

Survival of the fittest: allergology or parasitology?

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Allergologists have long recognized that only a small fraction of the environmental and food proteins that we are exposed to give rise to IgE responses. This has raised the intriguing and important question: What makes an allergen an allergen? Many protein allergens have close homologs in metazoan parasites, and as helminth genome information grows, it appears increasingly clear that not only are the immune mechanisms of allergy and anti-helminth immunity closely related, but so are the helminth and allergen proteins that induce them. This suggests that at least part of the secret of what allows some proteins to be allergens may actually lie in the long co-evolutionary relationship between worms and their vertebrate (and latterly human) hosts.

The allergy epidemic and the evolution of immunity against metazoan parasites

More than 20% of people in many 'Western' countries suffer from allergy [1], with conditions ranging from mild hay fever to fatal systemic anaphylaxis. It is caused by IgE-mediated type-I hypersensitivity responses to environmental antigens. The IgE antibody is part of a complex system in which high-affinity IgE-receptors on mast cells and basophils mediate antigen-dependent cell responses, especially release of potent inflammatory molecules. This leads to recruitment, proliferation and activation of eosinophils, a normally rare cell type that also express IgE-receptors and that are associated with inflammation and tissue damage in allergic responses. The existence of this complex and aggressive immune mechanism implies that there has been strong selective pressure to develop and retain it. It is very unlikely to have evolved to attack non-pathological environmental allergens. In fact, the most likely explanation is that IgE, its receptors and cellular responses, have evolved for another important immune function; defense against metazoan parasites; organisms that are themselves complex and highly evolved animals.

The most prevalent metazoan parasites are the worms and the ectoparasites (mites, ticks and insects), and there is plenty of evidence pointing to the importance of IgE in defence against parasitic worms. As early as 1964, passive transfer experiments led Dame Briget Ogilvie to conclude that reagin-like antibodies (IgE) were responsible for immunity to helminths in many animals [2]. Capron and colleagues demonstrated IgE-mediated killing of schistosomes *in vitro* and subsequent studies in human populations and experimental animals have correlated specific

IgE responses with resistance to schistosomes [3], hookworms [4], *Trichuris* [5], *Ascaris* [6] and *Echinococcus* [7]. IgE and eosinophil responses have also been associated with resistance to ectoparasites [8–10]. In particular, animal studies have demonstrated the importance of immediate hypersensitivity at sites of mite or tick attack, implicating mast cells, eosinophils, and IgE in resistance against repeat infestation [8,9]. Histamine release in the skin around a mite or tick may make feeding unpleasant for the parasite, and evoke a scratching response in the host, thus dislodging it.

Attack by metazoan parasites initiates a characteristic T helper cell type 2 (Th2) response in humans and experimental animals, resulting in a switch to IgE production as well as the recruitment, proliferation and activation of eosinophils and mast cells. This is thought to be a consequence of recognition of parasite molecules by the host's innate immune system [11]. The relationship between parasitic worms and their vertebrate hosts goes back a long time, and Jackson *et al.* [12] have recently provided a concise account of the presence of parasitic platyhelminths and nematodes during the evolution of vertebrate innate and adaptive immune systems and eventually, mammalian IgE. Present day relationships between humans and parasitic worms are often characterised by chronic and insidious host ill health, rather than acute disease with high morbidity. This does not imply that chronic worm infections cannot impose a selective pressure on their hosts. Such infections can cause anaemia and malnutrition, factors known to influence human reproduction by decreasing fertility and impacting on foetal and neonatal health [13–15].

In many of the worm–host relationships that have evolved, infection-restricting IgE-mediated effector mechanisms are accompanied by potent parasite-induced immunoregulation. It has been proposed that the moderation of aggressive inflammatory responses that result, increases fitness of both host and parasite [12,16]. The immunoregulatory mechanisms involved have been well characterised in many animal and human studies [17]. Molecular signals from the parasite increase numbers of T regulatory cells (Tregs) in conjunction with production of regulatory cytokines IL-10 and TGF- β . Chronic helminth infection induces a 'modified Th2 environment', characterized by cellular hyporesponsiveness and the production of IgG4 antibodies that can block binding of IgE to parasite antigens [18].

