Helminth-Induced IgE and Protection Against Allergic Disorders

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Abstract The immune response against helminths and allergens is generally characterized by high levels of IgE and increased numbers of Th2 cells, eosinophils, and mast cells, yet the clinical outcome with respect to immediate hypersensitivity and inflammation is clearly not the same. High levels of IgE are seen to allergens during helminth infections; however, these IgE responses do not translate into allergy symptoms. This chapter summarizes the evidence of the association between helminth infections and allergic disorders. It discusses how helminth infection can lead to IgE cross-reactivity with allergens and how this IgE has poor biological activity. This information is important for developing new diagnostic methods and treatments for allergic disorders in low-to-middle-income countries.

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Contents

1 Introduction

Over the past few decades, the prevalence of allergic disease has been on the rise in both developed and developing countries (Pawankar et al. 2011). In addition, many epidemiological studies have shown a higher prevalence of allergic disorders in subjects living in urban environments compared to rural areas, particularly in developing countries (Hamid et al. 2013; Perzanowski et al. 2002).

Such observations indicate that environmental factors, along with underlying genetics, play a key role in the development of allergic disease. Environmental influences can include exposures to microbes, parasites, and lifestyle factors (Burke et al. 2003; von Mutius 2002). Of particular interest have been infections with parasitic helminths that are highly prevalent in tropical regions of the developing world. It is estimated that a quarter of the World's population is chronically infected by helminths such as *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Necator americanus* or *Ancylostoma duodenale* (hookworms), schistosomes, and filarial worms (Bethony et al. 2006).

Despite the close parallels between immune responses that characterize helminth infections and allergic diseases, namely increased levels of immunoglobulin (Ig)-E, eosinophils, and mast cells along with T cells that preferentially secrete T helper type 2 (Th2) cytokines, the clinical outcome with respect to immediate hypersensitivity and inflammation is not the same (Yazdanbakhsh et al. 2001). Moreover, there is little geographical overlap worldwide between helminth infections and allergies. In fact, several studies have reported a negative association between the presence of helminth infections and allergic disorders (Feary et al. 2011). However, the relationship between allergic disorders and helminth infections does not show consistent results since there are also studies that show that helminth infections either have no effect or are associated with increased atopic disorders (Obihara et al. 2006; Palmer et al. 2002).

High levels of Th2 responses can lead to allergic diseases, and to prevent this, either a shift to Th1 or an increased anti-inflammatory response would be needed (Carvalho et al. 2006; Cooper 2009). A number of immune mechanisms have been

proposed to account for the negative association between helminths and allergies. The observations that chronic helminth infections are associated with higher suppressive responses, such as interleukin-10 (IL-10) and regulatory T cells, led to the proposal that a strong regulatory network induced by helminths might prevent the downstream effector phase of Th2 responses, preventing excessive inflammation (Satoguina et al. 2008; Wilson et al. 2005).

Another mechanism that might explain the inverse association between helminth infections and allergies could involve helminth-induced IgE. Early studies have suggested that polyclonal IgE that is stimulated by helminth infections might compete with allergen-specific IgE and therefore block degranulation of basophils and mast cells. However, a number of studies have refuted this IgE-blocking hypothesis. The idea that IgG4 antibodies associated with helminth infections might bind allergen and quench it from binding to IgE was put forward by Aalberse et al. (2009). An alternative idea that helminth infections may be associated with increased levels of allergen-specific IgE that are functionally poor and therefore cannot lead to basophil or mast cell degranulation (Yazdanbakhsh et al. 2002) is gaining support from several recent studies that indicate cross-reactive IgE might be associated with poor biological activity (Amoah et al. 2013b; Larson et al. 2012a).

Over the years, there has been increasing number of publications covering (a) the relationship between helminths and IgE, which started in 1969; (b) IgE and the issue of cross-reactivity; and (c) component-resolved diagnosis (CRD) (Fig. 1). In this chapter, we will focus on (1) the epidemiological studies of the relationship between helminth infections and allergic disorders and (2) the characterization of helminth-induced IgE and the possible application of new technologies in allergy diagnostics in low-to-middle-income countries.

2 Helminth Infections and Allergic Disorders

The prevalence of allergic diseases has been increasing mainly in the developed world where long ago changes have been seen in lifestyle and the environment characterized by increasing sanitation, hygienic measures, and urbanization. In less developed countries, the prevalence of allergic disorders is relatively low, but the allergic march is starting in these geographical areas due to ongoing dramatic changes in lifestyle and the environment. Although not as dramatic as in Western countries, an increase in allergic disorders has been reported in developing countries with the tendency for prevalences in urban centers to approach those seen in affluent countries.

In developed countries, the presence of IgE antibodies to allergens increases the risk of allergic disorders. These antibodies, which bind to high-affinity IgE receptors present on basophils and mast cells, can be cross-linked by allergens, an event that leads to mast cell degranulation and histamine release. The mast cell degranulation leads to inflammation and in target organs such as the airways can result in symptoms which can be recognized as an asthmatic attack. One of the diagnostic
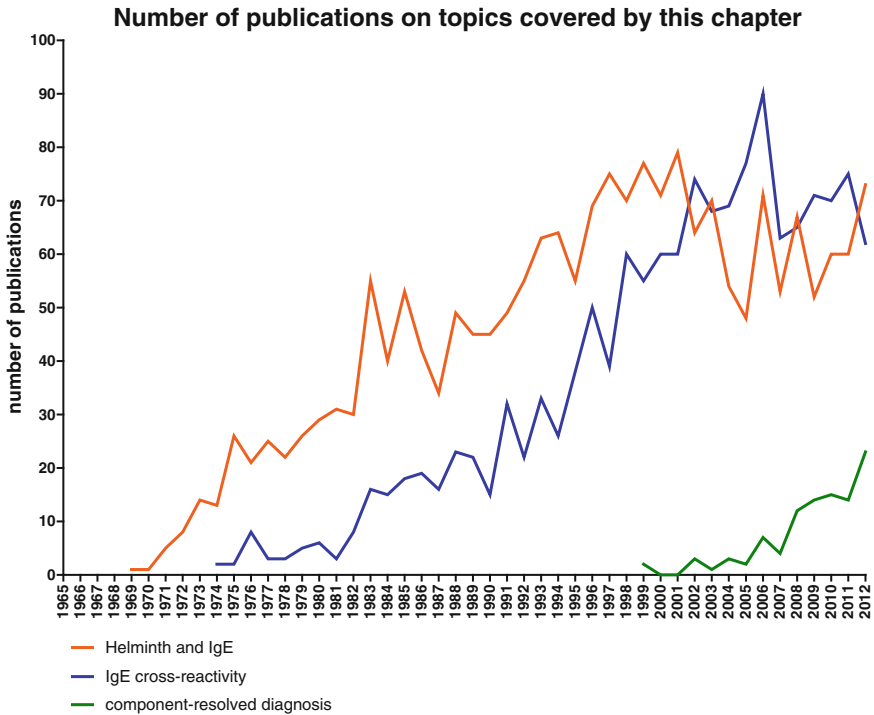
Fig. 1 The number of publications from 1969 to the end of 2012 in the major topics covered by this chapter. Specific search terms used in PubMed were "Helminth and IgE" (*orange*), "IgE cross-reactivity" (*blue*), and "component-resolved diagnosis" (*green*)

