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chapter 7



Summarizing Discussion

To de-worm or to re-worm:
the impact of helminth infections
on co-infections and on health
outcomes on Flores island, Indonesia

Abstract

Many countries are currently at various stages of an “epidemiological transition” in which infectious disease is replaced by cardiovascular disease (CVD) as the major cause of death. Evidence suggests that a sedentary lifestyle, with low physical activity and excessive caloric intake, are risk factors for metabolic syndrome and CVD. In addition to life style, low grade chronic inflammation seems to also be associated with CVD. In fact, the so called inflammatory diseases such as allergies, autoimmunities, inflammatory bowel diseases are all on the rise in affluent countries and in urban centers of low-to-middle income countries (LMIC). It is interesting, that certain infections, such as bacterial or protozoan, lead to inflammation, which when uncontrolled can lead to death, while others, such as helminth infections are generally believed to lead to anti-inflammatory responses. As helminth infections can induce host immune hyporesponsiveness, we hypothesized that this would affect co-infections, such as malaria, and inflammatory diseases, such as allergies, and that deworming would modulate these effects. The variability of the geographical and disease landscapes in Indonesia provided an opportunity to investigate whether helminth infections and a deworming program could have an impact on co-infections and other health outcomes. This thesis describes the results of a longitudinal study on a household-based cluster-randomized double-blinded placebo-controlled anthelmintic trial on malarial parasitemia, allergy and immune responsiveness, with particular focus on malarial parasitemia. In addition and as a starting point for future studies, a cross-sectional study in a subset population on the association between helminth infection and risk factors for CVD and insulin resistance is also reported.

Thesis outline

The previous chapters of this thesis described the findings of our study on the Island of Flores, Indonesia, where we explored the hypothesis that helminth infections, by inducing immune hyporesponsiveness, have an impact on co-infections (lead to higher malarial parasitemia and lower malaria disease) and on inflammatory diseases (suppress allergic reactions and allergic inflammation). We investigated whether periodic anthelmintic treatment, using single dose albendazole, can alleviate helminth-induced immune hyporesponsiveness. In **Chapter 1**, we reviewed and discussed current knowledge of major helminth infections in the shaping of host immune responses, consequences for other infections, and the outlook related to the increasing prevalence of allergy, autoimmune disease, type-2 diabetes (T2D) and CVD. In the context of malaria, while a host requires adequate pro-inflammatory responses as protection against malarial parasites (1), the inability to control inflammatory responses can be associated with pathology (2;3). The question can be posed as to whether deworming in areas where malaria is co-endemic can improve the clearance of malarial parasites but increase risk of unwanted clinical outcomes (**Chapter 2**). Using a household-clustered randomized double-blinded placebo-controlled anthelmintic trial, we investigated the effects of reducing helminth infections on the prevalence of malarial parasitemia in albendazole-treated group compared to a placebo-treated group (**Chapter 3**). We also asked whether we can alleviate helminth-induced immune hyporesponsiveness in the albendazole treatment group (**Chapter 4**). The household-clustered treatment randomization was aimed at reducing possible infection through other household members. As inflammation is a known risk factor for T2D and CVD (4;5), we explored the possibility that helminth-induced hyporesponsiveness might have a beneficial effect on preventing the development of T2D and CVD (6). We therefore investigated the association between helminth infection and risk factors for CVD and carotid intima media thickness (cIMT) (**Chapter 5**), as well as between the parasite and metabolic parameters and insulin resistance (IR) (**Chapter 6**).

Introduction

The term “epidemiological transition” is used to classify a population undergoing a transition from a state where infectious diseases (communicable diseases) as the major cause of death to a state in which CVD (non-communicable diseases) is the main cause of death (7). This transition is at different stages in different regions of the world. In industrial/developed countries (also known as high income countries [HIC]), infectious diseases are relatively well controlled compared to developing or low-to-middle income countries (LMIC), especially when compared to rural areas of LMIC. Causes of death in HIC are mainly due to degenerative diseases or to metabolic syndrome (MetS) related non-communicable diseases such as T2D and CVD (8). The results of a global disease burden assessment were reported recently and in some HIC regions, although still showing a high incidence, CVD is now better controlled (9). However, while the LMIC, which include the majority of the world’s population, are still struggling with infectious diseases, in their urban areas, they are also facing the emergence of health problems similar to those of HIC. Indonesia is one of the emerging world economies and covers a large geographic area that includes a constellation of islands between the Asian and the Australian

continental plates, forming a crossroad between the Pacific and Indian Oceans. In terms of lifestyle and disease landscape, the urban areas in Indonesia are reasonably comparable to developed countries and deworming programs in these areas have been reported (10). The recent improvements in infrastructure and control of infectious diseases in rural and semi-urban areas may represent a paradigm for the transition to a sedentary lifestyle coupled to a decline in infections (also including reduced helminth infections). A better understanding of the dynamic changes between communicable and non-communicable diseases could help guide a healthier transition.