The parasite-regulated response contrasts with atopy, a familial tendency to develop IgE-mediated allergic

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Table 1. Top 10 allergen families and super-families

Allergens in Allfam DB (May 2009)	Allergen families and Super-families	Are there helminth equivalents?
63	Prolamin super-family	? ^a Structurally diverse group of at least seven plant protein families with protein folds shared by many families
45	Profilins	<i>B. malayi</i> (ACD47109), <i>S. japonicum</i> (AAP06493), <i>S. mansoni</i> (EST Sm22625)
43	EF-hand proteins	Schistosome TAL proteins (AAA29856 [26] ^b , AAA29923 [26] ^b , AAC79130 [26] ^b , AAW49250 [27] ^b . Troponin C from <i>A. simplex</i> (Q9U3U5) ^b , <i>B. malayi</i> (ACD47109) and <i>N. americanis</i> (AI856935)
42	Tropomyosins	Tropomyosins in <i>A. simplex</i> (Y19221)[28] ^b , <i>S. mansoni</i> (A60607), <i>A. lumbricoides</i> (ABS82498)[29], <i>O. volvulus</i> (AAC28900) [30] ^b , <i>T. spiralis</i> (AAL14704), and <i>E. multilocularis</i> (CAC85552)
36	Cupin super-family	Helminth lipid binding proteins including <i>S. mansoni</i> Sm14 (P29498) and <i>E. granulosus</i> FABP1 (AAK12096)
32	Expansin super-family	? ^a Group of four structurally diverse plant families
20	PR-1 proteins	Venom like molecules <i>Brugia malayi</i> VAL-1(AAK12274), <i>S.mansoni</i> VAL-1 (AY994061)[31] ^b , <i>A. caninum</i> ASP-2 (AAC35986)[4] ^b and <i>O. volvulus</i> ASP-1 (AF020586)
20	Lipocalins	Fatty acid binding proteins <i>O. volvulus</i> Ov-FAR-1(L27686) <i>A. suum</i> ABA-1(AAB41117) and <i>S. japonicum</i> FABPc (AF044409)
19	Bet v1-related proteins	? ^a Three families of plant proteins
16	Trypsin-like serine proteases	Examples in most helminths including, <i>T. spiralis</i> TsSerP (ACA28932), <i>E. granulosus</i> Antigen 5 (A43933)[32] ^b and <i>S. mansoni</i> SmSP1 (CAA09692)

^a?, not known. These super-families are groups of families defined loosely by the nature of their protein folds. These folds are present in metazoan proteins, but the structure of the allergen databases and the current status of parasite genome annotation make it difficult to search for metazoan equivalents to the plant allergens listed.

^bKnown targets for IgE response [with references]

diseases. Atopy is marked by a strong Th2 response to environmental allergens and has been linked with polymorphisms in a number of genes associated with Th2 responses and levels of IgE [19]. There is increasing evidence that the Th2 hyper-responsiveness of atopic individuals is suppressed by helminth infections [20]. Allergic disease in atopic individuals may therefore be the unfortunate by-product of an IgE-mediated defence against metazoan parasites, in the absence of these infections and the absence of the regulatory mechanisms developed during host-parasite co-evolution. An intriguing possibility is that atopic individuals, with their predisposition to develop IgE responses, may be more resistant to metazoan parasites. In support of this, evidence is emerging that polymorphisms associated with increased asthma and allergy, are also linked with reduced helminth infection [21].

Whether the evolved hypersensitivity responses are parasite regulated (as in helminth infection) or unregulated (as in allergy), they target certain molecules as IgE antigens (or allergens). This begs the question: which properties make a molecule an allergen?

What makes an allergen an allergen?

