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The effect of parasitic co-infections on immune responses in Gabon : particular emphasis on malaria and helminths

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Chapter 8

General discussion

What we already knew

Helminth-induced modulation of *Plasmodium sp*. immune responses: What have we learnt from population based studies?

Studies using animal models have been pivotal to understand the effect of helminths on their host immune responses. In general they indicated that chronic helminth infections had the potential to skew the immune response toward a Th2 profile and to trigger the stimulation of a powerful regulatory network (1). Interestingly these immune modifications have been shown to lead to alterations of the host immune response to helminths in the first place, but also to others pathogens such as *Plasmodium sp.* (2). The implication of such findings is enormous given the geographical distribution of malaria worldwide. During the past years efforts have been put into epidemiological studies to verify these findings in affected populations. With regard to the humoral response, the main hypothesis was that in the context of coinfection the antibodies against *P. falciparum* which is dominated by cytophilic antibodies (IgG1 and IgG3) would be altered by the Th2 skewing of immune response observed in those chronically infected with helminths characterized by the predominance of IgG4 and IgE. To date only few studies have addressed this. Two of these studies were conducted in Senegal. The first one by Diallo *et al.* showed that IgG1 and IgG3 antibodies specific to PfMSP1-$_{19}$ and to *P. falciparum* schizont extract were significantly increased in children co-infected with *S. haematobium* and *P. falciparum* by comparison to those with *P. falciparum* mono infection only (3). Interestingly neither IgG2, IgG4 nor IgE levels specific to the same antigens were different between the two groups compared. These observations suggest that *S. haematobium* favours an efficient anti-malaria protective antibody response despite its Th2 skewing. In the second study the opposite observation was made by Roussilhon and co-workers (4). They showed that worm carriage was significantly correlated, on the one hand, with a decrease in cytophilic IgG1 and IgG3 antimalarial antibodies and, on the other, with an increase of non cytophilic IgG4 antibodies. Finally in a third study conducted in Mali no difference was observed between co-infected and mono-infected subjects in terms of their humoral response (5). These discrepancies in results obtained might indicate differences

in the study methodology or numerous environmental factors but most importantly indicates the need for further studies.

Antibody response although important is not enough to clear malaria infection on its own. Studies have shown that in contrast to normal mice, mice that are deficient in T cells cannot mount a sufficient immune response to *P. yoeli* infection after passive transfer of *Plasmodium* specific antibodies (6). These mice have a delayed time to appearance of microscopic parasitemia but cannot completely clear the parasite. This indicates that the humoral response might be important at the onset of the infection to limit the progression of the disease but that a complete clearance of the parasite is cell mediated. Cell mediated immunity specific to *Plasmodium spp.* has been shown to be complex and to involve a wide range of interacting innate and adaptive responses. Controlled human infection of malaria naive volunteers with *P. falciparum* has shown that both innate and adaptive immune cells contribute to the production of IFNγ a major pro-inflammatory cytokine (7,8) marking the so called Th1 response important for parasite clearance (9). Although in these studies no data were provided on the Th2 and regulatory responses the importance of the Th2 response in anti-malarial immunity as well as the need for an efficient regulatory response to avoid tissue damage has been suggested (reviewed in 10). There is evidence that some of the innate and adaptive effector mechanisms involved in immunity to malaria are modulated by helminths. For example in a study conducted in Sudanese children, it was observed that *S. haematobium* and *S. mansoni* were able to significantly impair the function of NK cells, a major source of IFNγ (11). In addition subjects chronically infected with helminths have been shown to have a lower frequency of dendritic cells (DC) which, also have an impaired function as indicated by their reduced capacity to respond to TLR ligands and to activate T cells (12,13). With regard to adaptive immune responses, it is now known that chronic helminth infections are linked to T cell hyporesponsivness mediated either through intrinsic mechanisms (14) or via their ability to induce regulatory cells that inhibit T cell responses (15–17). All these mechanisms are in the first place for helminths to evade the immune system, but as a off target effect may interfere with anti-malarial immunity.

