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chapter 8

Summarizing Discussion

Introduction

There is overwhelming evidence that reduced exposure to microorganisms or parasites during childhood may lead to the inadequate maturation of the regulatory component of the developing immune system. This immune dysregulation, which has been closely linked to lifestyle changes and urbanization, is thought to be one of the key factors driving the global rise in inflammatory conditions that include allergies. Helminth infections, which have strong effects on the immune system, are one of the exposures that have changed with lifestyle and urbanization. Indeed, these infections show little geographical overlap with allergic disorders and high burdens of helminth infections are often found in rural areas of developing countries where allergies are least common.

In this thesis, the complex dynamics between helminth infections and allergies among children in Ghana has been examined. Findings include insights into the relationship between helminth infections and allergies as well as urban-rural differences in allergy outcomes. In addition, the thesis explored the role of helminth-induced immunoglobulin E (IgE) cross-reactivity in explaining the lack of skin reactivity to allergens in the face of elevated IgE and also the underlying cellular immune mechanisms related to allergy.

Associations between helminths and allergies

In Chapter 2, the associations between helminth infections and allergy outcomes were examined. Helminth infections were seen predominantly among rural study participants and we observed that schistosome infection was inversely associated with house dust mite skin prick test (SPT) reactivity. Recent cross-sectional studies from helminth-endemic areas support our findings. One investigation conducted among urban children of low socioeconomic status (SES) living in Salvador, Brazil found that heavy infection with the soil-transmitted helminth (STH) *Trichuris trichiura* in early childhood was associated with reduced odds of SPT reactivity later in childhood [1]. Apart from demonstrating the importance of the timing of exposure to helminths, this study also showed that heavy helminth infections (compared to light ones) may have a protective effect against allergies. Similarly, an investigation from South Africa comparing allergy outcomes between rural children and urban low SES children of the same ethnicity, observed that current *Ascaris lumbricoides* infection was associated with reduced odds of SPT reactivity [2].

However, as shown in Chapter 2, the relationship is more complex as we found that although schistosome infection was protective, no association was observed between any of the STHs (*A. lumbricoides*, *Trichuris trichiura* or hookworm) and house dust mite SPT reactivity. The lack of an STH effect may in part be explained by the fact that these infections were not highly prevalent in our study areas. An investigation among Cuban children living in helminth-endemic areas had similar findings to our own in that current intestinal helminth infection was not associated with SPT reactivity [3]. In the Cuban study,

the prevalence of STH infections ranged from 6.3% to 10.6% [3] while ours ranged from 1.9% to 9.9%. Thus, results in Chapter 2 illustrate the importance of taking into account helminth species and degree of endemicity when investigating associations with allergy.

Chapter 2 also assessed whether helminth infections were associated with reported symptoms of allergy. We found that current helminth infection was not associated with reported wheeze or asthma. The effect of helminths on clinical allergy outcomes has been investigated in a number of studies and conflicting findings have been reported. For example, among Afro-Ecuadorian children, heavy *T. trichiura* infection was inversely associated with atopic wheeze [4], while a study in South African children observed that current *A. lumbricoides* infection was associated with increased odds of the asthma marker exercise-induced bronchoconstriction (EIB) [2]. However, in the same South African study, *A. lumbricoides* infection appeared to be associated with a decreased risk of a positive SPT to any aeroallergen [2]. In fact, research findings suggest that in areas with a heavy burden of *A. lumbricoides* infection, this helminth may induce an inflammatory response in the lungs that is independent of the parasite's effect on SPT reactivity [2]. Aside from inflammation associated with the lungs, current *A. lumbricoides* has been linked to a 4-fold reduction in the odds of atopic eczema in rural Cuban children [5]. Hence, it is difficult to conclude whether helminth infections also affect the expression of clinical allergy. Considering that clinical symptoms are relatively rare, it is possible that studies thus far have not been sufficiently powered. Therefore, much larger population studies are needed.

A number of studies have shown that infections other than helminths may play an important role in allergy outcomes. For example, an investigation among urban low SES children in Salvador, Brazil observed that in addition to the protective effect of *A. lumbricoides* infection, past exposure to *Toxoplasma gondii*, Epstein - Barr virus and herpes simplex virus (assessed by seropositivity) were each associated with a lower prevalence of SPT reactivity [6]. These findings highlight the importance of diverse childhood pathogens in reducing the risk of SPT reactivity. Furthermore, among a birth cohort of children from Ethiopia, *Helicobacter pylori* infection determined at 3 years was linked to borderline reduced odds of reported eczema and SPT reactivity to house dust mite [7].

