Opinion

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The *Anisakis* allergy debate: does an evolutionary approach help?

Alvaro Daschner[1], Carmen Cuéllar[2] and Marta Rodero[2]

[1] Servicio de Alergia, Instituto de Investigación Sanitaria, Hospital Universitario de la Princesa, Madrid, Spain
[2] Departamento de Parasitología, Facultad de Farmacia, Universidad Complutense, Madrid, Spain

Allergic phenomena share common pathways with the immune response against helminth parasites. The definitions regarding allergens and their related concepts have their roots in the area of allergy research. The experience with the fish parasite *Anisakis simplex*-associated allergic features still nurtures an open debate on the necessity of larvae being alive to induce allergic reactions such as urticaria or anaphylaxis. Conceptual definitions of allergen, major allergen, as well as putatively crossreacting antibodies, as are used in food allergy, depend on the clinical relevance of specific IgE and deserve careful interpretation in the various forms of *A. simplex*-associated allergic features. Conversely, an evolutionary based interpretation of the presence of specific IgE depends on the viability of *A. simplex*.

The *Anisakis* allergy debate

In 2002, a debate on the necessity of *Anisakis simplex* third stage larva being alive to elicit allergic symptoms was opened [1]. Meanwhile, several research areas in the field of *A. simplex* allergy have continued to publish their advances. It is, however, astonishing that clinical research has been the most neglected area, whereas laboratory studies have been a priority. These include *in vitro* diagnosis of *A. simplex* allergy, characterization of allergens and detection methods of antigens/allergens in food. With respect to these research areas, it has to be stated that they have all their major rationale in the supposed allergenic properties of several larval proteins and thus underpin the notion that *A. simplex* allergens would behave like food allergens. Although no study to date has scientifically confirmed that nonviable *Anisakis* material is able to induce acute allergic reactions in humans, the vast majority of all these laboratory studies claim their importance from the possibility of this type of allergy. Furthermore, *Anisakis* allergy has also been a focus in international food security agencies as to the possible allergenic potential and risk for the consumer of fishery products and has been included as an etiologic factor in the recent guidelines for the assessment of anaphylaxis [2,3] (see Glossary). It is nevertheless a typical feature of medical science that previously stated hypotheses persist unquestioned due to the impossibility to definitively discard them. Thus, in our case, efforts should focus on an estimate of the relative clinical importance of these statements.

Here, we would like to show that despite the potential quantitative power of all these scientific publications [4–8], claiming *a priori* the idea of *Anisakis* allergy as a food allergen in the past years, growing awareness of the scientific community about the relationship between parasites and allergy as well as a biologically plausible evolutionary based thinking enhances not only our understanding of *Anisakis* allergy and the role of immunoglobulin E (IgE) but also critically challenges some of the associated issues, such as the definition of allergens and major allergens, which are also frequently used in parasitology.

An historical overview

In 1990, *A. simplex* allergy was described for the first time in Japan [9]. Then, a boom of publications and research in the field of *A. simplex* allergy began after a new report from Spain on *A. simplex* induced anaphylaxis in 1995 [10]. Patients with acute allergic symptoms after consumption of parasitised fish displayed specific IgE against this nematode, and this parasite has from then on been handled as a potential food allergen. Further research has been performed following a classical protocol in food allergy. When patients display specific IgE against the source extract of a

