. If not, however, the molecular basis for propensity of specific proteins in the broader mixture to drive effector T cell responses remains a bit opaque. In the case of Der p 2/LPS, the adjuvant property of the complex is likely to be abetted by the fact that antigen and TLR ligand are, perforce, co-localized in the same phagosome. Such tight association (or, indeed, identity) between highly immunogenic proteins and activating ligands for PRRs has routinely been noticed for microbial antigens, including bacterial flagellin (a TLR5 ligand), profilin from *Toxoplasma gondii* (a TLR11 ligand) and the diverse lipid-decorated bacterial membrane proteins that signal through the TLR2 complex⁴⁴⁻⁴⁶.

Trompette's data also suggest that Der p 2-mediated facilitation of TLR4 signaling under conditions of very low ambient LPS exposure, those associated with increased rates of allergy, may act to shift the LPS-response curve into the Th2-inducing range. Further, the data suggest that Der p 2 is likely to promote LPS-driven exacerbation of established asthma by facilitating TLR4 signaling by airway cells. The fact that Der p 2 can reconstitute TLR4 signaling in the absence of MD-2 may well be especially important here as airway epithelial cells appear to express TLR4 but virtually no MD-2, at least under homeostatic conditions⁴⁷.

Greasing the way to generality (lipids, from A to P)

The Der p 2 studies suggest that, in this instance, allergenicity results from functional mimicry of a PRR complex protein, leading to Der p 2 engaging pathways that have evolved for the sensing of microbial infection and injury. Many questions remain, including the biologically relevant locus of action of TLR4 in the airway^{48, 49}, the natural ligand for Der p 2 (high resolution structures of MD-2 and Der p 2 reveal a narrower lipophilic cavity in Der p 2⁴⁰⁻⁴³), and the molecular requirements for Der p 2-dependent activation of TLR4 in the presence and absence of MD-2. A broader question is whether there is any reason to suspect that this is more than a unique oddity. In fact, in addition to the highly homologous allergen, Der f 2, several other major group II aeroallergens, including Eur m 2, Gly d 2, Tyr p 2 and Lep d 2, are ML proteins with considerable homology to Der p 2 and MD-2³⁸. Thus, mimicry of, and functional interaction with, the TLR4 complex may provide a basis for why several major aeroallergens are aeroallergens. There may also be generalizability along another axis as well. While some TLR ligands (e.g., TLR9 ligands) both prevent and inhibit experimental allergic asthma, with no facilitation at low doses⁵⁰⁻⁵³, TLR2 ligands have also been shown to be able to drive Th2 differentiation and allergic inflammation in the lung^{54, 55}. This is

consonant with studies linking *TLR2* mutations with protection from atopy and asthma⁵⁶. Interestingly, *TLR2* can signal the presence of non-enterobacterial LPS⁴⁶. Further, functional interactions with *TLR2* and both CD14 and MD-2 have been reported, although the latter observation remains controversial⁵⁷⁻⁶¹. Hence, it is possible that ML allergens may also interact functionally with *TLR2* signaling.

More generally, more than half of defined major allergens appear to be lipid-binding proteins⁶². Widely diverse protein families are represented, including, in addition to ML proteins, non-specific lipid transfer proteins (e.g., Pru p 3, Par j 1, and Tri a 14), 2S albumins (e.g., Ara h 2, Ses i 1, and Sin a 1), pathogenesis-related 10 family proteins (e.g., Bet v 1 and Pru av 1), lipocalins (e.g., Can f 1 and Equ c 1), secretoglobins (e.g., Fel d 1), and apolipoproteins (e.g., Der p 14). It is a reasonable, testable hypothesis that intrinsic adjuvant activity provided by the association of these proteins with their lipid cargo underlies their immunogenicity and/or allergenicity. Molecular definition of the lipids naturally bound to these proteins, the signaling pathways activated by such lipids, and the patterns of immune response thereby promoted are likely to provide important mechanistic insights. In addition to *TLR* activation, it should also be noted that diverse lipids are also known to stimulate activation of innate lymphocyte populations⁶³. Further, despite the traditional focus in immunology on pathways of immune activation, pathways of de-activation and/or resolution are just as important⁶⁴. It may well be that, in some instances, immunogenicity and allergenicity derive from interference with constitutive or induced pathways of immune counter-regulation.

Other components of allergens that mimic microbes

Even more broadly, a growing literature suggests that direct, maladaptive interactions with pathways of innate immune activation are likely to provide a unifying theme for the molecular basis of allergenicity⁶⁵. Particular notice has been paid to allergen-associated protease activity and carbohydrate structures.

