

Towards a differential definition of atopy: *Anisakis simplex* and the relationship between parasites and arthropods in respiratory allergy

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SUMMARY

Protective as well as enhancing effects of parasite infections on allergic disease have been postulated. Previous studies on this relationship focused frequently on skin test reactivity against aeroallergens, being house dust mites (HDM) the main agents responsible for a positive atopy outcome.

*We aimed to analyse the possible relationship between human parasite infection induced *Anisakis simplex* urticaria and respiratory allergy.*

*A total of 86 patients with gastro-allergic Anisakiasis and 203 patients with chronic urticaria sensitized against *A. simplex* were studied for sensitization against aeroallergens and evaluated for rhinoconjunctivitis or bronchial asthma (RCBA). We compared the results with a control group of 250 consecutive patients referred for evaluation of allergic RCBA and atopy prevalence data of our region.*

*Whereas no effect of *A. simplex* related disease on the overall allergic respiratory disease could be detected, a highly significant higher prevalence of RCBA associated HDM sensitization, but diminished allergy against other common aeroallergens (pollen, mould or dander) was observed in these groups.*

*The relationship between *A. simplex* parasitism-associated acute or chronic urticaria on one side and allergic respiratory disease on the other side depends on the definition of atopy. We propose a differential definition of atopy, with a special emphasis on arthropod related sensitization.*

Keywords allergic rhinitis, *Anisakis*, atopy, bronchial asthma, gastrointestinal nematode, urticaria

INTRODUCTION

The relationship between parasites and allergy is an intriguing matter in continuous debate in the last decades. One of the main, but still controversial, topics has been the hypothesis that parasite infection could be able to prevent allergic disease. In general, antiworm IgE antibodies, one of the Th2-features associated with parasite infections, are lower in continually re-infected children than in adults who remain uninfected (1). Evidence is accumulating that chronic helminth infections are inversely associated with immune disorders, including allergic disease (2,3). Thus, different studies show an inverse association between helminth infection and atopy, as well as allergic and nonallergic asthma (2,4,5). On the other side, other studies, mainly from regions with low prevalences of helminthiasis, have shown a positive association between asthma and *Ascaris* infection (6–8). Yazdanbakhsh *et al.* worked out an interesting hypothesis that could explain and unify the apparently controversial findings by postulating a relevant feature of IL-10 produced in chronic vs. sporadic parasite infections (9). One of the postulated features of this anti-inflammatory cytokine is its implication in the differential regulation of IgG₄, which may act as a blocking antibody preventing mast-cell degranulation (10). Thus, in contrast to chronic parasite infections in endemic regions, sporadic parasite infection would produce an enhancement of the allergic phenotype (11).

There is a lack of general agreement as to the precise definition of atopy and how the atopic individual can be identified: whereas the World Allergy Organization defines atopic disease as clinical allergic symptoms in combination with a positive allergy (skin prick test, SPT), the term atopy is frequently used to define a genetic predisposition to react by SPT to common aeroallergens, regardless of clinical symptoms. By analysing the different studies with respect to the outcome of protective or enhanced atopy or allergy, other variables in the study design or population sample are significant and summarized in Table 1.

Anisakis simplex parasitism is of worldwide distribution, being the most frequent clinical feature gastric Anisakiasis.

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Abbreviations: CU+, chronic urticaria with sensitization to *Anisakis simplex*; GAA, gastro-allergic Anisakiasis; RCBA, rhinoconjunctivitis or bronchial asthma; SPT, skin prick test

Table 1 When studying the relationship between parasite infection and the prevalence of allergic disease, the following variables have up to now been reported to be significant with respect to the outcome of protective or *enhanced* allergy by parasites (revised by Carvalho *et al.* (46))

Chronic vs. sporadic infection (frequent, infrequent) (11,46)
Parasite load (46)
Immunologic response is species specific (47)
Definition of protection or enhancement of allergy by atopy or asthma
Definition of atopy: specific serum IgE vs. SPT
The differences in the effects of geohelminth infections on allergic sensitization may relate to the endemicity of geohelminth infections between different populations (5)

Abdominal symptoms in acute gastric Anisakiasis result from the penetration of the gastric mucosa by the live larva of *A. simplex*. Gastro-allergic Anisakiasis (GAA) has been described as a *subgroup* of gastric parasitism, where the *acute infection* is accompanied by allergic symptoms like urticaria, angio-oedema or anaphylaxis (12,13). Chronic urticaria has also been related to previous parasitism by this ubiquitous fish-nematode (14,15). As the *A. simplex* cycle is not adapted to humans, these are accidentally infected and the parasite neither survives for more than a few days nor is reproductive. Due to the high rate of fish-consumption in Spain, of which the ingestion of raw, marinated anchovies play an important risk factor, the described *A. simplex*-induced allergic entities have a high prevalence in our area (Figure 1) (16). Gastric or gastro-allergic parasitism has been described as a model of acute parasitism, and is thus interesting to study with respect to the accompanying allergic phenotype (16).

