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Chapter 1

General introduction
Helminth and malaria co-infection in humans: Where do we stand now?

I. Epidemiology of helminths and malaria co-infection

Parasitic helminths encompass a variety of different pathogens including filaria, schistosoma and intestinal helminths. They are widely distributed in tropical and sub-tropical areas where, as shown in figure 1, they share the same spatial distribution as malaria and usually co-infect the same human host (1,2).

Figure 1: World map showing the geographical distribution of malaria and helminth infection or co-infection. Data on malaria are indicative of the percentage of population at risk in the world in 2013. These data were obtained from the WHO 2014 world malaria report. Data on helminths (lymphatic filariasis, onchocerciasis, schistosomiasis or soil-transmitted helminthiasis) were extracted from the following source (Lustigman, S. et al. A research agenda for helminth diseases of humans: the problem of helminthiases. PLoS Negl Trop Dis 6, e1582 (2012)).

Data are still lacking on the prevalence of malaria and helminth coinfection worldwide. However because more than 75% of malaria cases occur in sub-Saharan Africa (3), it is expected that this continent also bears the highest burden of helminths and malaria co-infection. Using a mathematical modelling approach it was recently estimated that approximately one quarter of African school-aged children were at risk of co-infection with malaria and hookworm (1). However because this study based its prediction on hookworm solely we can speculate
that a higher prevalence of co-infection would have been predicted if other helminth species were considered. Population-based studies conducted in different African countries have so far confirmed this (4–10) and have furthermore indicated an influence of helminths on malaria outcomes as depicted in Figure 2. For instance it was reported that helminth-infected individuals were more susceptible (5,6,11,12) or protected (7,8) against malaria and, had a higher (9,11) or a lower (10) carriage rate of malaria parasite depending on the species and the burden of helminth infection. Moreover in studies carried out in Thailand it was noted that malaria patients who were concurrently infected with helminths presented with less fever and were less likely to develop cerebral malaria or renal failure by comparison to their helminth-uninfected counterpart (13,14). However, there are contradictory findings in other settings (6,15). In addition, the effect of helminths on malaria induced anaemia, a leading cause of mortality in sub-Saharan Africa, has also been investigated. Although studies have suggested a protective effect of helminths on malaria-induced anaemia, a meta-analysis of all available published studies was not able to confirm these findings (16).

**Figure 2:** The possible influence of helminth infections on transmission and/or clinical outcomes of malaria parasites.
Many factors can influence the associations between helminths and malaria parasites or disease (Figure 3), among them helminth species involved infection pressure and burden, age of the study subjects and their nutritional status. *In utero* exposure to helminths antigens could also be added to this list (17,18). With respect to the latter, a birth cohort study conducted in Uganda showed that children who were followed from delivery till the age of five years had an increased risk of malaria infection and morbidity if their mother was infected with *M. perstans* and hookworm during pregnancy (19). Interestingly this result shows that not only current infection but also past exposure to helminth antigens might shape the host response to malaria parasite. Considerable efforts have been put into understanding the mechanisms whereby helminths can influence malaria infection. What is currently known is that helminths have the potential to manipulate the immune system of their host in order to ensure their survival, however, this immune modulation may have significant consequences on the host response to others pathogens such as *Plasmodium spp.* (20).

**Figure 3:** Infection with helminths or *P. falciparum* results in direct interaction between these parasites with the host immune system and metabolism. But co-infection can also result in direct or indirect interaction between these two parasites within the human host (depicted inside the red square). This interaction network might however be influenced by several factors shown here outside the red box. Because this thesis focuses on the effect of helminths on malaria we have emphasized the interference caused by helminths.
II. Immune response to *Plasmodium spp.* in the context of helminth co-infection