Glossary

Anaphylaxis: the most severe, potentially life-threatening, multisystem event of an allergic hypersensitivity reaction. It can affect the skin, the respiratory, gastrointestinal, nervous and cardiovascular system.
Excretory–secretory products (ESPs): these are metabolic products released by the larva and frequently elicit an immune response.
FcεRI: this serves as a high affinity IgE receptor. It is constitutively expressed on mast cells and basophils, is inducible in eosinophils and is necessary for the allergic hypersensitivity reaction.
Gastroallergic anisakiasis: an acute allergic reaction (urticaria/angioedema or anaphylaxis) which is produced in the context of an acute gastric parasitism by *A. simplex,* when the parasite attempts to penetrate the gastric mucosa.
IgE: immunoglobulin E is the least abundant immunoglobulin, present in mammals and is involved in the allergic response as well as in the immune response against parasites. When mast cell (or basophil) bound specific IgE recognizes the allergen, mast cell degranulation occurs. Depending on the site of action, the resulting allergic hypersensitivity reaction can elicit urticaria, angioedema, rhinoconjunctivitis, asthma or anaphylaxis.
Major allergen: denotes an antigen against which more than 50% of a selected group of patients (allergic to the allergen source in question) react.
Mast cell and/or degranulation: see also 'IgE' and 'FcεRI'. Crosslinking of two FcεRI via the antigen–IgE complexes on the surface of mast cells or basophils elicits a cascade of events, by releasing inflammatory mediators. These can elicit symptoms such as urticaria, angioedema or anaphylaxis, but also symptoms such as in rhinitis, asthma or gastrointestinal reactions.
Urticaria/angioedema: a skin reaction with changing, raised, itchy hives. When deeper skin layers are affected, the inflammatory reaction produces a swelling of the tissue (=angioedema). Acute urticaria/ angioedema can be a manifestation of food allergy, when exposed patients display specific IgE for a specific food agent.

Corresponding author: Daschner, A. (alvarodaschner@gmail.com).

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Table 1. Characterized allergens of *Anisakis simplex*

	MW	Compartment	Function	Positivity[a]	Major allergen?[b]	Pan-allergen?[c]	Refs.
Ani s 1	24 kDa	ESP	Kunitz-type trypsin inhibitor	85%	Yes		[46]
Ani s 2	97 kDa	Somatic	Paramyosin	88%	Yes	Yes	[52]
Ani s 3	41 kDa	Somatic	Tropomyosin	4%?		Yes	[64]
Ani s 4	9 kDa	ESP	Cystatin	27%			[25]
Ani s 5	15 kDa	ESP	SXP/RAL protein	25–49%			[48]
Ani s 6	7 kDa	ESP	Serpin	18%			[48]
Ani s 7	139 kDa	ESP	Glycoprotein	83–100%	Yes		[43]
Ani s 8	15 kDa	ESP	SXP/RAL protein	25%			[26]
Ani s 9	14 kDa	ESP	SXP/RAL protein	13%			[47]
Ani s 10	22 kDa	Somatic?	?	39%			[45]
Ani s 11	55 kDa	Somatic?	?	47%			[49]
Ani s 11-li	?[d]	Somatic?	?	?			[49]
Ani s 12	?	?	?	57%	Yes		[49]

Abbreviations: MW, molecular weight; ESP, protein from excretory–secretory products.

[a]Positivity: percentage of IgE reactivity in *A. simplex* sensitized patients.

[b]Major allergen: major allergens are recognized by >50% of patients displaying IgE against *A. simplex*.

[c]Pan-allergen: highly conserved proteins, which can explain crossreactive antibodies of other food sources.

[d]? = unknown.

food agent, such as *A. simplex* extract, it is usual that IgE is detected against several, often different proteins. Therefore, one research area in allergy focuses on the detection and characterization of allergens. In the case of *A. simplex*, several allergens of *A. simplex* have now been characterized. Some of them have been claimed major allergens and/or pan-allergens (Table 1).

With respect to crossreactivity, several studies have shown by different means possible crossreactivity by carbohydrates, phosphorylcholine, other anisakids, ascarids, mites, cockroaches or crustaceans [11–19]. This circumstance has led several authors to claim crossreactivity to be responsible for 'false–positive' specific IgE findings in subjects without a clinical history of *Anisakis* allergy. However, it has been shown that most of detectable, specific IgE is due to previous subclinical parasitic episodes with *A. simplex* in exposed subjects due to fish-eating habits [20,21]. Thermostability of several allergens as well as resistance to pepsin digestion or low pH were further necessary to underline the possibility of allergic reactions to nonviable larvae [5,22–26].