The purpose of this study was to analyse the atopy status as well as respiratory allergy in *A. simplex* related allergic disorders.

Our results show convincing evidence that other important factors, like the relationship between arthropod aeroallergen prevalence and house dust mite skin reactivity or a differential definition of atopy should be taken into account when analysing the interaction between parasites and allergy.

METHODS

We studied a total of 289 patients with *A. simplex* related allergic disorders: 86 patients with GAA and 203 patients with chronic urticaria sensitized against *A. simplex* (CU+). As a control group served another 250 consecutive patients with respiratory symptoms of suspected allergic origin.



Figure 1 A succulent fish meal?: Here we can observe a live filiform nematode (*Anisakis simplex*) emerging from anchovies (*Engraulis encrasicolus*). If parasitized fish is eaten crude or undercooked, the live larva, by penetrating into the gastric mucosa can produce gastric Anisakiasis, gastro-allergic Anisakiasis as well as a propensity to a chronic urticarial reaction.

All patients were studied by means of SPT for sensitization against common aeroallergens. We have available data about the existence or absence of RCBA in 92.5% of the studied patients.

Patients selection

All patients were consecutively recruited from the Allergy Section of the University Hospital La Princesa in Madrid in the centre of the city and from our peripheral allergy-unit in Coslada, a semi rural area. GAA was diagnosed as previously described (13). Shortly, diagnostic criteria were a compatible history of intake of raw or undercooked fish in the 24 h previous to the onset of acute urticaria, angio-oedema or anaphylaxis, with or without digestive symptoms, together with a positive SPT against *A. simplex* and a detectable specific serum IgE against *A. simplex*. In the case of an only doubtful implication of a suspected fishmeal, a descending total IgE and specific IgE several months after the acute episode helped us to confirm diagnosis as previously described (17). Criteria to include patients in the CU+ group were chronic recurrent wheals for more than 6 weeks, a positive SPT against *A. simplex* and detectable specific IgE against *A. simplex* (> 1.5 kU/L).

The control group was composed of consecutive patients referred to us for allergological evaluation of RCBA of physician-based suspected allergic origin. These were not assayed for *A. simplex* sensitization, assuming a known prevalence of *A. simplex* sensitization between 15% and 20% (16).

Skin prick test

SPT with *A. simplex* antigen (Laboratory IPI, Madrid, Spain). SPT against aeroallergens (Laboratory ALK-Abelló, Madrid,

Spain): animal dander (cat, dog), house dust mites (HDM, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*), five pollen mixtures of grass, tree and weed pollen, and mould (*Alternaria alternata*, *Cladosporium herbarum*). SPT was performed in standard technique and was considered positive with a mean wheal diameter of $\geq 3 \times 3$ mm. Histamine at 1% concentration and saline solution 0.9% (NaCl) were positive and negative controls. Sensitization to at least one aeroallergen group was evaluated. Our control group serves only for comparison of SPT reactivity of patients with RCBA.

Therefore, we also carried out an estimated comparison of our data with prevalence rates in the general population. We analysed data from a study based on the European Study of Asthma (18), a population-based study showing data about skin reactivity in subjects with ages between 20 and 44 years against pollen, HDM, mould and dander, comparable with our study and a similar SPT-method. Due to the wide variations in prevalence of atopy described in Spain (19), we only analysed data of a region in Central Spain with similar climatic conditions to our study population.

Laboratory determinations

Specific IgE against *A. simplex* was assessed by Cap-Feia (Phadia, previously Pharmacia, Uppsala, Sweden). Specific IgE was considered positive with results > 0.35 kU/L, but patients were included only if specific IgE was > 1.5 kU/L in order to minimize false-positive test results.

Clinical respiratory symptoms

Patients were asked about seasonal or persistent RCBA for at least 2 years, and were classified as having RCBA symptoms or not *regardless* of the posterior SPT results with respect to the physician-based suspicion of allergic RCBA.

Statistics

Statistical analysis was performed using SPSS 15.0 for Windows Version (SPSS Inc.).

Mean ages were calculated in all studied groups and compared by ANOVA.

Prevalences were calculated for gender in all studied groups and compared by χ^2 -test.

Prevalences were calculated for: sensitization to at least one *aeroallergen group*; (HDM; pollen, mould or dander (PMD) as one sensitization group), RCBA, and RCBA associated sensitization. χ^2 -test and Fisher's exact test were used for comparison of sensitized patients in the studied groups and control group.