Learning from animal models

The acquisition of a protective immune response to *Plasmodium spp.* has proven to be a complex process. It depends on a well-adjusted balance between different effector mechanisms of the host immune system at different stages of the infection (21,22). During the acute phase of malaria, the host immune response is essentially cell mediated (23,24) and involves innate mechanisms that rely on the recognition and uptake of sporozoite by antigen presenting cells (25,26) and by the release of pro-inflammatory cytokines, primarily by γδ T cells as well as Natural killer (NK) and NK T cells (27). This is followed by essentially, but not exclusively, the involvement of CD8 T cells at the liver stage (28,29) along with a variety of CD4 helper T cells subsets when the parasite reaches the bloodstream (21,23). The adaptive immune response observed during the acute phase relies on a fine equilibrium between a panel of inflammatory and anti-inflammatory cytokines that aim at stopping the replication of the parasite on the one hand and preventing host tissue damage on the other (21,30–32).

Understanding how helminths can influence anti-malaria immunity has been particularly of interest during the past decades. It is now well established that helminths have the potential to modulate the innate and adaptive immune response of their host (33,34). This immune modulation is characterized by an impairment of dendritic cell function (35), a Th2 skewed immune phenotype (36,37), and the induction of a potent regulatory network (38) leading to T cells hyporesponsiveness (39,40). In a mice model of *Plasmodium chabaudi* infection it was observed that the erythrocytic stage of *P. chabaudi* elicited a specific immune response characterized by the activation of IFN-γ producing T cells during the acute phase of the infection, followed by an increase of IL-4 producing T cells and of specific antibodies during the chronic phase. However in mice co-infected with *Heligmosomoides polygyrus* and *P. chabaudi* the presence of helminths resulted in a significantly lower production of IFN-γ along with an increase in the level of IL4, IL-10 and TGF-β in comparison to mice infected with *P. chabaudi* alone (41). As a consequence co-infected mice had a higher density of
parasitemia and higher mortality rate than those singly infected with \textit{P. chabaudi} only (41). In contrast, a study by Karadjan and co-workers showed no effect of \textit{Litosomoides sigmodontis} coinfection on the peak parasitemia of \textit{P. chabaudi} whereas a decrease was observed for \textit{Plasmodium yoelii} (42). Furthermore they did not report a difference in the level of IFN-\(\gamma\) or IL-10 between \textit{P. yoelii} single infected mice and those co-infected (42). Such heterogeneity in outcomes have been attributed to differences in study methodology, model of malaria, helminth species and the genetic background of mice used (43). However in order to distil the current body of knowledge, and take into consideration these factors, a meta-analysis was conducted. The meta-analysis focused on all available articles describing murine models of helminth and malaria parasite co-infection where the major pro-inflammatory cytokine IFN-\(\gamma\) was assessed (44). The meta-analysis confirmed a helminth-induced decrease of IFN-\(\gamma\) in malaria infected mice despite the heterogeneity observed when single reports were considered. It showed that this effect correlated with an increase of parasite density in co-infected mice but it was independent of IL-4 (42). Unfortunately their analysis did not include anti-inflammatory cytokines or the regulatory cells which are known to be increased by helminths and can down modulate the pro-inflammatory cytokine responses in murine models of malaria (45). In general, the reported impairment of the host immune response induced by helminths reflects their ability to influence anti-malaria cell mediated immunity. This impairment results from a modulation exerted both on the innate and adaptive components of the immune system. It was for example shown that schistosome antigens can effectively modulate DCs (46). In a model of \textit{H. polygyrus} and \textit{P. chabaudi} co-infection, DCs from co-infected host were less able to induce \textit{in vitro} proliferation or IFN-\(\gamma\) production by CD4\(^+\) T cells in response to malaria antigen stimulation when compared to mice infected with \textit{P. chabaudi} only (47). It should be noted that helminth infections can also result in intrinsic T cell hyporesponsiveness (48) as well as in induction of regulatory T (39,40) and regulatory B (49) cells able to dampen the function of effector T cells.

