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Chapter 9

General discussion

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The role of helminth infections in protection from atopic disorders

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Helminth infection and allergy: two diseases with similar immunological features

Helminth infections and allergies are both associated with so called T helper (Th)₂ type immune responses, characterized by eosinophilia, mastocytosis, high levels of immunoglobulin (Ig)E antibodies and the production of cytokines such as interleukin (IL)-4, IL-5 and IL-13. In some helminth infections, the Th₂ responses have been associated with parasite clearance and are therefore considered to be protective [98;298;299]. In contrast, the harmful outcomes of Th₂ responses are seen in allergic disorders, for instance in asthma, airway pathology and airway hyperresponsiveness, which are mediated by the effect of Th₂ cytokines and the accumulation of eosinophils and mast cells in lung tissue [454-457]. Interestingly, although the immune responses of these two diseases are similar, their distribution does not overlap geographically. While allergy is more prevalent in developed countries, helminth infections are more common in less developed ones. A full understanding of the factors that lead to the higher prevalence of allergic disorders in geographic areas where Th₂ responses are dominant may help us to design strategies that hopefully will prevent the global allergic march seen not only in developed countries but also in urban centers of less developed ones.

Allergic march: hypotheses that may explain patterns from developed to developing countries

Several hypotheses have been put forward to explain the rise in prevalence of allergies in the last few decades. These are mostly based on observations made in developed countries. However, the significance of various allergy-associated risk factors is known to differ according to geographical location. Therefore, it is important to study allergies and associated risks in different geographical locations and develop appropriate tools that can be used specifically in a given geographic region. A number of hypotheses put forward to explain the rise in allergic diseases are discussed below, before concentrating on studies of allergic disorders in Indonesia.

Hypothesis that pollution is responsible for the allergic march: although urban centers of the developing world are often highly polluted, the hypothesis that pollution can explain the increasing prevalence of allergic disorders has not been supported by studies carried out in East and West Germany. Prevalence of allergies in East Germany was considerably lower than in less polluted West Germany [458]. Although there is considerable evidence that exposure to respirable suspended (airborne) particles is involved in exacerbation of diseases such as asthma [459-461], it does not seem to be the only factor that has caused the recent rise in allergic diseases.

Hypothesis that changes in dietary intake explains the allergic march: it is thought that breast feeding [462], intake of 3-omega fatty acids [463] as well as intake of farm (non-Pasteurised) milk can influence the development of allergic disorders [208]. Food allergies are becoming a serious problem in the West, a prominent example being peanut allergy [464]. In several African and Asian countries however, peanut is a staple food and very few cases of peanut allergy, if any, have been reported in these areas. Food preparation and matrixes used in the food industry in the West are bound to have an important impact on the possible differences in peanut allergies seen in for example, the United Kingdom and Ghana. Indeed, one expects to find large differences in the dietary intake of urban compared with rural residents within developing countries; however, there are as yet, no large-scale studies of food consumption patterns that link detailed dietary intake to the prevalence of allergic disorders in, for example, Africa. In China, a recent study examined factors associated with prevalence of asthma in more than 10000 children living in 3 different regions. Frequent intake of raw vegetables was shown to be associated with reduced risk of wheezing [465]. The identification of molecular components in raw vegetables that drive the protective mechanism will be of great interest.

Hypothesis that increase in exposure to allergens explains the allergic march: it is clear that allergen exposure and increased IgE production is needed for the development of allergic disorders. The multi center study in 3 regions of China [465] reported the use of foam pillows as a risk factor for current wheeze, supporting the notion that synthetic bedding might increase the extent of exposure to house dust mite (HDM) and therefore lead to increased airway inflammation. The question remains whether the so-called allergic march can be explained solely by increasing exposure to allergens. Studies conducted in the 1980s in Papua New Guinea [466] suggested that introduction of blankets in some highland communities resulted in high exposure to HDM and high prevalence of asthma; however, higher prevalence of asthma were only seen in adults and not in children. The children, who presumably were also exposed to HDM in the same households, seemed to be protected from developing asthma by environmental factors; it is these factors that we are now seeking to identify by studying allergic disorders in developing countries. In more recent studies [208], in which lower prevalence of allergies were reported in traditional farming families or in rural areas of developing countries [467;468], exposure to allergens did not seem to explain the differences recorded in allergic disorders; in homes of traditional farmers, high concentrations of HDM were found with low sensitization rates [469]; in rural and urban areas of Ethiopia, differences in exposure to *Der p 1* could not explain skin prick test positivity to HDM [251]. Thus although use of carpets and mattresses is associated with increasing prevalence of allergies to house dust mite [470], sensitization to HDM can be prevented by unknown environmental factors [208]. Moreover, when we consider outdoor allergens, which would