methods for allergy is to perform a skin prick test (SPT). This is based on applying allergens to the skin, pricking the skin with a lancet and assessing whether a reaction develops to the allergen within 15 min. If a wheal and flare easily visible in lighter skin reaction is seen, which is the result of mast cell degranulation, then it is concluded that person being tested is sensitized to the allergen applied. In developed countries, the SPT is often interchangeable with the measurement of IgE antibodies to allergens by in vitro tests such as the ImmunoCAP assay. This means that allergen-specific IgE measured in serum can be associated with SPT reactivity and potentially with allergic symptoms. However, the relationship between IgE, SPT, and symptoms of allergy can be different in different geographical areas. For example, the proportion of SPT reactivity with clinical allergic asthma appears to be much smaller in some rural areas compared to urban centers in Europe (Priftanji et al. 2001) or in many developing nations compared to developed ones (Cooper et al. 2003a; Dagoye et al. 2003). Helminth infections have some interesting effects on the relationship between IgE, SPT, and clinical symptoms.

Several epidemiological studies have shown that helminth infections can be negatively associated with allergic outcomes (Feary et al. 2011). Some studies have

shown that having schistosome or filarial infections decreases the risk of SPT positivity (Supali et al. 2010; van den Biggelaar et al. 2001). Similarly, studies in Ecuadorian and Vietnamese children have demonstrated that having soil-transmitted helminth infections decreases the risk of SPT positivity (Cooper et al. 2004; Flohr et al. 2006). In addition, Cooper et al. (2003b) found that the presence of serological markers of chronic infections (elevated levels of total serum IgE or anti-*A. lumbricoides* IgG4) was independently negatively associated with allergen SPT reactivity. Araujo et al. (2000) reported a strong and inverse association between skin responses to allergens and infection with *Schistosoma mansoni*, among persons living in an area endemic for this helminth (Araujo et al. 2000). Similarly, a study in Gabonese children observed that the risk of a positive SPT was reduced by 72 % if a child was infected with *S. haematobium*, a blood-dwelling helminth (van den Biggelaar et al. 2001). Moreover, an investigation conducted among 1,385 urban and rural Ghanaian children aged 5–16 years showed a strong negative association between schistosome infection and SPT reactivity to mite but not with reported wheeze or asthma (Obeng et al. 2014). However, with regard to clinical symptoms of asthma, a case–control study in urban and rural Ethiopians aged 16–60 years determined that active hookworm infection reduced the risk of reported wheeze (Scrivener et al. 2001).

Although the majority of studies have shown a negative association between helminth infection and SPT, there are studies that show that helminth may increase the risk of asthma and atopic disorder. Obihara et al. (2006) found *Ascaris*-specific IgE may be a risk factor for atopic disease in populations exposed to mild *A. lumbricoides* infection (Obihara et al. 2006). In line with this, a study in China, in an area with a low burden of *A. lumbricoides*, demonstrated a positive association between helminth and allergen skin test reactivity as well as asthma risk (Palmer et al. 2002). Finally, there are also studies showing no significant association. A study in Brazil among patients aged 12–30 years with asthma or rhinitis living in an urban area endemic for geohelminth showed that individuals infected with a low parasite burden of *A. lumbricoides* did not differ on the frequency of positive SPT to dust mites from those *A. lumbricoides* negatives living in the same area (Ponte et al. 2006). A birth cohort of children from Ethiopia which investigated the effect of geohelminths on allergic symptoms, at 3 years of age, found that there was no association between helminth infections and wheeze nor with eczema (Amberbir et al. 2011).

Considering that cross-sectional studies can only show relationships, it is important to prove that helminths, and no other confounders, are responsible for any association seen with allergies. For this, interventional studies such as use of anthelmintics to remove helminths or intentional infection with helminths are needed. With respect to anthelmintic treatment, a study of soil-transmitted helminth-infected subjects demonstrated that regular anthelmintic treatment resulted in significant increase in skin test reactivity as well as serum level of IgE to aeroallergens (Lynch et al. 1993). In line with this, anthelmintic treatment of Gabonese children chronically infected with *S. haematobium* and soil-transmitted helminths resulted in increased SPT reactivity (van den Biggelaar et al. 2000). Three large interventional studies conducted in Ecuador, Vietnam, and Indonesia have shown different results.

In Ecuador, treatment with albendazole every 2 months, for 1 year, did not affect SPT nor clinical symptoms of allergy (Cooper et al. 2006), but in Vietnam, three-monthly treatment with albendazole resulted in a significant increase in SPT positivity but not in allergy symptoms (Flohr et al. 2010). The third study in Indonesia revealed that intensive community treatment of 3-monthly albendazole for 21 months over 2 years was not associated with increased risk of SPT to any allergen, but post hoc analysis showed that SPT to cockroach allergen was increased in the albendazole arm compared to placebo (Wiria et al. 2013). However, in agreement with the studies in Ecuador and Vietnam, there was no effect on reported clinical symptoms of allergy (Wiria et al. 2013). However, it has to be noted that one study in Venezuela showed that anthelmintic treatment resulted in improvement in all clinical indicators of asthma (Lynch et al. 1997). On the other hand, when considering anthelmintic treatment given during pregnancy, a large randomized, double-blind, placebo-controlled trial carried out in Uganda found that treatment of pregnant women with albendazole (compared with placebo) was strongly linked to an increased risk of doctor-diagnosed infantile eczema in their infants (Mpairwe et al. 2011).