However, from the year 2000 and as the result of a prospective clinical study, several critical analyses demonstrated that acute allergic symptoms such as urticaria, angioedema or anaphylaxis are produced only when the live larvae of *A. simplex* parasitise the gastrointestinal tract [27–29] causing gastroallergic anisakiasis (GAA). Anaphylaxis has been diagnosed in 35% of GAA patients [29]. The clinical implication for all these patients is the lack of necessity to follow a strict diet without fish. Deep-freezing of fish at –20 °C kills *A. simplex* larvae, and this simple dietary advice prevents patients with previous GAA from any further *A. simplex* associated allergic reactions [3,27,30].

Challenge tests with nonviable larvae of *A. simplex* were always negative in patients with GAA, even in those with previous anaphylaxis [31,32]. Nevertheless, it was possible that putative clinically relevant excretory–secretory products were not available in sufficient quantities in these studies; thus, another study used excretory–secretory products for the challenge tests again without any positive reaction [33]. Thus, the new concept of GAA implicated that the clinical hypersensitivity reaction be produced by the immune response against the acute parasitism by *A. simplex*. On the contrary, allergic reactions, including anaphylaxis, after intake of supposedly well-cooked fish have still been described and have been attributed to contact with nonviable *Anisakis* allergens [19]. It is important to recognize that no subsequent data has shown any positive challenge test with nonviable *A. simplex* material (Figure 1).

What role does IgE play within the allergic reaction?

The classical model in food allergy states that mast cell bound IgE, which is retained by the high affinity receptor of IgE, FcεRI, recognizes the allergen with its respective specificities. Mast cell and/or basophil degranulation in the tissues elicits the typical hypersensitivity type I symptoms, such as those seen in food allergy or GAA. Specific IgE can be detected by a skin prick test (SPT) against the eliciting food or it can be measured in serum.

What about specific IgE against *A. simplex*? Patients with a past episode of GAA display a positive SPT and specific serum IgE against *Anisakis*. It was shown that specificities are highly variable between patients, as well as the amount of response [29,34–36]. This can be detected by different means, such as IgE immunoblotting, or ELISA. Early studies in Japan on gastric or intestinal anisakiasis, however, showed that specific IgE is always produced, even in patients without any clinically overt allergic symptoms [37]. Yet, the epigastric pain suffered by patients with gastric anisakiasis has been postulated to be the correlate of an allergic reaction [38].

Surprisingly, all these patients tolerate well-cooked or deep-frozen fish, even if several studies have shown several of these allergens to be stable, especially allergens that are heat-stable or resistant to pepsin and deep-freezing methods [5,6,22–26]. The prevalence of *A. simplex* parasitising fish has been shown to be sufficiently high to infer a frequent contact with *Anisakis*-derived proteins and other