Given that most biological molecules do not appear to be targets for IgE, there has been a long and ongoing discussion into what makes an allergen an allergen [22]. It has been suggested that allergens may be characterised by size, or abundance, solubility, polymerisation, repetitive structure, enzyme activity, or by the presence of carbohydrate or hydrophobic surface patches [22–24]. However, none these properties is exclusive to allergen molecules, nor do they explain why allergens are common in some phyla and rare in others [22]. The cloning of numerous protein allergens has inspired allergologists to characterise these molecules according to their protein families (Pfam) generating a database (AllFam) that can be used

to study common structural and functional properties of allergens [<http://www.meduniwien.ac.at/allergens/allfam/>]. This has quickly confirmed that allergens are present in only a small number of protein families representing a very limited number of biochemical functions [25]. There are over 10000 protein families in the Pfam database [<http://pfam.sanger.ac.uk/>] and the number of these containing allergens is significantly lower than would be expected by chance [25]. Indeed, just ten of the protein categories contain nearly 45% of all known protein allergens [25] (Table 1). It should be noted that nearly all of these allergenic protein families have representatives in metazoan parasites. Indeed, such parasite proteins are probably the intended targets of the whole IgE system.

Environmental allergens and ‘metazoan-parasite-immunogens’

One of the most common single allergenic proteins is tropomyosin (Pfam PF00261). So far, tropomyosins (Tpm) from 42 different species have been identified as allergens (Table 1) and all of these are from invertebrates. Yet not all Tpm are observed to be allergenic. Jenkins *et al.* noted that non-allergenic Tpm from birds and mammals are usually more than 90% identical to human homologs, whereas allergenic Tpm from invertebrates are generally <55% identical [33]. From further *in silico* analysis of a number of animal food allergens, they observed that proteins with >63% sequence identity with human homologues were rarely allergenic. It seems that tolerance to self-antigens extends to almost identical molecules from closely related species, but that the immune system is able to mount an IgE-mediated hypersensitivity response against complex, but unrelated organisms. Presumably this is a mechanism to protect against metazoan parasites.

The list of invertebrate Tpm considered ‘allergens’ includes Ani s3 from nematode parasite *Anisakis simplex* and Pso o10 from *P. ovis* (sheep scab mite). Given the role

for IgE and mast cells in protection against arachnid re-infestation [8], and that the mite Tpm causes immediate hypersensitivity on contact with skin, Pso o10 is probably better described as a metazoan-parasite-immunogen, rather than an allergen. Tpm from parasitic nematodes *Ascaris lumbricoides* and *Onchocerca volvulus* induce IgE responses in infected people [30,34] and in experimental animals immunisation with species-specific Tpm has proved protective against challenge with filarial nematodes and with the trematode *Schistosoma japonicum* (reviewed in Ref. [30]).

Metazoan parasite-immunogens can be identified in other common families of protein allergens such as EF-hand proteins (43 allergens in Allfam database) and the PR-1 family (Table 1). The EF-hand is a helix-loop-helix structural motif that is found in many calcium-binding proteins including the *A. simplex* allergen, troponin C. Dunne *et al.* [26] reported that the principle IgE antigen in *S. mansoni* was a 22.6-kDa protein containing two EF-hand motifs. The molecule has been shown to be member of a family of proteins from the outer tegument layer of trematode parasites [35], which we term the 'tegument-allergen-like' (TAL) proteins. Binding of human IgE to many of these TALs has been confirmed [26] and, in infected communities, an IgE response to Sm22.6 (TAL1) has been associated with resistance to re-infection with *S. mansoni* [26]. Vaccination of mice with Sm22.6 (TAL1) has been shown to induce some protection against infection and the molecule has been proposed as a vaccine candidate [36].

The PR-1 proteins are another large group of protein allergens. They are characterised by the presence of SCP/TAPS domains (Pfam accession no. PF00188). Although they are named after the pathogenesis-related proteins of plants, most of the known PR-1 allergens are derived from insect venoms and more importantly, there are venom-like equivalents in the infectious stages of filarial nematodes [37,38], hookworm [4] and schistosomes [31]. These worm venom-like-allergens (VALs) are likely to be metazoan-parasite-immunogens. The hookworm examples, Ancylostoma-secreted-proteins (ASP), are targets for the human IgE response [4]. Furthermore, Bethony and colleagues showed that vaccination of naïve dogs with ASP-2 inhibited larval migration through the tissues and suppressed experimental hookworm infection [4]. These results led them to investigate the potential of *N. americanus* ASP-2 as a human hookworm vaccine. However, phase I trials in previously infected Brazilian subjects were halted when volunteers developed IgE-dependent generalized urticaria [39], demonstrating the potent allergen-like properties of this immunogen.

