Helminth infections, allergic disorders and immune responses: studies in Indonesia

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Chapter 1

General introduction

This thesis focuses on lymphatic filariasis and intestinal helminth infections and their relation to allergic disorders. In this introductory chapter, the life cycle of the major helminth parasites mentioned in this thesis will be discussed, the patterns of their distribution within households and families will be addressed followed by a description of immune responses mounted to these parasites. Next, allergic disorders will be considered alongside immune responses typifying allergies. The chapter will end by describing what is known about the interaction between helminths and allergic disorders.

1. Helminth infections: life cycle and disease

Lymphatic filariasis

Wuchereria bancrofti, Brugia timori and *Brugia malayi* are three species of tissue dwelling filarial nematodes that parasitize the lymphatic system of the human body and can cause a disease called lymphatic filariasis. Humans are the definitive host of *W. bancrofti* and *B. timori* whereas *B. malayi* has been reported to infect several wild and domestic animals [1] such as monkeys [1] and cats [2]. The intermediate host of filarial parasites is the mosquito. Microfilaria (mf) ingested by mosquitoes feeding on infected persons, penetrate the gut wall, and migrate to the flight muscles and moult twice to develop into infective L3 larva. The L3 is transmitted to humans via the biting site where infective mosquitoes take a blood meal. Once inside the human body, the larva migrate to the regional lymph nodes and large lymph vessels, where they mature into the young adult stages, L4. Within months larva will develop into mature, white thread like, adult worms. The adult worms can survive in the human lymphatic system for about 15 years. Males and females can pair in the lymphatic area, and the female worms can produce millions of mf. The mf then move to the blood stream and circulate in the periphery either at night or during daytime depending on the periodicity of the species involved [3]. When not circulating in the periphery, the parasites are located predominantly in the small vessels of the lungs [4]. The interval between infection and detection of mf in peripheral blood (pre patent period) has been estimated to be 7 months for *W. bancrofti* and 3.5 months for *Brugia* spp. [5].

The clinical pathology of lymphatic filariasis in humans is seen in a relatively small proportion of the affected population and is mainly caused by adult worms, which are present in the lymphatic system. Live adult parasites can affect the lymphatic system through their metabolic products and lead to recurrent attacks of acute lymphadenitis or lymphangitis. Dying and degenerating adult worms induce inflammatory responses locally and can decapacitate lymphatic vessels and cause lymph flow stasis. Localization and clinical manifestation of chronic obstructive disease caused by brugian filariasis is different from that seen in bancroftian filariasis. In bancroftian filariasis, hydrocele is the main clinical manifestation, which can be accompanied by lymphoedema and or enlargement of the whole arm, leg (elephantiasis), penis, scrotum or breast. On the contrary, hydrocele

is less common in brugian filariasis. The lymphoedema in brugian filariasis is mainly found in the leg below the knee and less frequently in other parts of the body [5]. Relatively little is known about the mechanisms underlying lymphatic pathology. Immunologically, high levels of pro inflammatory mediators such as tumor necrosis factor (TNF)-α are associated with increasing severity of disease [6]. Recently, new areas have opened up by studies that have shown that specific molecules involved in the regulation of growth of lymphatic vessels such as vascular endothelial growth factor (VEGF) and endothelin(ET)-1 are elevated in patients with filarial pathology [7], raising the possibility that suppression of such mediators may specifically help to improve filarial pathology. A relatively rare clinical manifestation of lymphatic filariasis is tropical pulmonary eosinophilia (TPE) or occult filariasis, which is the result of hypersensitivity reactions to mf [8]. TPE is marked by paroxysmal cough, wheezing, dyspnoea and eosinophilia [9]. Figure 1 shows the life cycle of a filarial infection.
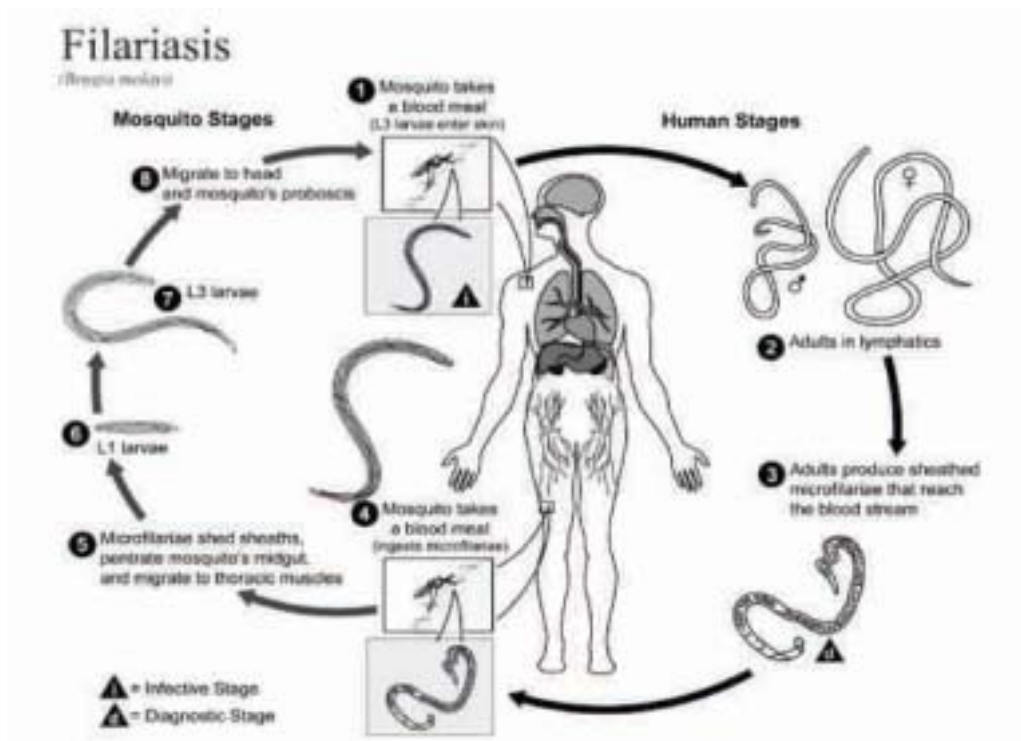

Figure 1. The life cycle of lymphatic filariasis (www.dpd.cdc.gov/dpdx).
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11

Intestinal helminthiasis

Ascaris lumbricoides, Trichuris trichuria and hookworm are three major intestine-dwelling nematodes [10]. The parasites have no intermediate hosts and their life cycles are simpler than tissue nematodes. The *A. lumbricoides* and *T. trichuria* infections start by ingestion of food or water contaminated with parasite eggs. Swallowed eggs will move down to the small intestine to hatch into larva and invade the small intestinal mucosa. The *T. trichuria* larva penetrates columnar epithelium to mature after 4 moulting steps and then move on to the large intestine to establish a chronic infection in that area by threading its anterior portion into the mucosa.

The *A. lumbricoides* larva penetrate the small blood vessels of intestinal mucosa and are then carried out into the portal system. Larva then follow the blood stream to the right ventricle and finally reach the small vessels of the lung where they grow to three times their original size within 10 to 14 days, and then they penetrate alveolar walls, ascend the bronchial tree to reach the throat to be swallowed a second time to reach maturation in the small intestine.

Infection of hookworms, *Ancylostoma duodenale* and *Necator americanus*, is initiated by active penetration of the filariform larva into the skin, which is facilitated by larval hydrolytic enzyme [11]. The larva enter capillaries to be carried by the blood stream to the lungs. From this site the development and travel pattern of hookworms is similar to that of *A. lumbricoides* where small intestine is their final destination.

Adult worms of *A. lumbricoides* and *T. trichuria* can survive in the intestine for 2 years while hookworms can live up to 5 years [12]. *Ascaris* requires 2-3 months and *Trichuris* 2 months from ingestion of the infective eggs to oviposition by the adult females. The adult females of *A. lumbricoides* can produce approximately 200,000 eggs per day and that of *T. trichuria* between 3000-20,000 eggs per day. The *A. lumbricoides* eggs that are passed in the stool become infective outside the body after 18 days to several weeks depending on the environmental conditions while the eggs of hookworms will hatch in 1 to 2 days giving rise to rhabditiform larva that after 5 to 10 days become filariform and are able to penetrate the human skin [12].

The clinical manifestation of intestinal helminth infections is highly dependent on the intensity of infection. Individuals, who carry light or moderate *A. lumbricoides* infections, respond negligibly to the parasite while heavy worm burdens can cause a malabsorption syndrome [13] and abdominal pain [14]. Inflammation of some of these organs caused by migration of adult worms such as peritonitis [15], hepatobiliaritis, pancreatitis [16] and hydronephrosis [17] have also been reported, but these are relatively rare. Light and moderate infections with *T. trichuria* are frequently asymptomatic, although there

