chapter 1

General Introduction

Global burden of allergic disease

Over the past few decades, there has been a sharp global increase in the prevalence of allergic disorders such as asthma, rhinoconjunctivitis, eczema and food allergy particularly among children [1]. Moreover, findings from the multi-centre International Study of Asthma and Allergy in Childhood (ISAAC) show large variations in the burden of allergic disease across continents and countries as well as between participating centres within the same countries [2]. The analysis of the global burden of allergic disease indicates a complex pattern over time. While most allergic conditions exhibit a general global rise [1], time trends point to a sharp increase in the prevalence of asthma symptoms in low to middle income countries (LMICs) while in high income countries, there appears to be a plateau or even a trend towards a decrease in symptom prevalence over time [4, 5] (Figure1).

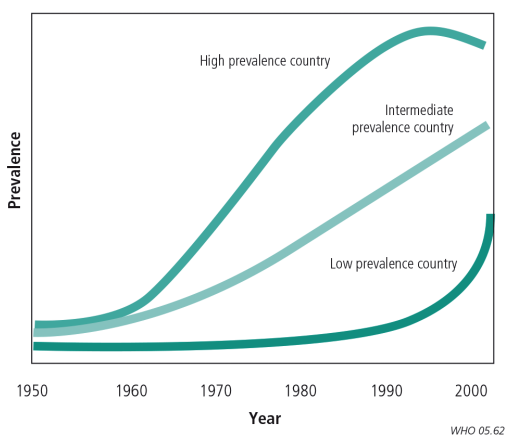

Figure 1: Global trends in the prevalence of asthma.

The lowest prevalence of asthma symptoms is seen in low to middle income countries where time trends are pointing to a sharp increase while in high prevalence countries which are also high income nations, there are indications of a plateau or decrease in prevalence over time.

Source: Bousquet J *et al.*, The public health implications of asthma. Bulletin of the World Health Organization. 2005 Jul;83(7):548-54.

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Although there is less information on the burden of food allergy from a global perspective, the point prevalence of self-reported food allergy in Europe is estimated to be about 6% with European children affected more than adults [6]. In addition, a national survey conducted in the United States estimated that among children 0 to 17 years, the prevalence of food allergy increased from 3.4% in 1997-1999 to 5.1% in 2009-2011 [7]. However, very little is known about food allergy outside of the United States, Europe and Australia. Given the growing problem of allergic disorders in relation to aeroallergy,

it would not be surprising to expect that food allergy might form the next wave of the 'allergic epidemic' [8]. Altogether, there is a rising awareness that allergic disorders need more attention in LMICs. In these countries, it is important that the extent of the burden is assessed and that there is adequate preparation to deal with the problem.

The development of allergic disease is known to be the result of complex interactions between genetic and environmental determinants [9]. In recent years, research has sought to determine the underlying factors that account for the trend towards the escalating global burden of allergic disease [1]. 'Allergic sensitization' – the production of serum-specific Immunoglobulin E (IgE) against innocuous antigens known as allergens, is a well-established factor in the pathogenesis of allergic disease [10]. Allergic sensitization can be determined by *in vitro* serological assessments as well as by *in vivo* skin tests [11] but without a positive clinical history of symptoms, it does not necessarily indicate allergic disease [12]. Interestingly, study findings show that the association between allergic sensitization and asthma symptoms in children varies greatly between populations worldwide and increases with economic development as measured by gross national income per capita [13]. In fact, for decades, economic development, urbanization and changes in lifestyle have been strongly linked to allergic disease. For example, studies from Asian economic hubs dating back to the 1970s illustrate how a higher prevalence of asthma among urban populations was associated with wealth and lifestyle changes in contrast with a lower prevalence of asthma in rural environments [14]. However, the specific factors associated with lifestyle changes and wealth which are responsible for the increase in allergies, remain unknown. In rapidly urbanizing developing countries currently, the reduction in infectious diseases especially among the affluent as well as improved hygiene and the adoption of a so-called "western lifestyle" which is also reflected in food intake, are all thought to be driving the increase in allergic disorders [15].

