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Emerging Antigens Involved in Allergic Responses

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Abstract

New allergic diseases can “emerge” because of exposure to a novel antigen, because the immune responsiveness of the subject changes, or because of a change in the behavior of the population. Novel antigens have entered the environment as new pests in the home (e.g., Asian lady beetle or stink bugs), in the diet (e.g., prebiotics or wheat isolates), or because of the spread of a biting arthropod (e.g., ticks). Over the last few years, a significant new disease has been identified, which has changed the paradigm for food allergy. Bites of the tick, *Amblyomma americanum*, are capable of inducing IgE antibodies to galactose-alpha-1,3-galactose, which is associated with two novel forms of anaphylaxis. In a large area of the southeastern United States, the disease of delayed anaphylaxis to mammalian meat is now common. This disease challenges many previous rules about food allergy and provides a striking model of an emerging allergic disease.

Introduction

Allergic disease can “emerge” because the subjects make IgE antibody responses against new antigens, because something else changes that results in a clinical response that was not previously apparent, or because physicians become aware of a syndrome that was previously not recognized. Over the last 150 years, allergic diseases have increased progressively, part of which can reasonably be ascribed to changes in public hygiene, and part to changes in lifestyle [1]. The progressive increases in allergic disease have actually occurred in several phases: hay fever emerged in Europe between 1870 and 1940 and was followed by a massive rise in allergic asthma in children between 1960 and 2000, and most recently, there has been an increase in peanut allergy that started around 1995 (Table I). Against this background, we identified a novel form of food allergy, where the primary antigen is the oligosaccharide galactose-alpha-1,3-galactose (alpha-gal) [2, 3]. We will focus on this condition, because it illustrates how complicated it can be to define the mechanisms and causes of a novel syndrome. It also provides an excellent example of a syndrome where the patients were initially dismissed because some features of these reactions were strikingly different from standard teaching. The IgE response to alpha-gal may also provide an opportunity to investigate the mechanisms controlling IgE antibody responses in humans.

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Delayed anaphylaxis to red meat

When the monoclonal antibody cetuximab went into clinical use, it rapidly became obvious that hypersensitivity reactions during the first infusion were more common than expected in an area of the southern United States. In some centers, these events were occurring in as many as 20% of patients treated [4], the reactions were generally so severe as to preclude subsequent use of the drug and occasionally proved fatal [5]. Investigation of this problem was made possible because:

- a. The oncology group in Nashville had 76 pretreatment sera from cancer patients who had been treated with cetuximab.
- b. ImClone had expressed the recombinant molecule in another cell line and had also published a detailed study on the glycosylation of cetuximab [2, 6].
- c. We had developed, together with Phadia, a technique to assay IgE antibodies against any molecule that can be biotinylated [7].

Our investigations established that the reactions to this mAb occurred in patients who had pre-existing IgE antibodies to the oligosaccharide on the Fab portion of the molecule [2, 8]. Alpha-gal is present at amino acid 88 on the Fab portion of the heavy chain. There is also a glycosylation site at amino acid 299 on the Fc portion of the heavy chain; however, this site does not usually carry alpha-gal or carries it in lower quantities on cetuximab [2, 6, 8]. Furthermore, it has now been established that oligosaccharides on the Fc site are not open to interact with IgE antibodies [8] (Figure 1). Our studies also provided clear evidence that these IgE antibodies were commonly present in sera of normal adult individuals in Tennessee, Virginia, and North Carolina [2, 9]. By contrast this IgE antibody is unusual in sera from Boston, California or Northern Sweden [2, 9].