could be an increased local inflammation such as eosinophilia and neutrophilia in sites where many worms are found [18]. Heavy infections of this whipworm can result in chronic diarrhea and rectal prolepses [19]. The most common symptom of hookworm infection is iron deficiency, anemia. One adult *A. duodenale* worm can be responsible for 0.1-0.2 ml blood loss/day whereas one adult *N. americanus* worm can result in a loss of 0.01-0.02 ml of blood/day [12]. Heavy infections, which lead to severe anemia, may cause lassitude, palpitation, dyspnoea and congestive heart failure [20]. Chronic anemia, especially in children can cause the retardation of growth and intellectual development [21]. Repeated penetration of hookworm larva may result in pruritic popular vesicular dermatitis at the site of larva entry. Respiratory symptoms can also be observed during pulmonary migration of *Ascaris* and hookworm larva [12]. Figure 2 shows the life cycle of intestinal helminth infections (*Ascaris, Trichuris* and hook worm).
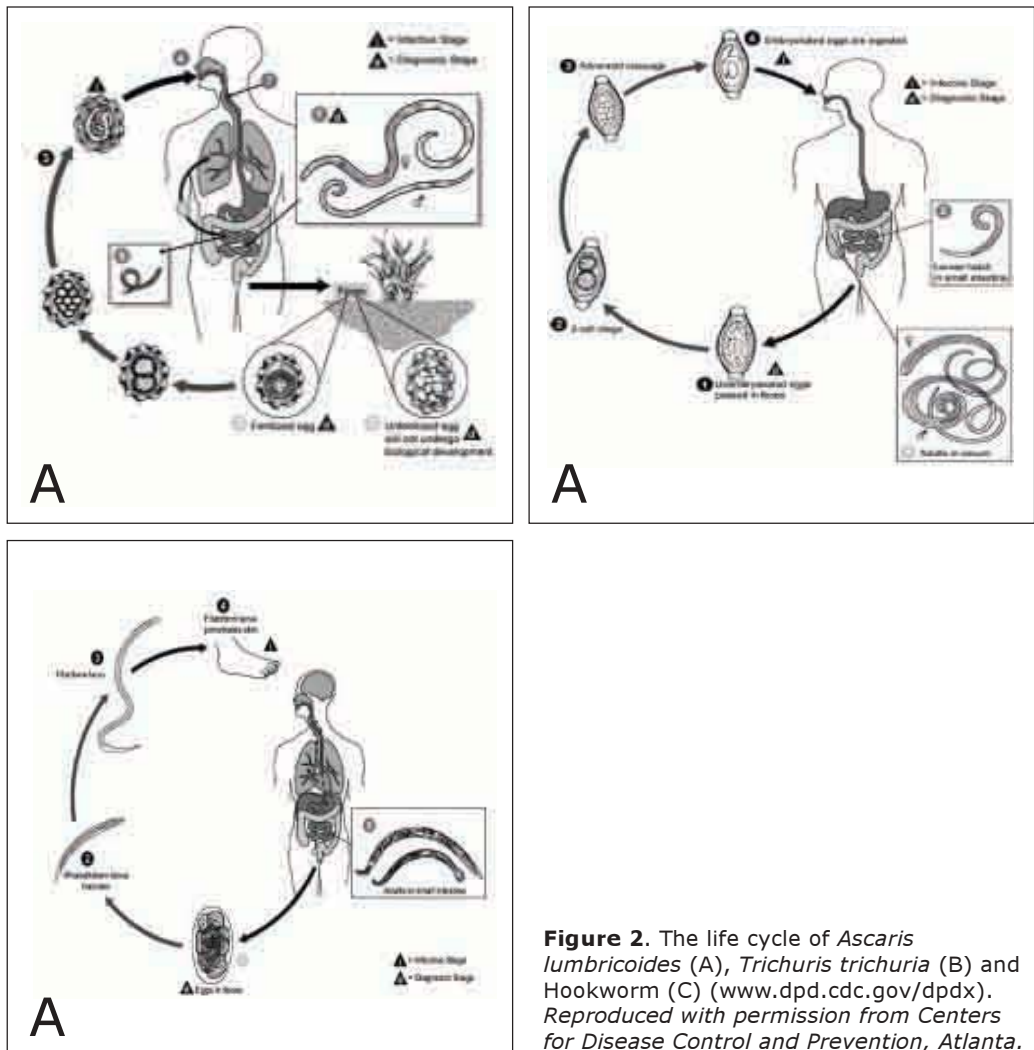

Figure 2. The life cycle of *Ascaris lumbricoides* (A), *Trichuris trichuria* (B) and Hookworm (C) (www.dpd.cdc.gov/dpdx). *Reproduced with permission from Centers for Disease Control and Prevention, Atlanta.*

2. Helminth infections in Indonesia

Lymphatic filariasis- epidemiology and treatment in Indonesia

When lymphatic filariasis was first reported in Indonesia in 1889 by Haga and Eecke [22] in a patient with scrotal elephantiasis, *W. bancrofti* had already been described as the cause of the disease in India [23]. Thirty-nine years later, Brug, a Dutch physician, reported the presence of mf in blood of a patient from Celebes which had features distinct from mf of *W. bancrofti* and named this nematode *Filaria malayi* [24] that later become known as *Brugia malayi*. Thereafter, Partono and co-workers [25] described a nematode which was similar to *B. malayi* but was larger in size with different morphology in several parts of the body. This species was then named *Brugia timori* because it was first found on Flores Island in Indonesia.

In urban areas, *Culex quenquefasciatus* [26] is the main vector of bancroftian filariasis, whereas in rural areas the diseases is transmitted by *Anopheles farauti and Anopheles punctulatis* [27]. For *B. malayi* the vectors vary depending on the periodicity of the parasite. The nocturnally periodic form, which is mainly found in Sulawesi, is transmitted by *Anopheles barbirostris* [28] whereas the sub-periodic biotype, which is found in Sumatra and Kalimantan, is transmitted by *Mansonia spp.* [2;29]. *B. Timori* has nocturnal periodicity [30] and is transmitted by *A. barbirostris* [31].

From extensive surveys on lymphatic filariasis that were conducted in Indonesia since 1970, it was reported that the prevalence of infection varied between 0-70 percent in different areas [32]. Due to control programs and changes in economy and infrastructure, Indonesian Health Minister in cooperation with University of Indonesia claimed that mf prevalence has declined to 0-19,6% [27], although in some remote and less accessible areas the prevalence of the infection may be higher [33]. However, as 22 species of mosquito are intermediate hosts for these parasites, it may not be surprising that WHO informal consultant meeting on South East Asia Region in India stated that this disease is endemic in 22 of 27 provinces in Indonesia with approximately 150 million people at risk of infection. That meeting also indicated Indonesia to be on top of the list of countries with the highest prevalences of lymphatic filariasis in South East Asia [34]. The studies reporting on filaria infections in different areas of Indonesia [35] in the period of 2000-2006 are summarized in figure 3.

The improvement in transportation which has led to people from endemic areas to travel to non-endemic ones and vice versa, may explain the increasing spread of the disease in Indonesia. However, the transmigration program which was started during Dutch domination, relocating groups of people from overcrowded islands such as Java and Bali, to those under-populated and endemic for filariasis, may also be

Figure 3. The distribution and the prevalence of lymphatic filariasis in several areas in Indonesia in the period of survey 2000-2006. *Data from Directorate General Communicable Diseases Control and Environmental Health of Indonesia.*

responsible for the increasing burden of this parasitic disease in Indonesia. Several studies reported differences in the pattern of clinical manifestations of lymphatic filariasis between transmigrants and indigenous population. It was found that individuals who had not been previously exposed, generally experienced clinical manifestations much earlier and with more severe outcome than the local population with life long exposure to filarial parasites [36-39]. Interestingly, transmigrants that were born in endemic areas reacted in a similar way to the indigenous population [40], indicating that exposure either intrauterine or very early in life might lead to modulation of immune responses in such a way that pathological reactions to infection are kept to a minimum.

Concerning age and gender, the prevalence of filarial infection in Indonesia, as determined by microfilaremia appears higher in males than in females [40-43] and increases with increasing age [40;43]. In terms of clinical manifestations, males seem to develop pathology earlier than females [36;44].

The current control programs in Indonesia recommend annual mass drug administration (MDA) using diethylcarbamizine 6 mg/kg body weight combined with albendazole 400 mg for at least four to five subsequent years [45]. This method has been shown to be effective in reducing mf prevalence of *B.timori* and intestinal helminth infections in Alor island, East Nusa Tenggara from 26.8% to 3.8% [46].

Intestinal helminths- epidemiology and treatment in Indonesia

The prevalence of intestinal helminth infections in Indonesia is generally high. Epidemiological surveys carried out over the period 1975 to 2003 found the prevalence of *A. lumbricoides*, *T. trichuria* and hookworms to vary from 14%-90%, 1%-90% and 18%-76%, respectively. The studies reporting on intestinal helminth infections in different areas of Indonesia over the period of survey from 1975-2003 are summarized in figure 4.
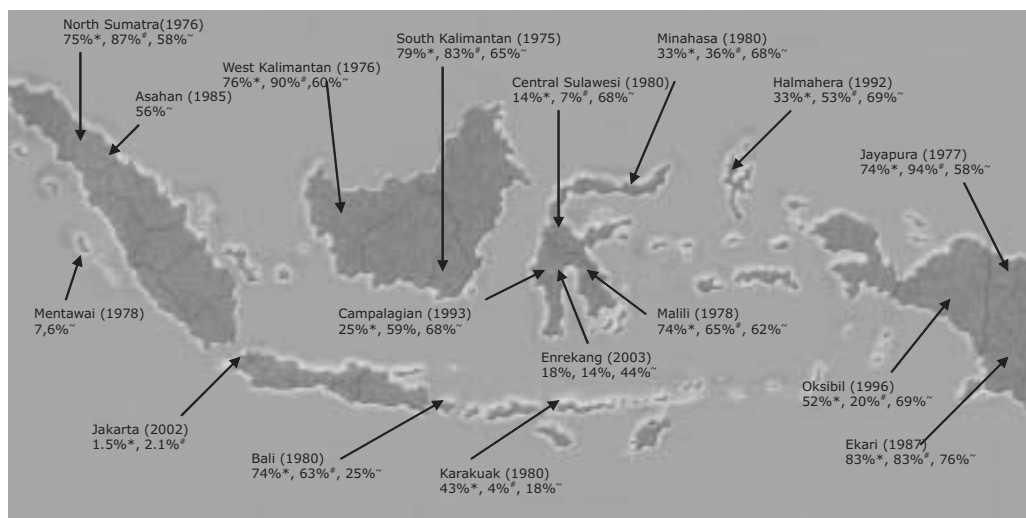

Figure 4. The distribution and the prevalence of intestinal helminth infections in several areas in Indonesia on the period of survey 1975-2003 (*data from www.pubmed.com*).
*Ascaris lumbricoides
#Trichuris trichuria
~Hookworm

Assuming that the methods used had similar sensitivity and specificity, the difference in prevalences might be due to variation in hygiene practices, temperature, altitude, soil type and time of screening. A Survey carried out on three occasions; July, March and August (dry, rainy and dry season, respectively) in Surabaya found that soil contamination rate was significantly lower in the dry season in July and August compared to the rainy season in March [47]. Moreover, a study conducted in Bali reported that the prevalence of *A. lumbricoides*, *T. trichiura* and hookworms infections was significantly higher in the wet highlands compared to wet lowlands, dry highlands and dry lowlands [48], suggesting that eggs survive better in high altitude and wet environments. Indeed, in terms of altitude, the transmission of hookworm seems to be predominant in highland areas as seen in high altitude areas in Enrekang, South Sulawesi [49], Bali [50] and Irian Jaya [51].