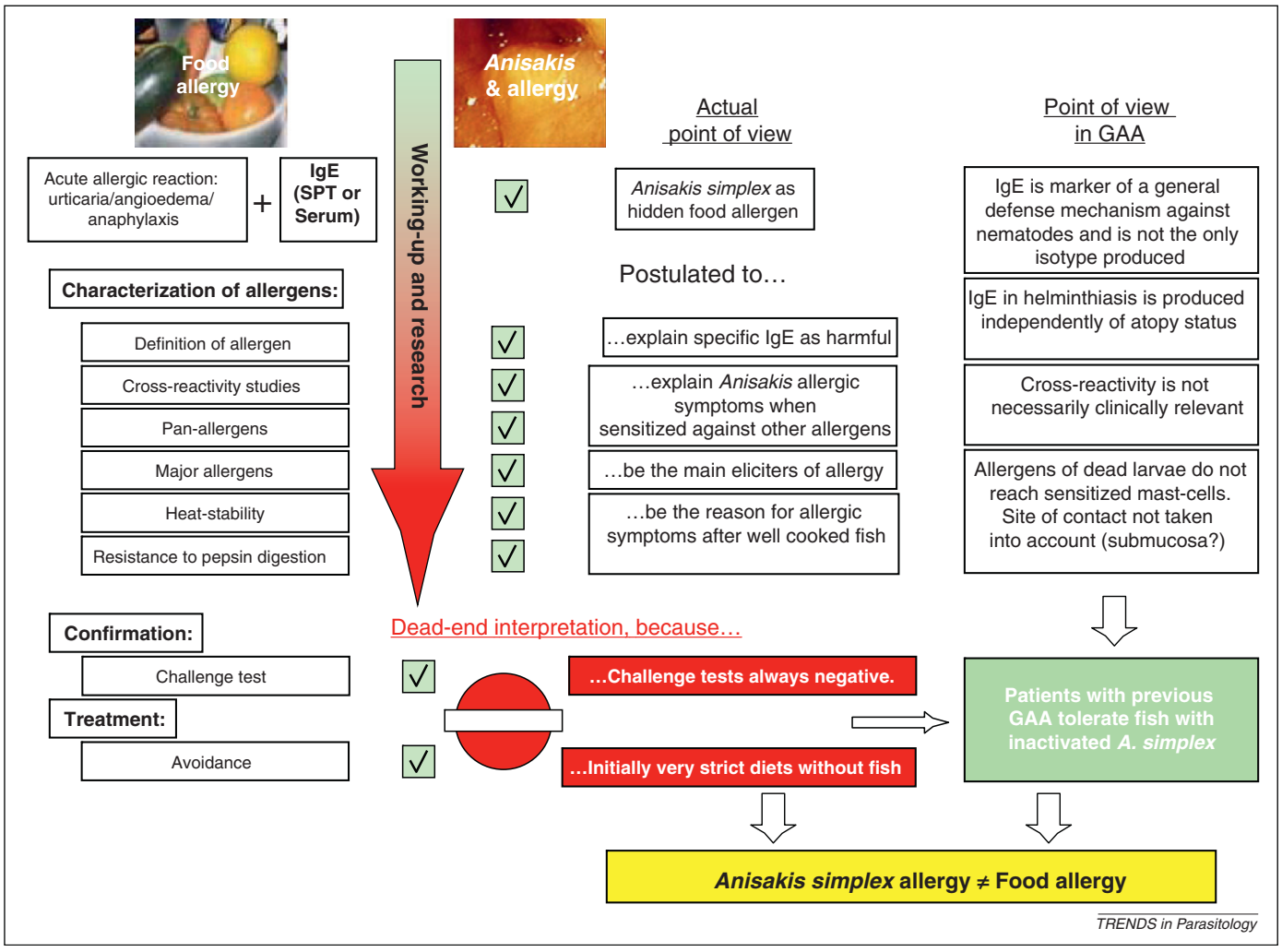

Figure 1. Food allergy versus *Anisakis simplex* allergy. When dealing with food allergy, allergic symptoms are accompanied by detection of specific IgE against the suspected food agent. However, owing to the possibility of clinically irrelevant specific IgE, the challenge test is the gold standard to confirm the suspected allergy. Research in food allergy includes characterization of allergens in the source material. Major allergens are recognized by >50% of confirmed allergic patients. Pan-allergens are highly conserved proteins, which can explain crossreactive antibodies of other food sources. When the allergens are heat stable and resistant to pepsin they are able to reach the gastrointestinal submucosa, where they can encounter specific mast cell bound IgE and elicit allergic hypersensitivity reactions. All these arguments have been applied to *A. simplex* allergy. However, challenge tests with inactivated *A. simplex* are always negative. Furthermore, more than 13 years of follow-up of patients with past GAA who have been eating well-cooked or deep-frozen fish, which is frequently parasitised with *A. simplex*, have been tolerating fish, which confirms that IgE against *A. simplex* allergens, which includes major allergens, heat stable allergens and allergens resistant to pepsin digestion and freezing procedures, is not to be dealt with as food allergy. In the right column, arguments are given for an alternative interpretation of IgE and 'allergens' in *A. simplex* associated allergy, as is the case in GAA. Crossreacting antibodies as assessed *in vitro* have not been shown to elicit allergic symptoms in challenge tests with nonviable larvae in patients after GAA [33]. This point of view does not exclude possible 'true' allergic reactions, as have been postulated to occur after contact with dead *Anisakis* allergens. Abbreviations: SPT, skin prick test; GAA: gastroallergic anisakiasis.

products and thus allergens [20]. Thus, if these patients display specific IgE, why do none of these antibodies act as a food allergen?