Another approach to investigate the effect of helminths on allergies would be to use data from studies where humans have been experimentally infected with helminths. Recent years have seen an increasing number of such studies where volunteers were infected with L3 larvae of hookworm *N. americanus* as well *T. suis* eggs (Jouvin and Kinet 2012; Wright and Bickle 2005). From these studies, it is possible to delineate whether helminth infections are associated with increased allergies or whether they can suppress allergic symptoms. Helminth infections lead to expansion of Th2 responses and upregulation of IgE as well as eosinophils (Wright and Bickle 2005). This expansion of Th2 responses was not associated with an increase in allergic symptoms even though the life cycle of the hookworm parasite involves lung passage. In two safety trials, small numbers of patients with rhino-conjunctivitis or asthma were treated with helminths. Ten L3 infective larvae of *N. americanus* were inoculated into allergic patients in UK who were followed up for 16 weeks and showed no worsening or improvement of their symptoms (Feary et al. 2010). Regarding *T. suis* ova therapy, a double-blind placebo-controlled trial conducted in Danish adults assessed the efficacy of *T. suis* ova therapy for the treatment of grass pollen-induced allergic rhinitis and found no therapeutic effect of this particular therapy (Bager et al. 2010).

Taken together, most studies, but not all, show that helminth infections are associated with decreased SPT, but there does not seem to be a strong effect on clinical symptoms with the possible exception of a beneficial effect on infantile eczema. Both for anthelmintic treatment studies and experimental helminth infections, it is possible that different helminths with their varying life cycles and locations in tissues would lead to different effects on allergic outcomes. Moreover, it should be noted that chronicity of infection as well as worm burden might be important parameters to take into account when studying the association between helminths and allergies. Chronic infections as well as higher worm burdens might have stronger regulatory effect on allergies than acute or light infections (Smits

et al. 2007). Finally, attention should be paid to the methods used to assess clinical symptoms of allergy as these could be a source of variability.

2.1 Mechanisms Behind the Association Between Helminths and Allergies

Given that most studies seem to show a negative association between chronic helminth infections and SPT reactivity, researchers have been looking for the mechanisms that could explain this. Although the mechanisms associated with this inverse relationship are not fully understood, it has been suggested that strong immune regulatory networks might be involved. This means that high levels of suppressive cytokines such as IL-10 and transforming growth factor-beta (TGF-β) as well as regulatory T and B cells (Smits et al. 2010), which seem to expand during chronic infections with helminth parasites, might downregulate allergic responses. The question as to where in the allergy cascade they exert their downregulatory activity is still unanswered. It is possible that regulatory responses affect Th2 and thereby IgE. However, immune regulation could also downregulate the effector phase of an allergic response which involves inflammation induced by mast cell degranulation (Larson et al. 2012b). This notion is supported by reports showing that IL-10 could inhibit basophil degranulation (Royer et al. 2001) and by the negative association between IL-10 and SPT (Macaubas et al. 1999; van den Biggelaar et al. 2000). Regarding antibodies, in the 1970s, the idea that polyclonal stimulation of IgE-producing plasma cells with many different specificities would compete with allergen-specific IgE for binding to high-affinity IgE receptors on mast cells was first proposed (Lancet editorial 1976; Godfrey 1975). This competition would reduce the chance of an allergen-dependent mast cell degranulation and therefore explain the absence of strong allergic responses in helminth-infected subjects (Lynch et al. 1998). However, a study by Mitre et al. (2005) showed that the high ration of polyclonal IgE to allergen-specific IgE did not inhibit basophil degranulation. Moreover, it has been shown that increases in IgE result in the upregulation of IgE receptors and that anti-IgE treatment is often accompanied by downregulation of IgE receptors which argues against the ability of high total IgE to compete out specific IgE (MacGlashan et al. 1997). Another hypothesis put forward is that high levels of IgG4 produced during parasitic infections could act as "blocking antibodies," since IgG4 is a Th2-dependent isotype not associated with clinical allergy (Aalberse et al. 2009; Arruda and Santos 2005). However, here, we will consider, yet another hypothesis, that helminths are associated with an IgE response that is cross-reactive with a low biological activity and therefore associated with less SPT reactivity to allergens and no strong increase in allergic symptoms in a Th2-skewed population.

3 Cross-Reactivity Between Allergen and Helminths

In general, cross-reactivity reflects the phylogenetic relationship between organisms that results in a high degree of homology in the primary structure of proteins and potentially in cross-reactivity (Aalberse et al. 2001). Cross-reactivity occurs when antibodies elicited to one epitope also recognize similar epitopes in other homologous molecules (Acevedo and Caraballo 2011). In allergy, the allergen that is supposed to induce the original allergic responses is named the primary sensitizer, and the others are considered cross-reactive allergens (Acevedo and Caraballo 2011). Two types of IgE cross-reactivities have been described: one cross-reactivity due to sugar moieties (glycans on glycoproteins) known as cross-reactive carbohydrate determinants (CCDs) and the other cross-reactivity due to proteins (Aalberse et al. 2001).

3.1 Cross-Reactive Carbohydrate Determinants (CCDs) and Helminths

The asparagine-linked carbohydrate components of plant and insect glycoproteins are highly cross-reactive and are known as CCDs (Altmann 2007). Two typical non-mammalian substitutions to N-glycans of plant glycoproteins are an α(1,3)-linked fucose on the proximal N-acetyl glucosamine and a β(1,2)-linked xylose on the core mannose (van Ree et al. 2000).

These epitopes are also found in helminth parasites. The existence of IgE antibodies directed to CCDs was first reported by Aalberse et al. (1981) in the early 1980s. This study demonstrated that serum IgE from European pollen or venom-allergic patients cross-reacted with extracts from various allergenic foods. However, treating the extracts with periodate, which destroys the carbohydrate structures, abolished the reactions, indicating the involvement of carbohydrates in this cross-reactivity (Aalberse et al. 1981). Another investigation by the same group observed elevated levels of IgE against peanut extract among grass pollen-sensitized European patients without peanut SPT reactivity or clinical symptoms of peanut allergy (van der Veen et al. 1997). Furthermore, among 91 % of those with a discrepancy between specific IgE to peanut and SPT, IgE against CCDs could be detected (van der Veen et al. 1997). In some of these patients, almost complete inhibition of IgE to peanut (as measured by competitive radioallergosorbent test) was possible with CCD. In addition, cross-reactive IgE directed against CCDs in this study was demonstrated to have poor biological activity (van der Veen et al. 1997).

