7 SUMMARIZING DISCUSSION

Partly based on:
Helminth-induced IgE and protection against allergic disorders

Firdaus Hamid[1,2], Abena S Amoah[2,3], Ronald van Ree[4], Maria Yazdanbakhsh[2]

1. Department of Microbiology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
2. Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands
3. Department of Parasitology, Noguchi Memorial Institute for Medical Research, Accra, Ghana
4. Department of Experimental Immunology and Department of Otorhinolaryngology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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WHAT WAS ALREADY KNOWN ABOUT THE RELATIONSHIP BETWEEN HELMINTH INFECTIONS, SOCIO-ECONOMIC STATUS AND ALLERGIC DISORDERS?

It has been reported in several epidemiological studies that the prevalence of atopic disorders is higher in developed countries than in developing countries[1,2]. In contrast, intestinal helminth infections such as *Ascaris lumbricoides, Trichuris trichiura* and/or hookworms (*Necator americanus* or *Ancylostoma duodenale*) are still highly prevalent in developing countries[3]. Interestingly, both helminth infection and allergens are potent inducers of Th2 responses that lead to high levels of IgE, tissue eosinophilia, as well as the secretion of Th2 cytokines such as IL-4, IL-5, IL-9 and IL-13[4,5]. Despite the similar immunological profiles associated with both helminth and allergies, the relation between helminth infections and atopic diseases is still unclear. Several studies have reported an inverse relation between helminth infections and atopic diseases[6-8] while other studies have shown that helminth infections might increase the risk of asthma or be associated with higher prevalence of atopy[9] (reviewed in **Chapter 1**). Interventional studies have also not been consistent; one large study in Ecuador found no changes in allergic disorders after one year treatment of intestinal helminths using albendazole[10], which is in contrast to an intervention study in Vietnamese children that suggested repeated anthelmintic treatment might increase the incidence of atopy[11].

Several studies have shown conflicting results with regards to the role of socio-economic status (SES) in the development of atopic disorders[12]. However, a recent systematic review has suggested that lower socio-economic levels are associated with higher prevalence of asthma, whereas the prevalence of other allergies such as food allergy and atopic dermatitis is associated with higher SES[12]. This systematic review might coffer from emphasis on data from Europe and the Americas, as well as data from high- and middle-income countries. Moreover, skin prick test (SPT) reactivity to any allergens has been reported to be more common in high-SES groups compared to low-SES groups within urban centers of developing countries[13,1].

In both developed and developing countries, a strong correlation is observed between allergen-specific IgE and symptoms of allergy among urban populations of high SES. However, in rural populations or urban population of developing countries with low SES, helminth-induced IgE cross-reactivity and regulatory networks may prevent the translation of allergen-specific IgE into skin reactivity or allergic symptoms as discussed in **Chapter 1**.

In addition, the isolation and characterization of allergen components, as well as their production by recombinant techniques, has led to significant progress in allergy diagnosis[15]. Component-resolved diagnostics (CRD) allows the detection of specific IgE against individual allergen molecules instead of against allergen extracts comprised of mixtures of allergen molecules that are commonly used in SPT and conventional specific IgE testing. The advantage of CRD is that distinction can be made between IgE antibodies against allergenic structures with different degrees of clinical relevance, ranging from irrelevant such as cross-reactive carbohydrate determinants (CCD)[16] to a risk factor for severe symptoms[17]. This

SUMMARY OF WHAT WAS ALREADY KNOWN

- There is an inverse geographical relationship between allergy and helminth infections.
- The prevalence of atopy and allergic diseases is increasing in both developed and developing countries.
- Low socio-economic status is associated with asthma symptoms and negatively with skin prick test (SPT) reactivity to any allergens.
- Anthelmintic treatment increases SPT in Vietnam but not in Ecuador.
- High levels of total IgE and allergen-specific IgE do translate into skin reactivity or clinical symptoms of allergy in most affluent countries.
- Component-resolved diagnosis of the specific IgE responses improves the specificity, sensitivity and clinical performance of laboratory assays used in the diagnosis of atopic disorders in developed countries.

method has been widely used in developed countries[15], but has still not been widely used in less affluent areas where helminth infections are highly prevalent.

HOW DID OUR STUDIES ADVANCE THE FIELD?

In order to move the field further, we designed a population study on Flores Island in Indonesia, detailed in **Chapter 3**.