Altogether, there is strong evidence for the protective effects of helminths on allergy outcomes in animal models [8] but the results of cross-sectional studies in humans vary greatly. Though it is generally agreed that helminth infections are often negatively associated with SPT, a lack of association or even positive associations have been observed with lung function. It is also essential to bear in mind that species of helminth as well as timing and burden of infection can all contribute to inconsistent findings in population studies particularly when the study outcome is as complex and multifactorial as clinical allergy. It is also imperative to consider that exposures other than helminths can play an important role in protection against allergy outcomes.

Urban versus rural comparisons and allergy

Part of Chapter 2 examined the urban-rural differences in aeroallergy outcomes and the role of parasitic infections as well as other factors in explaining these differences. In our study population, the most common allergen associated with SPT reactivity was house dust mite and this was most prevalent among urban high SES children (16.3%) followed by rural children (12.1%) and lastly urban low SES children (10.5%).

In other studies performed in central Ghana, the prevalence of SPT reactivity to any allergen was highest in a group of children attending an affluent urban school compared to their less affluent urban counterparts as well as rural children [9, 10]. Whereas the observed gradient in our study was urban high SES > rural > urban low SES, in central Ghana it was urban high SES > urban low SES > rural. Our findings indicate that rural children living in areas endemic for helminths are not always the most protected. In addition, our observations of lower SPT reactivity among urban low SES children who were not highly infected with helminths compared to our rural children, shows that protective factors aside from helminths that are present in urban low SES environments exist and have to be identified.

Another notable finding highlighted in Chapter 2 was that being overweight according to body mass index (BMI) was highest in the urban high SES category followed by rural and lastly, the urban low SES category. Furthermore, a strong association was observed between being overweight and SPT reactivity to house dust mite. These results appear to indicate that despite living in areas endemic for helminths and malaria, rural children in our study may have been of a better nutritional state, as measured by BMI, compared to their urban low SES counterparts. Therefore, rural children may have been more susceptible to allergic reactivity compared to urban low SES children. A relationship between BMI and allergy outcomes has been observed in some population studies. For example, among urban and rural South African children, increasing BMI was significantly associated with EIB as well as a greater strength of association between mite-specific IgE and SPT reactivity to house dust mite [11]. A subsequent investigation also from South Africa found that the consumption of an 'urban diet' partly explains the difference in the prevalence of SPT reactivity to allergens between urban and rural areas [12]. However, the relationship between urban diet and BMI specifically were not examined in this study.

Altogether, there is accumulating evidence to support the fact that determinants associated with lifestyle change such as increasing BMI are linked to allergy outcomes. Results outlined in Chapter 2 also illustrate the problem in labelling areas as rural or urban in rapidly developing countries since in some so-called rural areas, the living conditions and lifestyle may be transitioning to be more pro-allergic than expected. Consequently, there is a critical need for standardized definitions of what constitutes 'rural' and 'urban' environments in rapidly developing countries [13].

In Chapter 3 we reported the results of an in-depth analysis of markers related to food allergy in our study population and whether urban-rural differences existed. From

this cross-sectional study, we observed that the prevalence of SPT to food allergens was similar in urban and rural children but the proportion of rural children reporting adverse reactions was greater than among their urban counterparts which may in part reflect adverse reactions not related to allergy. Chapter 3 also described a nested matched case-control study in which cases were SPT positive for any food allergen and matched controls were SPT negative. A notable finding of this study was that the strength of the association between food-specific IgE and corresponding SPT was greater in the urban compared to rural area. This further demonstrates how environmental factors can modulate the link between IgE and SPT and possibly also the link to reported symptoms.

Helminth-induced IgE cross-reactivity

Although it is clear that different environmental factors play a role in the development of allergic disorders, there is evidence that the presence of certain helminth infections is an important factor associated with lower SPT to allergens. Therefore, a more in-depth understanding of mechanisms behind this association is imperative. In Chapter 4, the effect of helminth-induced IgE cross-reactivity on allergen-specific IgE in our study population was examined. Cross-reactivity is a reflection of the phylogenetic relationship between organisms that leads to a large degree of homology in the primary as well as three dimensional structures of glycoproteins [14]. Therefore, IgE directed against one allergen may recognize homologous structures from other sources. Two types of IgE cross-reactivity related to allergy have been recognized: cross-reactivity due to proteins and cross-reactivity due to glycans on glycoproteins known as cross-reactive carbohydrate determinants (CCDs) [14]. The first indication of possible helminth involvement in IgE cross-reactivity came from observations in population studies where elevated levels of allergen-specific IgE did not translate into skin reactivity or symptoms of allergy among helminth-infected children [15].