not be expected to change with lifestyle, increasing sensitization to birch and grass were reported over the last 11 year period in Greenlanders [471]. This indicates that the Inuit immune system is now reacting differently on allergen encounter, when compared to the previous decade. Taken together, there is much evidence that would argue against the theory that increasing exposure to allergens is the only factor that would explain differences in prevalence of allergic disorders or the rise in allergies worldwide.

Hypothesis that changes in patterns of infections and/or exposure to microbial and parasitic products explains the allergic march: one of the major factors that might explain the geographical variations in prevalence of allergies is the extent of exposure to viruses, bacteria and parasites. Indeed, advances in indoor plumbing, less crowded living conditions, infrequent contact with livestock and mud, and an increase in antibiotic use might have decreased our contact with microbes and pathogens in the 20th century. Together, these observations have led to the conclusion that an inverse relationship between exposure to microbes/pathogens and the development of allergies exists, and the promulgation of the hygiene hypothesis to explain the increase in atopic diseases. Substantiating this hypothesis, however, has proven difficult [210]. Recent epidemiological studies [472] have reported high prevalence of allergic disorders in Brazil, where about 20% of the population were reported to have asthma. A more extended, international study of asthma and allergy in children [473] showed the prevalence of “asthma”, to range from 5.5 to 28%. The authors concluded that in areas in which infections are rampant, there does not seem to be any protection from allergies. A number of issues need to be resolved before it is concluded that these studies dismiss the hypothesis of a negative association between microbial exposure and allergies. First, the study [473] was questionnaire-based and therefore highly culture sensitive. Secondly, there is often no indication of whether the study was conducted in urban or rural areas. Thirdly, no measurements of (past) exposure to infection or presence of (current) infection were reported. It is therefore too early to conclude that there are no negative associations between infections and allergies from such ecological studies.

The examples above show that more studies are needed to understand the risk and protective factors for allergic disorders in different parts of the world.

Study of allergy in Indonesia

The worldwide ISAAC study reported Indonesia to have the lowest prevalence of allergy in the world [214]. However, this study was performed in only one center in Java. A similar study conducted in 10 centers in India, showed a large variation in the prevalence of asthma (ranging from 3% to 17%) between different centers [214], indicating that the results of the 1998 ISAAC study in Indonesia may not be representative of the whole

country. With respect to investigating the inverse associations between allergy and helminth infection in Indonesia, it is most important to consider the risk factors of gain of helminth infections, as well as how allergy is to be defined and recorded.