The hygiene hypothesis

The hygiene hypothesis could provide an explanation for the observed increase in allergic disease and the relationship with improved living standards. The formulation of this hypothesis was based on observations from a national sample of British children that showed that smaller family sizes, higher standards of living and improved hygiene led to fewer childhood infections which in turn may have resulted in greater clinical expression of hay fever [16]. In immunological terms, reduced exposure to microbes during childhood is thought to lead to inadequate maturation of the immune system's regulatory arm resulting in uninhibited inflammatory responses towards harmless antigens [17, 18].

A relationship between environmental exposure to microbes and the development of allergic disease has been shown by farming studies conducted in Europe. These studies observed that European children growing up in microbe-rich traditional farming environments are less likely to suffer from asthma and allergic sensitization compared

to their urban counterparts [19]. Farming studies have been able to highlight the fact that early life exposures to microorganisms and parasites could educate the immune system in such a way that subsequent exposures to antigens that could potentially induce allergic reactions is tolerated and no allergies develop.

Helminths as immune modulators

In LMICs, parasitic infections remain highly prevalent and particularly widespread are chronic helminth infections. Helminths are eukaryotic parasites that have evolved the ability to down-regulate their host's immune responses and thus protect against their own elimination as well as reduce severe pathology in the host [20]. Over 1 billion people living in sub-Saharan Africa, the Americas and Asia are infected with one or more helminth species [21]. In these areas, helminth infections are linked to poverty and poor sanitation [22].

Interestingly, like allergic disorders, helminth infections are associated with strong T helper 2 (Th2) responses that lead to elevated levels of IgE (Figure 2) as well as increasing numbers of basophils, eosinophils and mast cells [23, 24].

Despite the similar immunological profiles, the resultant clinical outcomes are markedly different. During an allergic reaction, the immediate response to an allergen involves a cascade with mast cell and basophil degranulation, the release of immune

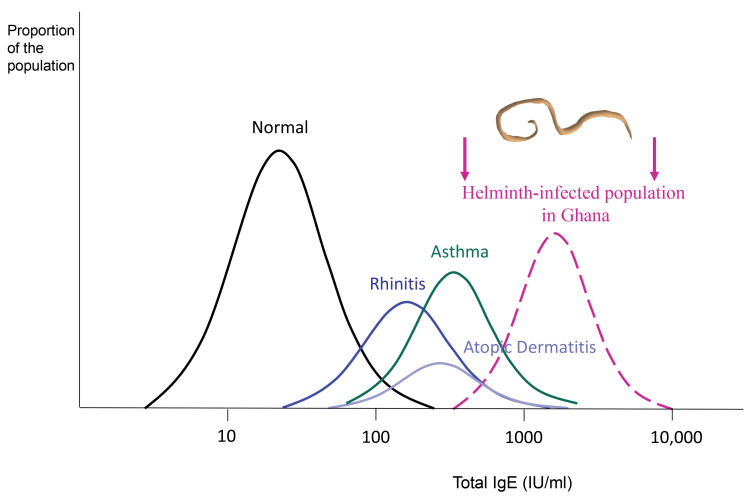


Figure 2: Total IgE in allergic disease and helminth infection.

Comparative total IgE levels in different allergic conditions and helminth infection. The figure shows a schematic representation of total IgE levels based on data from a comparative study among non-allergic and allergic Caucasian subjects in the United States (Wittig, H *et al.*, 1980 [3]). Data shown are from children aged 6 to 15 years. Figure 2 illustrates how children with rhinitis, asthma and atopic dermatitis are likely to have elevated total IgE levels compared to normal children. The figure also shows total IgE levels measured among helminth-infected children in Ghana aged 5 to 16 years.

mediators that cause an increase in vascular permeability and the contraction of smooth muscle (Figure 3A) [25]. On the other hand, these effector mechanisms are not seen in the immune response to chronic helminth infection.

Type 2 immune responses induced by helminths are characterized by the expansion of group 2 innate lymphoid cells [26] as well as Th2 cells that lead to increased production of cytokines such as interleukin 4 (IL-4), IL-5, IL-9 and IL-13 [27]. During a helminth infection, these factors are all key to the control of inflammation, enhancement of tissue repair and can result in worm expulsion [28]. Moreover, chronic helminth infections can induce an immune regulatory network in the host characterized by regulatory T cells, regulatory B cells and alternatively activated macrophages (Figure 3B) [27].