As described in Chapter 4, 17.5% of subjects in our study population were IgE sensitized to peanut (≥ 0.35 kU/L) yet 92.4% of those sensitized were peanut SPT negative. In addition, current infection with *S. haematobium* was strongly associated with peanut IgE sensitization and a strong correlation was observed between IgE against whole peanut extract and IgE against CCDs. Inhibition assays demonstrated that not only could this IgE against whole peanut extract be almost completely inhibited by the CCD marker bromelain, but also by *S. haematobium* soluble egg antigen which is enriched with N-glycans. Moreover, basophil histamine release assays showed that the IgE directed against peanut in this population had low biological activity. Findings in Chapter 4 provide a model which proposes that in helminth infections, primary sensitization may occur to carbohydrate moieties present in helminths which are also present in some well-characterized allergens such as peanut and that such IgE antibodies have low biological activity. Although the lack of clinical relevance of IgE antibodies against CCDs has been demonstrated in Europeans [16], in recent years, IgE directed against the carbohydrate

epitope galactose- α -1,3-galactose (α -gal) has been linked to two forms of anaphylaxis in the Southeastern United States [17]. Interestingly, in serum samples from children living in rural helminth-endemic communities in Kenya, Ecuador and Zimbabwe, positive IgE responses to α -gal have been observed [17, 18]. However, the clinical relevance of IgE to α -gal in helminth-endemic area is yet to be fully established.

Immune mechanisms

In Chapter 5, we focused on underlying immune mechanisms by examining the relationship between cellular immune responsiveness and SPT reactivity to house dust mite. In this chapter, *in vitro* whole blood culture cytokine responses to a panel of stimuli were used to assess general innate and adaptive immune responsiveness. We observed that higher innate as well as adaptive immune responses were associated with being a house dust SPT positive case. Similar observations were made among a cohort of urban low SES children living in Salvador, Brazil where past and current infections (helminth, viral and bacterial) were linked to reduced SPT reactivity [6]. In an immunological study in this Brazilian cohort, cytokine responses from whole blood cultures stimulated with mitogen were measured in 1127 children and different immunological phenotypes were defined: 'responsive' (characterized by generalized cytokine production above cytokine detection limits), 'under-responsive' (characterized by few responses above the detection limit) and 'intermediate' [19]. The responsive phenotype was associated with increased odds of SPT reactivity as well as allergen-specific IgE sensitization. Together, these studies indicate that overall immune hyperresponsiveness may be a characteristic of populations that are at increased risk of developing allergies.

In Chapter 5 it was also noted that this hyperresponsiveness extended to interleukin (IL)-10 production. In other words, high IL-10 in response to innate and adaptive stimulation was associated with increased SPT reactivity. These findings might have been unexpected as a number of investigations in humans have provided evidence that IL-10 plays a key role in the helminth-induced immune regulation of allergic responses [8]. For example, a study in Gabon, determined that IL-10 production by parasite-antigen stimulated peripheral blood mononuclear cells was higher in children infected with *S. haematobium* and elevated IL-10 levels were negatively associated with SPT reactivity to house dust mite [20]. In line with this, an anthelmintic trial conducted among Vietnamese children found that SPT reactivity was inversely associated with higher IL-10 in response to hookworm antigen and that after 12 months of deworming, there was a lower IL-10 response in the treated group although this was not statistically significant [21]. However, a study among Ecuadorian children living in a helminth-endemic area, observed no relationship between either *A. lumbricoides* antigen induced IL-10 determined in whole blood cultures or the frequency of IL10+ T cells and SPT reactivity [22].

Taken together, cellular immunological associations with allergy might be at two different levels. Firstly, general responsiveness and secondly, the type of response.

Future studies need to dissect this by examining responses at the single cell level to understand the sources of different cytokines and to determine the responsiveness of different cell types in allergic and non-allergics.

In Chapter 6, the examination of immune mechanisms was approached by investigating whether the dramatic environmental changes associated with urbanization were having an impact on the developing immune profile at the level of gene expression. The investigation described in Chapter 6 was performed in a subset of study participants and we observed that there were distinct differences in gene expression profiles of children attending schools in rural, urban low SES and urban high SES areas within one geographical region of Ghana. Specifically, higher gene expression levels of IgE, IL-10 and PD-1 were seen in rural compared to urban study participants. Although current *S. haematobium* infection could account for elevated IgE messenger RNA (mRNA) in the rural area, current helminth infection was not associated with elevated IL-10 and PD-1 mRNA in whole blood.

Given the urban-rural difference in IL-10 gene expression, we addressed whether there is a role for IL-10 genetic polymorphisms and found that underlying genetics could not explain the urban-rural difference in IL-10 mRNA. Therefore, high IL-10 mRNA in the rural area may have been a result of either undetected/past helminth infections or other chronic infections and factors. In addition, post-transcriptional regulation of the IL-10 gene has been reported [23] and therefore, IL-10 gene expression may be different from the production of the protein.