In the context of coinfection it has been suggested that the anti-inflammatory milieu induced by chronic helminth infections may dampen both the protective and immunopathological immune responses to *Plasmodium spp*.. As a consequence subjects living in endemic areas would be more likely to acquire new malaria infection but will develop fewer symptoms or have less severe disease. Researchers have tried to test this hypothesis in naturally exposed individuals who live in areas endemic for both parasites. From a clinical point of view, some studies reported that helminths could exert either a protective (18–20) or an aggravating effect (21,22) on malaria outcomes while others indicated no association (23). Finally whether the cell mediated immune response specific to *Plasmodium spp.* was affected by a concurrent helminth infection has also been assessed during the past few years. The studies looked at the cytokine profile of co-infected subjects and compared it to *Plasmodium spp.* mono-infected ones. They showed an increase (24,25), a decrease (26,27) or even an absence (28) of effect of chronic helminth infections on the levels of Th1, Th2 or regulatory cytokines in coinfected subjects. Several reasons were suggested to explain the observed differences such as methodology, study design, helminths species, environmental factors (29) and again, more data are needed to draw a definitive conclusion.

Key points regarding what we already knew:

Helminths and malaria overlap in their geographical distribution.

Chronic helminth infections induce a Th2 skewed immune response and a potent immune regulatory network in chronically infected subjects.

Helminths induced immune modulation can interfere with *Plasmodium spp.* specific immune response in mouse models.

Epidemiological studies show variable effect of helminths on malaria outcome and immune response to malaria parasites.

What this thesis adds to our knowledge

Chronic *S. haematobium* infection leads to an alteration of B cells function and changes in the distribution of B cell subsets

The role of B cells in immunity against pathogens is being actively examined and is currently shown to expand beyond the classical antibody production. B cells are at the intersection between innate and adaptive immune response. They can produce cytokines upon stimulation through pathogens recognition receptor (PRR) and B cell receptors (BCR) and activated B cells can act as antigen presenting cells (APC) and are able to deliver co-stimulatory signals leading to the differentiation of Th2 effector cells. Moreover in recent studies they have been recognized for their immune regulatory properties (reviewed in (30)). The multitude of B cell functions are mediated through a variety of subsets with distinct phenotypic and functional characteristics, however, data are still scarce on the extent to which their distribution and function are altered in subjects bearing chronic infection. This research question was addressed in a field study described in chapter 3. B cell subsets and B cell responses to BCR and Toll-like receptor 9 stimulation was assessed in infected school aged children and in their uninfected counterparts at baseline and 6 months after the removal of the trematodes by Praziquantel. We observed that while the percentage of total B cells was similar between infected and uninfected individuals the distribution of some B cell subsets was significantly altered by active *S. haematobium* infection. This alteration was characterised by a trend toward a decrease of the naive B cell population in infected individuals that was significantly increased upon praziquantel treatment. On the other hand, we noted an increased percentage of activated and atypical B cells in the schistosoma infected group along with an increase of double negative and switched memory B cells subsets. These findings should be interpreted to be in line with data suggesting that atypical and double negative memory B cells are increased in chronic infection or disease and are functionally impaired (31–33) . Recently, the function of atypical MBC was assessed in relation to classical MBC in Malian adults with lifelong exposure to malaria and Malian children developing clinical malaria (33). It was observed that in malaria exposed and infected individuals atypical MBC differentiated from classical MBC and these cells were