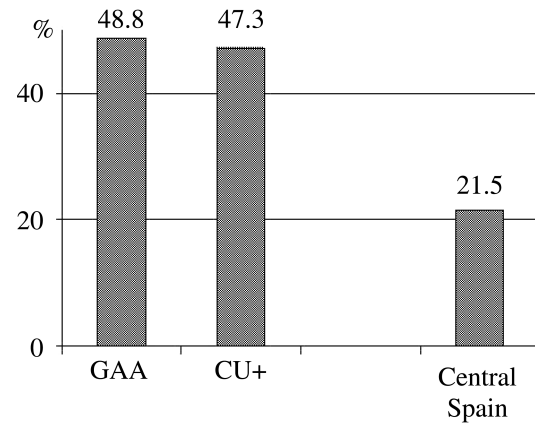


Figure 2 Prevalences of positive skin reactivity against aeroallergens. Patients are sensitized against at least one aeroallergen (house dust mites, pollen, mold or animal dander). The prevalence for the general population is shown as the mean value from a comparable study in the same region (18.3–24.6%). GAA: Gastro-allergic Anisakiasis, CU+: Chronic urticaria with sensitization against *Anisakis simplex*.

As mean age was significantly lower in the control group with respect to the studied groups, the control group was matched for age, and the comparisons were studied in a second statistical analysis.

RESULTS

A significant difference in the ratio male/female was observed comparing GAA patients (50/50) with CU+ patients (30/70; $P = 0.01$) and with the control group (36/64; $P = 0.03$).

Mean age was between 40 and 46 years in the studied groups and 35 years in the control group ($P < 0.0001$).

After matching for age, mean age was 42.7 years in the control group ($n = 166$).

Due to the inclusion criteria, all CU+ and GAA patients had a positive SPT against *A. simplex*. Median specific IgE against *A. simplex* was 14.5 (IQR 5.3–50.6) kU/L in CU+ and 54.2 (IQR 23.5–96.9) kU/L in GAA.

The prevalence of positive SPT against at least one *aeroallergen group* was clearly elevated in *A. simplex* related disorders, compared with the overall prevalence in the general population (Figure 2).

The prevalence of RCBA regardless of SPT data was 24.5% for GAA and 40% for CU+ patients, without significant differences within the studied groups. We have not any available useful data for comparison.

If only those patients were studied with sensitization *against* at least one *aeroallergen group* (= RCBA associated sensitization), the prevalence of RCBA was 21.1% in GAA

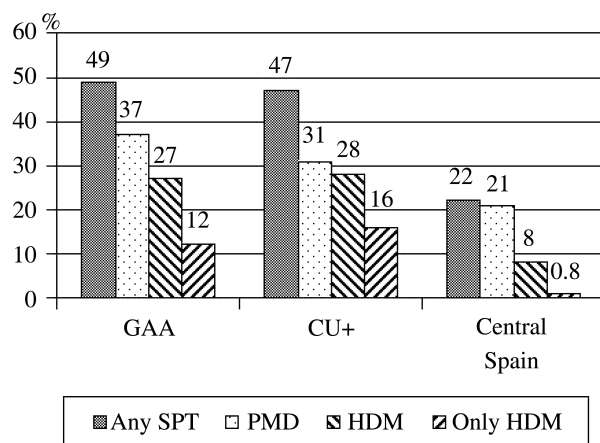


Figure 3 Differential prevalences of positive skin reactivity against aeroallergens in studied groups. Data for the general population are shown as an estimated comparison by combining the overall prevalence of positive SPT in our region (adapted data, see Methods-section) and our own differential results. GAA: Gastro-allergic Anisakiasis, CU+: Chronic urticaria with sensitization against *Anisakis simplex*. SPT: Skin Prick Test. PMD: pollen, mould or dander. HDM: house dust mites.

and 28.8% in CU+ patients (no significant differences within the studied groups). No useful data are available for a scientifically significant comparison.

The prevalence of positive SPT against aeroallergens in patients with RCBA was between 76.3% and 84.6% in our studied groups and similar with 75.6% in our control group. This means, that a similar proportion of patients with physician-based RCBA did not display any positive SPT.

We searched for a probable explanation of the high prevalence of positive SPT to aeroallergens in all studied groups of *A. simplex* related disorders: patients were above all sensitized against PMD, but interestingly HDM sensitization makes up about half of the sensitization rate in *A. simplex* related allergic disorders. On the other side in Central Spain only about 7.5% are sensitized to HDM (Figure 3).