Determination of allergy in different socioeconomic status (SES) backgrounds

Applying a questionnaire that can be used globally is crucial for international comparisons of allergy prevalence. In chapter 5 we investigated the prevalence of allergy in low- and high-SES schoolchildren using a modified ISAAC questionnaire that had previously been used worldwide [214]. The results from this study highlighted several important points: i) in contrast to a study by Stewart *et al.* which reported a positive association between lowest quartile of gross national product (GNP) per capita and lowest median positive responses to all the questions on symptoms of asthma, rhinitis and eczema [365], in our study the prevalence of wheezing, itchy-watery nose and itchy-skin rash in the last 12 months reported by parents was significantly higher in low-SES school children; ii) the difference between prevalence of parent reported allergies in children (asthma, rhinitis and eczema) attending the high- and low-SES schools disappeared when the question whether the disorder was confirmed by a health worker (HW) was used; iii) a higher agreement was found between parent reported symptoms and HW confirmed allergy in high-SES school; iv) skin prick test (SPT) positivity has shown to be positively associated with wheezing reported by parents and with asthma confirmed by HW in both schools; and v) poor associations between SPT positivity or specific IgE were found with all other allergic symptoms reported by parents, and although this improved when HW diagnosed symptoms were considered, the most frequent associations were found in the high-SES school. All these findings indicate that: i) the ISAAC questionnaire is not performing well; the terms to describe wheeze, itchy runny nose or itchy rash might not be accurate enough to classify allergic and non-allergic groups in Indonesia, therefore it needs further validation; ii) parent answered questionnaires may not be suitable for use in areas where education level of the parents is low; iii) from all the symptoms, wheezing or asthma has a better chance of being recognized/diagnosed by parents/health workers in all SES. Taken together we propose that in future studies, questionnaires need to be carefully assessed and if needed specifically designed in distinct geographical areas where cultural and socioeconomic differences may modify the responses obtained. The use of HW diagnosed data regarding allergic symptoms should be considered when embarking on epidemiological studies, particularly when populations of low socioeconomic status are the focus of the study. Finally, although calculation on attributable risk of asthma, rhinitis and eczema to SPT and specific IgE confirmed that in general, high-SES school showed higher percentages of attributable cases compared to low-SES school, these objective parameters (particularly SPT) are still required for optimal estimation of allergic symptoms in populations with low-SES and education.

Contribution of specific IgE on SPT in helminth endemic areas

In developed countries the presence of specific IgE to allergen is generally translated into SPT positivity [362;431]. In our study, carried out in an area endemic for lymphatic filariasis, we found high levels of mite-IgE to be associated with only a 6-fold increase in risk of responding to mite extracts on skin prick testing (Chapter 7). This is similar to a study in Gabon which observed an 8-fold increased risk [265], but much lower compared to data from the Netherlands showing a 39-fold increased risk of being SPT positive when high levels of specific IgE were measured (present) (van der Zee, unpublished data). It is very likely that populations in areas endemic for helminth infections are exposed to mites, but there is less concordance between high IgE levels and skin reactivity/clinical manifestation of allergy. The mechanisms behind this lack of concordance are not understood. Our study conducted in a high-SES school (chapter 6), showed children lightly infected with intestinal helminths to have high levels of mite-IgE - which had a strong predictive value for mite-SPT positivity (odds ratio (OR)=29). This further increased to an OR of 34 when infected children were excluded from analysis. This indicates that light helminth infection may play a minor role in decreasing the translation of IgE into SPT positivity (OR from 29 to 34). However, whether intense helminth infection or other environmental factors lead to the low risk of high IgE and SPT reactivity is not yet known. In mechanistic terms, experiments performed studying basophil degranulation indicated that sera from allergic Dutch subjects required low concentrations of house dust mite allergen to induce basophil degranulation, whereas sera from Gabonese children (infected with *Schistosoma*) with high IgE to mite, needed extremely high concentrations of allergen before degranulation was seen (van Ree, unpublished data). These data raise the possibility that IgE to mite in subjects living in areas endemic for helminth infection might have a low affinity and thus poor biologic activity in terms of basophil degranulation. This would explain the observation that in these subjects there is little SPT reactivity to house dust mite extracts. The question as to whether the IgE with poor biologic activity results from the effect of helminth infections or other factors such as malnutrition still needs to be explored in future studies.

Factors influencing allergic disorders in Indonesia

Studies in several countries, at two time points (5-6 years apart) using the ISAAC questionnaires, have revealed that allergic diseases are increasing in many parts of the world [342;345;347-349]. In some western countries asthma and allergies have reached alarming proportions, affecting up to one-third of children [214]. The prevalence of allergy is lower in developing than developed countries, however, great differences have been found between urban and rural areas in Asia [165;166;350] and Africa [169;170;249;351]. Although inheritance has been shown to be a primary risk factor [191], it does not explain the great differences between developed and developing

countries, nor between rural and urban communities, yet it is important to realize that genes, by environment interaction, could have a pronounced effect on disease outcome in these different settings [352].