Parasite proteases play vital roles in the interaction between metazoan parasites and their hosts. They are required for invasion, migration and feeding [40,41], and are likely, therefore, to be important parasite immunogens. Many known allergens are proteases, including the major dust mite allergen, Der p1. The Allfam database [<http://www.meduniwien.ac.at/allergens/allfam/>] lists 15 subtilisin-like, 16 trypsin-like and 11 papain-like proteases, and similarly, there are IgE-binding proteases in a number of helminth species [40].

In addition to proteases, other major allergen families, the profilins and lipocalins include examples from insects, mites and nematodes. Sensitivity to members of these families found in innocuous environmental agents may also represent a misdirected anti-metazoan parasite response. We believe that this is because the IgE system has evolved to target molecules that are derived from relatively large, complex, multi-cellular creatures but that are detectably different from the host itself.

Evolution of IgE targeting

In protecting itself from other metazoan species the host must target parasite antigens without developing auto-reactive responses. Metazoan parasites are much more closely related to their mammalian hosts than bacterial, viral, or fungal pathogens, so the range of target proteins, which are available for anti-metazoan parasite IgE responses, but are not closely related to self, will be limited. We suggest that, over evolutionary time, this targeting of a restricted range of antigens would have conferred selective advantage by enabling a host to defend itself, without exposing itself to self-harm or inducing tolerance. Thus, the potential of environmental and food allergens to be allergens, is restricted to those proteins that are structurally related to a limited range of metazoan parasite proteins, which are themselves sufficiently different to host's proteins to be the targets for a 'safe' deployment of IgE and its dependent effector mechanisms.

As would be expected from a response that targets metazoan parasites, IgE targeting appears to exclude bacterial antigens. Indeed, it has been noted that, unlike many other eukaryotic proteins, known allergens are molecules that do not have bacterial homologues [42]. IgE responses to bacterial antigens have been reported, but usually only in association with severe hypersensitivity to other allergens [43,44]. This may represent a bystander effect in a highly skewed Th2 environment [43,44]. There are also reports of IgE responses to viral antigens in humans and experimental animals. This has included responses to respiratory syncytial virus [45], dengue virus [46] and influenza [50]. Interestingly, reported allergic symptoms following influenza vaccination are often due to egg-derived contaminants in the vaccine [47].

Concluding remarks

Whether immune responses are skewed towards IgE production by metazoan parasite attack or allergy, it seems that a very limited number of molecules have the properties to become IgE antigens. Allergens have been defined as 'non-parasitic antigens capable of stimulating a type-I hypersensitivity reactions in atopic individuals' [48]. We now need an additional term to describe the IgE antigens from metazoan parasites that also crosslink IgE receptors on effector cells evoking a type-I hypersensitivity response that is protective against the invader. These molecules are the 'metazoan-parasite-immunogens' that the system probably evolved to detect.

The IgE response clearly did not evolve to cause allergy. The evidence that it did evolve to limit metazoan parasite burden is strong. With the advent of genetically modified foods, allergologists are very concerned about what are the

structural features that make a protein (or a new GM protein) an allergen. However, the allergy literature rarely refers to the parasitic origins of the allergic response, even in discussions on the evolutionary relationships of allergens. With further research and the increasing availability of helminth genome data, it should be possible to identify many more immunogens. Comparing these will provide additional structural information, including which types of protein fold are targeted by IgE, and this may, for example, help us to understand why certain plant proteins are allergens. If we can learn how the host-parasite relationships have evolved to suppress and regulate anti-immunogen responses, it will help in the design of specific immunotherapy for allergy. To understand the IgE response more fully, including its targets and regulation, it is essential to consider how it coevolved with our long-time adversaries: the metazoan parasites.

Perhaps 200 years after the birth of Charles Darwin, parasitology can help introduce evolution into allergology.

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