While the cellular immune response is important to reduce high patent parasitemia to low levels during the acute phase of malaria the final effective mechanism for clearance of blood stage parasites was suggested to be antibody dependent. It was indeed shown that B cell
knockout mice are unable to clear *P. chabaudi* despite their ability to reduce parasitemia during the acute infection (50). This situation led to a chronic relapsing parasitemia that was only controlled by a subsequent adoptive transfer of B cells. The role of *Plasmodium spp.* specific antibodies in anti-malaria immunity has been shown to range from blocking liver or erythrocyte invasion by parasites, preventing sequestration of infected erythrocytes in micro vasculature, mediating antibody dependent cellular immunity (ADCI) as well as preventing fertilisation of gametes (22). In the context of co-infection humoral response to *Plasmodium spp.* can be influenced by chronic helminth infections that are usually marked by Th2 skewing and regulatory responses along with an increased production of IgG4 and IgE (reviewed in (51,52)). This Th2 profile can interfere with the ADCI necessary to control *Plasmodium spp.* parasites, which rely on cytophilic antibodies (53,54). Experimental data are still scarce on how helminths influence B cells function and distribution in general, and more particularly in the context of malaria infection. Understanding this would be of particular importance given that new B cell subsets, such as regulatory B cells, implicated in immune tolerance (55), might help shed light on anti-malaria immunity.

**Learning from population-based studies**

Although studies in mice have been instrumental to understand how helminths can affect anti malaria specific immunity, they nevertheless represent a questionable model for helminth and malaria co-infections in humans (discussed in (56)). For this reason significant efforts have been devoted to determine the extent to which the findings in experimental animal models would translate into population-based studies. In helminth endemic countries where the force of the infection is relatively high, exposure to antigens can start in utero (18,57) and the first active helminth infection generally followed by repeated re-infection, happen early in life (58,59) leading to chronicity at a relatively young age. In the case of *S. haematobium*, chronic infection is marked by the release of eggs by the female worms into the bladder that cause local damage resulting in haematuria. From the perspective of the host immune response, chronic *S. haematobium* infection is characterized by a Th2 skewed immune phenotype (60) that leads to B-cells class switching towards IgG4 and IgE. The balance between these
two antibodies is thought to be important in anti-schistosoma immunity, contributing to resistance or susceptibility to re-infection. When considered in detail, studies have indicated that schistosome antigen specific IgM, IgE, IgA and IgG1 are rather protective against re-infection whereas IgG2 and IgG4 are mainly associated with susceptibility (61–64). Although the role of humoral immune response in anti-schistosoma immunity is well understood it is not very clear why it takes years for naturally exposed individuals to develop a protective antibody response. While this might result from a general state of hyporesponsiveness mediated by regulatory cells (65), a helminth induced impairment of B cell function cannot be formally ignored and needs to be assessed given the existence of reports indicating an increased proportion of exhausted B cells in individuals with chronic viral infections (66,67). Addressing this question might also help to extend our knowledge on how helminths interfere with anti-malaria immunity.