Risk factors for atopic disorders and interactions with socio-economic status

In **Chapter 2 and 4**, evidence was provided that socio-economic status is an important factor in atopic disorders in Indonesia. We found that high parental education, which is one of the indicators of high-SES, was associated with skin reactivity to house dust mite (HDM) (**Chapter 4**). In addition, we also found that the prevalence of skin prick test reactivity to any allergens was higher in high-SES compared to low-SES schoolchildren (**Chapter 2**) or higher in a semi urban compared to a rural area (**Chapter 4).** This result is in line with other studies conducted in Ghanaian children, which found that the prevalence of SPT to any allergens was higher in urban rich compared to urban poor children[13,14]. We also observed that there was a strong association between specific IgE and SPT reactivity to the same aeroallergen in the children from a high-SES school (**Chapter 2**), a result that is consistent with what is found in developed countries[18,19]. However, no such association was seen in the low-SES group (**Chapter 2**) living in the same urban area. Similar dissociation between specific IgE (sIgE) to aeroallergens and skin prick test reactivity to the same allergen has been reported by us in a rural area of Indonesia (**Chapter 4).** The prevalence of allergic symptoms was higher in low-SES children than in high-SES children which is in contrast to what is often reported[20,21]. This might be due to the inability to distinguish between symptoms that arise

due to respiratory infections rather than allergy. Indeed, we observed that SPT positivity is a risk factor for reported symptoms of allergy in high-SES but not in low-SES children (**Chapter 2**). This result implies that SPT can be used as a good predictor for allergy symptoms in high-SES children, comparable to the situation in developed countries, but not for children of low-SES. Altogether, the high-SES life-style which is associated with western diet and less physical activity may contribute towards atopy compared to low-SES environments.

Environmental factors are closely related to socio-economic status. One of the important environmental factors is thought to be helminth infection. In **Chapter 2 and 4** we provide evidence that helminth infections are more prevalent among low SES groups compared to high SES groups. Important contextual determinants for helminth infection are poverty, lack of sanitation and poor hygiene practices in low-SES population[22]. With regard to the association between helminth infections and atopic disorders, in a meta-analyses, Feary *et al.* have shown that hookworm infections determined by microscopy are significantly associated with lower SPT reactivity to HDM[23]. In chapter 4, we used a highly sensitive and specific method (*polymerase chain reaction*, PCR) for detection of soil transmitted helminth infections and were able to demonstrate that high load of hookworm infection decreased the risk of being prick test positive to HDM in the semi-urban area only (**Chapter 4**). However, the situation was different when SPT to cockroach was examined. In the same semi-urban area, children with access to piped water coming from non-farming families had reduced risk of SPT to cockroach (**Chapter 4**). The precise cause of this association for skin reactivity to cockroach is unknown but it is thought to be related to higher exposure to this aeroallergen in low-SES houses, which might defy the mechanisms that are responsible for the lower prevalence of SPT to HDM. However, it is also possible that different mechanisms govern HDM versus cockroach SPT reactivity.

In summary, within the same urban centres of a developing country; our study found that depending on the level of SES of a child, the prevalence of different allergy outcomes or risk factors for atopic disorders may be very different. Our study also indicates that the lower prevalence of skin reactivity to HDM in a rural area of Indonesia might be due to a lower level of education (as part of the SES indicators) and a greater degree of helminth infections compared with the semi urban area. Moreover, the risk factors for sensitization to HDM are distinct from those for sensitization to cockroach. Together, these results highlight that environmental as well as socio-economic factors should be considered by clinicians and researchers working on prevention, diagnosis and treatment of atopic disorders in low-to-middle income countries. In addition, many factors in the environment contribute to the development of allergies (e.g. diet, immunisation, pets, tobacco smoke, and air pollution), therefore, more research is needed to evaluate the possible risk and protective factors in more detail as well as to pinpoint elements of SES that matter in the development of atopic disorders.