In terms of gender dependent differences in prevalence/intensity of infection, results are not consistent. In Sumatra, *Ascaris* infection was more prevalent in females compared to males but this was not the case for *Trichuris* and hookworm infections [52]. In Bali, the prevalence of hookworm infections was higher in males, but this was not the case for *Ascaris* and *Trichuris* [48]. In South Kalimantan *Ascaris* was more prevalent in females whereas hookworm was more often found in males [53]. Altogether, *Ascaris* seems to be more prevalent in females whereas hookworm appears to be more common in males. The differences may reflect different personal hygiene practices and differences in occupation. Age is also an important determinant of infection. Different age distribution curves have been seen for *Ascaris* and *Trichuris* infections on the one hand and hookworm infections on the other. The prevalence of *Ascaris* and *Trichuris* infections is higher at younger age, whereas hookworm infections are more prevalent in older age groups [48;49;54-56]. This might be due to the route of parasite entry; for *Ascaris* and *Trichiuris* the oral route and for hookworm the skin which means that differences in age related behavior will affect the degree of exposure to infection. In Indonesia, a high prevalence of hookworm infections is seen in those involved in farming [48;49;54-56]. In most farming areas, no latrines are available and therefore high degree of contact with contaminated soil is expected.

Directorate General Communicable Diseases Control and Environmental Health of Indonesia recommends the use of 400 mg albendazole every 6 month to control intestinal helminth infections. In areas which is also endemic for lymphatic filariasis, this drug is given annually together with diethylcarbamizine 6 mg/kg body weight [45]. Recently, it was reported that combination of 400 mg albendazole with 210 mol vitamin A increased hemoglobin concentrations and decreased anemia prevalence which is linked to hookworm infections [57].

3. Immune response in helminth infections

Helminths are extracellular parasites living in the lymphatics, bloodstream, or in the gastrointestinal tract. Helminths do not replicate in the human host and need some time to reach target organs to reproduce. Infections with parasitic helminths are often asymptomatic, and most hosts seem to ignore the presence of parasites for considerable lengths of time. The immune response of the host to helminth infections is characterized by T helper (Th)2-like responses with the production of the cytokines interleukin (IL)-4, IL-5, and IL-13, as well as elevated immunoglobulin (Ig)E and the expansion and mobilization of specific effectors cells, such as mast cells, eosinophils, and basophils [58]

Skewing of cellular immune responses studied in populations living in areas endemic for lymphatic filariasis

On the basis of the presence or absence of mf and presence or absence of pathology, the population residing in an endemic area can be classified into three different groups: 1) those who are exposed, but seem to be un-infected and show no sign of infection, the-so called endemic normal (EN) or asymptomatic amicrofilaremic subject (mf negative) [59;60]; 2) those with mf positive generally showing no outward signs of filarial disease; and 3) those with chronic pathology (CP), who are generally mf negative representing with lymphedema, hydrocele and elephantiasis. The fact that all these three groups are exposed to filarial infection [61], but not everyone becomes infected or develops chronic obstructive disease suggests that host specific immune responses to filarial infections is diverse.

Natural immunity to lymphatic filariasis may be acquired over time. Analyses of an age-stratified population and quantification of worm burden by measuring circulating antigens in Papua New Guinea showed an increase in circulating antigens (CA) in individuals under 20 years of age but not in those older than 20 [62]. However, there are also studies that argue that immunity does not develop in filariasis [63]. Although it is not clear whether strong immunity develops to filarial infections, cellular and humoral immune responses have been studied in EN and have been compared with those of mf positive subjects. In EN higher interferon (IFN)-γ production and lower IL-4 and IL-5 have been measured compared with mf positive subjects [64]. However, a study conducted on an island endemic for *W. bancrofti* found the adult residents who remained putatively immune (mf negative or CA negative) for 17 years showed cellular immune responses marked by high cellular proliferation; generation of IL-2, IL-5, IL-10, IFN-γ, and granulocyte-macrophage colony-stimulating factor (GMCS-f) to adult- and mf-antigens, compared with infected subjects [65], suggesting that in asymptomatic mf negative individuals both Th1 and Th2 responses are higher than in microfilaremics.

The immune responses of the mf positive subjects have been extensively documented. These individuals show in addition to T cell hyporesponsiveness (which is discussed in the next section), modified Th2 responses. Modified Th2 responses are characterized by skewing of cytokine profiles towards Th2 but with concomitant elevated IL-10 [66;67].

In individuals exhibiting chronic pathology comparison of cellular responses with those infected subjects that show no signs of any pathology, has indicated that indeed higher responses are found to filarial antigens when pathology is present without microfilaremia [68;69]. Some studies have found that although IL-4 responses are slightly higher in CP patients, the IFN-γ is strongly up-regulated in this group resulting

in ratios of IFN-γ:IL-4 that are significantly higher in CP [70], indicating skewing towards Th1.

Hallmarks of the humoral immune responses in filariasis, IgG4 and IgE are differentially regulated in the different clinical categories described. In general, filarial-specific IgG4 responses are elevated during active infection, thought to be driven by the modified Th2 responses [71], whereas IgE antibodies are associated with the absence of active infection [66;72]. Thus, the EN group has generally high levels of IgE and low levels of IgG4 whereas CP group, depending on whether they are mf negative or mf positive will show very high levels or some IgE responses to filarial antigen, respectively [73;74].

In summary, the mf positive subjects are immunologically the best characterized group and are found to have low T cell proliferative responses as well as low cytokine production, but with some skewing towards Th2. The EN group, responds to filarial antigens by both Th1 and Th2 cytokine production, however, more studies seem to favor a stronger skewing towards Th1 in this group [75;76]. Finally, the CP group is hyper responsive to filarial antigens. In a sub group of CP patients, mf is found in peripheral blood and this group tends to have lower immunological reactivity to filarial antigens. It is not clear why this group would have developed pathology if their immune responses are low to filarial antigens. Along the same line, it is not clear why strong immunological responses in some subjects leads to infection-free status without pathology (the EN group) while in another it drives the development of immunopathology (the CP group).

Immune response to filarial infection studied in animal models
The skewing towards Th2 in filarial infections has also been reported in animal models and this is seen with all life cycle stages to which the human immune system is exposed to. Using different inbred strains of mice it was shown that the L3 elicits a significant IL-4 response within 12–14 days of infection [77;78] suggesting L3 are potent stimulators of IL-4. Moreover, implantation of BALB/c mice with adult worms clearly elicits high levels of IL-4 [79]. Although infection of mice with mf showed to induce an IFN-γ response at early time points after infection [79-82], this was followed by an increase in levels of IL-5 and a subsequent decline in IFN-γ at later time points [80]. Moreover, high levels of IgE and a switch in cytokine profiles from IFN-γ to IL-4 was seen when mice were given multiple mf infection via intravenous route (chronic exposure) [82]. Therefore not only the antigenic make up of a lifecycle stage but also repeated exposure might drive Th2 responses.

IL-4 in filarial infected mice appears to downregulate polyclonal proliferative as well as IFN-γ responses. Experiments in BALB/c mice infected subcutaneously with L3 showed a suppression of polyclonal (but not antigen-specific) proliferative and IFN-γ responses. When IL-4 was neutralized *in vitro*, a partial restoration of the polyclonal proliferative

response, and increased IL-2 and IFN-γ responses to mitogen was seen whereas this had no effect on antigen-specific cytokine production [81]. Similarly, mice were implanted intra peritoneally with adult *B. malayi*; the peritoneal exudate cells (PEC) were unable to support the proliferation of a conalbumin-specific T cell clone [83] and the generation of these defective antigen-presenting cells (APC) was dependent on IL-4 [84]. Therefore, in animal models, the Th2 cytokine, IL-4, seems to be involved in proliverative hyporesponsiveness. In animal models, *Litomosoides sigmodointis* infections in BALB/c, the only model of filariasis which allows the complete development of the parasite with an immunecompetent mouse, attempts have been made to understand mechanism of immunity to infection. Parasite clearance in this model seemed to be mediated by IL-5 and IFN-γ as determined by use of cytokine gene knock out (KO) mice [85;86].

In human, the prevalence and intensity of *A.lumbricoides* and *T.trichuria* infections is age dependent, typically convex, peaking in children and adolescents [87;88] and thereafter declines with increasing age. It is thought that resistance is acquired slowly as a function of increasing exposure [89].

A study carried out in an area endemic for *A. lumbricoides* in rural Ecuadorian communities and uninfected subjects from an urban environment reported greater frequencies of IL-4 and IL-5 relative to IFN-γ secreting cells, and a greater absolute production of IL-5 in peripheral blood mononuclear cells (PBMC) of exposed individuals stimulated with *A.lumbricoides* antigens compared to the urban population [90], indicating the expansion of Th2 type response in intestinal helminth infection. Similar type of immune responses were also reported in other studies [90-92] and it has been postulated that these cytokines have a protective role against infection. In a study of PBMC from young adults with moderate *A. lumbricoides* infections, higher antigen specific IL-4 and IL-5 but lower IFN-γ was found compared to non-infected controls resulting in a high ratio of Th2/Th1 in infected subjects compared to the non infected group [90]. It was hypothesized that the expulsion of parasites may be under the influence of exposure-acquired Th2 cytokines. In a study in Cameroon of 150 individuals aged 2-36, an increasing magnitude of IL-9, IL-10, and IL-13 in response to parasite antigens was inversely correlated to the intensity of *A. lumbricoides* infection in individuals aged >11 years [91;92]. For *T. trichuria* infection alone, there are no data on the relationship between Th2 skewing and immunity. However, in mixed infections of *A. lumbricoides* and *T. trichuria* in Cameroon, it was reported that 89 months after de-worming the susceptibility to re-infection was related to poor IL-13 and IL-5 responses to *A. lumbricoides* as well as *T. trichuria* parasite antigens [93;94].