It has been shown that patients with GAA show a dynamic polyclonal stimulation after the parasitic contact. Specific IgE rises after 1 month, and immunoblotting studies have shown that IgE with new specificities are produced [35,39]. But IgE is not the only immunoglobulin isotype produced. Also specific IgG, IgG_4, IgA and IgM antibodies can be detected against *A. simplex* in sera of GAA patients [29]. Thus, all these features – production of IgE, polyclonal expansion of IgE and other immunoglobulin isotypes – belong to a typical immunological response against helminth parasites [40]. In contrast to food allergy, this response is not associated with an atopic predisposition; it belongs to a general and evolutionary conserved response against an invading helminth [41]. Again, the IgE response is independent of overt allergic symptoms. This line of argument moves us away from allergology and brings us nearer to a parasite-induced perspective of the IgE response.

IgE: link between allergy and parasitology

Allergies and the immunologic response against helminth parasites share common pathways. Both are typical Th2-activated responses with production of interleukin 4 (IL-4), IL-13 and IL-9. IL-9 has its major effect on mast cells, is responsible for intestinal mastocytosis and sensitizes mast cells for further action of other cytokines or the effect of specific degranulation and mediator release. IL-4 and IL-13 have several activities and are paradigmatic of the Th2-deviated immune response, but IL-4 is responsible for the switch to IgE production and has effects on the smooth muscle, epithelial cells and goblet cells of the