As reported in Chapter 6, significant differences in the expression of genes associated with pattern recognition receptor signalling were observed between the two urban schools. Elevated expression of these genes was seen among urban high SES children compared to their urban low SES counterparts. As underlying variations in genetic polymorphisms did not explain observed differences between the two urban groups when it came to TLR-2 and TLR-4, these findings show how even within an urban setting, lifestyle affects gene expression patterns associated with the recognition of microbial products. Thus, Chapter 6 appears to support studies that emphasize the influence of environmental exposures on inflammatory disorders. In fact, the notion that diverse microbial exposures in childhood may protect against the development of allergies and other inflammatory disorders has formed the basis for the 'biodiversity hypothesis'. According to this hypothesis, reduced contact with natural environmental microbes as a result of urbanization and lifestyle change may affect the immune-modulatory capacity of human commensal microbiota thus leading to more inflammatory conditions [24, 25].

Helminth and allergies: insights from population studies

Chapter 7 provided a review of the recent literature on helminth infections and allergies from observational and intervention studies. The conflicting findings in the

recent literature were discussed extensively in this chapter. Chapter 7 also covered research areas beyond the scope of this thesis but which are relevant to understanding the relationship between helminths and allergies in childhood.

Limitations and future directions

Limitations of the Study Design

One of the major limitations of the overall investigation described in this thesis is the cross-sectional study design. Cross-sectional studies examining associations between helminths and allergy outcomes are prone to the problem of temporality [26] since all parameters of interest are determined at the same time. Therefore, prospective studies are needed to fully investigate causality. Another important future direction would be to examine the effect of treatment with anthelmintics on allergy outcomes among Ghanaian children. In addition, the studies described in this thesis focused on allergy outcomes in children aged 5-16 years. It is imperative that future investigations in Ghana include other age-groups such as children under 5 years as well as adults.

Markers of urbanization and lifestyle change

As more investigations in developing countries such as Ghana examine the effects of urbanization on health outcomes, better characterization of urban and rural areas based on standardized indicators are needed. In addition, standardized markers of individual socioeconomic status are imperative to properly investigate the impact of lifestyle change on health outcomes.

Molecular diagnostics in helminth detection

A limitation of the study described in this thesis was that the diagnosis of helminth infections was based on a single sample. This may have led to an underestimation of the helminth burden in our study population. Therefore, future investigations would not only have to incorporate the collection of multiple samples but also molecular diagnostic techniques such as real-time PCR [27] and circulating antigen tests to detect schistosome infection [28].

Recombinant allergen technology

IgE cross-reactivity between helminth antigens and allergens clearly demonstrates the limitations associated with measuring IgE responses against whole allergen extracts in helminth-endemic populations. In recent years, *in vitro* allergy diagnostics in industrialized countries has moved towards component-resolved diagnosis in which purified natural or recombinant allergens are used to detect IgE sensitization to individual allergen molecules [29]. 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 component-resolved

diagnosis [30]. Recombinant allergen technology for the evaluation of IgE responses to allergens in helminth-endemic populations is much needed for better specificity and to improve diagnostic accuracy.

Novel allergens relevant in the tropics

A notable finding highlighted in Chapter 5 was that in a subset of our study participants with elevated IgE responses to whole peanut extract, a few had elevated IgE to the recombinant form of the peanut allergen Ara h 9. Furthermore, IgE antibodies against Ara h 9 were biologically active at low allergen concentrations as determined by basophil histamine assays. Ara h 9 is a member of the nonspecific lipid transfer protein (LTP) family of allergens and appears to play a role in peanut allergy among patients in the Mediterranean region [31]. It is also believed that the peach LTP allergen Pru p 3 may act as primary sensitizer among peanut allergic subjects in Spain [32]. The origin of sensitization to LTPs in areas of the tropics such as Ghana and the role of helminths remain unknown but provide future directions for further research. Aside from the case of Ara h 9, other novel allergens found in the tropics exist that require better characterization [33] including the mammalian carbohydrate α -gal. More in-depth studies are needed to assess the prevalence of sensitization to α -gal in populations in different geographical areas and the relationship between sensitization and clinical outcomes.

Concluding Remarks

The intersection between helminths and allergies in Ghana is an interesting area of research which has shed light on immune responsiveness, on cross-reactivity as well as on variations in allergy phenotypes / outcomes in different geographical locations within one region of Ghana. Future studies have to build on these findings in order to generate the tools to diagnose, treat and prevent allergic disorders in developing countries such as Ghana where allergies are emerging as chronic diseases of public health importance.

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