At the humoral level, a study in lightly and heavily infected Bangladeshi children reported that the levels of IgG1, IgG4 and IgE to *A.lumbricoides* antigens were significantly higher

in heavily- compared to lightly-infected group, suggesting that these antibodies simply reflect the intensity of infection [95]. However, another study reported that susceptibility to *A. lumbricoides* infection was negatively associated with levels of specific IgE [96;97]. In another study looking at *T. trichuria* infections, specific IgE was inversely correlated to the intensity of infection as defined by egg output [92]. These results together tend to suggest that Th2 responses are associated with protection from intestinal helminth infections

Th2 and Th2 responses in animal model of intestinal helminth infections

The host-protective effects of Th2 type cytokines against intestinal helminth has been shown in animal models. Elevated IL-4 levels have been demonstrated in mice infected with *Trichuris muris* and *Heligmosomoides polygyrus*, and anti-IL-4 or anti-IL-4R antibodies block host immunity to a challenge *H. polygyrus* infection, as demonstrated by increased adult worm survival and egg production [98]. Moreover, increased numbers of muscle larvae was reported in IgE-depleted, *T. spiralis* infected rats whereas rapid expulsion was induced when the rats were treated by purified IgE antibody [99-101]. These data support the protective role of IL-4, as nematode infection-induced IgE production is IL-4-dependent [102]. The protective role of IL-5 in natural immunity in animal models of infection is not as strong as IL-4, although this cytokine induces eosinophilia [103;104], which has been reported to be able to kill some parasites in vitro [105]. In vivo study of *T. spiralis* infected mice treated with anti-IL-5 mAb to prevent eosinophilic responses however did not show an increase of worm burden [106]. Similarly, anti-IL-5 mAb had no effect on control of *T. muris*, *H. polygyrus*, or *Nippostrongylus brasiliensis* infections in mice, even though it prevented blood and tissue eosinophilia [98;104]. The evidence that IL- 9 plays a role in host resistance came from a study of mice infected with *T. muris*. In the study the elevated levels of IL-9 was correlated with the enhancement of intestinal mastocytosis and the production IgE, which was shown to be responsible for worm expulsion [107]. The data were in agreement with the demonstration of extremely rapid expulsion of the parasites in IL-9-transgenic infected mice, which constitutively overexpress IL-9 [107]. The last of Th2 type of cytokine, IL-13, has been shown to play a protective role in animal models as IL-13 deficient mice infected with *T. muris* fail to clear the infection [108]. However, the protective role of IL-13 seems to depend on TNF-α as TNF-α receptor KO mice failed to expel *T. muris* [109]. In vivo blockade of TNF-α in normally resistant mice showed no alteration of IL-4, IL-5, or IL-13 production in the draining lymph nodes despite significant delayed worm expulsion. But, TNF-α receptor KO mice, produced high levels of parasite-specific IgG2a, the antibody under the control of IFN-γ, and low IgG1 [110].

In contrast to Th2, Th1 type responses in animal models were shown to relate to susceptibility or chronic infection. Unlike strains that produce a predominantly IL-4 response, the mouse strains that produce a strong IFN-γ response to a primary *T. muris* infection develop

chronic infections with this parasite and neutralizing IFN-γ using mAb at the time of *T. muris* inoculation resulted in expulsion of larvae before they developed into fecund adults [111]. In addition, a significant positive correlation was found between worm burden and parasite-specific IgG2a indicating that IFN-γ plays an important role in the establishment of chronic trichuriasis [112]. Other Th1 type cytokine, the IL-12, also seem to have similar effect to IFN-γ. A study that treated *N. brasiliensis* infected mice with IL-12, starting at the time of parasite inoculation, showed an enhancement of egg production several fold and prolonged the course of infection. When IL-12 treatment was discontinued, adult worms are expelled [113].

To conclude, the data generated from population studies but particularly from animal models indicate that Th2 responses can be generated to intestinal helminth antigens and such responses seem to be associated with effective immunity to these parasites. The situation is less clear for filarial infections, where both only one fully permissive animals model exists and no re-infection studies can give us robust data on important immune responses that can be protective.

4. Immune hyporesponsiveness during chronic helminth infections: adaptive and innate immune responses

Although Th2 skewing is observed in filarial as well as intestinal helminth infections, some level of immune hyporresponsiveness is also associated with chronic helminth infections.

Immune hyporesponsiveness in filarial infection

In filarial infections, individuals with mf in peripheral blood fail to proliferate to filarial antigens [65;114;115], while in EN as well as CP, proliferation exists to varying degrees [72]. Interestingly, administration of diethylcarbamazine citrate (DEC) to mf positive individuals restores T-cell responses [116]. Analysis of the cytokine profiles in PBMC showed that in filarial infections IL-4 predominates with little IFN-γ production [70;117] and diminished IL-5 production [115]. Similar to T-cell proliferation, when DEC was administered to mf positive patients, IFN-γ production was up-regulated [116]. Although in one study IL-4 level after DEC treatment remain unchanged, in another study de-worming during *W. bancrofti* infections resulted in the reversal of IL-5 production [118] indicating that not only Th1 but also Th2 responses are partially down regulated during active filarial infection.

Production of IL-10, an immunesuppressory cytokine, has been seen in subjects living in areas endemic for lymphatic filariasis. PBMC from mf positive subjects spontaneously secreted

10-fold more IL-10 than did PBMC from patients with CP. When PBMC were incubated with parasite antigen, mf positive subjects also showed higher IL-10 production compared to CP patients [67]. Mf positive subjects tended to have increased levels of both IL-10 protein and mRNA in comparison to CP and EN, and the reduced capacity of PBMC from mf positive individuals to proliferate was correlated with IL-10 mRNA levels [119]. Blocking IL-10 production using antibodies to IL-10 has been shown to reverse cellular proliferative responses in vitro [70;119] and to enhance IFN-γ secretion in response to *B. malayi* antigens [120]. Besides IL-10, transforming growth factor (TGF)-β, another suppressory cytokine, is also produced during filarial infection [70]. A study carried out in an area endemic for *W. bancrofti* showed that TGF-β mRNA is produced in 80% of mf positive subjects compared to 50% in CP and neutralization of this cytokine significantly enhanced lymphocyte proliferation to filarial antigens in mf positive individuals [70]. Recent experiments in the *B. malayi*-mouse model showed that both CBA/Ca and C57Bl/6 mice treated with anti-IL-10 clear intravenous mf infections more rapidly than control mice. In addition, IL-10-deficient mice implanted intra peritoneally with adult worms clear mf, but not the adult worms, more rapidly than wild-type mice (Gray *et al*., unpublished as indicated in [121]).

Immune regulation in intestinal helminth infection

In intestinal helminth infections, T cell hyporesposiveness has also been documented. A study conducted in an area highly endemic for *Ascaris* and *Trichuris* infections showed that PBMC responses to parasite antigens were higher in the control group compared with the group of individuals infected with *Ascaris* only or infected with both *Ascaris* and *Trichuris*, and lower IL-12, IFN-γ and TNF-α productions were measured in infected subjects compared to controls [122]. Indeed, elevated IL-10 production was also observed during chronic infection with *A. lumbricoides* [91]. The levels of IL-10 tended to increase in those with high *A.lumbricoides* infections even if they had lower *T.trichuria* infections [94]. The possibility that IL-10 may be responsible for the lower IFN-γ production in infected individuals is supported by experiments in mice infected with *T. muris* where in IL-10 deficient mice significantly higher levels of IFN-γ are produced in response to *T.muris* infection [123]. Recently, Turner and co workers reported the presence of TGF-β when whole blood was stimulated with *A. lumbricoides* extracts, particularly in individuals with heavy burdens of infection in Cameroon (unpublished data reported in [124]).

Regulatory T cells (Tregs) and helminth infections

IL-10 and TGF-β are two cytokines that have strong down regulatory properties and have the capacity to switch off inflammatory and protective immune responses [125-127]. These two molecules are produced, among others, by Tregs [128]. Tregs or suppressive T-cells are important in controlling excessive activation of effector T-cells in an immune response [129]. Various Subsets of natural and induced Tregs have been reported in

human and rodents including Tr1 cells, Th3 cells, NK Tregs, CD4+CD25+ Tregs, CD4+CD25- Tregs, CD8 Tregs and CD8CD25-cells [130]. Tregs work by cell-to-cell contact and by producing inhibitory cytokines such as IL-10 and TGF-β [128;131], which decrease proliferation and cytokine production by Th1 and Th2 cells [132]. Blocking of IL-10 and TGF-β production can result in the reversal of the suppressive effects of Tregs [133].

Studies in animal models of infection with filarial worms have shown that indeed, infection with *L. sigmodontis* leads to the development of regulatory T cells. Removal of these cells using antibodies, resulted in reduction of the worm burden [134] which indicates that these cells play a role in worm survival. In models of intestinal helminth infections the importance of regulatory cells in controlling pathology was provided in inflammatory bowel disease studies. Some strains of mice and rats develop severe colitis after transrectal challenge with trinitrobenzene sulfonic (TNBS) or dinitrobenzene sulfonic (DNBS) acid and this colitis is characterized by infiltration of the mucosa with CD4+ IFN-γ-producing T cells [135]. Treatment with recombinant IL-10 [136] inhibits TNBS colitis and more interestingly, colonization of mice with *T. muris* or *H. polygyrus* reduces spontaneous TNBS as well as DNBS induced colitis [137;138]. These data indicate colitis results from a dysregulated immune response and helminth infections that induce regulatory cytokines, for example IL-10, can protect mice from developing colitis.