In another study, about 42 % of pollen-allergic European patients were found to have specific IgE to the CCD marker bromelain without skin reactivity to this molecule (Mari et al. 1999). In line with these observations, 23 % of a large group of 1,831 subjects with symptoms of allergic respiratory disease were IgE sensitized to bromelain without SPT to the same molecule (Mari 2002). Taken together, these

studies demonstrate the role of anti-CCD IgE in false-positive in vitro allergy test responses. One could argue that this lack of biological activity was due to the fact that bromelain is substituted with a single IgE-binding glycan, making effective cross-linking highly unlikely. The most convincing proof of the complete lack of clinical relevance of CCD-specific IgE was reported by Mari et al. (2008) in a study where grass pollen-allergic patients with high titers of CCD-specific IgE were skin-tested and subjected to an oral challenge with human lactoferrin expressed in rice kernels. This transgenic molecule was substituted with multiple IgE-binding glycans, but both SPT and oral challenge were completely negative (Mari et al. 2008). This study shows that a single IgE-binding glycan is not the reason for lack of activity of IgE to CCD.

With regard to helminth-induced IgE cross-reactivity, a recent study among schoolchildren (aged 5–16 years) in Ghana, West Africa, demonstrated how cross-reactivity between helminth antigens and allergens can affect IgE sensitization patterns and clinical expression of allergy (Amoah et al. 2013b). In this study, the overall prevalence of peanut–IgE sensitization was 17.5 % (233 out of 1,328). However, none of the peanut–IgE-sensitized children had either SPT reactivity to peanut or much reported adverse reactions to peanut. In this study, the presence of *S. haematobium* infection was positively associated with an increased risk of having peanut-specific IgE. In a subset of this study population, both the CCD marker bromelain and *S. haematobium*-soluble egg antigen (SEA) inhibited IgE binding to peanut extract. This study also showed that peanut-specific IgE was strongly correlated with CCD-specific IgE. Furthermore, these results indicate that much of IgE to peanut in Ghanaian children could be directed against CCD which is also present in the schistosome SEA. In addition, basophil histamine release assays demonstrated that the IgE directed against peanut in this population had low biological activity (Amoah et al. 2013b).

This study provides a model which proposes that parasite-induced IgE against CCDs that are carried by parasites might account for high IgE levels to food allergens, and the finding that this IgE does not lead to reactivity to allergenic extracts either in vitro (in basophil release assay) or in vivo (in skin prick testing) further confirms that these IgEs to CCDs are clinically irrelevant (Amoah et al. 2013a).

Although a number of studies have demonstrated that IgE antibodies directed against CCDs are not of clinical relevance, IgE direct against the mammalian carbohydrate epitope galactose-α-1,3-galactose (alpha-gal) has been linked to anaphylactic reactions.

The first cases of anaphylactic reactions associated with alpha-gal were among cancer patients in the southeastern USA receiving therapy with the monoclonal antibody cetuximab (Chung et al. 2008). This antibody carries alpha-gal structure. These patients were shown to have pre-existing IgE against alpha-gal, and immediate-onset anaphylaxis followed the first infusions of cetuximab (Chung et al. 2008). Further analysis indicated that IgE directed against alpha-gal was possibly induced by the lone star tick *Amblyomma americanum* commonly found in the southeastern USA (Commins et al. 2011). Aside from immediate-onset anaphylactic

reactions, delayed onset reactions 3–6 h following the consumption of mammalian meat have also been linked to IgE directed against alpha-gal, present on this food (Commins and Platts-Mills 2013).

In addition, positive IgE responses to alpha-gal have been observed in samples from children living in helminth-endemic areas of Ecuador and Kenya (Commins and Platts-Mills 2013). The involvement of helminths in the induction of IgE responses to alpha-gal has been indicated by an investigation conducted in Zimbabwe. In this study, IgE responses to alpha-gal were measured in urban cat-allergic patients as well as in rural helminth-infected subjects (Arkestal et al. 2011). The study observed that 85 % of the parasite-infected group had IgE against alpha-gal and 66 % had IgE against the cat allergen Fel d 5 found in cat dander extract which has been demonstrated to have alpha-gal epitopes (Gronlund et al. 2009). Moreover, in this study, IgE to alpha-gal and IgE to Fel d 5 were highly correlated. Among the urban cat-allergic patients, only a few had IgE responses to Fel d 5 and alpha-gal, while 74 % had responses to the recombinant form of the cat allergen Fel d 1. By contrast, only two of 47 of the parasite-infected had IgE to Fel d 1 which lacks alpha-gal epitopes. Taken together, these observations suggest that in helminth-endemic areas, the IgE to alpha-gal may not be clinically relevant. However, given that no information was collected on reactions to mammalian meat in the helminth-endemic areas (Commins and Platts-Mills 2013), additional in-depth studies are needed to assess the prevalence of sensitization to alpha-gal in different populations worldwide and the relationship between IgE sensitization to this oligosaccharide and clinical outcomes.

3.2 Peptide Cross-Reactivity and Helminths

Cross-reactions between allergens from invertebrates such as mite and snail; cockroach and ascaris; mite, shrimp, and cockroach; and mites and schistosomes (Aalberse et al. 2001) have been reported and involve protein cross-reactivity. Three of the proteins that are involved in these examples are tropomyosin, glutathione S-transferase (GST), and paramyosin (Aalberse et al. 2001).

Tropomyosins are proteins involved in the contraction of muscle cells along with actin and myosin (Arruda and Santos 2005). Not only are tropomyosins major allergens of seafood, mite, and cockroach but are also highly immunogenic helminth proteins (Sereda et al. 2008). Tropomyosins from invertebrates are strong inducers of IgE antibody responses in human (Jenkins et al. 2007). Santiago et al. (2011) demonstrated that there was 72 % identity at the amino acid level between the tropomyosin from the filarial parasite *Onchocerca volvulus* (OvTrop) and the house dust mite tropomyosin Der p 10 (Santiago et al. 2011). A strong correlation between specific IgE to Der p 10 and IgE to OvTrop was shown. In addition, histamine release from basophils sensitized with the sera of individuals IgE positive to Der p 10 could be triggered by either the OvTrop or Der p 10. It is, however, important to realize that such biological activity is not proof of clinical allergy.

In the study by Mari et al. (2008) with transgenic lactoferrin, histamine release was also reported at relatively high protein concentrations, but SPT and oral challenge with the same molecule were negative. The study by Santiago et al. (2011) does, however, confirm that the anti-tropomyosin antibodies induced in filarial infection are cross-reactive with those allergenic tropomyosins of invertebrates (mite) that may affect sensitization and regulation of allergic reactivity. As expected, no clinical mite allergy was reported for the subjects studied, indicating that these cross-reactive responses are of no clinical relevance.