Exclusive HDM sensitization in the studied groups is frequent and could explain in part the overall elevated SPT prevalences (Figure 3) in these groups.

We then analysed the possible clinical relevance of the different sensitization rates. Thus we looked for the distribution of specific aeroallergen sensitization ONLY in patients with respiratory symptoms (RCBA) (Figure 4). We detected in our control group a higher sensitization rate against PMD (96%) compared with our studied groups (66–76%) but a higher sensitization rate against HDM in *A. simplex* related disorders (55–59%; $P = 0.001$).

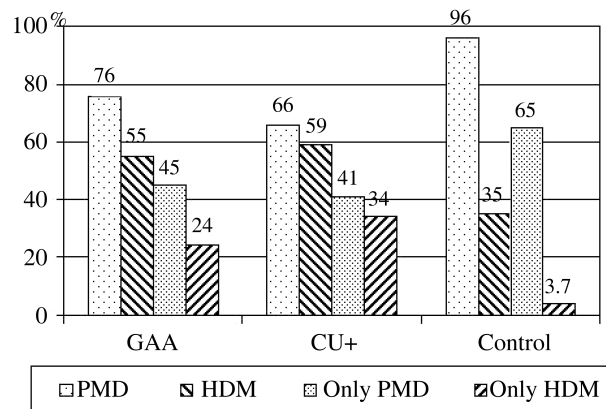


Figure 4 Differential sensitization prevalences in patients with allergic rhinoconjunctivitis or bronchial asthma. This analysis includes patients with sensitization to at least one aeroallergen (HDM: house dust mites; PMD: pollen, mould or dander). GAA: Gastro-allergic Anisakiasis, CU+: Chronic urticaria with sensitization against *Anisakis simplex*. The differences between all studied groups versus control group are highly significant (p between 0.001 and 0.0001).

Analysing respiratory allergy for sensitization only against HDM, we find only about 4% sensitization in our control group (patients with RCBA associated sensitization), but a significant higher percentage between 24% and 34% in our studied groups ($P = 0.0001$). In parallel, sensitization only against PMD allergens is diminished in *A. simplex* related disorders (41–45%) compared with our control group (65%; $P = 0.001$) (Figure 4).

After matching the control group for age, no differences in the commented results could be stated.

DISCUSSION

Before analysing our data, and comparing with previous studies in the same fields, for a more comprehensive reading, some special aspects of this study have to be highlighted:

- 1 Our study and control population in Central Spain live in a predominantly dry climate at about 600 m and are exposed to low levels of HDMs.
- 2 No chronic parasitic disease is worth mentioning with respect to a significant prevalence.
- 3 High sensitization rates against *A. simplex* in the general population explain the endemic character of contact of our population with this parasite, but GAA and Gastric Anisakiasis are acute expressions of this parasitism, whose larvae do not survive in humans.
- 4 In our evaluation of atopic disease, we do not only take into account only bronchial asthma, but also allergic rhinoconjunctivitis.

The prevalence of positive SPT to any aeroallergen was estimated to be more than two times higher in our studied groups than previously described in a comparable population based sample. When we analysed for differential sensitization, a high proportion of positive SPT could be attributed to exclusive HDM sensitization. This fact is considerable, as sensitization to HDM is infrequent in Central Spain, with a predominant dry climate and an altitude of the meseta (plateau of Castile) of > 600 m. Exclusive HDM sensitization in the general population has been estimated at about 0.8%, but between 12% and 16% in our studied groups, reflecting the possibility, that this HDM sensitization without sensitization to other aeroallergens could be the main responsible for the overall elevated SPT reactivity.

The main problem in the evaluation of SPT with mites is a possible proteolytic irritancy of some allergenic extracts or the putative degranulation capacity of mite elastase (20,21). Whereas all patients were studied by the same method, it could be argued, that urticaria patients would be more prone to irritancy or nonspecific results by skin testing. Previous results also showed an association of chronic urticaria with predominant HDM skin reactivity (20,22). In spite, two aspects of our results rest importance to this idea. First, there is a high proportion of patients with previous respiratory allergy exclusively sensitized to HDM. More importantly, patients with GAA (only acute urticaria or angio-oedema of mainly less than 24 h duration) do not have chronic cutaneous symptoms or hyperreactivity and were evaluated for *A. simplex* sensitization by means of SPT several weeks or months after the acute episode. These patients were comparable with respect to the HDM sensitization rate to those with CU+ patients.