The result is an anti-inflammatory environment typified by elevated levels of IL-10 and transforming growth factor (TGF)-β as well as general T-cell hyporesponsiveness [29] which is thought to enhance survival of the worms within their immunocompetent host. Therefore, in populations chronically infected with helminths, there is an attenuation of responsiveness to so-called 'bystander antigens' that include vaccines and allergens [30].

Several epidemiological studies conducted in helminth-endemic countries have reported an inverse association between the presence of helminth infections and allergic disease [31, 32]. The picture is not very clear since some investigations have observed no effect while others have shown positive associations between helminths and allergies [31, 32].

Helminths and allergies in Ghana

Despite recent control efforts, Ghana, in West Africa, remains endemic for helminths that include soil-transmitted helminths as well as both *Schistosoma haematobium* and *S. mansoni* [33, 34]. At the same time, the process of urbanization in Ghana is leading to dramatic environmental, social and lifestyle changes. Although there is insufficient information on the national burden of allergies in Ghana, recent studies indicate that the prevalence of allergic diseases is on the increase [35-37] with factors associated with urbanization being implicated in this rise [36, 38].

This is illustrated by two surveys conducted 10 years apart in one region of Ghana that showed that the prevalence of allergy markers (exercise induced bronchospasm and allergic sensitization based on skin prick test reactivity) almost doubled over the period among schoolchildren aged 9-16 years [37]. In addition, both surveys observed that markers of allergic disease were more common among affluent urban children compared to poor urban children and compared to rural participants [35, 37]. However these investigations did not examine the role of helminth infections in observed differences in allergy outcomes.

Therefore, questions still remain on the nature of the relationship between helminth infections and allergies among children in a rapidly developing LMIC like Ghana.

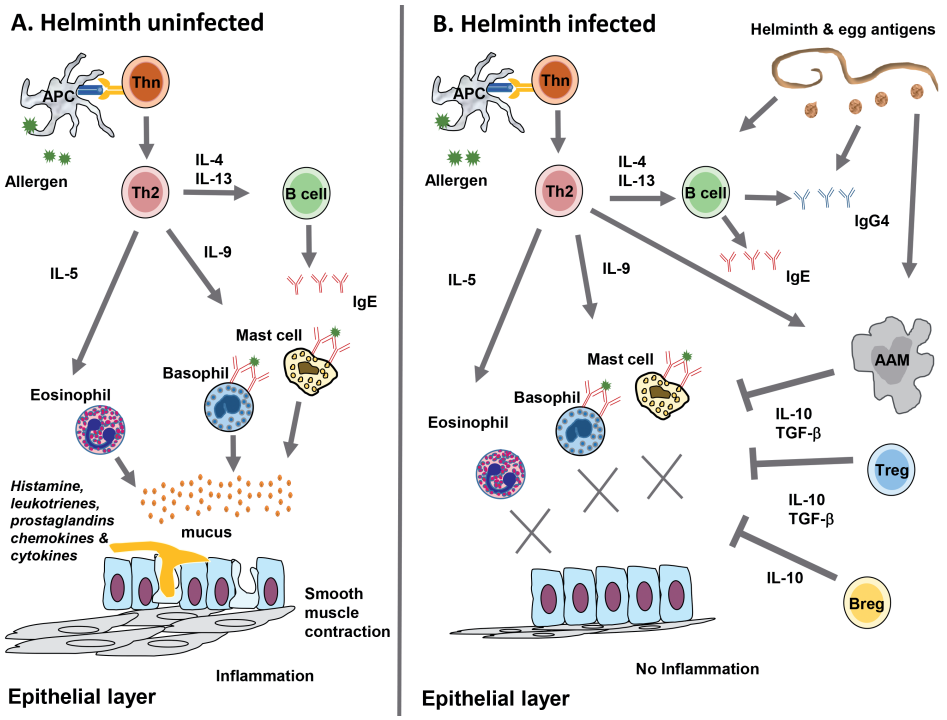


Figure 3: Allergic sensitization and airway inflammation in helminth uninfected and helminth infected individuals

A. In an uninfected individual predisposed to allergy, initial exposure to an allergen leads to the uptake and processing of the allergen by antigen presenting cells and the differentiation of CD4+ naïve T-cells into Th2 cells. Th2 cells secrete cytokines that induce immunoglobulin class switching to IgE in B cells. This process is termed allergic sensitization. Re-exposure to the sensitizing allergen triggers a cascade of events that lead to the activation of effector cells (mast cells, basophils and eosinophils) which release immune mediators such as histamine, leukotrienes, prostaglandins, chemokines and cytokines. These immune mediators induce airway inflammation in the lung epithelial layer characterized by vascular permeability, smooth muscle contraction and mucus production by goblet cells.