Using the same study group as in chapter 5, we investigated the influence of some environmental factors on asthma and atopy (chapter 6). Compared to children from high-SES, children in the low-SES school were characterized by low parental education, high number of siblings, higher exposure to tobacco smoke, low nutritional status and more infections with helminths. For allergy, children in the high-SES school had a higher prevalence of SPT than children from the low-SES school. In logistic regression analysis, performed separately for each school, and adjusted for sex and age, we found that in the low-SES school neither parental education, nutritional status, SES, the presence of a smoker, animals in the house or helminth infection exerted a significant influence on asthma, specific-IgE or SPT positivity. Interestingly, in high-SES school some factors had a tendency to be inversely associated with allergy: helminth infections and asthma, low socioeconomic level as well as low nutritional level and SPT or specific-IgE and the presence of animal in the house and SPT. Regarding helminth infection, we only saw a weak protective effect on asthma in high-SES school while in other studies, an inverse association was reported for wheezing [170] but not for asthma [255]. It is not clear whether this was just found by chance or the number of children that were infected with helminths in this school was too few for the study to have sufficient statistical power. However, the following aspects of this study should be considered: i) children from the low-SES school are more homogeneous in terms of parental education level, socioeconomic level, helminth infection positivity, while in the high-SES school there was some degree of heterogeneity. Therefore, the cut off points used to dichotomize some factors were not suitable for use in both schools; ii) a more accurate measure of how often children are exposed to animals or to tobacco smoke may need to be obtained when determining the influence of these factors on allergy and iii) as we have discussed before, socioeconomic status, in particular educational background, needs to be taken into account when assessing allergy in Indonesia. Together, our findings indicate that in developing countries factors influencing allergy and atopy are complex, not only due to the endless number of variables in terms of genetic and environmental factors, but also due to social and cultural differences that may complicate acquisition of accurate data via questionnaires.

Risk factors regulating gain of helminth infection

Helminths are complex eukaryotic organisms, some species are endowed with the ability to live for decades in the human host, sometimes without causing any symptoms. Although exposure to the parasite is the major factor to gain the infection, immunological and hereditary factors may also affect susceptibility to infection and resistance. Moreover,

with the finding that children born to microfilariaemic mothers had higher prevalence of microfilaria than children whose mothers were amicrofilaremic [338;474], the influence of intrauterine events should not be ignored.

In the study detailed in chapter 3, we found that in the young age group, the filarial infection status (as assessed by anti-filarial IgG4) of mother and child were correlated, whereas from 5 years on this association extended to the father as well, indicating the possibility that the influence of intrauterine sensitization is predominantly manifested in young children up to four years of age, after which it becomes overruled by other factors such as genetic constitution and/or environmental and household factors. These studies were extended by using a statistical method developed by Houwing-Duistermaat *et al.* [339] showing that a genetic, household and environmental model could explain the clustering of filarial infection within communities in Indonesia [160;339]. We studied the population both as a whole, and also after separating into adults and children (chapter 4). As expected, clustering analysis of 583 inhabitants, belonging to 35 families and spread over 133 households showed genetic and household factors to exert a considerable influence on filarial infection. Interestingly, when the analysis was applied to adults and children separately, the clustering of infection can only be explained by genetic factors in children alone ($p=0.02$), while in adults, household and environmental factors were important ($p=0.01$ and $p=0.03$, respectively). Furthermore, while genetic variance in both children and adults was shown to be 0.07, the household variance only existed in adults and not in children (0.09 and 0.00, respectively). These data indicate that individual behavior or environmental factors overrule the genetic predisposition in the adult population, whereas in children with a relatively homogenous behavior/environment, a stronger effect of genetics can be seen. These findings suggests that in order to confirm the role of specific genes in resistance/susceptibility to filarial infection, in independent studies it may be important to concentrate on genotyping subjects that are below the age of 20 years.

In order to investigate the relationship between the two Th2 associated conditions - allergy and helminth infection, we decided to look at how allergic disorders are influenced by genetic and household/environmental factors in an area where helminth infections are prevalent.