While our findings largely explained the reactions to cetuximab, it left open two questions: why there were IgE antibodies present in this area and whether they were associated with any other disease. Screening sera from the allergy clinic in Virginia identified a group of patients who had presented with anaphylaxis or severe episodes of hives. In several cases, these patients were convinced that their episodes occurred 4-5 hours after eating beef or pork. Despite the inherent unlikelihood of an "immediate hypersensitivity" reaction occurring 4-5 hours after oral exposure, there was the rationale that this oligosaccharide (alpha-gal) is a blood group substance of the non-primate mammals [10]. In keeping with that, the patients were consistent in saying that they had no problems with chicken, turkey, or fish. We initially published the description of 24 cases of reactions to red meat, and subsequently published a paper documenting the serum IgE antibody levels in 208 cases [3, 11●●]. We are now aware of at least 1,000 cases in Virginia and have estimated that there must be more than 5,000 cases in the southeastern United States. Preliminary data from food challenges have confirmed the delay in symptoms after eating beef or pork. Furthermore, cases of this kind have now been reported from France, Sweden, Spain, Germany, Japan, and Australia, as well as the United States (Table II) [12●, 13, 14, 15●●, 16, 17, 18, 19●●].

The presence of these IgE antibodies in a defined area of the United States was initially an enigma. There did not appear to be any consistent relationship with pollen, fungal, dust mite or insect sensitivity. There were strong correlations with IgE antibodies to cow's milk, cat, and dog, but these could be explained by the presence of alpha-gal in the extracts [2, 3]. The first clue to the cause came from the observation that the area of these reactions coincided with the maximum prevalence of Rocky Mountain Spotted Fever (RMSF) [9]. That observation led to intensive investigation of the possible role of ticks bites in the IgE response to alpha-gal. The results showed i) a close correlation between the distribution of

Amblyomma americanum and the maximum incidence of IgE ab to alpha-gal. ii) A highly significant correlation between a history of prolonged itching after tick bites and IgE ab to alpha-gal. iii) Prospective evidence of an increase in IgE ab after tick bites in these cases. iv) Significant correlation between IgE ab to *A. americanum* and IgE to alpha-gal. Taken together the results argue that bites from the lone star tick *A. americanum*, were a, or the, primary cause of IgE ab responses to alpha-gal in the United States (9). We also became aware that Dr. Van Nunen in Australia had reported to the Sydney Allergy Society that some patients who were bitten by ticks in “the bush” had developed allergy to meat (18). However none of the results from USA or elsewhere explain why certain ticks give rise to IgE antibodies to this particular oligosaccharide.

Tick bites as a cause of IgE antibody response

Despite the worldwide prevalence of ticks and the wide range of diseases that can be transmitted by these ectoparasites, there is only a limited amount of evidence about the human immune response to tick bites. It was already known that ticks can induce an IgE antibody response, because there are ticks that can give rise to anaphylaxis at the time of the bite. Bites from *Argas reflexus* in Germany, *Ixodes holocyclus* in Australia, and *Ixodes pacificus* in the USA have been reported to cause anaphylaxis occasionally [20–22]. Furthermore, the sera from these patients have been shown to contain IgE antibodies to tick derived proteins [20]. Our results with *A. americanum* show that IgE antibodies to alpha-gal are common, can be very high titer, and can contribute 10-50% of the total IgE [9, 11●●].

The immune response to ticks has been extensively studied in mice, guinea pigs, and farm animals [23, 24]. In guinea pigs, the local response in the skin can be heavily infiltrated with eosinophils and basophils, and it has been argued that this cellular infiltrate can play an important role in preventing successful attachment and feeding by the ticks [25]. While there is extensive evidence about the biological activity of the components of tick saliva, very little of this information relates to *A. americanum* [26]. Bites of the lone star tick are often severely pruritic, and may persist for weeks [9]. By contrast, the bites of *I. scapularis* that transmit Lyme disease are generally not pruritic [27]. We would now add the response to tick bites as further evidence that the skin is a particularly effective route for producing specific IgE antibody responses.

Relevance to understanding the control of IgE responses

IgE antibody responses are generally considered to be T dependent; however, there is extensive evidence that the switch to IgE production can take place outside organized germinal centers [28, 29●]. IgE production to alpha-gal does not occur in children who are exposed to this epitope from gut bacteria or by eating or inhaling it. This leaves open the question of whether the switch of B cells to IgE production occurs in the skin or in the local lymph nodes (Fig 2). We have recently analyzed the evidence about IgE production and argued that extensive recombination of IgE B cells in germinal centers (GC) is not an essential part of the human IgE response [29●]. By contrast, a recent paper based on murine data argued that the production of IgE antibodies relevant to allergic disease requires extensive rearrangement to achieve high enough affinity [30]. In fact, the evidence from human studies suggests that binding of IgE on a basophil to repeated or multiple epitopes on a large molecule does *not* require high affinity of the IgE molecules [29●, 31]. What is certain is that patients immunized under conditions that favor formation of GC (i.e., high dose with an adjuvant) do not make high titer or persistent IgE antibody responses. Taken together, we would argue that the likely route for switch from IgM to IgE is outside fully formed GC, and also that much of the IgE production occurs from IgE plasma cells in protected sites within the bone marrow [29●].