Effect of anthelmintic treatment on atopic disorders

Considering that cross sectional studies can only show relationships and do not demonstrate causality between helminths and allergies, interventional studies where anthelmintics are

used 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 IgE in serum to aeroallergens[24]. In line with this, anthelmintic treatment of Gabonese children chronically infected with *Schistosoma haematobium* and soil transmitted helminths resulted in increased SPT reactivity[7]. Two large interventional studies previously mentioned conducted in Ecuador and Vietnam showed different results. In Ecuador, treatment with albendazole every two months for one year, did not affect SPT nor clinical symptoms of allergy[10], but in Vietnam, three monthly treatment with albendazole resulted in a significant increase in SPT positivity but not in allergy symptoms[11]. In **Chapter 5**, our randomised placebo controlled trial showed that intensive community treatment of 3 monthly albendazole for 21 months 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. However, in agreement with the studies in Ecuador and Vietnam, there was no effect on reported clinical symptoms of allergy (**Chapter 5**). It has to be noted that one study in Venezuela showed that anthelmintic treatment resulted in improvement in all clinical indicators of asthma[25]. It is important to mention that in our study, helminth infections were not completely cleared; therefore the effect to anthelmintic treatment on allergy outcome could not be fully seen. A study in Ecuador, looking at communities receiving anthelmintic treatment with ivermectin for 15-17 years compared to non-treated communities showed an increase in the prevalence of allergen skin test reactivity in treated communities[26]. This suggests that longer anthelmintic treatment in our study may have been needed to see the effect of treatment on atopic disorders. 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[27].

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. For anthelmintic treatment studies, 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[28]. Another mechanism that might explain the inverse association between helminth infections and atopy may involve helminth-induced IgE cross-reactivity. Current helminth infections are associated with increased levels of allergen-specific IgE that do not translate into skin reactivity or clinical symptoms. This helminth-induced IgE may be of low affinity; this might explain why it does not lead to SPT reactivity. Finally, attention should be paid to the methods used to assess clinical symptoms of allergy as these could be an important source of variation.

Microarray and Helminth-induced IgE

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 used in developed countries to provide additional information on IgE profiles of poly-sensitized allergic patients to improve the management of their conditions[15,29,30].

In a group of children (described in chapter 4), high levels of IgE to house dust mite were found, but these did not translate into skin prick test reactivity. In this 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 cut-off ≥ 0.35 kU_A/L) was compared to semi-quantitative IgE analysis using a commercially available microarray chip (ImmunoCAP-ISAC). We found that the prevalence of IgE sensitization to whole house dust mite extract (Der p) was 74% while sensitization to purified recombinant and natural house dust mite allergens such as Der p 1 and Der p 2 as assessed by the microarray biochip was generally weak and only up to 5% (**Chapter 6**). This investigation also demonstrated that among these same children, there was IgE reactivity to purified natural (glycosylated) allergens of Bermuda and Timothy grass pollen but not to (non-glycosylated) recombinant grass pollen allergens on the chip (**Chapter 6**). As shown in Figure 1, the microarray technique used on a 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 (such as natural glycosylated pollen allergens) that have been shown to be clinically irrelevant. Essentially, helminth-infected Indonesian children recognize purified natural pollen allergens because of having carbohydrate-specific IgE antibodies but do not recognize non-glycosylated recombinant pollen (and house dust mite) allergens. Helminth-induced cross-reactivity is most likely at the basis of these IgE responses. European children on the other hand clearly react with the latter category of allergens supporting true sensitization. Also, the high prevalence of positive IgE to peanut in the Indonesian children was shown to be caused by IgE against CCD, as demonstrated by inhibition with bromelain (a commonly used marker glycoprotein for CCD reactivity) and SEA (soluble egg antigen of *S. haematobium,* a source of helminth-derived CCD). The commonly recognized major peanut allergens (Ara h 1, 2, 3 and 6) were not recognized.

As described in **Chapter 6**, we also found high reactivity to recombinant venom allergens among school children in Indonesia. This cannot be explained by CCD. The source of sensitization to these venom allergens in children who do not appear to have clinical venom allergy is yet not clear. This unexpected IgE detection can be explained in two possible ways; there is a true sensitization to some kind of venom allergens (not necessarily the common wasps or bees represented by the recombinant allergens on the chip), or the majority of cases of double positivity to honey bee/wasp allergens may be due to sensitization to cross-reactive proteins of non-insect venom origin (e.g. of helminths).