Clustering of allergic disorders in areas endemic for helminth infections

In western populations, genetic factors have been shown to influence allergic disorders. Not only asthma [191] but also related phenotypes such as total-IgE [407;408], allergen specific-IgE [409;410], SPT positivity [409;410] and bronchial hyperresponsiveness [411;412] have been shown to be influenced by several genes. In helminth infections,

genetic factors play an important role in controlling the outcome of an infection. We have found evidence for genetic effects in filarial infection (chapter 4). Detailed whole genome analysis in schistosomiasis patients in Brazil has identified chromosome 5q31-q33 as a locus responsible for controlling the intensity of *Schistosoma mansoni* infection [145] and there is also evidence for genetic control of pathology by a region containing the gene for the interferon-gamma receptor 1 subunit in this disease [146]. For *Ascaris lumbricoides*, recent studies conducted in Nepal have found a locus controlling the infection intensity on chromosomes 1 and 13 [147]. Such studies are yet to be carried out for filariasis.

We have taken the cluster analysis of filarial infections a step forward and applied a similar statistical test to allergic disorder in an area endemic for filariasis. As presented in chapter 7, both genetic and household factors could explain clustering of specific and total IgE. But, when the test was used to examine the clustering of mite-SPT, only household effect was significant. These data indicate that like in western countries, in areas where encounter with helminths is frequent, IgE antibodies are under genetic control. However, for skin reactivity to house dust mite (which in areas of low pathogen exposure seems to be under genetic control) in areas where exposure to microorganisms and helminths is high, the clustering within families could only be explained by environmental factors. The next question is, what environmental factors overrule the genetic influences - could exposure to microorganisms or helminths be responsible? Although it is interesting to note that none of the microfilaria positive subjects were SPT positive, larger studies in areas of high microfilaremia prevalence are needed to substantiate the possible influence of filarial infections in overruling the genetic factors modulating the development of positive SPT reactions.

Association between helminth infections and allergies

There is considerable evidence for inverse association between helminth infections and allergic disorders. In Ethiopia, hookworm infections were shown to decrease the risk of wheeze [170], while in Venezuela [248] and in Ecuador [250] intestinal helminth infections were inversely associated with atopy (skin prick test positivity to environmental allergens). There is also evidence that another class of helminths, schistosomes, have a protective role on atopy in Gabon and Brazil [241;242]. However, not all studies have shown a protective effect for helminth infections on allergy. A large study [253] conducted in the rural area of Anqing province in China, documented an increased risk of asthma as well as skin prick test positivity to allergens in children with *Ascaris lumbricoides* infections. In this area, however, the prevalence of *Ascaris* infections (history of or current stool positivity) was only 24.6%, which is considerably lower than has been reported in areas where a negative association was found between this infection and allergic disorders:

63.4% in Ecuador [259] or 74% in Gabon [241]. It is also important to note that the history of *Ascaris* infection was used in the Chinese study [253] which is expected to have a high likelihood of generating inaccurate results regarding present *Ascaris* infection. It is important to try and obtain data on the actual presence of the intestinal worms and intensity of infections by parasitological examination of stool samples.

Recent publications from Africa report somewhat inconsistent findings. In a case-control study carried out in Ethiopia [182], the association between parasitic infections and atopic dermatitis (AD) was investigated and it was reported that intestinal helminth infections, in particular *Trichuris*, increased the risk of AD. In addition, the history of malaria infection was a significant risk factor for development of AD. The case definition determined by the ISAAC questionnaire, however, might have been problematic as acknowledged by the authors. More than 60% of AD cases were not atopic to the allergens tested. Further studies are needed to firmly establish the association between AD and parasitic infections. With respect to airway allergies, studies in South Africa [475] have indicated that there has been a dramatic increase in bronchial hyper-responsiveness (BHR) over the last two decades with no current association between atopy and BHR. In this study, the authors concluded that *Ascaris* infection had no modifying effect on BHR; however, they had only measured serological responsiveness to *Ascaris*, which has a relatively poor specificity and sensitivity as a marker of infection.