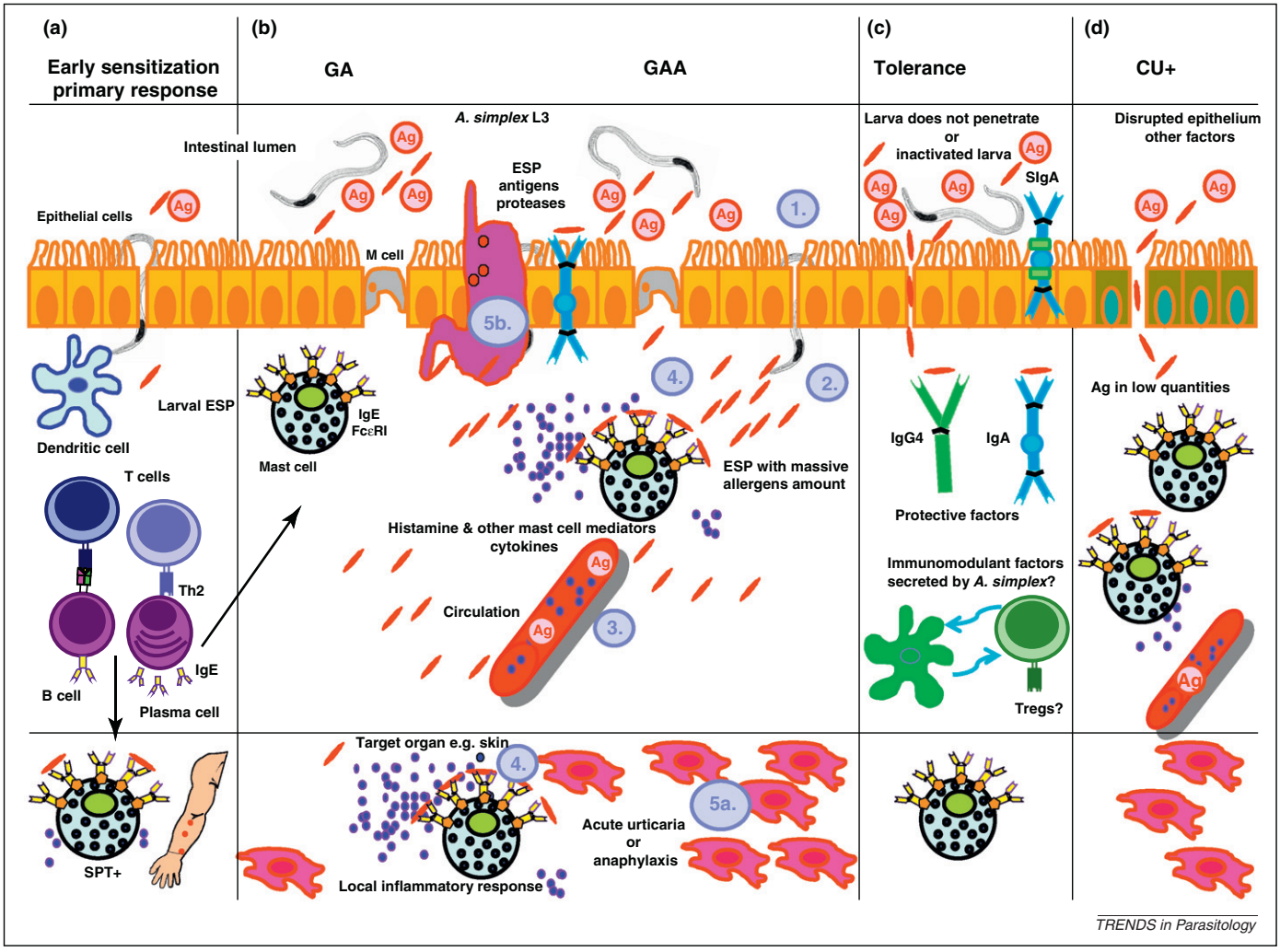

Figure 2. *Anisakis simplex*, alive, inactivated and their allergens. **(a)** Early sensitization occurs when the live *A. simplex* L3 larva penetrates the gastrointestinal mucosa, and within a Th2-biased milieu finally produces specific IgE against *A. simplex* excretory–secretory products (ESPs) and bystander (surface or somatic) antigens. This results in circulating IgE, as well as mast cell bound IgE to the high affinity receptor for IgE, FcεRI, localized in the submucosa, but also in other target organs, such as the skin. Positive skin prick tests (SPTs) demonstrate sensitized mast cells after a first parasitic episode. **(b)** Gastric and gastroallergic anisakiasis: a live L3 larva penetrates after sensitization in a new episode the gastric epithelium **(1)**. Parasite proteases and other products help the larva to migrate through the epithelium and the immune exclusion mechanisms; for example, secretory specific IgA are bypassed and massive amounts of ESPs gain access to the submucosa **(2)** and then via circulation to target organs, such as the skin **(3,5)**. Some of them are allergens, which bind to the IgE molecules on the mast cell, and by crosslinking FcεRI molecules, trigger degranulation of these cells and histamine. Other mediators and cytokines are released **(4)** and begin a series of events leading to allergic symptoms in susceptible patients such as urticaria or anaphylaxis (gastroallergic anisakiasis, GAA) **(5a)** or in gastric anisakiasis to a local inflammatory response (5b). **(c)** Tolerance: when the live larva does not penetrate the gastric mucosa, or inactivated *A. simplex* larvae or their allergens remain in the gastric lumen, protective factors keep sensitized mast cells in the submucosa and other target organs from coming into contact with putative allergens. These factors could include secretory IgA (sIgA), circulating and tissue dwelling IgA or IgG_4, which compete for the allergens, or immunomodulatory factors secreted by *A. simplex*. Low level access of allergens into the submucosa could thus be possible without resulting clinical symptoms. It is also possible that cofactors necessary for the acute allergic reaction are only present when they are released in the context of penetration by the L3 larva. Unlike in food allergy, the epithelial barrier function is not necessarily disrupted [69]. It is rather the live larva, which actively bypasses all barrier functions. **(d)** Chronic urticaria: a relationship of *Anisakis* sensitization-associated chronic urticaria (CU) with previous parasitism by this nematode has been postulated. The pathomechanism has not been elucidated, but low or missing specific IgG_4 or sIgA production as well as a disrupted mucosal epithelium could account for allergens in low quantities gaining contact with submucosal mast cells and producing a prolonged or chronic urticarial reaction. Abbreviations: GA, gastric anisakiasis; GAA, gastroallergic anisakiasis; ESP, excretory–secretory products; Ag, *A. simplex* antigens; FcεRI, high affinity receptor of IgE; SPT+, positive skin prick test; Tregs, T regulatory cells; CU+, *Anisakis* sensitization-associated chronic urticaria.

gastrointestinal tract to produce the 'weep and sweep' response; this is an evolutionary maintained response to eliminate larvae or minimize helminth burden [42]. The same mechanisms are responsible for the allergic reaction. Depending on the site of contact with allergens, these induce the asthmatic response, an intestinal reaction or a cutaneous one as in IgE-mediated urticaria or angioedema.