Helminth parasites generally cause chronic infections in their host, which results from their ability to influence the immune system of the host leading to immune hyporesponsiveness. This ensures the long term survival of the parasites in the human host while possibly benefiting the host by preventing immune pathological reactions (11). As it can be assumed that both the host and the helminth evolve within this relationship, a sudden deworming process might disrupt this relationship. It is important to note that helminth induced immune hyporesponsiveness may also affect host responses to bystander antigens (12). In rural LMIC, helminth infections are commonly co-endemic with malaria, tuberculosis (TB) or HIV/AIDS (13) and therefore the question what the consequence of deworming will be on co-endemic infections needs to be answered. Helminths are also thought to be associated with a lower incidence of allergy (14) and autoimmune disease (15) again raising the question whether these diseases patterns would change in the absence of helminth infections. The prevalence of T2D and CVD in rural LMIC, while increasing, are lower than urban areas of LMIC or in HIC (9). The evidence of the possible association between helminth infections and T2D and CVD in experimental models is gradually becoming available but in humans this has remained largely unexplored (6).

The large geographic area of Indonesia and the widespread population, with a declining helminth burden in some places, provides an opportunity to investigate whether helminth infections and deworming have an impact on co-infections such as malaria and on other health outcomes such as allergy, T2D and CVD. Specifically, here, we discuss our investigation of the relationship between soil-transmitted helminths (STH) and malaria, allergy, IR (a marker for T2D) and atherosclerosis (a marker for CVD).

Albendazole treatment reduced but did not eliminate helminth infections

In **Chapters 3** and **4**, we showed that a two-year course of intensive albendazole treatment reduced but did not entirely eliminate helminth infections. Other longitudinal, three to four monthly interval deworming trials, using various drugs against STH have also reported difficulties in eliminating helminths (16-18). These poor results could be related to highly contaminated environment and reinfection but also to the limited efficacy of available anthelmintic drugs or the possible emergence of drug resistance (19). Altogether, in terms of egg load, these earlier studies (16-18) reported significant decreases that were similar to our own findings. Using a sensitive PCR diagnostic method, we have also shown that evaluation of deworming by microscopy can fail to identify submicroscopic infections. This represents a possible source of continuing transmission when treatment is discontinued. Given that global campaigns are underway to control and eliminate STH, our findings and those of others suggest an urgent need for new approaches in helminth elimination programmes.

The impact of deworming on malarial outcomes

Despite a significantly reduced load of helminth infections following deworming, these parasites remained, in part due to persisting *Trichuris* worms, which appear not to be affected by albendazole used as a single dose at three monthly intervals. In addition, compared to our preliminary findings during the piloting phase of the program, the prevalence of malarial parasitemia in our study population dropped at the beginning of the study and gradually declined during the study period, which led to the low level of malarial prevalence in our co-infection study. This situation affected the study power and the statistical analyses, especially at the end of the study. Nevertheless, we found that compared to placebo, the prevalence of malarial parasitemia was transiently increased in the albendazole group, when there was sufficient transmission. Findings regarding clinical symptoms were not significant (**Chapter 3**). Malaria-related clinical symptoms were also not paralleled by the malarial parasitemia findings during the study, suggesting that most malaria in the population is asymptomatic. This might be related to the previously high rates of malaria transmission in the area, which may have led to a certain degree of immunity to malaria in this population.

Regarding the recent fall in the prevalence of malarial parasitemia, a model has shown that the loss of protective immunity is gradual (22). Our malaria-infected subjects were found to be relatively asymptomatic, but malarial parasitemia prevalence was higher in the younger age group. Therefore, while both immunity to disease severity and parasitemia might be well established in the older age group, in younger children an earlier malaria exposure may have only resulted in immunity to clinical disease. The low malaria transmission may have played a role in our study, resulting in new infections that were insufficient to provoke clinical symptoms. This was indeed shown in a study in five communities in Kenya and Gambia with differing levels of malaria transmission, in which higher malaria transmission was related to greater severity of malaria (23).