Thus, cross-reactivity of HDM and *A. simplex* could be the primary factor, that would explain our findings and has previously been documented by immunoblotting inhibition, but was more pronounced between *A. simplex* and storage mites (23). Other studies showed cross-reactivity between *A. simplex* and *D. pteronyssinus* (23,24). Helminth parasites and HDM are known to share several structures like glutathione S-transferase, paramyosin or tropomyosin (25). But, more interesting, N-glycans, like $\alpha(1,3)$ -linked fucose is present on glycoproteins of arthropods and helminths and could act as pattern recognition receptors (9,26,27). The clinical relevance has not yet been clarified, as is the question if this cross-reactive IgE is of low affinity. Hence, by analogy with cross-sensitization from pollen to foods, sensitization to parasites might induce clinically irrelevant cross-reactions to HDMs (26).

On the other side, if we take into regard only patients with rhinoconjunctivitis or bronchial asthma (RCBA), we find a very high sensitization rate against HDM in *A. simplex* related disorders. Therefore, the association of sensitization between *Dermatophagoides* spp. and *A. simplex* could be of clinical relevance.

Further, arguments extracted from our own study diminish the importance of cross-reactivity as main explanatory factor. While the frequency of the overall sensitization against HDM is elevated, RCBA-associated SPT reactivity against the PMD group is diminished in our patients with *A. simplex* related urticaria. This inversion is not a simple reflection of the change in the relationship of HDM/PMD sensitization, as in our estimated comparison with the general population, the prevalence of the overall RCBA associated sensitization is diminished.

It is conceivable, that infection with IgE-inducing parasites results in stimulation of dormant allergic manifestations in atopic subjects (28). If cross-reactivity between *A. simplex* and HDM is clinically relevant, this could explain the apparently different prevalences of especially clinically relevant HDM related respiratory disease in our studied groups. Threshold levels for sensitization as well as for eliciting clinical symptoms have been proposed for HDM (29). Thus, generally low levels of HDM-allergens in our studied region do not give rise to primary sensitization, but cross-sensitization could elicit symptoms by HDM or other arthropods, as threshold levels for clinical symptoms could be lowered.

Previous studies, which showed a negative correlation between atopic asthma and parasites, have mainly been carried out in populations with a high prevalence of helminthic infections (4,30). These are supposed to be more prevalent in tropical regions, where there are also favourable climatic conditions for the growth of different mite species (31). This is important to mention here, because for the proposed protective effect of an atopic status on parasitic disease, or the proposed protective effect of chronic parasitic infection on asthma or skin reactivity, the definition of atopy has widely been the presence of positive skin reactivity to at least one common aeroallergen. If we thoroughly analyse these studies, we find, that the predominant 'atopy marker' is HDM sensitization, frequently finding only weak sensitization rates against nonarthropod allergens (4,31–34). Van den Biggelaar *et al.* showed a decreased reactivity to house dust mite in *Schistosoma haematobium* infected children in a region where sensitization to other aeroallergens (pollen, dander) was extremely infrequent (35).

We only could compare our parasite linked atopy prevalence by means of an estimated comparison. Therefore we cannot reach any powerful valid conclusion about the direct association of *A. simplex* related disorders and atopy. However, using a rapid undifferentiated definition of atopy, defined as a positive SPT to at least one common aeroallergen, we would have concluded an enhancing effect of *A. simplex* on atopy. The 21.5% prevalence of clinically confirmable allergic rhinitis in a cross-sectional, population based study in Spain (36), percentage, which has to be slightly elevated, if we take

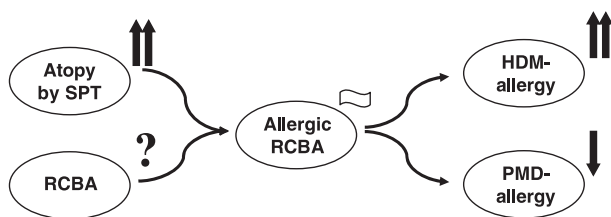


Figure 5 Protective or enhancing effect of *Anisakis simplex* related disorders on atopy and allergy. Opposite results depend on the definition of atopy. If the general atopy status is assessed, we detect an enhancing effect *A. simplex* parasitism on atopy (see Figure 2). If only RCBA associated sensitization (RCBA plus positive SPT) is assessed no significant effect can be detected (see Discussion), whereas, if we finally apply a differential atopy definition (see Figure 4), an enhancing effect for HDM related RCBA can be stated, but a slightly protective effect for RCBA associated sensitization against the pollen, mould or dander group (PMD).

into account the prevalence of allergic asthma without rhinitis, places a comparable prevalence near our own prevalence of allergic RCBA between 21.1% and 28.8%, expressing the absence of an effect of *A. simplex* on the prevalence of allergic RCBA. But with our innovative analysis, when differentiating HDM from other aeroallergens (PMD), the prevalence of RCBA associated PMD sensitization is slightly diminished, whereas RCBA associated HDM sensitization is clearly elevated (Figure 5).