In GAA, we do not know which of the multiple IgE specificities is clinically relevant and at which site and under which conditions. We only know that acute allergic symptoms appear only in the context of the acute parasitism and can suspect that one of the excretory–secretory products has to be actively secreted to the submucosa when the larva penetrates the gastrointestinal tract [29]. One candidate of the characterized allergens is *Ani s 7*, as it is an excretory–secretory protein actively produced by *A. simplex* [43]. However, even if nearly 100% of patients with GAA display IgE antibodies against this allergen, patients tolerate well-prepared fish. Nevertheless, if the necessary site of contact or secretion is the submucosa, a clinically relevant allergic reaction is only to be expected when the live larva penetrates the gastric or intestinal mucosa. Another possibility derived from the demonstration of parallel specific IgA and IgG_4 production would also

Table 2. Definition of allergen

Term	Definition	Refs.
Allergen	An agent (e.g. pollen, dust, animal dander) that causes IgE-mediated hypersensitivity reactions.	[65]
	Any substance stimulating the production of immunoglobulin IgE in a genetically disposed individual (but is synonymous with antigen, a term usually used to describe a substance that generates immunoglobulin responses other than IgE or a cellular immune response).	[66]
	An antigen that induces an allergic or hypersensitivity response.	[67]
	A nonparasitic antigen capable of stimulating a type I hypersensitivity reaction in atopic individuals.	[68]

be their activity as inhibitory or blocking antibodies (Figure 2) [20,29]. Hymenoptera allergy is elicited by stinging insects and could be a model for comparison. Interestingly, specific IgG_4 has been associated with protection even when specific IgE is present [44].

Allergens, major allergens and pan-allergens

The definition of an allergen is not uniform (Table 2). Whereas all definitions are uniform with respect to the statement that an allergen is an antigen able to produce IgE antibodies, most, but not all, incorporate a further requirement: an atopic status is necessary to respond with an IgE response. Most important, however, is that the definition rarely includes the necessity of the allergen to be a nonparasitic antigen. This is logical, as the IgE response against helminths is universal in vertebrates. Nevertheless, IgE-producing parasite-derived antigens have been isolated and characterized. Even the international nomenclature consensus has been applied to these proteins and, in our case, IgE-producing antigens for *A. simplex* have been called *Ani s 1* through *Ani s 12* [23,26,45–52]. Even so, two *A. simplex*-derived proteins have been designated pan-allergens *Ani s 2* and *Ani s 3*, but unlike in food allergy, where the presence of pan-allergens explains clinically overt symptoms with different antigen sources containing these pan-allergens, it has not been shown to date that *A. simplex*-derived pan-allergens are clinically relevant [53,54]. A recent study has shown tropomyosin to be a candidate pan-allergen able to produce immunological crossreactivity in human filarial infection that might affect sensitization to house dust mites [55]. *Ani s 3*, the tropomyosin of *A. simplex*, has been characterized as an allergen, but only a very small proportion of *Anisakis* allergic sera has been found to recognize this allergen [54].

Another point is the definition of major allergen in relation to the 'allergic' episode. It has been shown that the detection of different 'major' allergens is dependent on the time elapsed between the gastroallergic episode and the serum analysis [43]. Recent data show that the first described major allergen *Ani s 1* is a major allergen after GAA but is detectable in only 42% of cases when specific IgE against *Anisakis* is associated with chronic urticaria (M. Rodero *et al.*, unpublished). This is contrary to *Ani s 7*, which is highly (>90%) recognized after GAA as well as in *Anisakis* associated chronic urticaria, a fact that underlines a previous parasitic contact.