In a large cross-sectional study conducted in the rural area of Butajira in Ethiopia by Britton and co-workers [255], no evidence of a protective effect of intestinal helminth infections against wheeze or asthma was found, a finding that seems to contradict their previous data obtained in Jimma, another rural area in Ethiopia [170]. It was noted that, in Butajira, the prevalence and intensity of helminth infections were lower (33.8%) than the prevalence found in Jimma (77.3%). So, again in Butajira [255], lower prevalences of *Ascaris* infections, which would predict light infections, did not protect against allergies, emphasizing the possible importance of intensity of helminth infections in modulating allergic responses [476]. Indeed, it has been argued that high intensity of helminth infections might be associated with protection whereas light helminth infections might exacerbate allergic disorders [258]. The mechanisms behind this are purely speculative; with light infections, helminth-associated molecules that drive Th2 responses might potentiate IL-4, IL-13 and IgE, whereas only with heavy infections, molecules that lead to regulatory immune responses might reach a sufficient level to modulate the immune system and down-regulate Th2 responses [259].

A relatively small-scale randomized placebo-controlled anti-helminth treatment trial in Gabonese school children [241] has shown that a reduction in *Ascaris* and *Trichuris*

worm burdens results in a significant increase in the rate of developing skin reactivity to house dust mite. This study is in agreement with the findings by Lynch and co-workers [477], suggesting that helminth infections contribute to the lower prevalence of atopy in tropical populations. However, a recent study in Ecuador has reported that anti-helminth treatment has no effect on SPT positivity nor on allergic symptoms [478]. The latter study was a one-year anti helminth treatment study in contrast to the studies in Gabon and Venezuela which assessed allergies following a longer period of 30 and 24 months treatment schedule, respectively.

In the study we carried out in Indonesia (chapter 6) children from a school where 92% of the pupils had high intensity of intestinal helminth infection were compared with children from another school where light infections were detected in 23% of the children. The prevalence of skin prick test positivity to environmental allergens was 5.7% and 16.4%, respectively. In the school with high intensity of infections, it was not possible to accurately determine the association between helminth infections and atopy or allergic symptoms because of the very high prevalence, leaving us with too few truly negative subjects. In the school with lower prevalence of helminths, the presence of helminth infections was not protective against atopy. The latter might have been due to the intensity of infections being very light. Ideally, such association studies would be carried out in an area where prevalence of helminth infections is high enough (> 50%) with a good range in intensity to allow the determination of the effect of both presence and intensity of infection on allergic disorders.

In contrast to human studies, several studies in animal models have shown consistent results where helminth infections suppress allergic inflammation. Bashir and colleagues [479] have demonstrated that both allergen-specific IgE and IL-13 production to peanut is down modulated by infection with the nematode *Heligmosomoides polygyrus*, which is a natural helminth infection in the mouse. Another set of studies with the same parasite but in an allergic airway inflammation model [260] have indicated that this helminth infection results in a strong reduction in OVA-driven eosinophilic airway inflammation. Several other helminth infections have been shown to inhibit airway inflammation. Mice infected with *Schistosoma mansoni* show a clear infection intensity-dependent decrease in eosinophilic inflammation (H.H. Smits, unpublished results). *Nippostrongylus braziliensis*, is also able to inhibit lung inflammation [480], a finding that was also reported with *Ascaris suum* extract implants, which inhibited lung inflammation and airway hyperresponsiveness [261]. Whereas the well controlled conditions in animal models of infections and allergy seems to generate consistent findings, the relevance of animals models to the allergic diseases in humans is constantly debated.

Effect of helminth infections on third party antigens, consequences and solutions

The studies of the interaction between helminth infections and allergies are based on the immunological observations that chronic helminth infections are associated with immune hyporesponsiveness to specific parasite antigens primarily but also to some unrelated, third party antigens. The question of whether helminth infections result in cellular hyporesponsiveness to allergens and lower Th2 inflammation has been investigated in one human study [429] and in several animal models of allergies and helminth infections [260;261;479;480]. There is some evidence that the suppressory immune mechanisms might indeed play a role in inhibiting the development of allergies. Although considerable part of the studies described in this thesis has been based on investigations of the risk factors associated with gain of helminth infections and allergic disorders, we have also addressed the question of how we could modulate the hyporesponsiveness in helminth-infected subjects. This issue is of importance for the prospect of vaccinations (new and old ones) in communities where helminth infections are highly prevalent and immune hyporesponsiveness would be expected to affect responses to vaccines, particularly weak vaccines. Indeed there is evidence for a detrimental effect of helminth infections on responses to vaccines such as tetanus or bacillus Calmette-Guerin (BCG). T-cell proliferation as well as cytokine production in response to BCG [278;279] as well as to tetanus toxoid (TT) [280-282] is lower in helminth infected subjects compared to non-infected ones, and anti-helminth chemotherapy before or after vaccination increased BCG-vaccine efficacy by inducing T- cell proliferation as well as IFN- γ production [277].