The increased risk of malarial parasitemia was not expected. However, a recent review by Nacher suggested that *Ascaris* infection is associated with protection against malaria disease or parasitemia (20), perhaps indicating that the increased risk for malarial parasitemia in our study participants may have been caused by a reduced level of *Ascaris* infection. However, as our study was not designed to stratify the analyses based on helminth species at baseline, the power of the study was insufficient to address this specific question. The same problem precluded the investigation of the effects of deworming on different malaria species.

Another question is why the effect of albendazole treatment was primarily confined to individuals >15 years of age. A possible explanation is that the lower *Ascaris* infections in the older age group compared with the younger, may mean better reduction of infection and therefore more profound effect on malarial parasitemia in those older than 15 years of age. Similar findings were reported from a small study in Madagascar where higher malarial parasitemia was seen in older age group, the group in which *Ascaris* egg loads were strongly decreased following anthelmintic treatment (16;21).

Deworming to improve immune responsiveness

Through investigations in children infected with STH, we showed that depletion of regulatory T cells (Treg) improved responses to in vitro malaria antigen stimulation (18). Indeed, we found that in vitro immune responses were enhanced after albendazole treatment and significant

increase in malaria-specific and mitogen-induced tumor necrosis factor (TNF) and interferon (IFN)- γ cytokine production was seen (**Chapter 4**). However, our hypothesis that alleviation of immune suppression by helminth infections would stimulate a better clearance of malarial parasites (and therefore result in lower prevalence of this parasite in our study population), in parallel with an increase in clinical outcomes, was not clearly resolved. As mentioned previously, albendazole treatment had neither a beneficial nor a detrimental impact in terms of clinical outcomes (**Chapter 3**). Despite the possibly already established immunity to clinical malaria, one can speculate that the increased pro-inflammatory responses to the malarial parasite observed during the trial may not be sufficient to cause clinical symptoms. This could be due to the maintenance of a certain level of anti-inflammatory responses, because of incomplete deworming that resulted in a sufficient immune suppression to prevent the development of clinical symptoms.

Two years of deworming has a minimal impact on allergy outcomes

In brief, we also found no significant impact of albendazole treatment on allergy outcomes. Although not statistically significant, the trend for increased skin prick test (SPT) reactivity was in line with our hypothesis. The risk of SPT reactivity increased incrementally with longer treatment and raises the question of whether even longer deworming periods are needed to produce more pronounced effects on allergic outcomes, as seen in a study where 15-17 years of ivermectin treatment for onchocerciasis in Ecuador resulted in increased prevalence of SPT reactivity (24). The question of whether this is related to differences in the prevalence and type of helminth infections remains unanswered. As with investigations of helminth-malaria co-infection outcomes, further research is needed before sufficient information is available on whether helminths may provide protection or actually increase the risk for the development of allergies as suggested by some investigations (25;26).

Helminth infection is negatively associated with risk factors for T2D and CVD

We have shown in cross sectional manner, in **Chapter 5**, that STH infection was associated with lower risk factors for CVD. Individuals with helminth infections had a significantly lower body mass index (BMI) or waist-to-hip ratio, and lower lipid levels, compared to uninfected individuals. However, a direct association between helminth infection and CVD markers, such as cIMT as a marker of atherosclerosis (**Chapter 5**), was not clear cut. In addition, no significant difference in fasting blood glucose (FBG) was found between infected and uninfected individuals. Indeed, the cIMT of our study participants was relatively low compared to individuals of the same age living in HIC (27;28). It is possible that cIMT level and FBG are still within the normal range, and therefore could not be affected by helminth infections.

In **Chapter 6** we have shown that helminth infection was associated with improved insulin sensitivity, despite this, there was no significant difference in FBG level as already alluded to. The improvement in insulin sensitivity seemed to be related, in part, to lower levels of insulin. This lower insulin level might be due to less insulin being needed to maintain normal blood glucose in individuals with helminth infections.