The problem arises from the false interpretation of allergens as possible allergy producing agents. We have now argued that none of the 12 characterized allergens produces allergic symptoms after an episode of GAA in frequent fish consumers who freeze the fish to be consumed and are thus exposed to *A. simplex* allergens. But the possible misinterpretation of applying definitions of the allergy field to parasitology becomes even worse; several of the characterized allergens are designated major allergens. These are recognized by more than 50% of patients sensitized against *Anisakis*, as detected by serum specific IgE or SPT [56]. This should not mean that these antibodies are clinically relevant. In food allergy, when it has been demonstrated that a major allergen is stable to heat and digestion, this should be clinically relevant, as it can reach the gastrointestinal mucosa undamaged. Yet, when dealing with well-prepared fish containing *Anisakis* allergens this is not the case. A further requirement is necessary. One first requirement for specific IgE to elicit symptoms, such as urticaria, is that the encounter with the corresponding allergen must be at a specific site (possibly the submucosa). Further necessities, such as the IgG_4/IgE ratio, or other unknown necessities have not been thoroughly addressed when evaluating allergenicity issues related to *A. simplex* allergy and should be studied further (Figure 1).

A different focus on this dilemma renders a better explanation: *Anisakis* is a helminth parasite and humans have evolved general defense mechanisms against helminths of this order, such as *Ascaris lumbricoides* or *Toxocara* spp. [57]. One of these mechanisms is the production of IgE. Even if *Anisakis* is not a natural human parasite, why should evolutionary shaped biological laws not apply to this nematode? In fact, whereas all patients with the different forms of gastric or intestinal anisakiasis produce specific IgE, the high prevalence of clinically overt allergic conditions in *A. simplex* parasitism has been postulated to the fact that humans are not a natural host for this parasite, and *Anisakis* parasitism is only acute or 'intermittent' (repeated acute parasitism by *Anisakis*). Therefore, it lacks immunoregulatory features typical of other chronic helminthiasis [58]. In this sense, urticaria has even been proposed to be the exaggerated clinical outcome of a beneficial immunologic mechanism [59].

Concluding remarks and future directions

Here, the acute, putatively IgE-mediated reactions that accompany GAA have been analyzed; however, chronic urticaria has also been associated with previous parasitic episodes, and the pathogenic mechanisms have not been elucidated. It is interesting to denote that low IgG_4 responders have been associated with a prolonged acute or a chronic urticarial reaction [20,60]. It can therefore not be ruled out, that in this case, and together with an altered intestinal permeability, such as in food allergy, allergens could bypass the parasite-driven regulatory network and reach the final target organs to elicit allergic symptoms such as urticaria (Figure 2) [61].

Thus, we show that IgE production against *Anisakis* is a normal, evolutionary maintained feature, where acute urticaria/angioedema or anaphylaxis can accompany the acute parasitic episode. However, when dealing with IgE-inducing antigens in laboratory research, the knowledge of allergy research is not sufficient. Cautious handling with the definition of allergen, major allergen or pan-allergen should prevent allergists and parasitologists from drawing a direct line from IgE to allergy.

In *A. simplex* allergy and GAA, after obtaining a thorough clinical history, the etiologic diagnosis is partially based on detection of IgE antibodies in serum or by SPT against an extract of the larva. When gastroscopic examination has been performed, most cases are diagnosed by a visual microscopical morphological classification. It would now be interesting to apply available molecular tools to test the hypothesis that different *Anisakis* species could be responsible for a distinct clinical outcome (only gastrointestinal symptoms/GAA/*Anisakis* allergy) [62,63].

Further issues to be resolved are the role of the other immunoglobulin isotypes produced, especially IgG_4 and IgA, and if these are really associated with the regulatory network. As acute allergic reactions associated with non-viable larva are also described, epidemiological studies could help to clarify the relative quantitative and qualitative importance of the distinct forms of *Anisakis* allergy. Challenge tests with inactivated *A. simplex* containing the different characterized allergens in those patients claimed to be allergic to *A. simplex* (without live larva) could be useful. It is to be expected that research in allergology will still benefit from knowledge in the field of parasitology.

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