Epidemiological studies conducted in endemic areas have investigated the influence of helminths on either the cellular or the humoral immune response of individuals co-infected with *P. falciparum*. Unfortunately these studies have so far generated contradictory results that are difficult to reconcile. For example infection with *S. haematobium* in malaria co-infected children in Senegal was associated with an increase of IgG1 and IgG3 cytophylic antibodies specific to MSP119 *P. falciparum* antigen (68) whereas in Mali it did not influence the level of total IgG to AMA1 or MSP1 (69) and in Zimbabwe it was negatively correlated with IgG1, IgG4 and IgM antibodies against schizont extracts, MSP3 and GLURP (70). More studies are still needed to get a comprehensive picture of the influence of helminths on anti-malaria humoral immunity. It will also be important to determine the effect of helminths on *P. falciparum* sexual stage antibody responses. Indeed the few studies that are currently available have only focused on antibody response to *P. falciparum* asexual stage antigens whereas some data indicate that carriage of *P. falciparum* gametocytes can be increased in helminth infected subjects (71,72) possibly as a consequence of an impairment of the host immunity to gametocyte antigens. An effect of helminths has also been reported on the cellular immune responses of malaria infected subjects. However its direction is still a matter of debate. In the case of the Th1 mediated anti-malaria immunity, some studies reported an increase (73,74) or a decrease (74,75) in the level of
Th1/pro inflammatory cytokines such as IFN-\(\gamma\) and TNF in helminths and malaria co-infected subjects. However, there are also studies that show no association between helminths and immune responses to \textit{P. falciparum} infection\(^{(76–78)}\). Similar discrepancies have similarly been observed in studies where other arms of anti- malarial cellular immunity such as the Th2, Th17 or Regulatory T cell responses were assessed. It is possible that the observed differences in study outcomes reflect underlying heterogeneities in the study sites and methodologies: 1) different epidemiological features of malaria and helminth infections; 2) age and history of exposure to infection; 3) other helminth infections not considered. As indicated in table 1 all the current available immuno-epidemiological studies were also characterized by the diversity of immunological assays used to assess the influence of helminths on cellular immune responses during \textit{P. falciparum} infections. For example, many different methods were used to measure the cytokines involved, in plasma, in serum, in supernatants or intra-cellularly following stimulation of peripheral blood mononuclear cells or whole blood by different stimuli. These differences might explain, to some extent, the observed discrepancies in the direction of the effect of helminths on cellular immunity upon \textit{P. falciparum} infection. It is also important to underline that in most cases studies on this topic have only given an incomplete picture of the interaction between helminths and cellular immunity during malaria by focusing on only few cytokines or few parameters to characterise the Th1, Th2, Th17 or regulatory T cells immune responses. Adding to this is our limited knowledge of innate immunity in the context of co-infection. Majority of studies have indeed focused on malaria specific adaptive immunity and none have so far assessed both the innate and adaptive immune responses in the same cohort.

\section*{I. Scope and aims of this thesis}

The main objective of this thesis is to improve our understanding of helminth and \textit{Plasmodium spp.} co-infection within their human host. We aimed to assess how helminths manipulate the immune system of their human host and how such a manipulation could affect immune response in subjects infected with malaria parasites. Our study population was selected in an area where the burden of helminths (particularly \textit{S. haematobium}, filaria, intestinal helminths) and malaria
Table 1: Summary table of epidemiological studies that assessed the effect of helminth infections on the immune response of individuals infected with *P. falciparum*