In the clinical evaluation about the relationship between allergy and parasites, it is asthma or bronchial hyperresponsiveness as unique expression of respiratory allergy, which have mainly been analysed in previous studies. This is probably due to the fact that the association of asthma and skin reactivity as a risk factor in industrialized countries has mainly been reported for HDM (37). But allergic rhinoconjunctivitis in industrialized countries is much more frequent than asthma. Therefore, in spite of the less objective measurement of physician-based evaluation of rhinoconjunctivitis, we were able to have at our disposal an ample sample size in our studied groups if we investigated not only asthma symptoms.

Our results, taking into regard only SPT positive associated respiratory allergies, are in accordance with the hypothesis of enhanced atopic respiratory disorders associated with only sporadic contact with parasites, in this special case of *A. simplex* infection induced urticaria, but only taking into account HDM sensitization (26,38). A higher frequency of *Dermatophagoides* sensitization has been reported among children who become infected with *Ascaris* (7). In one study, the allergen content of dust samples was measured, and despite a lower *Der p 1* allergen exposure in the rural population, a high frequency of *D. pteronyssinus* sensitization was described and associated with high-intensity parasite infec-

tion (4). This is consistent with our idea, that atopy definition should be differentiated with respect to arthropod (as are HDMs and probably cockroaches) and parasite related allergens on one side and pollen, dander or mould allergens on the other side. Cross-reactivity between nematodes and arthropods, as has been documented previously (24,26,39–41), and our own results encourages us to this differential definition of atopy and the conclusion, that we are dealing with two different entities of allergic aeroallergen sensitization.

Anisakis simplex is acquired as short-term infection and thus explains the more frequent generation of allergic responses. But this allergic response has been postulated to help expelling the worm. Thus, the allergic phenomenon in the gastric mucosa and the gastric symptoms, which include vomiting, would help to get rid of the larva (12,42–44), as has also been postulated in early reports from Japan on Gastric Anisakiasis and possibly restricted to genetic factors (42,45). The accompanying cutaneous reaction in GAA could be seen as an accompanying phenomenon of this gastric reaction. Thus, our results reflect the idea that a genetic predisposition to a differential sensitization against HDM or related allergens, protects against *A. simplex* infection, where the accompanying urticaria helps with the expulsion of the larva (12).

As *A. simplex* larvae do not survive, develop or multiply in humans as accidentally hosts, there is no evolutionary selection factor to be supposed in the relationship between both: neither is it to be expected, that humans have developed a response directed specifically against *A. simplex*, nor is it to be expected, that, like in other host–parasite relationships, it is the fish–parasite *A. simplex* that has evolved mechanisms to induce specific immunologic mechanisms in humans. Thus, immunologic features are to be interpreted in the sense of a general nematode or perhaps helminth parasite response, as could be the above mentioned pattern recognition receptors, mimicking a response to other parasites that in turn have a co evolutionary relationship with humans.

Again, these facts underline our idea, that *Anisakis* related disorders, that are predominantly Th2 related, are probably associated with a propensity not merely to an atopic status, but possibly to a restricted HDM-reactivity. This reactivity could in turn reflect a facilitated immune reaction to other arthropods and nematodes. In this respect it is notable, that nearly all patients with HDM reactivity showed positivity against *D. pteronyssinus* and *D. farinae*, and most also against cockroach (data not shown). Our question remains: does contact with *A. simplex* have an influence on an arthropod specific allergic sensitization to aeroallergens or does an allergic predisposition against specific arthropod allergy confer protection against parasites cross-reacting with *A. simplex*?