Chronic helminth infections and innate immune responses

In terms of innate immune responses, recent work has indicated that some helminth infections may also be associated with an altered innate immune response. The innate immune response is able to sense invading micro organisms and to react rapidly to contain infection allowing time for the more sophisticated adaptive immune system to develop. Specialized receptors present on cells of the innate immune system interact with molecular patterns specific to micro organisms. A well known family of such receptors is the toll like receptor (TLR) which initiate a cascade of signals leading to release of cytokines and chemokines that set the anti microbial events into action [139]. Recently, specific helminth derived molecules have been identified that can stimulate the innate immune system via TLR [140-142]. However, chronic infections with schistosomes and filarial parasites have been shown to result in a lower responsiveness of monocytes or B cells to TLR ligands [143;144]. Thus, helminth infections not only act to down regulate adaptive immune responses, but also seem to interfere with the innate immune system

To conclude, the ability of IL-10 and TGF-β to down regulate excessive immune responses in mf positive subjects may explain the ability of filarial worms to survive for long periods of time without inducing extensive pathology. The same might be true for intestinal helminth infections, although given their localisation, one might expect less need for a pronounced regulatory responses. In addition to adaptive immune responses, evidence is emerging that

innate responses may also be modified during helminth infections. The regulatory response both at the innate and adaptive level might influence responses to third party antigens

5. Studies of risk factors involved in acquisition of helminth infections

Although most people in endemic areas are facing the same parasitic infections, the chance to become infected or to develop clinical pathology is not similar, suggesting the existence of genetic factors as underlying mechanisms responsible for the pattern of susceptibility to infection and disease. For schistosomiasis and ascariasis it has been shown that the outcome of infections are controlled by certain genes. A genome scan for human schistosomiasis carried out in Brazil 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 IFN-γ receptor 1 subunit in this disease [146]. A recent study conducted in Nepal has found a locus controlling *A. lumbricoides* intensity of infection on chromosomes 1 and 13 [147]. Unfortunately, such information is lacking for lymphatic filariasis with the exception of non reproducible association between certain HLA class I and II alleles and development of elephantiasis [148-150]. Attempts are being made to understand pattern of transmission and the nature of risk factors governing filarial infection epidemiology.

Family make up has been put forward to account for susceptibility to filarial infection. A large family study in India involved 946 families reported that mf prevalence among offspring born to mf positive parents was higher compared to the ones who were born to mf negative parents [151] suggesting that parental infection is the important risk factor for infection susceptibility in children. However, as it was also concluded by the authors, since the families live in the same household and therefore with equal environmental exposures, not only familial but environmental factors may explain the high risk of infection within a family.

The role of the household exposure has been investigated in Brazil. A parasitological survey performed by a 'door-to-door' approach showed that the presence of mf in adults is a risk factor for pediatric infection that lived in the same household [152]. Another study that was also performed in the same area in Brazil detected no increased risk of being a mf positive adult, if another adult in the same household was mf positive [153]. From these two studies it seems that in childhood there is a window of opportunity to become infected and this is determined by household factors that encompass genetic and environment component. For intestinal helminth infections, the investigation into household effect came from a survey 428 households in a shanty town in Coatzacoalcos, Mexico. The study found that individuals with heavy *A. lumbricoides* and *T. trichiura* infections clustered together and lived in the same households [154]. This pattern may result from focal transmission in the vicinity of the house. However, since the members of the houses also often belong to the one family, the genetic similarities among family members may also influence their susceptibility to infection.

The data above show how difficult it is to dissect genetic and environmental factors since people sharing the same genes often live in the same house therefore exposed to the same environment, same diet and other habits.

A better approach to analyze clustering is to use the information of all possible relationships, i.e. for a genetic effect not only parent-offspring pairs but also aunt/ uncle-niece/nephew pairs and for a household effect not only parent-offspring pairs but also pairs of spouses. Such a multivariate analysis can be performed by using generalized linear mixed models [155;156]. The influence of each type of effect (genetic, household, and environmental) on the outcome can be tested. In the second step the contribution of a certain effect on the outcome can be estimated. It is noteworthy that not all variables can be tested using multivariate analysis, but only the ones that are relevant to study objectives and shown to have a tendency to influence biologically the disease output [157].

Walter in 1973, using a statistical test that was developed by him, studied the clustering of filarial infection. He found that mf presence was aggregated within households [158]. In 1981, using segregation analysis, Ottesen and coworkers compared the evidence for genetic factors and environmental factors influencing infection susceptibility in Polynesian bancroftian filariasis. They found that genetic effects were more dominant compared to environmental factors in terms of affecting infection susceptibility [159]. Those two studies used the presence of mf as infection determinant. A study that used both mf and anti-filarial IgG4 was conducted in a village in Indonesia and explored familial, household and environmental relationships between subjects by constructing pedigrees and household mapping in the village. When the multivariate analysis was used, the results showed genetic, household and environment factors influenced the clustering of anti-filarial IgG4, but only genetic factors could explain the transmission of mf [160].

Multivariate analysis in *A. lumbricoides* infections has also been done in a study in Nepal to investigate the risk of re-infection one year after treatment with albendazole [161]. The variance component analysis of the familial data provided unequivocal evidence for a strong genetic component accounting for between 30% and 50% of the variation in worm burden whereas shared environmental (i.e., common household) effects account for between 3% and 13% of the total phenotypic variance. From these data it appeared multivariate analysis can be used to estimate the contribution of different factors on disease output and determine which one is more dominant when several factors exist together. Moreover, using this analysis it is possible also to exclude prenatal influence (if it there) by selecting the age of the population chosen.

6. Allergy: increasing prevalences worldwide

The increase of allergic diseases in many parts of developed countries has been alarming. A study conducted in Sweden reported a two-fold increase in asthma, allergic rhinitis and eczema in schoolchildren in the period between 1979 to 1991 [162]. Two surveys conducted 15 years apart in Aberdeen also found increased respiratory symptoms and atopy in schoolchildren [163]. In less developed countries, although not as dramatic as in Western countries, increase in prevalences of allergic disorders has been reported from Asia [164-167], Africa [168-170] and South America [171] with the tendency for prevalences in urban communities to approach those seen in Western countries.

Migration from less to more developed countries seems to increase the risk of developing allergies. In Melbourne, the prevalence of hay fever and asthma in Asian immigrants was strongly associated with their length of residence in Australia [172]. Similar findings have been reported for migrants of Turkish origin in Berlin [173]. These studies indicate that affluence, urban environment as well as exposure to new allergens may be responsible for the increase in allergic disorders. Several leading factors in developed countries or urban areas, namely the increase in exposure to outdoor pollutants [174;175], the increased indoor allergen load [176], altered diet [177;178] and changes in exposure to microbes [179;180], all parts of adoption of Western lifestyle, have been hypothesized to explain the increase in allergic disorders. The lack of consistency in the results from studies that have attempted to examine these factors may indicate that either other factors are important or that methods utilized to obtain population data need improvement, for example the use of questionnaires. Can they be used universally to describe allergic symptoms and to classify individuals into ones with and without disease? International study of asthma and allergy in children (ISAAC) and American thoracic society (ATS) questionnaires have been compared in ethnically homogenous inner city communities in Kuala Lumpur to examine asthma and related allergy symptoms, to find that the ISAAC questionnaire was more reliable than the ATS questionnaire [181]. Similarly, different questionnaires were used to estimate the prevalence of atopic eczema in Ethiopian children, but compared to doctor diagnosed eczema, neither ISAAC nor Unite Kingdom (UK) refinement of Hanifin and Rajka's diagnostic criteria performed well in predicting the cases of atopic eczema [182]. These examples question the reliability of the data obtained via questionnaire. What about the diverse ethnic backgrounds which use the same national language?

A study in northern Norway reported a higher prevalence of asthma, allergic rhino conjunctivitis and atopic dermatitis in Sámi children than in white Caucasian Norwegian children [183]. Is this related to genetic or environmental diversity? or as a result of

different interpretation of the questionnaire? A study in Singapore, a small country with high gross nation product (GNP) and a homogeneity in terms of geography, air pollution and language showed that using English questionnaires administered to Chinese, Malay, and Indian ethnic groups reported differences in the prevalence of asthma as well as rhinitis in the different ethnic groups. Interestingly, while asthma was reported to be highly prevalent among Indians [184], rhinitis was more frequently reported in the Chinese population [185]. These data raise the question whether ethnicity (genes as well as life style differences) may explain the different outcomes regarding allergic symptoms rather than the interpretation of the questions asked.

Objective parameters such as bronchial hiperresponsiveness (BHR) may help diagnose asthma, but it can only be performed in areas where sophisticated medical facilities are available. Simple tools such as audiovisual presentation of asthma may be useful in the field. Indeed, this tool has been reported to have a good agreement with BHR measurements in diagnosing asthma in a mixed ethnic population in Sidney [186]. However, when this audiovisual tool, together with questionnaires was applied in a large study that involved 99 centers from 40 countries [187], much diversity in its performance was seen. In that particular study, adolescents seemed to interpret the written questions about wheezing differently from the audiovisual presentation and this varied among the centers involved. Another multi centre study conducted in English speaking countries found a limited agreement between the questionnaires and the audiovisual presentation of asthma; this poor agreement could not be explained by issues such as language, culture, or literacy [188].

The issue of translation is also important as shown in a study from Thailand. The study found that the perception of wheeze was different among asthmatic as well as control children from the words chosen by the medical personnel. Children were shown the video of asthma and were asked to give a name for the symptoms in their own language. After the local words of wheeze were listed, they were used to screen the prevalence of wheeze in the appropriate population. When the results were compared to ISSAC questionnaire translated into Thai language, there was a 67% reduction in the number of cases [189]. This study indicates that the actual local term that describes wheeze and other symptoms of asthma is more accurate in estimating prevalence than the terms translated directly from the ISSAC questionnaire.

Taken together these data show that in order to accurately compare prevalence of diseases from one country to another, additional tools are needed to cross the borders of nationality, ethnicity, language and literacy.