<table>
<thead>
<tr>
<th>No</th>
<th>Authors (year of publication)</th>
<th>Type of immunological assay performed</th>
<th>Type of stimuli used if cells were stimulated</th>
<th>Type of read out</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Noone et al. (2013)</td>
<td>Plasma cytokine</td>
<td>NA</td>
<td>Plasma cytokines</td>
</tr>
<tr>
<td>2</td>
<td>Courtin et al. (2010)</td>
<td>Plasma cytokine</td>
<td>NA</td>
<td>Plasma cytokines</td>
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<tr>
<td>3</td>
<td>Diallo et al. (2010)</td>
<td>Whole blood culture</td>
<td>MSP1 antigens and <em>P. falciparum</em> schizont lysate</td>
<td>Cytokines measured in the cell culture supernatant</td>
</tr>
<tr>
<td>4</td>
<td>Dolo et al. (2012)</td>
<td>Plasma cytokine</td>
<td>NA</td>
<td>Plasma cytokines</td>
</tr>
<tr>
<td>5</td>
<td>Hartgers et al. (2009)</td>
<td>Whole blood culture</td>
<td><em>P. falciparum</em> infected red blood cells (iRBCs)</td>
<td>Cytokines measured in the cell culture supernatant</td>
</tr>
<tr>
<td>6</td>
<td>Lyke et al. (2006)</td>
<td>Serum cytokine</td>
<td>NA</td>
<td>Serum cytokine</td>
</tr>
<tr>
<td>7</td>
<td>Metenou et al. (2009)</td>
<td>Whole blood culture</td>
<td>iRBCs</td>
<td>Cytokines measured in cell culture supernatant</td>
</tr>
<tr>
<td>8</td>
<td>Metenou et al. (2011)</td>
<td>Whole blood culture</td>
<td>iRBCs</td>
<td>Intracellular cytokines</td>
</tr>
<tr>
<td>9</td>
<td>Muok et al. (2009)</td>
<td>Whole blood collected and stained without stimulation</td>
<td>T cells characterized by surface markers</td>
<td></td>
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<tr>
<td>10</td>
<td>Nmorsi (2009)</td>
<td>Plasma cytokines</td>
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<td>Plasma cytokines</td>
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<tr>
<td>11</td>
<td>Wilson et al. (2009)</td>
<td>Plasma cytokines</td>
<td>NA</td>
<td>Plasma cytokines</td>
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<tr>
<td>12</td>
<td>Lyke et al. (2012)</td>
<td>Surface staining of PBMC</td>
<td>NA</td>
<td>T cells characterized by surface markers</td>
</tr>
<tr>
<td>13</td>
<td>Metenou et al. (2012)</td>
<td>Whole blood culture</td>
<td>iRBCs</td>
<td>RNA mDCs, pDCs</td>
</tr>
<tr>
<td>14</td>
<td>Panda et al. (2013)</td>
<td>Plasma cytokine</td>
<td>NA</td>
<td>Regulatory T cells characterized by surface markers</td>
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<tr>
<td>15</td>
<td>Wammes et al. (2010)</td>
<td>Cell isolation, depletion and phenotyping</td>
<td>iRBCs</td>
<td>T cells characterization</td>
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<tr>
<td>16</td>
<td>Wilson et al. (2008)</td>
<td>Whole blood culture</td>
<td>SEA, SWA and PHA</td>
<td>Cytokines in cell culture supernatant</td>
</tr>
<tr>
<td>17</td>
<td>Hartgers et al. (2008)</td>
<td>Whole blood culture</td>
<td>iRBCs</td>
<td>Cytokines measured in the cell culture supernatant</td>
</tr>
<tr>
<td>18</td>
<td>Lyke et al. (2012)</td>
<td>PBMC culture</td>
<td>SEA, SWAP, AMA1, MSP1</td>
<td>Antibodies, Memory B cells response to malaria or schistosoma antigens</td>
</tr>
<tr>
<td>19</td>
<td>Diallo et al. (2004)</td>
<td>Plasma cytokines</td>
<td>NA</td>
<td>Plasma cytokines</td>
</tr>
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</table>
are remarkably high. Characterizing immune responses of subjects living within this context is of special interest. Indeed whereas experimental studies inform us on how the immune system works in optimal conditions, field studies involving naturally exposed individuals might give a valuable insight into the immunological profiles encountered in endemic regions. We set out to assess the different arms of the immune system. On the one hand we assess the humoral response to *P. falciparum* sexual and asexual stages antigens and the effect of *S. haematobium* on B cell subsets and function. On the other, we evaluate the cellular immune reactivity by analysing the host innate and adaptive immune response in *P. falciparum* infected subjects in the context of concurrent chronic helminth infections.

II. Outline of this thesis

This thesis is divided into height chapters:

The **first chapter** serves as Introduction.

The **second chapter** describes the study population, the study area, as well as the study procedures and different immunological assays that were used.

In the **third chapter** we investigate the effect of *S. haematobium* infection on the different B cells subsets and their response to B cell receptor engagement and TLR ligands.

In the **fourth chapter** we expand our studies to assess the effect of helminths on the antibody response specific to *P. falciparum* sexual and asexual stage antigens.

In the **fifth chapter** we assess the *in utero* effect of filarial infection on the Th1, Th17 and regulatory T cell responses in newborns.

In the **sixth chapter** the innate and adaptive immune responses of *S. haematobium* and *P. falciparum* co-infected school age children were compared to children with *P. falciparum* infection only.
The **seventh chapter** consist of a meta-analysis that extends our assessment of the effect of helminths on the cellular immune responses of subjects infected with *Plasmodium spp.* to all available results of such studies in the literature.

Finally in the **eighth chapter** we discuss the main findings of this thesis.

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