In order to develop strategies to overcome helminth-induced hyporesponsiveness (with the possible potential by use) in vaccine formulations, we carried out the studies in chapter 8 by looking for microbial component that have the ability to induce a high pro-inflammatory response. Toll like receptors (TLR), are an important class of innate immune receptors that interact with products from microorganisms and stimulate the immune system, setting the scene for the shaping of the adaptive immune responses. We have tested a number of well-known ligands for TLRs in children with intense helminth infections, and in those with no or light infections. We have shown that Resiquimod-848 (R-848) is the ligand that has the highest absolute amount and ratio of pro-/anti-inflammatory responses in both groups of children suggesting that such a ligand might be useful in formulations of vaccines to be used in areas endemic for helminth infections. We also noted an interesting difference in the dynamics of pro- and anti-inflammatory cytokines produced in children with or without helminth infections. We found that in 24 hours stimulated culture supernatants, higher levels of the pro-inflammatory cytokine TNF- α were produced (in response to LPS) in children with heavy helminth infections. At this time IL-10 was also produced but the level was relatively lower compared to TNF- α level. When the stimulation was continued to 72 hours, the level of TNF- α spontaneously

decreased in all groups of children while IL-10 was maintained at a high level in only group of children with intense helminth infections. Thus, although early after stimulation pro-inflammatory cytokine production was higher in children with high intensity helminth infections, the response ultimately switched to an anti-inflammatory cytokine profile after 72 hours. The question remains, and needs to be addressed in future studies, what is the consequence of an early versus late cytokine production during the innate immune response, on the adaptive responses that develop subsequently. Also, we need to understand what causes this difference. Are the cells that produce IL-10 at a later time point different from the cells that produced this cytokine at the early time point? And are these cells present only in children that are chronically infected with intense helminths infections? Does a high TNF- α production lead to a feedback high IL-10 production from the same cell or another cell? Do children with intense infections express low level of IL-10 receptors, which would result in higher unbound IL-10 to be measured in the supernatants? Unfortunately, we have no information at 72 hours regarding the TLR-7 ligand which we propose to be suitable for use in vaccine formulations because of its strong pro-inflammatory response. The stimulations with R-848 need to be prolonged to 72 hours and the ratios of pro- and anti-inflammatory cytokines need to be measured. It is interesting to note that this ligand is also considered as a promising vaccine adjuvant in newborns, because it has been reported to have a strong inflammatory property whereas other TLR ligands are poor stimulators of innate immune responses in newborns [446]. Moreover, the receptor of this ligand is located in the intracellular compartment of a cell and might not be affected by (extracellular) helminth infections. Future studies using a larger set of TLR ligands as well as different time courses should help the selection of a broader array of compounds for use to overcome immune hyporesponsiveness in individuals living in helminth endemic areas.

Conclusion

The current thesis has raised more questions than it has answered. The complex relationship between helminth infections and allergies need to be addressed in future studies taking into account the findings in this thesis. The question of intensity and chronicity of infections needs to be addressed as well as the species of helminths. Moreover, the role of other factors on allergies such as nutritional status has to be explored further. The same applies to factors that govern gain of helminth infections. It would be important to get more detailed insight into how genetic polymorphisms control allergies and helminth infections in Indonesia. Lastly, we have to consider not only the protective effects of helminths, but also detrimental effects of these infections on issues such as co-infection or vaccine efficacy. This means that we also need to focus on the question of how we can reverse hyporesponsiveness seen in children with helminth

infections who are also often malnourished. For this, the avocation of anti-helminth treatment on the one hand and research into identification of structures that stimulate the immune system strongly on the other hand, are options that we need to consider in our future investigations in Indonesia.