In another investigation, Santos et al. (2008) showed that the predicted structure of *A. lumbricoides* tropomyosin was similar to that of *Periplaneta americana* tropomyosin. The same study compared IgE responses to these proteins in Brazilian children aged 3–6 years living in a helminth-endemic area and cockroach-allergic patients aged 2–52 years also from Brazil (Santos et al. 2008). A strong correlation was also found for IgE antibodies to tropomyosin from *A. lumbricoides* and from *P. Americana* in sera from both populations. Seventy-six percent (90 out of 119) of subjects from the parasite-endemic area had positive IgE antibodies against cockroach tropomyosin without allergy to cockroach (Santos et al. 2008). In line with this study, Acevedo et al. (2009) have also demonstrated high allergenic cross-reactivity between *Blomia tropicalis* tropomyosin (Blo t 10) and *Ascaris* tropomyosin in Colombian asthmatic patients (Acevedo et al. 2009).

The glutathione S-transferases (GSTs) are detoxification enzymes found in most living organisms (Sheehan et al. 2001). The important known sources are cockroaches, house dust mites, and molds; however, GSTs from invertebrates including helminths are known to be strong inducers of IgE (Acevedo et al. 2013). Moreover, *Blattella germanica* GST caused positive immediate skin tests in cockroach-allergic asthmatic patients, suggesting that GST from cockroach is a clinically relevant allergen (Arruda et al. 1997). Regarding helminth-induced IgE cross-reactivity, Santiago et al. (2012) showed that the GSTs from the filarial worm *Wuchereria bancrofti* (WbGST) and cockroach GST (Bla g 5) were 30 % identical at the amino acid with marked similarity in the *N*-terminal region (Santiago et al. 2012). Interestingly, mice infected with *Heligmosomoides bakeri,* a parasite that contains a GST that was 32 % identical to Bla g 5, developed immediate hypersensitivity reaction in the skin to cockroach GST (Bla g 5), suggesting that some parasite-induced cross-reactivity may induce in vivo reactivity to the cross-reactive allergen in a common allergen source like house dust mite (Santiago et al. 2012).

Paramyosin is another allergen family from invertebrate muscle that is targeted in IgE responses against helminths (Fitzsimmons et al. 2014). A study among patients reporting symptoms of allergy and *ascaris*-infected subject in Philippines showed evidence of cross-reactivity between paramyosin from mite (*B. tropicalis*) and paramyosin from *A. lumbricoides* (Valmonte et al. 2012). This study observed that IgE to mite extract among allergic patients can be inhibited, up to 92 %, by *ascaris* antigen, while mite extract could inhibit up to 54 % of *Ascaris*-sIgE among *Ascaris*-infected subjects. Of note, IgE responses to the recombinant form of the paramyosin *Blomia* allergen (Blo t 11) were seen in 80 % of allergic patients and 46 % of *Ascaris*-infected subjects (Valmonte et al. 2012).

In general, IgE cross-reactivity between helminth antigens and allergens demonstrates the limits to diagnostic value of examining IgE responses to whole allergen extracts in helminth-endemic populations. Establishing the molecular basis of cross-reactivity between helminths and common allergen sources is essential to evaluate whether sensitization to the latter is true primary sensitization or cross-reactivity induced by helminths.

4 Component-Resolved Diagnosis (CRD) in Allergy Diagnosis

For the past few decades, in vitro allergy diagnostics has been largely based on the detection of specific IgE to whole extracts comprised of allergenic and non-allergenic components (Treudler and Simon 2013). However, this approach has been problematic since the allergenic content of whole extracts is often difficult to standardize and also the specific allergic reaction inducing components in whole allergen extracts can be hard to identify (Valenta et al. 1999).

Such issues in in vitro allergy diagnostics led to the development of CRD in which purified natural or recombinant allergens are used to detect IgE sensitization to individual allergen molecules (Treudler and Simon 2013).

The use of molecular techniques and recombinant DNA technology has allowed the sequencing, synthesizing, and cloning of allergenic proteins leading to the production of recombinant allergens for CRD (Gadisseur et al. 2011). Recombinant allergens can be generated as defined molecules with consistent quality and without biological variation (Valenta and Niederberger 2007).

The molecular biological techniques underlying CRD were initially employed for the determination of the primary structures and molecular identities of allergens (Valenta et al. 1999). The sequence analysis of allergens allowed the identification of structurally related allergens and also revealed how closely linked cross-reactive molecules may not be differentiated by the immune system (Valenta et al. 1999). CRD involves the use of specific marker allergens to diagnose real sensitization toward a particular allergen source and to discriminate from sensitization to CCD or other homologous allergens (De Knop et al. 2010). It also allows the differentiation between clinically important and irrelevant specific IgEs (Treudler and Simon 2013).

In terms of the application of CRD to research in helminth-endemic populations, the study on peanut allergy among Ghanaian schoolchildren found that in a subset of study subjects with elevated IgE to whole peanut allergen, responses to recombinant forms of the major peanut allergens (rAra h 1, 2 & 3) were generally very low (Amoah et al. 2013b). In addition, among Brazilian children living in a helminth-endemic urban area, Carvalho et al. (2013) evaluated the use of IgE responses to *B. tropicalis* allergens (rBlo t 5 and rBlo t 21) in improving the specificity of determining mite allergy in this population. This study showed that

the assays using recombinant allergens exhibited lower IgE cross-reactivity with *A. lumbricoides* antigens and therefore conferred higher specificity in detecting genuine mite IgE sensitization than crude mite extract.

4.1 Microarray

In recent years, microarray biochips have been developed to allow the simultaneous measurement of specific IgE to multiple recombinant and natural allergen components using a small amount of serum. These microarray biochips are increasingly being used in developed countries to provide additional information on IgE profiles of polysensitized allergic patients to improve the management of their conditions.

