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

Wahyuni, Sitti

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# Summary

Lymphatic filariasis is a vector born disease with a wide distribution throughout the tropics and subtropics. In Indonesia, although the prevalence is not high, data from Indonesian ministry of health revealed that none of the areas screened since the first year of the third millennium were free of this infection. Indeed, mass treatment implemented since the early eighties showed a significant decrease of the prevalence compared to the earlier three decades, but obviously no eradication.

In endemic areas, most individuals show cellular and humoral immune responses to the parasite. However, the responses may differ within the population depending on infection status and clinical manifestations, which range from asymptomatic infected subjects to those exhibiting chronic pathology.

Innate immunity and acquired immunity, linked to the genetic makeup of a host are thought to regulate gain of filarial infection and disease outcome. However, efforts to identify genes responsible for different outcomes of infection and disease have given inconsistent results. To improve genetic studies of complex traits, it is important to have good insight into the influence of environmental factors that might play an important role in gain of infection and progression to disease. There is relatively little information on the influence of family and household factors on the clustering behavior of lymphatic filariasis in endemic villages.

There is an interesting link between helminth infections and allergy, both share strong T helper (Th)<sub>2</sub> responses but their distribution worldwide does not seem to overlap, at a macro level. At a more detailed level, studies on the association between helminths and allergies carried out in many countries have reported positive, negative and no association, indicating the variability of environment factors and possibly genetic factors that may determine the nature of the association. The question how these two diseases might associate or dissociate at the village level is worth exploring to start gaining insight into the genetic and environmental control of helminth infections and allergies and their interactions.

Using a standard questionnaire of allergy the International Studies of Asthma and Allergy in Childhood (ISAAC) investigated the worldwide prevalence of asthma, allergic rhinoconjunctivitis, and atopic dermatitis. Indonesia was also a participant country in this study and it was found that Indonesia was one of the countries with lowest prevalence of allergy in the world. Since the study was done only in one area in Java, the data might not represent the prevalence in the whole country. Moreover, Indonesia has highly diverse populations and the questionnaires that were used may only be understood by some of the population but not by the others. Additional tools such as objective parameter may

also be needed to assess allergy in a country with heterogeneity in language, socioeconomic status and culture such as in Indonesia.

Several investigations on the association between helminth infections and allergy in humans have found that helminth infections protect from the development of allergies [170;241;242;244;250;251]. These findings have to be confirmed in trials with anti helminth treatment [96;248;264] and as already underway, in trials where infectious or non infectious parasitic helminths are given. The question of how the presence of helminth infections affects innate immune responses to possible adjuvants or to bacterial/viral infections, needs to be addressed to pave the way for optimal policy development regarding anti helminth treatment campaigns

The work presented in this thesis was carried out in two areas in South Sulawesi, Indonesia. The first was in the Mamuju district where total populations in communities in the area were surveyed and the second was carried out in Makassar where school children from two schools situated in different parts of town were recruited into the study. The studies presented in this thesis aim to: i) evaluate risk factors determining the outcome of filarial infections, ii) seek association between the prevalence of helminth infection and allergy, iii) measure the influence of several factors on allergy and atopy, iv) investigate the clustering of allergy in communities where helminth infections are highly prevalent and v) analyze the innate immune responses of children with and without helminth infections to respond to microbial products.

The thesis begins with general introduction as described in **chapter one**. This covers lifecycle of several helminth infections of relevance to this thesis that are prevalent in Indonesia and also cites most recent data on the prevalence of the infections in this area. The ability of helminth parasites to influence immune responses in both humans and experimental animal models is considered next and the possible mechanism by which these parasites down-regulate immune responses is described in detail. Further, factors regulating gain of helminth infection as well as how these factors are investigated in epidemiological studies is described. The second part of the introduction provides information on allergy worldwide and in particular the situation in Indonesia followed by the description of what is known as the immunological basis of allergic disorders. The interaction between helminth infection and allergy and possible mechanisms whereby helminth infections can suppress allergic disorders is given in some detail. The adverse effect of immune regulation induced by helminth infections is described and in the last part of this chapter the account of how adjuvants may enhance immune responses by engaging certain components of the innate immunity is given.

The subsequent chapters that describe the results of the investigations fall into two broad areas: the first focusing on filarial infection, Ig (immunoglobulin)E levels and risk factors for infection while the second deals with the possible association between nematode infections and allergy.

Enzyme linked immunosorbent assay (ELISA) and radioallergosorbent test (RAST) are two methods that can be used to detect specific IgE in both helminth infections and allergy. In **chapter two**, the two methods for detection of IgE antibodies to *Brugia malayi* were compared using a large number of sera from individuals from Salubarana and Kalia villages where lymphatic filariasis is endemic. A strong correlation was found between RAST, a less field applicable method, and the user friendly ELISA. The sensitivity of RAST was greater compared to ELISA caused by the superior ability of RAST to detect very low levels of IgE. This indicates that for immunoepidemiological studies it is possible to employ the ELISA method. However, due to its relatively low sensitivity, in areas with a low intensity of transmission or in areas where control programs are implemented, the RAST method would have to be the method of choice.