The patients who present with delayed anaphylaxis or urticaria to red meat have several other features that may help to understand the mechanisms of sensitization. Firstly, many of these patients are over 60 years old when they present, and secondly, at least half of them do not have a significant allergic history. The implication is that an ectoparasite injecting truly foreign antigens into the skin can induce an IgE antibody response, regardless of atopic status or age.

Novel antigens, novel syndromes, and new diseases

Exposure to novel antigens can occur from new animals infesting the home or from novel food sources [32]. In addition, different extraction techniques (e.g., wheat isolate), genetically modified foods, or the addition of prebiotics or probiotics can change exposure. More recently, a supposedly benign cleaning agent used on the skin, triclosan, has been shown to have systemic effects related to allergy [33]. As we have learned from the alpha-gal story, a new syndrome may “emerge” either because the novel exposure causes sensitization or because the new food or medication identifies a pre-existing sensitization. In an allergy clinic, there are many patients who describe syndromes that do not make sense. It is difficult to investigate individual cases, but some significant syndromes have been identified correctly on the basis of as few as 5 cases. On the other hand, there are some “new” diseases, which have all the hallmarks of being allergic but are not well understood.

Short-chain galacto-oligosaccharide [GOS] is a prebiotic that has proven benefits as an additive to cow’s milk-based infant formula. In 2012, several cases of anaphylaxis to formula were reported from Singapore in subjects who were not allergic to cow’s milk proteins [34●●]. The evidence strongly suggested that these cases had become allergic to GOS for some reason, which appears to be restricted to Southeast Asia.

Eosinophilic esophagitis (EoE) is an important “new” disease in which inflammation of the esophagus gives rise to symptoms, including obstruction. There are good reasons for saying that diet is germane, but it has proved difficult to work out which food substances are relevant. Equally, it is not clear what form of sensitization is relevant, because some patients have eosinophilia and eosinophil infiltrates in the esophagus, *without* any skin test or serum evidence for an IgE response [35]. The question here is whether the increase in EoE could be related to changes in diet or to a new exposure that has allowed sensitization to common food substances. The most significant food sources appear to be wheat and cow’s milk, and as many as 50% of the patients have low titer IgE antibodies to one or both of these sources. However, it remains unclear which components [ie specific proteins] of these extracts are relevant [36, 37●●].

Several years ago, investigators in France and Belgium reported patients who had “immediate” reactions to pork that were severe [38, 39]. Investigation of those cases demonstrated that the reactions were related to IgE antibodies to pork albumin, which were cross reactive with cat Albumin [39]. This pork-cat syndrome has recently “emerged” in the USA [40].

Conclusions

The enthusiasm of the lone star tick and its larvae for biting humans has not only given rise to two novel forms of anaphylaxis, but also provided a model for understanding the ways in which parasites induce IgE responses without creating a risk for inhalant allergic disease [11]. Bites from this tick can cause high titer IgE antibodies to alpha-gal and also major increases in total IgE. Further, these responses often occur in adults over age 60 years and in subjects with no history of inhalant allergy. This is in keeping with the known ability of

helminth parasites that go through the skin to induce high levels of IgE, including IgE specific for oligosaccharides [41, 42●]. What is not clear is how this response is controlled. At present, we do not know about the role of T cells in this response or the mechanisms of isotype switch. The possibility remains that parasite-induced IgE responses to oligosaccharides are relatively T cell-independent. We do know that IgG1 antibodies to alpha-gal are part of the response to ticks, while the naturally occurring IgG response to alpha-gal is predominantly IgG2 [43]. Whether the natural antibody response to alpha-gal is influenced by the IgE response to the same epitope is less clear. Thus, it is possible that the allergic subjects have two different antibody responses against the same epitope that are not related.