In a previously published cohort of children in Indonesia, high levels of IgE to house dust mite were found, but this did not translate into SPT reactivity (Hamid et al. 2013). In the study, helminth-induced IgE cross-reactivity was implicated as a possible explanation for the elevated levels of clinically irrelevant allergen-specific IgE. In a subset of these children, the specific IgE to *Dermatophagoides pteronyssinus* (Der p) determined by the ImmunoCAP method (sensitization cutoff ≥0.35 kUA/L) was compared to semiquantitative IgE analysis using a commercially available microarray chip (ImmunoCAP ISAC). It was found that the prevalence of IgE sensitization to whole house dust mite extract (Der p) was 74 %, while sensitization to recombinant and natural house dust mite component allergens as assessed by the microarray biochip was only up to 5 % (Hamid et al. unpublished). This investigation also observed that among these same children, the highest IgE reactivity was to natural allergens of Bermuda and Timothy grass pollen and not much to recombinant grass pollen allergens on the chip indicating that these chips can possibly provide valuable additional information when studying sera with less known IgE specificities. For example, as shown in Fig. 2, the microarray slide contains natural forms of major peanut and grass pollen allergens that contain glycan structures as well as recombinant allergen components that are not glycosylated. The microarray technique used for serum sample from a helminth-infected individual compared to a European allergic patient can differentiate between IgE directed against protein structures that may be biologically active and IgE directed against carbohydrate moieties on glycosylated allergens that might be clinically irrelevant.

5 Future Directions

The studies of helminths and IgE have shed much light but also uncertainty about different aspects of the association between IgE, SPT, and allergy symptoms. Further research into this area should consider the importance of refining and preparing new diagnostic methods for the developing world where allergies are
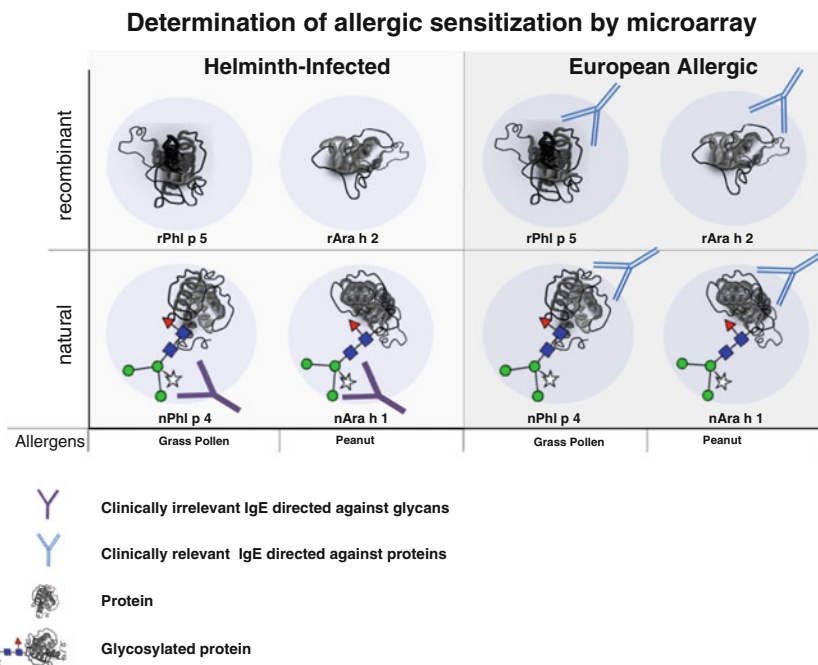
Fig. 2 An illustration of typical component-resolved diagnosis results generated using the ImmunoCAP ISAC™ microarray. The microarray slide shown contains natural grass pollen (nPhl p 4) and recombinant grass pollen (rPhl p 5) as well as natural peanut allergen (nAra h 1) and recombinant peanut allergen (rAra h 2). For the allergic European, the IgE recognizes and binds to the protein structures on both the natural and recombinant allergens for both grass pollen and peanut. This IgE is biologically active and also clinically relevant. In the serum of the helminth-infected subject, elevated levels of IgE are observed that recognize and bind to carbohydrate moieties on the natural allergen components. This IgE does not bind to the protein structures of these components, is clinically irrelevant, and shows poor biological activity

increasing, but the diagnosis is hampered by the complexity of the IgE antibodies. Moreover, further understanding of how IgE cross-reactivity develops and how this affects the biological activity of the antibody is needed. This could help devise interventions to stimulate IgE with poor biological activity which could hamper the development of high-affinity IgE, mast cell degranulation, and allergy symptoms.

References

Aalberse RC, Koshte V, Clemens JG (1981) Immunoglobulin E antibodies that crossreact with vegetable foods, pollen, and Hymenoptera venom. J Allergy Clin Immunol 68:356–364

Aalberse RC, Akkerdaas J, van Ree R (2001) Cross-reactivity of IgE antibodies to allergens. Allergy 56:478–490

Aalberse RC, Stapel SO, Schuurman J et al (2009) Immunoglobulin G4: an odd antibody. Clin Exp Allergy 39:469–477

Acevedo N, Caraballo L (2011) IgE cross-reactivity between Ascaris lumbricoides and mite allergens: possible influences on allergic sensitization and asthma. Parasite Immunol 33:309–321

Acevedo N, Sanchez J, Erler A et al (2009) IgE cross-reactivity between Ascaris and domestic mite allergens: the role of tropomyosin and the nematode polyprotein ABA-1. Allergy 64:1635–1643

Acevedo N, Mohr J, Zakzuk J et al (2013) Proteomic and immunochemical characterization of glutathione transferase as a new allergen of the nematode *Ascaris lumbricoides*. PLoS ONE 8: e78353

Altmann F (2007) The role of protein glycosylation in allergy. Int Arch Allergy Immunol 142:99–115

Amberbir A, Medhin G, Erku W et al (2011) Effects of Helicobacter pylori, geohelminth infection and selected commensal bacteria on the risk of allergic disease and sensitization in 3-year-old Ethiopian children. Clin Exp Allergy 41:1422–1430

Amoah AS, Boakye DA, van Ree R et al (2013a) Parasitic worms and allergies in childhood: insights from population studies 2008–2013. Pediatr Allergy Immunol 25:208–217

Amoah AS, Obeng BB, Larbi IA et al (2013b) Peanut-specific IgE antibodies in asymptomatic Ghanaian children possibly caused by carbohydrate determinant cross-reactivity. J Allergy Clin Immunol 132:639–647

Araujo MI, Lopes AA, Medeiros M et al (2000) Inverse association between skin response to aeroallergens and Schistosoma mansoni infection. Int Arch Allergy Immunol 123:145–148

Arkestal K, Sibanda E, Thors C et al (2011) Impaired allergy diagnostics among parasite-infected patients caused by IgE antibodies to the carbohydrate epitope galactose-alpha 1,3-galactose. J Allergy Clin Immunol 127:1024–1028

Arruda LK, Santos AB (2005) Immunologic responses to common antigens in helminthic infections and allergic disease. Curr Opin Allergy Clin Immunol 5:399–402

Arruda LK, Vailes LD, Platts-Mills TA et al (1997) Induction of IgE antibody responses by glutathione S-transferase from the German cockroach (*Blattella germanica*). J Biol Chem 272:20907–20912