B. In a helminth-infected individual, the sensitization phase may occur but during chronic helminth infection, a strong regulatory network is activated involving regulatory T cells, regulatory B cells and alternatively activated macrophages. The induction of the regulatory network leads to the release of cytokines interleukin-10 (IL-10) and transforming growth factor (TGF)-β and an anti-inflammatory environment in which Th2 effector mechanisms are suppressed. Re-exposure to the sensitizing allergen does not lead to the release of immune mediators in the lung epithelial layer and therefore there is reduced airway inflammation.

Abbreviations: APC, antigen-presenting cell; Thn, CD4+ naïve T-cell; Treg, regulatory T cell ; Breg, regulatory B cell, AAM; alternatively activated macrophage;

Scope and objectives of the thesis

This thesis investigates the relationship between helminth infections and allergies among schoolchildren living in one region of Ghana.

The specific objectives are:

i. To determine urban-rural differences in allergy outcomes in Ghana
ii. To examine the association between helminth infections and allergies
iii. To characterize IgE responses associated with helminth infections and allergies
iv. To profile cellular immunological responses and their relationship with helminths and allergies in Ghana

Study design

The work described in this thesis is based on a cross-sectional study in Ghanaian children to establish the association between parasitic infections and allergy outcomes. For this investigation, urban and rural schools were approached to participate and study subjects were recruited from these schools. The urban schools were categorized as either being 'urban high socioeconomic status (SES)' which were private fee-paying schools or 'urban low SES' which were government-funded public schools. Of particular interest for the investigation, were schools in rural areas where parasitic infections were known to be prevalent and where no school-based mass deworming programmes had been implemented in recent years.

Study area and population

Out of the 10 administrative regions of Ghana, the Greater Accra Region in which the capital city is located was selected for the investigation. It is the second most populous region in the country with an estimated population of 4,010,054 [39].

According to the 2010 national household census, the proportion of Ghana's population living in urban areas is 50.9% [39] with the Greater Accra Region having the highest level of urbanization in the country [39]. At the time of the study, the region was divided into six districts and for the investigation, one urban district (Accra Metropolitan) and three rural districts (Ga West, Ga East and Dangme East) were targeted. The target age-group was children between the ages of 5 and 16 years. This age-group was sought because of its particular vulnerability to allergic disease.

Outline of the thesis

The prevalence of parasitic infections and allergy outcomes are analyzed in **Chapter 2**. In this chapter, aeroallergy as well as reported symptoms of asthma and wheeze were examined and their relationship with helminth infections was determined. In addition, the effects of body mass index as a marker of nutritional state as well as urban versus rural residence on allergy outcomes were assessed.

In **Chapter 3**, adverse reactions to food and food sensitization in Ghanaian schoolchildren were investigated for the first time. In a matched case-control analysis in a subset of participants, food sensitization based on skin prick test reactivity to food allergens and specific IgE sensitization to the same foods were examined. Reported adverse reactions to food among cases and matched controls were also assessed.

Chapter 4 focuses on peanut allergy in Ghana where the consumption of peanuts is known to be high. Adverse reactions to peanut and peanut sensitization based on serum-specific IgE as well as skin reactivity were studied. Associations between helminth infections and peanut allergy outcomes were also assessed. In a subset of the study population, the nature of peanut-specific IgE was examined through the analysis of specific IgE responses to recombinant peanut allergens as well as IgE to cross-reactive carbohydrate determinants. The chapter also explores the biological activity and cross-reactive nature of peanut-specific IgE.

The association between cellular immune responsiveness and skin prick test reactivity to house dust mite is addressed in **Chapter 5**. Immune responsiveness described in the chapter was based on cytokine responses as determined by *in vitro* whole blood culture assays.