It is already clear that the major increases in hay fever, asthma, and most recently, peanut allergy cannot simply be ascribed to hygiene [1, 44]. However, none of these diseases are an important feature of life in a pre-hygiene village [45, 46●, 47, 48●]. The important thing at this point is to realize that there are many different changes in lifestyle, diet, household exposure, etc. that could influence or encourage allergic disease (Table II). The alpha-gal syndrome or delayed anaphylaxis to red meat as we now understand it has broken almost all of the “rules” about food allergy, and there are undoubtedly other syndromes that will emerge. In the meanwhile, there are serious questions about the route of sensitization, the immunological control, and the reasons for the apparent increase in alpha-gal, nut allergy, pork-cat syndrome, GOS reactions, and EoE that will only be sorted out by studying the patients.

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Highlights

Tick bite induced, IgE to alpha-gal is related to two novel forms of anaphylaxis.

IgE to alpha-gal provides a model of a parasite-induced IgE response not related to asthma.

Foreign antigens entering the skin can induce high titer IgE antibodies.

Fig 1.

Glycosylation sites on the Monoclonal Antibody Cetuximab [2]

Asparagine N-43 on the light chain is not glycosylated. Asparagine N-88 on the FAB portion of the heavy chain is often or usually glycosylated with galactose- α -1,3-galactose [6]. Asparagine N-299 on the heavy chain can be glycosylated with α -gal, but the sugars are enclosed by the FC chains [8].

**IgG₂ to alpha-gal from
gut organisms:**

IgG₂ is common in antibody response to sugars and may be T-independent

IgE & IgG response to tick bites:

Local inflammation with the possibility of local switch due to multiple seed tick bites

IgE responses are generally considered to be T-dependent, but the specificity of T cells related to tick bites is not known

Fig 2.

IgG and IgE antibody responses to the oligosaccharide galactose alpha-1, 3-galactose: potential for distinct/unrelated control mechanisms

Table I

Explanations for the “emergence” of an allergen

A.	Changes in human existence, primarily related to hygiene, that have dramatically increased our IgE responses to common proteins in the environment.
	<ul style="list-style-type: none"> • Pollens, 1870 – 1940: skin tests • Dust mite, 1960 – 2000: skin tests, IgE antibodies • Peanut, 1995 – present: IgE antibodies
B.	Changes in the indoor environment that increased exposure to indoor allergens.
	<ul style="list-style-type: none"> • Increased temperature in houses – dust mite • Increased domestic pets, etc.
C.	Novel proteins or antigens in food that can either give rise to sensitization or reveal pre-existing antibodies to an epitope
	<ul style="list-style-type: none"> • Short-chain galacto-oligosaccharides (GOS)
D.	New or increased exposures to arthropods
	<ul style="list-style-type: none"> • Asian lady beetles and stink bugs • Tick bites from <i>Amblyomma americanum</i> – sensitivity to galactose-α-1,3-galactose

Table II

Diseases associated with IgE antibodies to galactose-alpha-1,3-galactose: USA and the world

	Year	Cases (n)	Tick Species	Reference
Anaphylaxis to cetuximab				
Cancer patients in NC and TN	2007	19	N/A	[4]
Delayed anaphylaxis or urticarial with red meat [†]				
Virginia	2009	24	<i>A. americanum</i>	[3]
	2013	~1,000	<i>A. americanum</i>	§
Nancy, France	2012	14	<i>I. ricinus</i>	[12]
Stockholm, Sweden	2013	39	<i>I. ricinus</i>	*
Germany	2013	21	"ticks"	[15]
Australia	2009	25	<i>I. holocyclus</i>	[18]
	2013	400	<i>I. holocyclus</i>	‡

[†] Other reports from Spain, Japan, and China [14,17].

[§] Published and unpublished data collected at the University of Virginia and in two private practice clinics in Lynchburg, VA [9,11].

* Manuscript submitted for publication.

[‡] Personal communication with Dr. van Nunen.