Bager P, Arnved J, Ronborg S et al (2010) Trichuris suis ova therapy for allergic rhinitis: a randomized, double-blind, placebo-controlled clinical trial. J Allergy Clin Immunol 125:123–130

Bethony J, Brooker S, Albonico M et al (2006) Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367:1521–1532

Burke W, Fesinmeyer M, Reed K et al (2003) Family history as a predictor of asthma risk. Am J Prev Med 24:160–169

Carvalho EM, Bastos LS, Araujo MI (2006) Worms and allergy. Parasite Immunol 28:525–534

Carvalho KA, de Melo-Neto OP, Magalhaes FB et al (2013) Blomia tropicalis Blo t 5 and Blo t 21 recombinant allergens might confer higher specificity to serodiagnostic assays than whole mite extract. BMC Immunol 14:11

Chung CH, Mirakhur B, Chan E et al (2008) Cetuximab-induced anaphylaxis and IgE specific for galactose-alpha-1,3-galactose. N Engl J Med 358:1109–1117

Commins SP, Platts-Mills TA (2013) Delayed anaphylaxis to red meat in patients with IgE specific for galactose alpha-1,3-galactose (alpha-gal). Curr Allergy Asthma Rep 13:72–77

Commins SP, James HR, Kelly LA et al (2011) The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose-alpha-1,3-galactose. J Allergy Clin Immunol 127:1286–1293

Cooper PJ (2009) Interactions between helminth parasites and allergy. Curr Opin Allergy Clin Immunol 9:29–37

Cooper PJ, Chico ME, Bland M et al (2003a) Allergic symptoms, atopy, and geohelminth infections in a rural area of Ecuador. Am J Respir Crit Care Med 168:313–317

Cooper PJ, Chico ME, Rodrigues LC et al (2003b) Reduced risk of atopy among school-age children infected with geohelminth parasites in a rural area of the tropics. J Allergy Clin Immunol 111:995–1000

Cooper PJ, Chico ME, Rodrigues LC et al (2004) Risk factors for atopy among school children in a rural area of Latin America. Clin Exp Allergy 34:845–852

Cooper PJ, Chico ME, Vaca MG et al (2006) Effect of albendazole treatments on the prevalence of atopy in children living in communities endemic for geohelminth parasites: a cluster-randomised trial. Lancet 367:1598–1603

Dagoye D, Bekele Z, Woldemichael K et al (2003) Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. Am J Respir Crit Care Med 167:1369–1373

De Knop KJ, Bridts CH, Verweij MM et al (2010) Component-resolved allergy diagnosis by microarray: potential, pitfalls, and prospects. Adv Clin Chem 50:87–101

Feary JR, Venn AJ, Mortimer K et al (2010) Experimental hookworm infection: a randomized placebo-controlled trial in asthma. Clin Exp Allergy 40:299–306

Feary J, Britton J, Leonardi-Bee J (2011) Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 66:569–578

Fitzsimmons CM, Falcone FH, Dunne DW (2014) Helminth allergens, parasite-specific IgE, and its protective role in human immunity. Front Immunol 5:61

Flohr C, Tuyen LN, Lewis S et al (2006) Poor sanitation and helminth infection protect against skin sensitization in Vietnamese children: a cross-sectional study. J Allergy Clin Immunol 118:1305–1311

Flohr C, Tuyen LN, Quinnell RJ et al (2010) Reduced helminth burden increases allergen skin sensitization but not clinical allergy: a randomized, double-blind, placebo-controlled trial in Vietnam. Clin Exp Allergy 40:131–142

Gadisseur R, Chapelle JP, Cavalier E (2011) A new tool in the field of in-vitro diagnosis of allergy: preliminary results in the comparison of ImmunoCAP(c) 250 with the ImmunoCAP(c) ISAC. Clin Chem Lab Med 49:277–280

Godfrey RC (1975) Asthma and IgE levels in rural and urban communities of the Gambia. Clin Allergy 5:201–207

Gronlund H, Adedoyin J, Commins SP et al (2009) The carbohydrate galactose-alpha-1,3-galactose is a major IgE-binding epitope on cat IgA. J Allergy Clin Immunol 123(5):1189–1191

Hamid F, Wiria AE, Wammes LJ et al (2013) Risk factors associated with the development of atopic sensitization in Indonesia. PLoS ONE 8:e67064

Jenkins JA, Breiteneder H, Mills EN (2007) Evolutionary distance from human homologs reflects allergenicity of animal food proteins. J Allergy ClinImmunol 120:1399–1405

Jouvin MH, Kinet JP (2012) *Trichuris suis* ova: testing a helminth-based therapy as an extension of the hygiene hypothesis. J Allergy Clin Immunol 130:3–10

Lancet editorial (1976) Editorial: IgE, parasites, and allergy. Lancet 24:894–895

Larson D, Cooper PJ, Hubner MP et al (2012a) Helminth infection is associated with decreased basophil responsiveness in human beings. J Allergy Clin Immunol 130:270–272

Larson D, Hubner MP, Torrero MN et al (2012b) Chronic helminth infection reduces basophil responsiveness in an IL-10-dependent manner. J Immunol 188:4188–4199

Lynch NR, Hagel I, Perez M et al (1993) Effect of anthelmintic treatment on the allergic reactivity of children in a tropical slum. J Allergy Clin Immunol 92:404–411

Lynch NR, Palenque M, Hagel I et al (1997) Clinical improvement of asthma after anthelminthic treatment in a tropical situation. Am J Respir Crit Care Med 156:50–54

Lynch NR, Hagel IA, Palenque ME et al (1998) Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. J Allergy Clin Immunol 101:217–221

Macaubas C, Sly PD, Burton P et al (1999) Regulation of T-helper cell responses to inhalant allergen during early childhood. Clin Exp Allergy 29:1223–1231

MacGlashan DW Jr, Bochner BS, Adelman DC et al (1997) Down-regulation of Fc(epsilon)RI expression on human basophils during in vivo treatment of atopic patients with anti-IgE antibody. J Immunol 158:1438–1445

Mari A (2002) IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. Int Arch Allergy Immunol 129:286–295

Mari A, Iacovacci P, Afferni C et al (1999) Specific IgE to cross-reactive carbohydrate determinants strongly affect the in vitro diagnosis of allergic diseases. J Allergy Clin Immunol 103:1005–1011

Mari A, Ooievaar-de HP, Scala E et al (2008) Evaluation by double-blind placebo-controlled oral challenge of the clinical relevance of IgE antibodies against plant glycans. Allergy 63:891–896