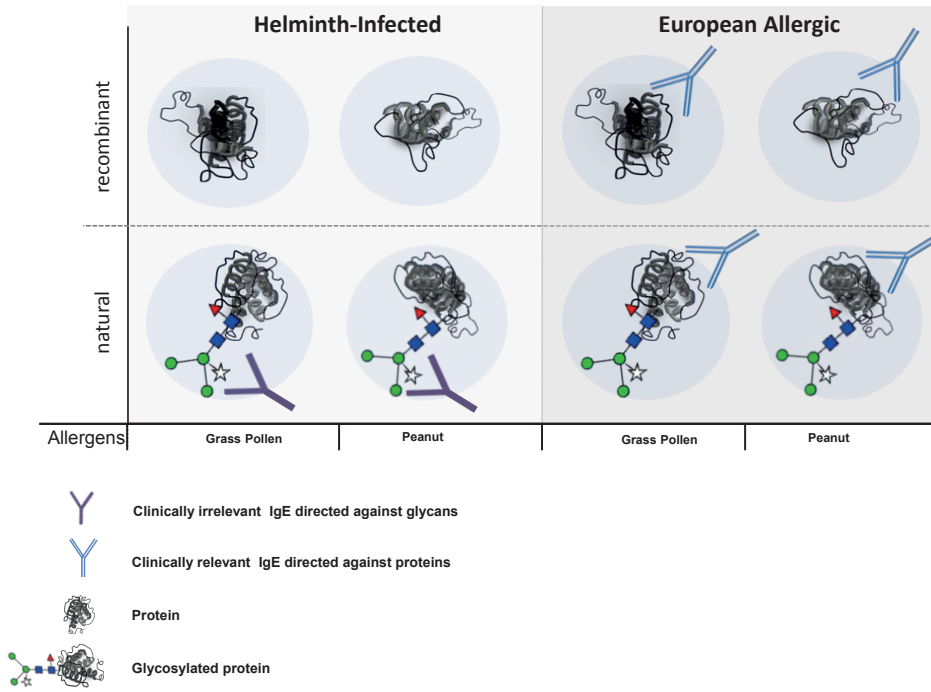

Figure 1. Determination of allergic sensitization by microarray. *An illustration of typical component-resolved diagnosis results generated using the ImmunoCAP ISAC™ microarray. The microarray shown contains natural and recombinant grass pollen as well as natural and recombinant peanut allergens. For an 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 a 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.*

In summary, the study presented on the profile of IgE antibodies using a biochip array and plasma from a helminth-endemic area has provided evidence that there is a strong IgE reactivity to some of the natural allergen components from pollen which carry CCDs. This reactivity is probably helminth-induced. Our study also found unexpectedly high reactivity to recombinant venom allergens in children from a helminth-endemic area, but the origin of this sensitization still needs to be elucidated. We provide evidence of cross-reactivity between IgE to allergens and CCD markers, suggesting that using natural and recombinant allergens on microarray might help to better differentiate between real allergic sensitization and cross-reactivity for diagnosis of atopic disorders in low-to-middle income countries where helminth infections are prevalent. Further research into helminths and IgE should focus on refining and preparing new diagnostic methods for the developing world where allergies are increasing but the diagnosis is hampered by the lack of knowledge on locally important allergen sources and the complexity of the specificities and characteristics of IgE antibodies. Furthermore, a better understanding of how IgE cross-reactivity develops and how this affects the biological activity of the antibody is needed.

SUMMARY OF THE FINDINGS

In this thesis, we investigated the risk factors for atopic sensitization and allergic disease in some areas of Indonesia where the socio-economic status and life style are very different from affluent countries. We studied the effect of anthelmintic treatment on atopic disorders. Furthermore, we characterized the IgE antibody profiles among children who are living in a helminth-endemic area. The main findings are:

- Specific IgE was a risk factor for being SPT positive and SPT positivity was a major risk factor for reported clinical symptoms of allergy in high-SES children from an urban area but not in low-SES (**Chapter 2**).
- High load of hookworm (*Necator americanus*) infection based on PCR method was an independent protective factor for HDM skin reactivity (**Chapter 4**)
- Cockroach skin reactivity was increased in children from farmer families, which are presumed to have higher exposure to cockroach (**Chapter 4**)
- Repeated three-monthly treatment with single dose albendazole over 21 months reduced but did not eliminate helminth infection (**Chapter 5**)
- Two years anthelminthic treatment increased skin reactivity especially to cockroach (**Chapter 5**).
- In an helminth-endemic area, carbohydrate-bearing allergens were more recognized than recombinant allergen components (**Chapter 6**)
- High reactivity to recombinant venom allergen was observed in children from a helminth-endemic area (**Chapter 6**)

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