The focus of **Chapter 6** is on urban and rural differences in the gene expression profiles of a subset of children in the study population. The role of parasitic infections in observed differences in expression profiles was also investigated. The contribution of genetic versus environmental factors in IL-10 as well as Toll-like receptor 2 and 4 expression patterns was assessed.

Chapter 7 is a review of the recent literature on helminths and allergies in childhood

In **Chapter 8**, the main study findings of the thesis are summarized and discussed along with the study limitations and future directions.

References

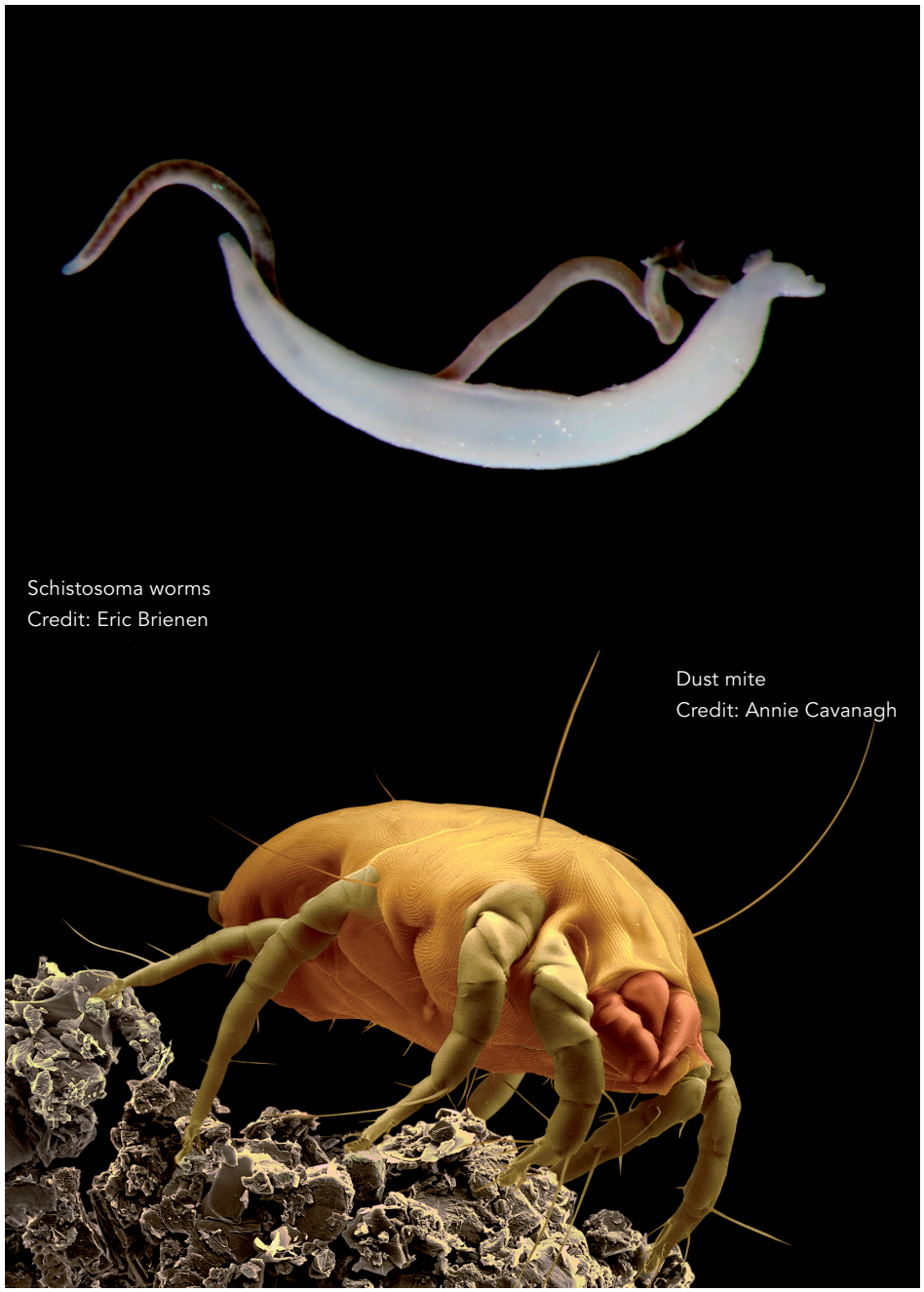
1. Pawankar R, Canonica GW, Holgate ST, Lockey RF, WAO White Book on Allergy Update 2013. In: WAO ed. Milwaukee, Wisconsin: World Allergy Organization, 2013.
2. Mallol J, Crane J, von Mutius E, Odhiambo J, Keil U, Stewart A, The International Study of Asthma and Allergies in Childhood (ISAAC) Phase Three: a global synthesis. Allergologia et Immunopathologia 2013;41: 73-85.
3. Wittig HJ, Belloit J, De Fillippi I, Royal G, Age-related serum immunoglobulin E levels in healthy subjects and in patients with allergic disease. The Journal of Allergy and Clinical Immunology 1980;66: 305-13.
4. Asher MI, Recent perspectives on global epidemiology of asthma in childhood. Allergologia et Immunopathologia 2010;38: 83-7.
5. Bousquet J, Bousquet PJ, Godard P, Daures JP, The public health implications of asthma. Bulletin of the World Health Organization 2005;83: 548-54.
6. Nwaru BI, Hickstein L, Panesar SS, Muraro A, Werfel T, Cardona V, Dubois AE, Halken S, Hoffmann-Sommergruber K, Poulsen LK, Roberts G, Van Ree R, Vlieg-Boerstra BJ, Sheikh A, The epidemiology of food allergy in Europe:

a systematic review and meta-analysis. Allergy 2014;69: 62-75.

7. Sicherer SH, Sampson HA, Food allergy: Epidemiology, pathogenesis, diagnosis, and treatment. The Journal of Allergy and Clinical Immunology 2014;133: 291-307 e5.
8. Prescott S, Allen KJ, Food allergy: riding the second wave of the allergy epidemic. Pediatric Allergy and Immunology : official publication of the European Society of Pediatric Allergy and Immunology 2011;22: 155-60.
9. Cookson W, The alliance of genes and environment in asthma and allergy. Nature 1999;402: B5-11.
10. Wu LC, Zarrin AA, The production and regulation of IgE by the immune system. Nature Reviews Immunology 2014;14: 247-59.
11. Salo PM, Arbes SJ, Jr., Jaramillo R, Calatroni A, Weir CH, Sever ML, Hoppin JA, Rose KM, Liu AH, Gergen PJ, Mitchell HE, Zeldin DC, Prevalence of allergic sensitization in the United States: Results from the National Health and Nutrition Examination Survey (NHANES) 2005-2006. The Journal of Allergy and Clinical Immunology 2014.
12. Hamilton RG, Allergic sensitization is a key risk factor for but not synonymous with allergic disease. The Journal of Allergy and Clinical Immunology 2014.
13. Weinmayr G, Weiland SK, Bjorksten B, Brunekreef B, Buchele G, Cookson WO, Garcia-Marcos L, Gotua M, Gratziou C, van Hage M, von Mutius E, Riikjarv MA, Rzehak P, Stein RT, Strachan DP, Tsanakas J, Wickens K, Wong GW, Atopic sensitization and the international variation of asthma symptom prevalence in children. American Journal of Respiratory and Critical Care Medicine 2007;176: 565-74.
14. Asher MI, Urbanisation, asthma and allergies. Thorax 2011;66: 1025-6.
15. Linneberg A, The increase in allergy and extended challenges. Allergy 2011;66 Suppl 95: 1-3.
16. Strachan DP, Hayfever, hygiene and household size. BMJ 1989: 1259-60.
17. Yazdanbakhsh M, Kremsner PG, van Ree R, Allergy, Parasites, and the Hygiene Hypothesis. Science 2002;296: 490-94.
18. McSorley HJ, Hewitson JP, Maizels RM, Immunomodulation by helminth parasites: defining mechanisms and mediators. International Journal for Parasitology 2013;43: 301-10.
19. Ege MJ, Mayer M, Normand AC, Genuneit J, Cookson WO, Braun-Fahrlander C, Heederik D, Piarroux R, von Mutius E, Exposure to environmental microorganisms and childhood asthma. The New England Journal of Medicine 2011;364: 701-9.
20. Maizels RM, Yazdanbakhsh M, Immune regulation by helminth parasites: cellular and molecular mechanisms. Nature Reviews Immunology 2003;3: 733-44.
21. Lustigman S, Prichard RK, Gazzinelli A, Grant WN, Boatin BA, McCarthy JS, Basanez MG, A research agenda for helminth diseases of humans: the problem of helminthiases. PLOS Neglected Tropical Diseases 2012;6: e1582.
22. WHO, Soil-transmitted helminth infections. Geneva: World Health Organization, 2014.
23. Voehringer D, The role of basophils in helminth infection. Trends in Parasitology 2009;25: 551-6.
24. Stone KD, Prussin C, Metcalfe DD, IgE, mast cells, basophils, and eosinophils. The Journal of Allergy and Clinical Immunology 2010;125: S73-80.
25. Janeway CA, Travers P, Walport M, M. S, Immunobiology: The Immune System in Health and Disease: 5th (Fifth) Edition. New York Garland Science, 2001.
26. Licona-Limon P, Kim LK, Palm NW, Flavell RA, TH2, allergy and group 2 innate lymphoid cells. Nature Immunology 2013;14: 536-42.
27. Girgis NM, Gundra UM, Loke P, Immune regulation during helminth infections. PLOS Pathogens 2013;9: e1003250.
28. Gause WC, Wynn TA, Allen JE, Type 2 immunity and wound healing: evolutionary refinement of adaptive immunity by helminths. Nature Reviews Immunology 2013;13: 607-14.
29. Smits HH, Everts B, Hartgers FC, Yazdanbakhsh M, Chronic Helminth Infections Protect Against Allergic Diseases by Active Regulatory Processes. Current Allergy and Asthma Reports 2010;10: 3-12.
30. McSorley HJ, O'Gorman MT, Blair N, Sutherland TE, Filbey KJ, Maizels RM, Suppression of type 2 immunity and allergic airway inflammation by secreted products of the helminth Heligmosomoides polygyrus.