Mitre E, Norwood S, Nutman TB (2005) Saturation of immunoglobulin E (IgE) binding sites by polyclonal IgE does not explain the protective effect of helminth infections against atopy. Infect Immun 73:4106–4111

Mpairwe H, Webb EL, Muhangi L et al (2011) Anthelminthic treatment during pregnancy is associated with increased risk of infantile eczema: randomised-controlled trial results. Pediatr Allergy Immunol 22:305–312

Obeng BB, Amoah AS, Larbi IA et al (2014) Schistosoma infection is negatively associated with mite atopy, but not wheeze and asthma in Ghanaian Schoolchildren. Clin Exp Allergy 44:965–975

Obihara CC, Beyers N, Gie RP et al (2006) Respiratory atopic disease, Ascaris-immunoglobulin E and tuberculin testing in urban South African children. Clin Exp Allergy 36:640–648

Palmer LJ, Celedon JC, Weiss ST et al (2002) Ascaris lumbricoides infection is associated with increased risk of childhood asthma and atopy in rural China. Am J Respir Crit Care Med 165:1489–1493

Pawankar R, Canonica GW, Holgate ST et al (2011) Introduction and executive summary: allergic diseases as a global public health issue. In: Pawankar R et al (eds) World Allergy Organization (WAO) white book on allergy. pp 11–20

Perzanowski MS, Ng'ang'a LW, Carter MC et al (2002) Atopy, asthma, and antibodies to Ascaris among rural and urban children in Kenya. J Pediatr 140:582–588

Ponte EV, Lima F, Araujo MI et al (2006) Skin test reactivity and Der p-induced interleukin 10 production in patients with asthma or rhinitis infected with Ascaris. Ann Allergy Asthma Immunol 96:713–718

Priftanji A, Strachan D, Burr M et al (2001) Asthma and allergy in Albania and the UK. Lancet 358:1426–1427

Royer B, Varadaradjalou S, Saas P et al (2001) Inhibition of IgE-induced activation of human mast cells by IL-10. Clin Exp Allergy 31:694–704

Santiago HC, Bennuru S, Boyd A et al (2011) Structural and immunologic cross-reactivity among filarial and mite tropomyosin: implications for the hygiene hypothesis. J Allergy Clin Immunol 127:479–486

Santiago HC, LeeVan E, Bennuru S et al (2012) Molecular mimicry between cockroach and helminth glutathione S-transferases promotes cross-reactivity and cross-sensitization. J Allergy Clin Immunol 130:248–256

Santos AB, Rocha GM, Oliver C et al (2008) Cross-reactive IgE antibody responses to tropomyosins from Ascaris lumbricoides and cockroach. J Allergy Clin Immunol 121:1040–1046

Satoguina JS, Adjobimey T, Arndts K et al (2008) Tr1 and naturally occurring regulatory T cells induce IgG4 in B cells through GITR/GITR-L interaction, IL-10 and TGF-beta. Eur J Immunol 38:3101–3113

Scrivener S, Yemaneberhan H, Zebenigus M et al (2001) Independent effects of intestinal parasite infection and domestic allergen exposure on risk of wheeze in Ethiopia: a nested case-control study. Lancet 358:1493–1499

Sereda MJ, Hartmann S, Lucius R (2008) Helminths and allergy: the example of tropomyosin. Trends Parasitol 24:272–278

Sheehan D, Meade G, Foley VM et al (2001) Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem J 360:1–16

Smits HH, Hammad H, van Nimwegen M et al (2007) Protective effect of *Schistosoma mansoni* infection on allergic airway inflammation depends on the intensity and chronicity of infection. J Allergy Clin Immunol 120:932–940

Smits HH, Everts B, Hartgers FC et al (2010) Chronic helminth infections protect against allergic diseases by active regulatory processes. Curr Allergy Asthma Rep 10:3–12

Supali T, Djuardi Y, Wibowo H et al (2010) Relationship between different species of helminths and atopy: a study in a population living in helminth-endemic area in Sulawesi, Indonesia. Int Arch Allergy Immunol 153:388–394

Treudler R, Simon JC (2013) Overview of component resolved diagnostics. Curr Allergy Asthma Rep 13:110–117

Valenta R, Niederberger V (2007) Recombinant allergens for immunotherapy. J Allergy Clin Immunol 119:826–830

Valenta R, Lidholm J, Niederberger V et al (1999) The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). Clin Exp Allergy 29:896–904

Valmonte GR, Cauyan GA, Ramos JD (2012) IgE cross-reactivity between house dust mite allergens and Ascaris lumbricoides antigens. Asia Pac Allergy 2:35–44

van den Biggelaar AH, van Ree R, Rodrigues LC et al (2000) Decreased atopy in children infected with *Schistosoma haematobium*: a role for parasite-induced interleukin-10. Lancet 356:1723–1727

van den Biggelaar AH, Lopuhaa C, van Ree R et al (2001) The prevalence of parasite infestation and house dust mite sensitization in Gabonese schoolchildren. Int Arch Allergy Immunol 126:231–238

van der Veen MJ, van Ree R, Aalberse RC et al (1997) Poor biologic activity of cross-reactive IgE directed to carbohydrate determinants of glycoproteins. J Allergy Clin Immunol 100:327–334

van Ree R, Cabanes-Macheteau M, Akkerdaas J et al (2000) Beta(1,2)-xylose and alpha(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. J Biol Chem 275:11451–11458

von Mutius E (2002) Environmental factors influencing the development and progression of pediatric asthma. J Allergy ClinImmunol 109:S525–S532

Wilson MS, Taylor MD, Balic A et al (2005) Suppression of allergic airway inflammation by helminth-induced regulatory T cells. J Exp Med 202:1199–1212

Wiria AE, Hamid F, Wammes LJ et al (2013) The effect of three-monthly albendazole treatment on malarial parasitemia and allergy: a household-based cluster-randomized, double-blind, placebo-controlled trial. PLoS ONE 8:e57899

Wright V, Bickle Q (2005) Immune responses following experimental human hookworm infection. Clin Exp Immunol 142:398–403

Yazdanbakhsh M, van den Biggelaar A, Maizels RM (2001) Th2 responses without atopy: immunoregulation in chronic helminth infections and reduced allergic disease. Trends Immunol 22:372–377

Yazdanbakhsh M, Kremsner PG, van Ree R (2002) Allergy, parasites, and the hygiene hypothesis. Science 296:490–494