7. Studies of risk factors for allergic disease

Similar to helminth infections, allergy is influenced by many factors. Nevertheless, compared to helminth infections the risk factors for allergy are better investigated in numerous studies. The role of family history or genetic background has been known to be a risk factor for allergic disorders [190]. Searching for genes that are responsible for susceptibility to asthma and other phenotypes of allergy such as rhinitis, eczema, IgE antibodies, skin reactivity to allergens and bronchial hyperresponsivenes have resulted in many candidates which have been extensively reviewed elsewhere [191].

Apart from genetic studies, there are data on cord blood (CB) to try and understand early life events and intrauterine exposures that may predict whether a child will develops allergy or not. Total-IgE and specific-IgE to egg and milk were detected in CB [192] as were total and specific IgE to aeroallergens [193]. It has been known that infants from atopic families have a higher risk to develop atopy compared to children without a positive family history. However, the absence of correlation between paternal-IgE and CB-IgE [193-195] indicates that IgE in CB may not be influenced by genes only but also by intrauterine exposure. The CB-IgE has been reported to be associated with atopy in infants at ages 9, 12 and 18 months [196-199], to eczema at age 9 months [200] and to bronchial asthma at five years of age [201]. Therefore it appears that maternal influence on allergic predisposition of an infant is strong and elevation of CB-IgE might be a good predictor of early-onset of allergy in children.

Several environmental factors have been indicated to play a significant role in allergy, for example, indoor allergens [176;202;203], air pollution [174;175;204] tobacco exposure [205] antibiotic use [206], altered diet [177;178] and western life style [207]. In contrast, exposure to farming environmental [208], expose to animals [209] and microbial infection [179;180;210] have been shown to reduce the prevalence of allergy. These data indicate that although genetic background as well as intrauterine factors has a strong influence on allergy, environmental factors can not be ignored. Moreover, allergic diseases show a great variation in prevalences between developed and non-developed countries and between urban and rural communities within one country, which probably reflects the difference in environments between one area and another.

The influence of infections on allergy has been the matter of much discussion and debate in the last two decades [211]. In terms of helminth infection the effect becomes particularly interesting since both of the diseases are marked by Th2 skewing. Interestingly, genes that are associated with asthma and some of the other allergic phenotypes, have also been shown to play a role in controlling helminth infections such as 5q31-33 in *Ascaris* infections and signal transducer and activators of transcription (STAT)-6 in *Ascaris* [212]

and *Hymenolepis diminuta* infections [213]. These findings suggest the possibility that individuals resistant to helminth infections might have genes that predispose them to have allergic disorders. To study these issues, it would be interesting to use multivariate approach which can test for and estimate the contributions of household as well as genetic factor (as already applied to helminh infection) also to allergic disorders. Such studies may be able to detect the interaction between helminths and allergic disorders spatially within a village taking into account genetic and environmental factors.

8. Allergy in Indonesia

Phase I of ISAAC study reported that the prevalence of (12-months reported) asthma, rhinitis and allergy symptoms in Indonesia was 1.6%, 5.2% and 1.6% respectively. This study, which was also conducted in 56 countries all over the world, placed Indonesia as one of the countries with the lowest prevalence of allergy [214]. Whether this study underestimated the real prevalence or not is not clear. The Indonesian national health survey in 1996 showed that in rural areas the average asthma prevalence was 4.3% while in urban areas it was 6.5%. In Jakarta, the capital of Indonesia, the prevalence was very high (16.4%) [215]. In an earlier survey in 1992 it was found that respiratory problems including asthma, was seventh in importance among major diseases that caused mortality [216]. But, it should be noted that the study participants covered by Indonesian national health survey represented the whole population (100000), while the ISAAC study was based on 2249 children aged 13-14 years old. However, the Indonesian national survey did not clarify whether the estimates were based on history of asthma or current asthma. Moreover, in Indonesia, only one centre was involved in the ISAAC study while in India 10 centres took part in the ISAAC screening [214]. A large variation was reported in the prevalence of asthma in the different areas of India ranging from 1 to 17%. Thus the Indonesian ISAAC performed in one area, might not represent the prevalence in the whole country.

Concentrations of carbon monoxide and particulate matter with diameters less than or equal to 10 micron in several large cities in Indonesia reach "very unhealthy" and "hazardous" levels, as defined by the Pollution Standards Index [217], and could play an important role in exacerbation of allergic disorders in large metropolitans in Indonesia. Skin prick tests done in 107 asthmatic patients in Jakarta reported that 77.6% were reactive to either: *Dermatophagoides pteronyssinus* (77.57%), *Blomia tropicalis* (71.96%), *Austroglycyphagus malaysiensis* (33.64%) *Elaeis guineensis* (22.43%), *Acacia auriculiformis* (12.15%), *Dicranopteris spp* (11.21%), *Curvularia fallax* (8.41%) and *Exserohilum rostratum* (13.08%) indicating that regional allergens are also important in triggering allergic reactions in Indonesia [218]. Another study in a health centre in Jakarta recorded house dust mite (HDM) as the major allergen

(54%) in asthmatic children. This study also reported genetic background to be an important risk factor, as it found 95.2% of the104 asthmatics to have a positive family history of atopy and 79.80% a positive family history of asthma [219].

With the exception of data from the ISAAC study [214], it has not been possible to find reports on rhinitis and eczema. However, with the large difference between ISAAC and Indonesian national survey findings, one has to assume that the other two allergic disorders would also have different prevalences than the ISAAC if measured in a larger population and in wider age groups.

9. Th2 and regulatory responses in allergy

According to European Academy of Allergology and Clinical Immunology (EAACI), the definition of allergy is a hypersensitivity response initiated by exposure to environmental agents, termed allergens, at a dose that is tolerated by normal subjects. Atopy is a personal tendency to produce IgE antibodies to minimal doses of allergens that can be detected by the presence of serum IgE binding to the allergens or by skin reactivity to the allergen deposited into the epidermis of the skin. Atopic individuals can develop symptoms of allergy such as rhino-conjunctivitis, eczema, asthma and food allergy [220].

An atopic person produces IgE antibodies when she or he encounters an allergen. This IgE can bind to IgE receptors (FcεRI) present on mast cells, basophils and eosinophils which are granular cells containing preformed inflammatory mediators within their granules. Whenever an allergen is encountered, it will cross link IgE, leading to activation of the FcεRI that results in degranulation and release of inflammatory mediators mainly from mast cells [221]. Mast cell degranulation leads to the release of mediators like histamine, leukotrienes and chemokines with both pharmacological and immunological effects on surrounding tissues. Chemotactic factors generate secondary inflammation by recruiting eosinophils, monocytes, neutrophils and lymphocytes to the inflammatory site [222]. These recruited cells, in turn, release large quantities of cytokines, which further disrupt tissue homeostasis; the tissues affected being the airways, the skin and the gastrointestinal tract mainly.

The allergic inflammatory cascade is orchestrated by Th2 cytokines which promote the production of IgE by B lymphocytes (through IL-4 mainly), promote the growth and de-granulation of mast cells (through IL-4 and IL-9) [223] and induce differentiation, activation and in situ survival of eosinophils (through IL-5) [224]. The combined interaction between IgE and mast cells/eosinophils, can lead to cascade of events that translate into an allergic reaction as shown in figure 5.
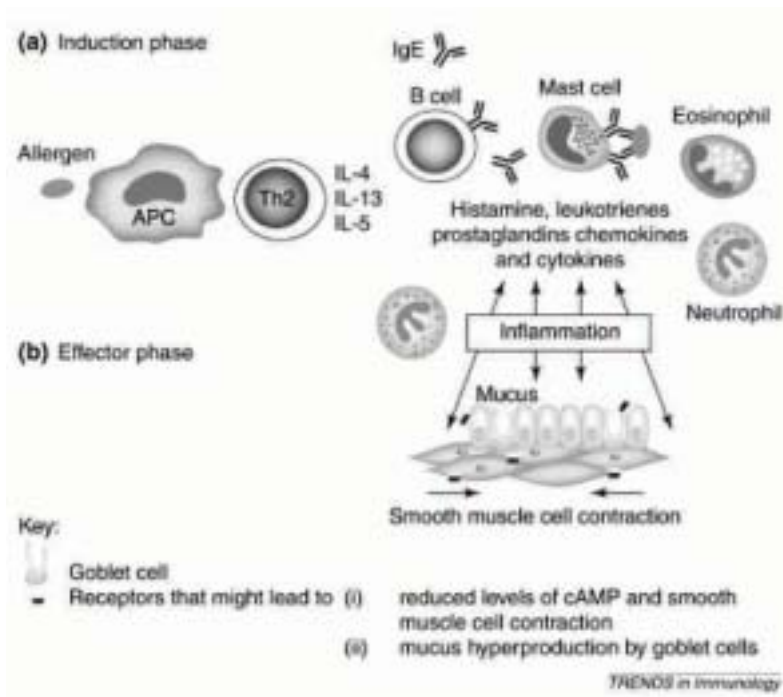
Figure 5. The development of allergic disease [476].
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Allergic reactions can be prevented by avoiding contact with allergens, a process that can prove to be very difficult as exposure to minute amounts of allergen could still precipitate an attack. Medication is often used to block the inflammation, for instance by using corticosteroids, or to limit the inflammation after IgE-induced activation of mast cells by using antihistamines. However, continuous use of drugs such as corticosteroid can lead to unacceptable side effect. Another method that was introduced in 1911, allergen specific immunotherapy (SIT), was applied to allergic rhinitis caused by grass pollen [225]. This method was reported in a well controlled study to be reasonably safe and effective [226] and so far has been applied to allergic rhinitis, allergic conjunctivitis, allergic asthma and allergic reaction to *Hymenoptera* venom, and the allergens for which immunotherapy is known to be effective are *Hymenoptera* venom, pollen, cat dander, dust mites, cockroaches and fungi [227]. The immunological mechanism behind prevention/treatment of allergies by immunotherapy is not fully understood but many groups report it to be via induction of Tregs [134;228].