In line with our study, where we showed helminth infections were associated with lower lipids, in apoE^{-/-} mice, factors released from *Schistosoma mansoni* eggs appeared to have

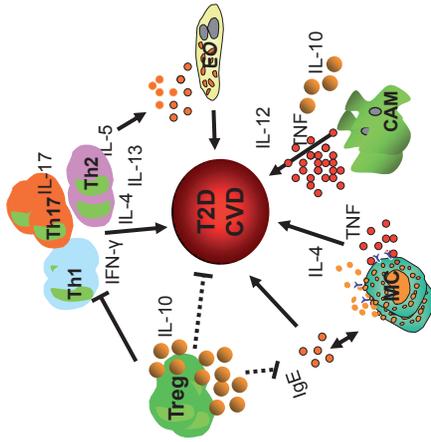
lipid-lowering effects (29). Indeed, it was recently shown in an experimental hookworm model that infection with *Nippostrongylus brasiliensis* could prevent obesity, as well as reduce lipid levels and improve insulin sensitivity (30). The authors showed that the protective effect was associated with eosinophilia induced by helminth infection and was related to interleukin (IL)-4 mediated alternatively activated macrophages in adipose tissue. Moreover, Bhargava et al. also demonstrated that lacto-N-fucopentose III, an immunomodulatory glycan that can be found in human milk and in parasitic helminths (in *S. mansoni* soluble egg antigen), could improve insulin sensitivity by enhancing white adipose tissue insulin signalling through induction of IL-10 production (31). Whether helminth infections lead to similar changes in humans still requires further investigation (Fig. 1).

C-reactive protein (CRP) is commonly measured as a marker of inflammation (32). Measuring high sensitivity (hs)-CRP in our study participants, we found a very low level of hs-CRP despite the probable high infection pressure in the community. As blood samples were analyzed in a laboratory facility in the Netherlands that carries out routine measurements using a gold standard assay, differences in laboratory protocols cannot account for the low hs-CRP level. Although studies in Philippines (33) and Ghana (34) show also low level of CRP in rural areas where high infection rates are expected, in the Tsimane Indians in Bolivia, high levels of CRP were measured (35). These variable findings need to be further investigated. Interestingly, total IgE (TlgE) levels have been reported to be inversely correlated with growth and the hs-CRP level in LMIC (36). The level of TlgE is thought to reflect the length of helminth exposure during the host lifetime (37). The inverse association is suggested to be related to a concept of trade off in the use of limited energy available to the host defence (36;37). Blackwell and colleagues have put forward the following explanation: when facing high burden of different infections, there would be a trade-off between defence against helminths (with production of TlgE) and defence against other infections, such as bacterial or protozoan, that are associated with elevated CRP (36). This argument is not entirely satisfactory, as helminth infections are altogether considered less life threatening than bacterial or protozoan infections. Alternative explanations such as the ability of helminth infections (high IgE) to suppress inflammation (low CRP) needs to be tested.

Is there a similarity in the association of helminths and CVD to that of helminths and allergy?

It might be interesting to test whether helminths or their products can be used as a treatment for CVD, as extensively highlighted in the allergy field. Around two decades ago, an elevated TlgE was shown to be associated with risk for CVD (myocardial infarction, stroke or peripheral arterial disease) in studies conducted in affluent countries (38-40). A recent study by Wang et al. (41) showed that IgE was most elevated in patients with myocardial infarction, followed by unstable angina pectoris, and least elevated in stable angina pectoris. This suggests that different types of CVD are associated with specific differences in the level of TlgE. A number of these authors have also reported increased levels of IgE and FcεR1 in the atherosclerotic lesions of patients. To demonstrate the role of IgE and FcεR1 in atherosclerotic lesions, an apoE^{-/-} mice model deficient for the FcεR1α receptor (apoE^{-/-}, FcεR1α^{-/-}) has been used and it has been demonstrated that these mice have lower occurrence of atherosclerotic lesions. Interestingly, it was also mentioned that the reduced levels of atherosclerotic lesions in apoE^{-/-}, FcεR1α^{-/-}