The potential biological importance of the fact that many allergens have protease activity has received considerable experimental attention over the years. Such proteases have been shown to be able to: (a) facilitate access of allergens to the innate immune system (e.g., by disrupting airway epithelial cell tight junctions⁶⁶ or by degrading lung surfactant proteins thought to play an important role in allergen clearance⁶⁷); (b) induce the production of epithelial-derived mediators that drive dendritic cell recruitment and/or activation^{68, 69}; (c) activate the protease-activated receptor (PAR) 2, a G protein-coupled receptor expressed by both epithelial cells and other innate immune cells that exhibits cooperativity with *TLR4* signaling^{69, 70}; and (d) cleave molecules important in regulating relevant adaptive immune responses such as CD25 and CD23⁷¹. Which of these functions may be biologically central to allergenicity is, of course, difficult to disentangle experimentally. More recently, Medzhitov's group has proposed an elegant model of potential wide generality. Cysteine proteases secreted by tissue helminths are essential for maintenance of the life cycles of these parasites that drive robust, Th2-polarized immune responses in infected hosts⁷². Given this, they hypothesized that the innate immune system evolved of a method of detecting soluble pathogen-derived protease activity, a pathway activated incidentally, to host's detriment, by allergens⁷. Notably, they found that the occupational allergen papain, acting through an as-yet unidentified sensor, activates basophils to function as Th2-polarizing antigen-presenting cells^{7, 73}.

The immunostimulatory properties of complex polysaccharide structures such as β -glucans and mannans, expressed by diverse microbes and engaged by PRRs of the CLR family such as Dectin-1, Dectin-2 and DC-SIGN, have also been appreciated for some time. Of note, for the present discussion, both dendritic cells and airway epithelial cells express CLR family members. Some can signal directly; others modulate signaling by *TLRs*⁷⁴. Glycans present in

whole extracts of allergenic organisms, including dust mites and *Aspergillus*, clearly have relevant bioactivity⁷⁵⁻⁷⁷. A variety of allergens are directly decorated by glycans. Recent evidence suggests that glycans on some allergens—including Der p 2 and the Bermuda grass allergen, BG60—may bind to and/or signal through the CLRs, DC-SIGN and L-SIGN⁷⁸. Glycans can act as strong Th2-polarizing signals. Indeed, β -glucan structures present in Ara h 1, the major glycoprotein peanut allergen, have been shown to be able to drive Th2 polarization through engagement of DC-SIGN on dendritic cells⁷⁹. Furthermore, chitin, a polysaccharide polymer present in abundance in helminths as well as numerous allergen sources—including fungi, insects and crustaceans—directly drives the tissue recruitment of IL-4-expressing eosinophils and basophils⁸⁰. While the responsible signaling receptor remains unclear, the fact that basophils are fully able to initiate allergen-driven Th2 responses^{73, 81, 82} suggests an obvious mechanism for the promotion of immune recognition and immune class polarization of chitin-associated proteins. It will be noted that this would still appear to beg the question of why *specific* chitin-associated proteins have the propensity to be recognized as allergens. Perhaps this is a function of differential protein adsorption to immunogenic chitin polymers.

Summary

Growing evidence suggests that allergenicity arises as an incidental if pernicious result of mistaken identity—that proteins tend to generate allergic responses when they directly intrude on and activate innate immune pathways that evolved to signal the presence of infection. This gift (*German*: poison) of mimicry is clearest for the major house dust mite allergen Der p 2, which is a functional mimic of an essential TLR4 complex molecule. It appears likely, however, that similar intrinsic adjuvant activity provided by the hydrophobic cargo of lipid-binding allergens, carbohydrate structures on glycoprotein allergens, and allergen-associated protease activity has considerable generality as a molecular substrate of allergenicity. A better molecular understanding of the relevant ligands, receptors and signaling pathways has clear translational promise for novel preventive and therapeutic approaches to allergic disease.

What do we know?

- The recent revolution in innate immunology has led to a highly productive re-framing of fundamental questions in allergy research, driving a paradigm shift in our understanding of the molecular and cellular substrates of allergenicity.
- The Th2-polarized T cell immune responses that mark and underlie both adaptive responses to helminth infection and maladaptive, allergic responses can be induced by several, apparently unrelated pathways of innate immune activation.
- The intrinsic adjuvant activity provided by direct engagement of these diverse pathways of innate immune activation appears central to allergenicity and allergic disease.

What is still unknown?

- The lipids associated with most lipid-binding allergens under conditions of natural exposure, and the receptors and the signaling pathways engaged by such protein/lipid complexes.
- The innate immune sensor for helminth- and allergen-associated soluble protease activity, and the structural requirements (and receptors) for Th2-promoting carbohydrate structures on glycoprotein allergens.
- Which of these conserved pathways will be most tractable for targeting for translational development of novel preventive and therapeutic approaches to allergic disease.

Acknowledgments

Supported by a Senior Investigator Award from the American Asthma Foundation, and by NIH grant AI088372.

Abbreviations used

CLR	C-type lectin receptor
LPS	lipopolysaccharide
NRL	nucleotide-binding domain and leucine repeat-containing receptor
OVA	ovalbumin
PRR	pattern recognition receptor
RLR	RIG-I-like helicase
TRL	Toll-like receptor

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