SIT or desensitization has been shown to lead to an increase Tregs which express IL-10 [229]. SIT is accompanied by the induction of a strong allergen-specific IgG4 response [230]. These antibodies have been implicated to block IgE-facilitated antigen presentation to allergen-specific Th2 cells [231]. In addition, they have been shown to inhibit activation

of effector cells of the immediate allergic response, i.e. basophils and mast-cells [232-234]. Direct competition between IgG4 and IgE for binding of allergen has been proposed as the mechanism of this inhibitory effect. Moreover, mixed complex of allergen-specific IgE and IgG4 bridged by allergen was shown to cross-link high-affinity IgE receptors (FcεRI) and low-affinity IgG-receptors (FcγRIIb), resulting in inhibition of allergen-induced mediator release from basophils [235-237]. In addition, alpha-1 protease inhibitor (α1PI) has been reported to be a potent co-stimulus for IgE versus IgG4 synthesis and the equilibrium between protease/protease inhibitor may participate in the control of human IgE and IgG4 synthesis [238]. Thus, inflammatory response driven by Tregs is a crucial element of successful SIT and the humoral arm of the immune system most likely synergizes with it. The regulatory responses expressed as supressory cytokines, Tregs or high IgG4:IgE ratios seems to present pathways that can alter the course of allergic disorders.

Altogether, SIT is the only causal treatment available, unfortunately is not completely safe due to potential consequence of injecting allergens that could lead to anaphylaxis [239]. Thus in addition to being burdensome (years of monthly injections) it can cause severe, potentially life-threatening side-effects. High concentrations of complex allergen extracts are administered parentally for up to five years, each monthly injection giving the risk of a serious allergic reaction. Although oral and sublingual immunotherapy has been shown to be a possible route, the effects have not been as impressive as injection of allergen subcutaneously [240]. Thus searching for other alternatives such as molecules that may specifically induce regulatory cells should be considered.

10. Interaction between helminth infections and allergy

Both helminth infections and allergies are associated with elevated levels of IgE, tissue eosinophilia, mastocytosis and CD4+ T cells that preferentially secrete the Th2 cytokines IL-4, IL-5, and IL-13 [58;222]. However, the distribution of both diseases do not overlap geographically. While allergic disorders are more prevalent in developed countries, helminth infections are more common in less developed ones.

Studies carried out in areas with high or low prevalence of different helminth infections and their relationship to allergic disorders are summarized in table 1. This table shows that the effect of helminth infections on allergy varies depending on the study area and the species of parasite. While schistosome infections have consistently shown a negative association with skin prick test positivity and asthma severity [241-244], intestinal helminth infections can have no, negative or even a positive association with atopic disorders. So far there are no published data on the association between filarial infections and allergies with the exception of tropical pulmonary eosinophilia that has been described

in areas endemic for filarial infections, exhibiting clinical manifestations like asthma [245]. This symptom however, occurs in subject who are hypersensitive to filarial antigens and is more common in those sporadically exposed and often amicrofilaremic [246].

Species	Outcome	Effect	Infection	Study area
Ascaris (14%) Trichuris (16%) Necator (83%)	Asthma	-ve	direct	Papua New Guinea [247]
Intestinal helminth	SPT/IgE	-ve	deworming	Venezuela [248]
Ascaris (38%) Hookworm (10%)	Wheeze	-ve	direct	Ethiopia [170]
Ascaris/hookworm (12%)	SPT	-ve	direct	Gambia [249]
Ascaris (54%) Trichuris (46%) Ancylostoma (3%)	SPT	-ve	direct	Ecuador [250]
Ascaris (31%) Hookworm (10%)	Wheeze	-ve	direct	Ethiopia [251]
Ascaris	Asthma/rhinitis/IgE	+ve	IgE-seropositive	East Germany [252]
Ascaris (12%) Ascaris (16%) Ascaris (24.6%)	Asthma/SPT/ BHR	+ve	Direct History Past/current	China [253]
Trichuris (26%)	Eczema	+ve	direct	Ethiopia [254]
Ascaris (17%) Hookworm (2.5%)	Asthma/Wheeze/SPT	No	direct	Ethiopia [255]
Ascaris (17%) Necator (14%)	SPT	No	IgE seropositive	Ethiopia [256]
Ascaris (77%) Schistosome (55%)	Asthma (patient based)	No	direct	Tazmania [257]
Schistosome (87%)	Course of astma (patient based)	-ve	direct	Brazil [243]
Schistosome (40%)	SPT	-ve	direct	Gabon [241]
Schistosome (24%)	SPT	-ve	direct	Brazil [242]
Schistosome (24%)	SPT	-ve	direct	Brazil [244]

Table 1. Associations found between helminth infections and allergy

Schistosomes are tissue dwelling parasites, which may have a more profound and systemic effect compared with intestinal nematodes which are more isolated anatomically by being localized in the gut. Moreover, how infection status is determined, may also influence the associations found. In a Chinese study [253] where a positive association was reported between *Ascaris* and asthma/SPT, infection was determined by history of having had an infection or by the presence of antibodies to *A. lumbricoides* antigens; both are non specific criteria and most probably do not represent active infection. Moreover, the chronicity of helminth infections may play an important role in whether it is associated positively or negatively with allergic disorders has argued that the complexity of the association between geohelminth infections and allergies may be due to variation in chronicity [258]. In this paper Cooper has proposed that when the prevalence of geohelminth infections is low because of sporadic and seasonal transmission, an acute allergy-enhancing effect might be seen. As seen in the table 1, in areas where the prevalence of geohelminths is low, for

example in East Germany (IgE-Ascaris seropositivity 57%, which is expected to highly overestimate infection), China (24%) and Butajira, Ethiopia (26%), a positive association has been reported. In contrast, when the prevalence of helminth infection was high presumably because of high and continuous transmission, for example in Equador (54%) [259] and in another area of Ethiopia (31%) [170], the infections appeared to be negatively associated with allergic disorders. The protective effect of helminth infections against allergic inflammation has been strengthened by several studies in animal models [138;260-263], and is also supported by the reports that removal of parasites by chemotherapy results in increased skin-test reactivity to HDM [96;248;264]. In figure 6, the possible relationship between geohelminth infection prevalences and atopic disorders is depicted.
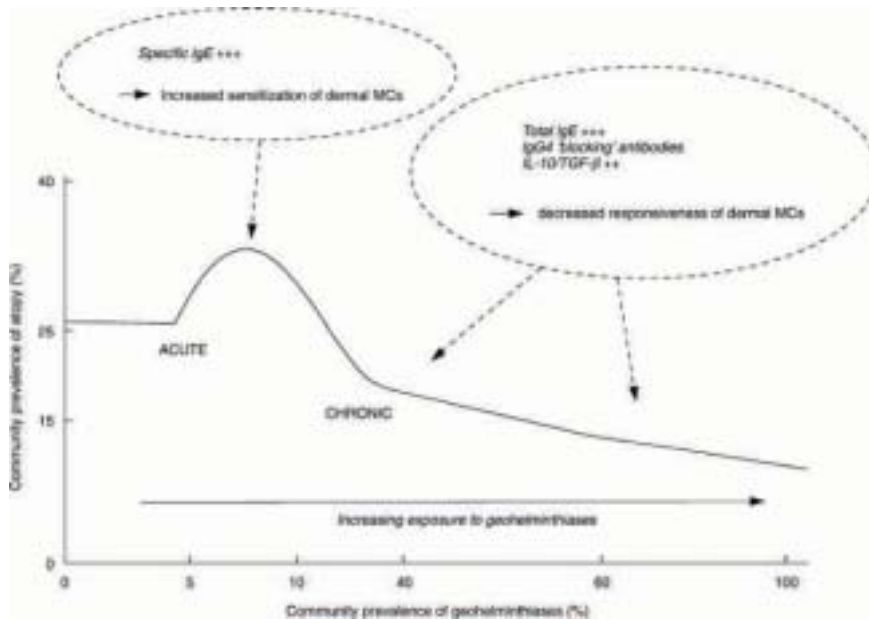

Figure 6. The effect of 'acute' *versus* 'chronic' geohelminth infections on allergy [256]. *Reproduced with permission from Blackwell Publishing, Inc.*

With regards to the fact that allergy and helminth infection are both marked by Th2 and production of IgE, several mechanisms have been put forward to explain the negative association between them:

Mast cell saturation. Total non specific-IgE is highly up regulated during helminth infections. This polyclonal antibody may saturate FcεRI on mast cells and make it impossible for allergen specific IgE to bind to high affinity IgE receptors [248;249]. However, two studies from Africa failed to show any contribution of total IgE to parasite-mediated suppression of atopic disorders [251;265]. Moreover, mast cells can accommodate additional binding when concentration of circulating IgE increases [266]

IgE cross-reactivity. Cross-reactivity between parasite antigens and allergens is regarded to be another possible mechanism whereby allergic reactivity is not seen to allergens in helminth infected subjects. A cross-reactivity between *Anisakis simplex* and several dust mites has been reported [267], and the antigenic and allergenic cross-reactivity between this worm and other nematodes is a well-known phenomenon [268;269]. Helminth parasites and HDM are known to have several structures in common such as gluthatione-S-Transferase, paramyosine and tropomyosine [270]. Moreover, such cross reactive antibodies are thought to be of low affinity and not able to lead to mast cell degranulation [271]. The IgE reactivity to HDM in infected subjects might then be as a result of cross reactive IgE antibodies. Thus the high IgE to HDM seen in some parasitized individuals who have no skin prick test reactivity to HDM, might indeed represent cross reactive biologically poorly reactive IgE. However, this theory is not fully supported by a study in Gabon where no cross reactivity between HDM and extract antigens was found (van den Biggelaar *et al.*, unpublished as indicated in [265]).