REFERENCES

- 1 Weiss ST. Parasites and asthma/allergy: what is the relationship? *J Allergy Clin Immunol* 2000; **105**: 205–210.
- 2 Selassie FG, Stevens RH, Cullinan P, *et al.* Total and specific IgE (house dust mite and intestinal helminths) in asthmatics and controls from Gondar, Ethiopia. *Clin Exp Allergy* 2000; **30**: 356–358.
- 3 Brunet LR, Hunt J & Rook GA. Dangers of therapeutic manipulation of the Th–Th2 balance. *Trend Immunol* 2002; **23**: 127–128.
- 4 Scrivener S, Yemaneberhan H, Zebenigus M, *et al.* Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case–control study. *Lancet* 2001; **358**: 1493–1499.
- 5 Cooper PJ. Intestinal worms and human allergy. *Parasite Immunol* 2004; **26**: 455–467.
- 6 Hunninghake GM, Soto-Quiros ME, Avila L, *et al.* Sensitization to *Ascaris lumbricoides* and severity of childhood asthma in Costa Rica. *J Allergy Clin Immunol* 2007; **119**: 654–661.
- 7 Dold S, Heinrich J, Wichmann HE & Wjst M. *Ascaris*-specific IgE and allergic sensitization in a cohort of school children in the former East Germany. *J Allergy Clin Immunol* 1998; **102**: 414–420.
- 8 Mao XQ, Sun DJ, Miyoshi A, *et al.* The link between helminthic infection and atopy. *Parasitol Today* 2000; **16**: 186–188.
- 9 Yazdanbakhsh M, Kremsner PG & van Ree R. Allergy, parasites, and the hygiene hypothesis. *Science* 2002; **296**: 490–494.
- 10 Capron A, Dombrowicz D & Capron M. Helminth infections and allergic diseases: from the Th2 paradigm to regulatory networks. *Clin Rev Allergy Immunol* 2004; **26**: 25–34.
- 11 Hagel I, Di Prisco MC, Goldblatt J & Le Souef PN. The role of parasites in genetic susceptibility to allergy. IgE, helminthic infection and allergy, and the evolution of the human immune system. *Clin Rev Allergy Immunol* 2004; **26**: 75–83.
- 12 Daschner A, Alonso-Gomez A, Caballero T, Barranco P, Suarez-De-Parga JM & Lopez-Serrano MC. Gastric anisakiasis: an underestimated cause of acute urticaria and angio-oedema? *Br J Dermatol* 1998; **139**: 822–828.
- 13 Daschner A, Alonso-Gomez A & Cabañas R, Suarez-De-Parga JM & Lopez-Serrano MC. Gastroallergic anisakiasis: borderline between food allergy and parasitic disease-clinical and allergologic evaluation of 20 patients with confirmed acute parasitism by *Anisakis simplex*. *J Allergy Clin Immunol* 2000; **105**: 176–181.
- 14 Daschner A, Vega de la Osada F & Pascual CY. Allergy and parasites reevaluated: wide-scale induction of chronic urticaria by the ubiquitous fish-nematode *Anisakis simplex* in an endemic region. *Allergol Immunopathol* 2005; **33**: 31–37.
- 15 Gracia-Bara MT, Matheu V, Zubeldia JM, *et al.* *Anisakis simplex*-sensitized patients: should fish be excluded from their diet? *Ann Allergy Asthma Immunol* 2001; **86**: 679–685.
- 16 Daschner A & Pascual CY. *Anisakis simplex*: sensitization and clinical allergy. *Curr Opin Allergy Clin Immunol* 2005; **5**: 281–285.
- 17 Daschner A, Alonso-Gomez A, Caballero T, Suarez-De-Parga JM & Lopez-Serrano MC. Usefulness of early serial measurement of specific and total immunoglobulin E in the diagnosis of gastro-allergic anisakiasis. *Clin Exp Allergy* 1999; **29**: 1260–1264.
- 18 Cortés X, Soriano JB, Sanchez-Ramos JL, Azofra J, Almar E & Ramos J. European study of asthma. Prevalence of atopy in young adults of 5 areas in Spain. Spanish Group of European Asthma Study. *Med Clin (Barc)* 1998; **111**: 573–577.
- 19 Burney P, Malmberg E, Chinn S, Jarvis D, Luczynska C & Lai E. The distribution of total and specific serum IgE in the European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1997; **99**: 314–322.
- 20 Caliskaner Z, Ozturk S, Turan M & Karaayvaz M. Skin test positivity to aeroallergens in the patients with chronic urticaria without allergic respiratory disease. *J Invest Allergol Clin Immunol* 2004; **14**: 50–54.
- 21 Reed CE & Kita H. The role of protease activation of inflammation in allergic respiratory diseases. *J Allergy Clin Immunol* 2004; **114**: 997–1008.
- 22 Mahesh PA, Kushalappa PA, Holla AD & Vedanthan PK. House dust mite sensitivity is a factor in chronic urticaria. *Indian J Dermatol Venereol Leprol* 2005; **71**: 99–101.
- 23 Johansson E, Aponno M, Lundberg M & van Hage-Hamsten M. Allergenic cross-reactivity between the nematode *Anisakis simplex* and the dust mites *Acarus siro*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, and *Dermatophagoides pteronyssinus*. *Allergy* 2001; **56**: 660–666.
- 24 Bernardini R, Mistrello G, Novembre E, *et al.* Cross-reactivity between IgE-binding proteins from *Anisakis simplex* and *Dermatophagoides pteronyssinus*. *Int J Immunopathol Pharmacol* 2005; **18**: 671–675.
- 25 Thomas WR & Smith W. Towards defining the full spectrum of important house dust mite allergens. *Clin Exp Allergy* 1999; **29**: 1583–1587.
- 26 Allen JE & Maizels RM. Immunology of human helminth infection. *Int Arch Allergy Immunol* 1996; **109**: 3–10.
- 27 Tawill S, Le Goff L, Ali F, Blaxter M & Allen JE. Both free-living and parasitic nematodes induce a characteristic Th2 response that is dependent on the presence of intact glycans. *Infect Immun* 2004; **72**: 398–407.
- 28 Buijs J, Borsboom G, Renting M, *et al.* Relationship between allergic manifestations and *Toxocara seropositivity*: a cross-sectional study among elementary school children. *Eur Respir J* 1997; **10**: 1467–1475.
- 29 Platts-Mills TA, Ward GW, Sporik R, Gelber LE, Chapman MD & Heymann PW. Epidemiology of the relationship between exposure to indoor allergens and asthma. *Int Arch Allergy Appl Immunol* 1991; **94**: 339–345.
- 30 Van den Biggelaar AHJ, Rodrigues LC, van Ree R, *et al.* Long-term treatment of intestinal helminths increases mite skin-test reactivity in Gabonese schoolchildren. *J Infect Dis* 2004; **189**: 892–900.
- 31 Van den Biggelaar AH, Lopuhaa C, van Ree R, *et al.* The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. *Int Arch Allergy Immunol* 2001; **126**: 231–238.
- 32 Yemaneberhan H, Bekele Z, Venn A, Lewis S, Parry E & Britton J. Prevalence of wheeze and asthma and relation to atopy in urban and rural Ethiopia. *Lancet* 1997; **350**: 85–90.
- 33 Cooper PJ, Chico ME, Rodrigues LC, *et al.* Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. *J Allergy Clin Immunol* 2003; **111**: 995–1000.
- 34 Cooper PJ, Chico ME, Bland M, Griffin GE & Nutman TB. Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. *Am J Respir Crit Care Med* 2003; **168**: 313–317.
- 35 Van den Biggelaar AH, van Ree R, Rodrigues LC, *et al.* Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. *Lancet* 2000; **356**: 1723–1727.
- 36 Bauchau V & Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* 2004; **24**: 758–764.