One possible factor that may influence outcome of helminth infections is prenatal sensitization. In **chapter three**, by conducting two large studies, the first only mothers and their offspring, while the second included both parents, specific antibodies in mothers and their offspring were correlated. The results showed that levels of specific IgE and IgG4 in children up to 10 years were correlated with maternal antibody levels and support the hypothesis that children can become sensitized to filarial antigens in utero. In contrast, no association was observed between IgG4 levels of children and their fathers in children up to the age of four, supporting the intrauterine effects rather than environmental/household ones. The importance of the environmental factors in influencing IgG4, is supported by the strong correlation between antibody levels in older children and their fathers and between maternal IgG4 and paternal IgG4.

Previous epidemiological studies on clustering of filariasis had shown that family, household and environmental factors play a role in controlling the acquisition of these pathogens and subsequent outcomes of the host-pathogen interaction. However, how these factors affect pattern of infection in young versus old had never been addressed. In **chapter four**, using a sophisticated statistical analysis, it was shown that both genetic and environmental factors could contribute to the clustering of filarial infection in the whole population. By analysing the data in an age-structured manner, it was shown that genetic factors had a more pronounced effect in children than in adults. The data indicate that genotyping studies that are to be conducted on populations affected by brugian filariasis could concentrate efforts and resources on the typing of younger age groups where

environmental effects have not yet overruled the genetic influences on gain/loss of infection.

Compared to non tropical countries, the prevalence of allergies in tropical areas is lower. In contrast, helminth infections in the tropics are more prevalent raising the possibility that an inverse association exists between the two diseases. To start exploring this association in Indonesian populations, the performance of modified ISAAC questionnaire translated into Bahasa Indonesia to measure the incidence of asthma and other atopic diseases was assessed. Two schools of children from families with different socioeconomic status (SES) were studied. As shown in **chapter five**, the study highlighted a poor correlation between symptoms reported by parents and diagnosis of allergic disease by health workers with the exception of the association between wheezing and asthma in the high SES school, which underscores the need for the use of objective measurements, such as skin prick test (SPT) and IgE levels when conducting epidemiological studies of allergic diseases in Indonesia. Furthermore, the assessment of SPT and IgE levels in the school children indicated that these parameters have a better predictive value for allergic diseases in the high-SES school compared with those in low-SES school.

Several factors have been hypothesized to be responsible for the increase in allergic disorders seen in developed countries as a result of large-scale studies carried out in many of the industrialized nations. However, investigations of risk and protective factors associated with variation in the prevalence of allergic disorders in developing countries are limited. **Chapter six** analyses the same cohort of children described in chapter 5 with a view to determining the incidence of asthma and atopy in relation to various environmental factors, nutritional status and helminth infection. The incidence of asthma was similar in both schools, but children from the low-SES school were significantly less likely to be SPT positive. Further breakdown of the high-SES school demonstrated that a lower category of SES was associated with a significant decrease in incidence of atopy but not of asthma. Although many of the factors considered (family size, presence of animal, helminth infections and etc.) showed no significant effect on asthma and atopy in the low-SES school, there was a tendency in the high SES-school for children with no helminth infections to have more asthma. In addition, children in the high-SES school showed a significant negative correlation between nutritional status and SPT or IgE positivity. The study did not show a statistically significant role for helminths in inhibiting SPT responses.

There is strong evidence for the ability of genetic factors to influence allergic disorders as documented from many studies mainly in western countries but whether this is also observed in areas where helminth infections are endemic and can influence immune

responses by inducing Th2 skewing has never been investigated. In **chapter seven**, using the same population and statistical methods as in chapter 4, the clustering of allergy within families and households in areas endemic for filarial infection was analyzed. It was found that genetic factors contribute significantly to both total and allergen-specific IgE, whereas environmental factors influence the clustering of SPT positivity. There was some indication that the presence of filarial infection as defined by microfilaria in the blood, but not by elevated anti-filarial IgG4 levels, protects against SPT reactivity to mite allergens. This raises the interesting question of whether the level of helminth infection must reach a threshold before a protective effect on SPT to allergens is observed.

Due to the capacity of helminths to down-regulate the immune responses not only to helminth antigens but also to third party antigens, the question whether these infections alter also responses to ligands that engage toll like receptor (TLR)s and which ligands are particularly strong in stimulating a pro inflammatory response was addressed in **chapter eight**. Using various ligands for different TLRs, the ratio of pro-(TNF(tumor necrosis factor)- $\alpha$ ) and anti-inflammatory (IL(interleukin)-10) cytokines was measured in children infected with high load of intestinal nematodes versus lightly infected/uninfected controls. The major findings were that i) TLR-7 ligand induced the strongest pro-inflammatory response in both uninfected and infected children and ii) IL-10 levels in response to lipopolisacharyde (LPS), a TLR-4 ligand were sustained at 72 hrs of stimulation in children with heavy worm burdens compared to those with light burdens or with no infection. The latter results demonstrated that the dynamic of cytokine production following the innate immune stimulation can be different according to infection status. The data generated on the different pro inflammatory capacity of the different TLR ligands, can have implications for optimal vaccine design in helminth-infected children