IgG blocking antibody. IgG antibodies are thought to be able to inhibit IgE binding to allergens by neutralizing allergen molecules before they could interact with IgE antibodies bound to FcεRI on mast cells and basophils [272] or inhibit mast cell signalling by cross-linking the FcεRI to the immunoreceptor tyrosine inhibition motif-containing inhibitory receptor FcγRIIb [235-237]. Indeed IgG4 antibodies to parasite antigen that are associated with asymptomatic helminth infection have been reported to block IgE-mediated allergic response to parasite antigen [9]. IgG4 isotype is also produced during allergen immunotherapy, and the success of immunotherapy is marked by IgG4 production even though at the same time the patient also produces specific IgE to allergens [273]. It then has to be postulated that in helminth infections, where IgG4 antibodies are up-regulated, IgE mediated mast cells degranulation is inhibited by IgG4. However, in a study carried out on Gabonese sera, IgG4 antibodies to allergens were not elevated in helminth infected individuals (van den Biggelaar *et al.*, unpublished as indicated in [265]).

Other possible mechanisms whereby helminth infections can suppress allergic disorders

Immune hyporesponsiveness has been reported in helminth infections, as an immunological state that may be beneficial to the parasite because this would allow long term parasite survival in the human host. For the host, immune hyporesponsiveness may also be beneficial as it will prevent excessive inflammation and resultant tissue damage as a consequence of the presence of parasites. Indeed, it is known that clinical pathology like elephantiasis in filarial infections only affects individuals who over react to the parasite. The data indicating that helminth infections might suppress allergic inflammation as well as autoimmune diseases like diabetes mellitus type 1, exacerbate multiple sclerosis and crohn diseases [274-276], raises the question whether immune

hyporesponsiveness could play a role in the negative association between chronic helminth infection and allergic disorders.

It has been noted that helminth antigen specific immunological hyporesponsiveness may spill over to non-related antigens. Several studies in areas endemic for helminth infections have reported that T-cell proliferation as well as cytokine production in response to vaccines such as bacillus Calmette-Guerin (BCG) [277-279] as well as toxoid tetanus (TT) [280-282] is lower in helminth infected subjects compared to non-infected ones, and that anti-helminth chemotherapy before or after vaccination increased BCG-vaccine efficacy by inducing T- cells proliferation as well as IFN-γ production. This spill over suppression might also affect reactions to allergens. The precise mechanisms whereby allergic disorders can be suppressed during chronic helminth infections are not completely understood. It has been proposed that Tregs may play a role although the question remains as to whether suppression is antigen specific or not and long lasting or not. The suppressory cytokines such as IL-10 and TGF-β exert bystander suppression. However, it is also possible that immune saturation/exhaustion as a result of chronic helminth infection no longer allows strong inflammatory reactions to take place.

11. Adverse effect that immune regulation induced by helminth might harm

The other side of the coin is that down-regulated inflammatory response would be expected to result in compromised ability for the host to react vigorously to invading pathogens; a serious problem particularly in areas where infections are rampant. Individuals who are chronically infected with helminths, when invaded by another organism, may react less strongly in terms of Th1 or Th2 under the influence of regulatory mechanisms. Thus microorganisms may be attacked insufficiently. However, under the influence of a strong regulatory network these infections may not lead to strong inflammation or tissue damage therefore not become severe/fatal. Some support for this possibility comes from Thailand, where helminth infections are associated with high *Plasmodium falciparum* infections but prevalence of severe malaria is low in helminth-infected individuals [283]. The fact that helminth infected subjects may be at a higher risk of contracting infections emphasizes the need for effective vaccines. Given that it was mentioned above that responses to vaccines such as BCG and TT are weaker in helminth infected subjects, extra efforts have to be made to find new formulations that are effective in helminth positive subjects.

12. Adjuvants to enhance immune response

A prerequisite for a good immune response to a vaccine is a state of inflammation. Pure proteins lead to poor immune responses, thus contaminating pure antigens with adjuvants such as killed pathogens or microbial products to magnify the acquired immune responses

is important. Natural infections as well as artificial ones are recognized by the immune system via pattern recognition receptors (PRRS) such as TLR receptors. These receptors are expressed on the surface or intracellular compartments of the resident cells of the innate immune system [284]. Immune responses can be effectively initiated if receptors such as TLRs are cross-linked to their ligands (figure 7).
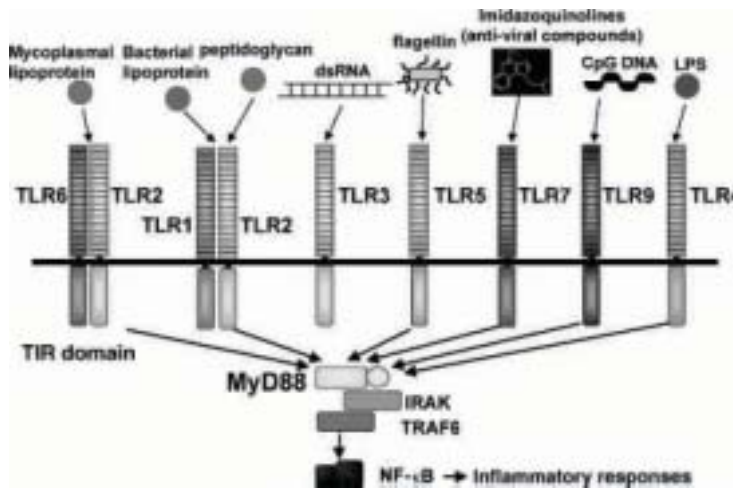

Figure 7. TLRs recognize molecular pattern associated with bacterial pathogens and induce immune responses [482].
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So far there are eleven members of the TLR family identified in humans: TLR2 recognizes peptidoglycans, in addition to the lipoproteins and lipopeptides of gram-positive bacteria and mycoplasma lipopeptide [139;285]; TLR2 collaborates with its relatives TLR1 and TLR6 to discriminate between the molecular structures of diacyl and triacyl lipopeptides, respectively [139]; TLR3 recognizes double-stranded (ds)RNA generated during virus replication [286]; TLR4, the first TLR member to be discovered, is a receptor of LPS, the outer membrane component of Gram-negative bacteria [139]; TLR5 recognizes flagellin, a protein component of bacterial flagella [287]; TLR7 is close relative to TLR8 and both can recognize single-stranded (ss)RNA [288]; TLR9 mediates the recognition of the unmethylated 2' -deoxyribo cytidine-phosphate-guanosine (CpG) DNA motifs found in bacteria and DNA viruses [289]; the ligand of TLR10 has not been identified yet, but genomic studies indicate that TLR10 is in a locus that also contains TLR1 and TLR6 [290] and the last, TLR11 was found to be involved in recognition of uropathigenic bacterial products [291]. Following pathogen recognition, TLR initiate intracellular signal transduction that results in the expression of genes involved in inflammation, anti-microbial

responses and maturation of dendritic cells [139]. Each TLR activate common and unique transcription factors through different signaling pathways to drive specific biological responses against microorganisms

Since helminth infections are accompanied by anti-inflammatory cytokines, the induction of pro-inflammatory responses may be compromised. There is very little known about the TLR activity in helminth infected subjects. As already discussed earlier, two studies in schistosome and filarial infections have shown that innate responses may be affected during these infections [143;144]. It is important to consider the immune responses of individuals residing in areas endemic for multiplicity of infections when testing novel adjuvants such as TLR ligands. Such studies might help to develop new formulation for vaccines that are effective in the population living in environments with high microbial burdens that are most in need of improved or novel vaccines.

Scope of this thesis
In general the studies described in this thesis focus on helminth infections and allergy in Indonesian populations in South Sulawesi.

Chapter 2. Antibody IgE response is one of the hallmarks of both helminth infections and allergies. While in helminth infections, IgE is commonly measured using ELISA, in the field of allergy the IgE antibody production in response to allergens is mostly measured by RAST. Due to the importance of IgE in both diseases we compared the ELISA versus the RAST method in measuring specific IgE in areas endemic for lymphatic filariasis to assess and compare the performance of these tests.

Chapter 3. Prenatal sensitization is one possible factor that may influence outcome of helminth infections, in this chapter we addressed this issue by measuring the correlation between specific and polyclonal IgG4 and IgE antibodies in children and their mothers. To gain a better understanding of parental influence, in a second set of samples we compared maternal and paternal influences on the development of specific IgG4 as a surrogate marker of filarial infection in children

Chapter 4. Epidemiological surveys in endemic areas have documented the influence of household and family factors on filarial infection; such studies may help shed light on elements that control the acquisition of pathogens and subsequent outcomes of host-pathogen interaction, which in the long term might help design appropriate control strategies. Previous studies on filariasis clustering had shown that family, household and environmental play a role. The question of how differently these factors affect pattern of infection in young versus old was examined. This knowledge is important when deciding

which subjects should be used for genomic analysis to identify genes that link to susceptibility or resistance to infection.

Chapter 5. Information on allergy in Indonesian populations is rather limited, therefore we measured allergy in two urban schoolchildren with different socioeconomic status. Using ISAAC questionnaires and objective parameters of allergy we compared the prevalence of allergy in both schools. The appropriate of using ISAAC in developing country is discussed.

Chapter 6. Several factors have been hypothesized to explain the difference of allergy prevalences between developed-developing countries or between urban-rural areas within a country. However, how these factor influence allergy and atopy in Indonesia has never been addressed. To investigate this, we initiated a study at two schools with children from different socioeconomic backgrounds. Parental education, number of siblings, exposure to animals, presence of smoker in the house, nutritional status and helminth infections were measured to see how these factors influence the allergic phenotype in the study population.

Chapter 7. With the finding of several genes that are linked to allergy, it is clear that allergy is influenced by genetic factors. In helminth endemic areas, the Th2 stimulating activity of helminth infections may interfere with genetic studies that aim to link genes to allergy in such areas. We set out to determine the extent to which the genetic models could explain occurrence of allergies in an area where filarial infections are endemic.

Chapter 8. Lower efficacy of vaccination had been observed in several areas endemic for helminth infections, and this might be under the influence of anti-inflammatory cytokines that are produced during chronic helminth infections. It is therefore important to search for candidate adjuvants that have strong pro-inflammatory properties and that perform well in such areas. In this chapter we compared pro- and anti-inflammatory responses to several TLR ligands in children heavily and lightly infected with helminths to start identifying TLR pathways that perform well in areas endemic for helminth infections.