European Journal of Immunology 2012;42: 2667-82.

31. Feary J, Britton J, Leonardi-Bee J, Atopy and current intestinal parasite infection: a systematic review and meta-analysis. Allergy 2011;66: 569-78.
32. Flohr C, Quinnell RJ, Britton J, Do helminth parasites protect against atopy and allergic disease? Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2009;39: 20-32.
33. Soares Magalhães RJ, Biritwum N-K, Gyapong JO, Brooker S, Zhang Y, Blair L, Fenwick A, Clements ACA, Mapping Helminth Co-Infection and Co-Intensity: Geostatistical Prediction in Ghana. PLOS Neglected Tropical Diseases 2011;5: e1200.
34. Lodh N, Naples JM, Bosompem KM, Quartey J, Shiff CJ, Detection of parasite-specific DNA in urine sediment obtained by filtration differentiates between single and mixed infections of Schistosoma mansoni and S. haematobium from endemic areas in Ghana. PLOS ONE 2014;9: e91144.
35. Addo Yobo EO, Custovic A, Taggart SC, Asafo-Agyei AP, Woodcock A, Exercise induced bronchospasm in Ghana: differences in prevalence between urban and rural schoolchildren. Thorax 1997;52: 161-65.
36. Addo-Yobo EOD, Custovic A, Taggart SCO, Craven M, Bonnie B, Woodcock A, Risk factors for asthma in urban Ghana. The Journal of Allergy and Clinical Immunology 2001;108: 363-68.
37. Addo-Yobo EOD, Woodcock A, Allotey A, Baffoe-Bonnie B, Strachan D, Custovic A, Exercise-Induced Bronchospasm and Atopy in Ghana: Two Surveys Ten Years Apart. PLOS Medicine 2007;4: e70.
38. Stevens W, Addo-Yobo E, Roper J, Woodcock A, James H, Platts-Mills T, Custovic A, Differences in both prevalence and titre of specific immunoglobulin E among children with asthma in affluent and poor communities within a large town in Ghana. Clinical and Experimental Allergy : journal of the British Society for Allergy and Clinical Immunology 2011;41: 1587-94.
39. Ghana Statistical Service, 2010 Population and Housing Census; summary report of final results. Accra: Ghana Statistical Service, 2012.



Schistosoma worms
Credit: Eric Brienen

Dust mite
Credit: Annie Cavanagh