A. Animal models without the present of helminths or human living in non-helminth endemic areas.



B. Animal models with the present of helminths or human living in helminth endemic areas.

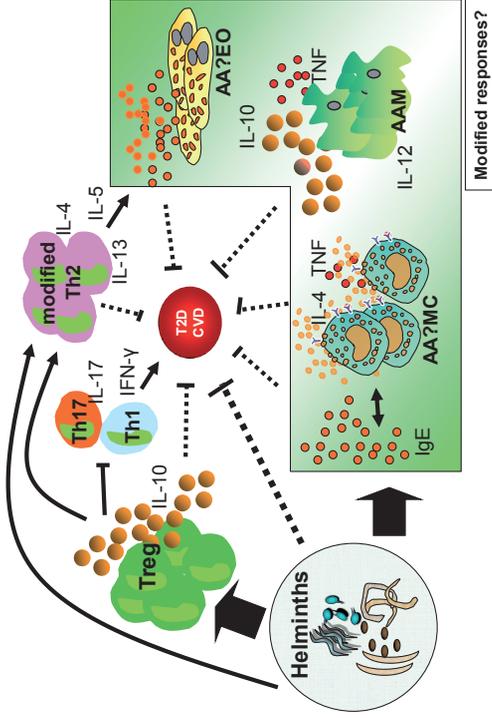


Figure 1. A possible schematic role of inflammatory cells network on type-2 diabetes and cardiovascular diseases with or without involvement of helminth infections. Various cells of the immune system seem to be involved in cardiovascular disease (CVD) or type-2 diabetes (T2D). In high income countries or in experimental models, inflammatory conditions created by the activity of inflammatory T cells such as T helper (Th) 1, Th17 and even Th2 and their cytokines, has been shown to increase the risk of T2D and CVD. Moreover, classically activated macrophages (CAM), mast cells (MC) and eosinophils (EO) have been shown to take part in the pathogenesis of the disease in target organs. However, the activity of regulatory T cells (Treg) and/or anti-inflammatory cytokines such as interleukin (IL) 10, might be able to prevent T2D and CVD (A). The situation seems to be different in rural areas of low-to middle income countries where helminth infections are highly prevalent. The same applies to animal models where the effect of helminth infections has been studied on atherosclerosis or diabetes. Helminths lead to the expansion of Treg, IL-10 and Th2 cells. During helminth infections, inflammatory cells might be modified: modified Th2, alternatively activated macrophages (AAM) have anti-inflammatory properties while eosinophils and mast cells seem no longer to behave as pro-inflammatory immune cells. Thus in presence of helminth infections, the immune system is in an anti-inflammatory mode which is considered to be beneficial against development of T2D and CVD (B). The solid lines represent associations based on data available, while dotted lines represent theoretical associations that are yet to be tested.

mice did not affect total cholesterol (TC) or LDL levels, although they were associated with increased serum triglyceride (TG) and HDL levels (41). Altogether, these results seem to be in contrast to our findings: while in western countries IgE levels were positively associated with FBG, in our study in Indonesia, a high level of TlgE was associated with lower FBG. Moreover, in contrast to studies of atherosclerosis mentioned above, we noted a negative association between TlgE, which in our studies is related to helminth exposure, and risk factors for CVD that included TC, LDL and HDL.

The question arises whether findings in CVD may have some analogy with studies of allergic disorders. In areas where helminths are not endemic, allergy is associated with high levels of TlgE, while in helminth endemic populations, high levels of TlgE are associated with helminths, but inversely correlate with allergy (14). It is tempting to speculate that the possible protective effect of living in areas where helminths are highly endemic against development of CVD is based on similar mechanisms to the protection against the development of allergy, namely, the presence of strong anti-inflammatory and modified responses (Fig. 1).

Conclusions and future perspectives

We have shown in this thesis that helminth infections were associated with hyporesponsiveness. Yet we observed no consistent beneficial or detrimental effect regarding malaria or allergy after two years of community based anthelmintic therapy, which reduced but did not eliminate helminths. We also showed that helminth infections were negatively associated with risk factors for CVD, as well as being possibly beneficial for insulin sensitivity. The role of helminths in T2D and CVD needs to be followed up.

Altogether long-term, well-powered, placebo controlled anthelmintic trial are needed with better anthelmintics to investigate the causal relationship between helminth infections and malaria and allergy. A number of issues need to be considered for such future studies:

1. more adequate anthelmintic drugs and/or schedules
2. longer deworming period
3. areas where the endemicity of malaria is high
4. use of molecular methods to determine *Trichuris* worm burden

Only then will it be possible to bridge the gap between the findings in animal models that show a beneficial effect of helminths on a number of diseases and the situation in humans.

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