- 37 Sporik R, Holgate ST, Platts-Mills TA & Cogswell JJ. Exposure to house-dust mite allergen (Der p I) and the development of asthma in childhood. A prospective study. *N Engl J Med* 1990; **323**: 502–507.
- 38 Yazdanbakhsh M & Wahyuni S. The role of helminth infections in protection from atopic disorders. *Curr Opin Allergy Clin Immunol* 2005; **5**: 386–391.
- 39 Cooper PJ, Chico ME, Sandoval C & Nutman TB. Atopic phenotype is an important determinant of immunoglobulin E-mediated inflammation and expression of T helper cell type 2 cytokines to *Ascaris* antigens in children exposed to ascariasis. *J Infect Dis* 2004; **190**: 1338–1346.
- 40 Pascual CY, Crespo JF, San Martin S, *et al.* Cross-reactivity between IgE-binding proteins from *Anisakis*, German cockroach, and chironomids. *Allergy* 1997; **52**: 514–520.
- 41 Moncayo AL & Cooper PJ. Geohelminth infections: impact on allergic diseases. *Int J Biochem Cell Biol* 2006; **38**: 1031–1035.
- 42 Asaishi K, Nishino C, Ebata T, Totsuka M, Hayasaka H & Suzuki T. Studies on the etiologic mechanism of anisakiasis. Part 1. Immunological reactions of digestive tract induced by *Anisakis* larva. *Gastroenterol Jpn* 1980; **15**: 120–127.
- 43 Bell RG. IgE, allergies and helminth parasites: a new perspective on an old conundrum. *Immunol Cell Biol* 1996; **74**: 337–345.
- 44 Finkelman FD, Shea-Donohue T, Goldhill J, *et al.* Cytokine regulation of host defense against parasitic gastrointestinal nematodes: lessons from studies with rodent models. *Annu Rev Immunol* 1997; **15**: 505–533.
- 45 Shimakura K, Miura H, Ikeda K, *et al.* Purification and molecular cloning of a major allergen from *Anisakis simplex*. *Mol Biochem Parasitol* 2004; **135**: 69–75.
- 46 Carvalho EM, Bastos LS & Araujo MI. Worms and allergy. *Parasite Immunol* 2006; **28**: 525–534.
- 47 Britton J. Parasites, allergy, and asthma. *Am J Respir Crit Care Med* 2003; **168**: 266–267.