characterized by the expression of an array of inhibitory receptors and by an impairment of BCR signalling. These changes were associated with an impaired B cell proliferation, cytokine production and antibody secretion upon stimulation. In our study we noted some functional changes as well in that the total B cell population of *S. haematobium*-infected children had a lower expression of Ki-67, a marker of cell proliferation, and produced less TNF, somehow resembling what might be considered as an exhausted profile. Taken together these findings might indicate that as expected infection with *S. haematobium* leads to B cells activation and immunoglobulin class switching of B cells. However in chronically infected individuals the repeated stimulation of B cells results in a state of B cells exhaustion or hyporesponsiveness that is partially restored upon specific anti-schistosoma treatment. This phenomenon might help the parasite to prolong it survival within the definitive host and explain why acquisition of protective humoral immunity takes time to develop in individuals living in endemic areas. It can also contribute towards limiting tissue damage in the host. However it is still unclear whether and how this down regulation of the B cell response due to *S. haematobium* infection can also affect the host response to other pathogens.

Individuals chronically infected with helminths have an impaired antibody response to *P. falciparum* sexual stage antigens

Sexual stage of *Plasmodium spp.* plays a central role in malaria transmission. Epidemiological studies have indicated that helminth co-infected individuals are more likely to carry *P. falciparum* gametocyte (34). Unfortunately not much is known about the mechanisms underlying such association. Gametocytogenesis can be influenced by various stress-inducing factors including the *Plasmodium* specific immune responses (reviewed in (35)). On the other hand helminths are well known for their ability to modulate the immune system of their host and question might arise as to whether in coinfected individuals this helminth induced immune modulation might interfere with anti-gametocyte immunity. This question was addressed for the first time in a cross-sectional study described in chapter 4. Our principal objective was to determine the influence of *S. haematobium* and filaria co-infection with malaria parasite on the antibody responses to *P. falciparum* sexual stage antigens. The study was conducted in an area

endemic for both helminths and for malaria as described in chapter 2. Total IgG to sexual stage antigens Pfs230 and Pfs48/45 was measured and their levels compared between individuals coinfected with helminths and *P. falciparum* or with only with *P. falciparum.* It was observed that IgG to Pfs48/45 sexual stage antigen but not Pfs230 was significantly lower in co-infected individuals even after adjustment for confounding factors such as age, haemoglobin level or the density of *P. falciparum* asexual stage parasite. This is the first time that helminth infection is reported to influence an immunological response to a *P. falciparum* sexual stage antigen. Additional studies will be needed to test this finding in different geographical areas. If confirmed, this could have implications for malaria transmission. Indeed anti Pfs48/45 IgG is known to exert transmission blocking activity by inhibiting the fertilisation of *P. falciparum* gametes in the mosquito gut (36). Hence a lower antibody response to this antigen due to helminths might result in an increased multiplication of *P. falciparum* in the mosquito gut and an increase of its transmission to humans. This hypothesis will need to be tested in future studies.

From the two helminth species considered in this study only *S. haematobium* but not filarial parasites was associated with a significant decrease in antibodies to *P. falciparum* sexual stage antigens. Although this has to be confirmed in further studies, we hypothesize that while *L. loa*, *M. perstans* and *S. haematobium* are all located in the blood vasculature of their host, their effect on the immune response or on host metabolism might be more pronounced for *S. haematobium*. For example, anaemia was more frequent in subjects infected with *S. haematobium*. The selective effect of *S. haematobium* infection on Pfs48/45 and not Pfs230 is difficult to explain. It is possible that the modulatory effect exerted by helminths is not sufficient to impair the humoral response to Pfs230 antigens. Data from field studies (35,36) have shown Pfs230 to be more immunogenic than Pfs48/45, however, another explanation would be that our study was underpowered to detect such an effect.

Despite the fact that *S. haematobium* infection was associated with an impaired response to Pfs48/45 antigens, we did not observe a decrease in gametocyte carriage in subjects infected with schistosoma and/or filarial parasites. This suggests that Pfs48/45 plays a limited role in the

development of gametocytes within the human host. This is supported by a study where sera from individuals naturally exposed to *P. falciparum* were used to assess the capacity of sexual stage antigens to induce complement-mediated lysis of *P. falciparum* gametes (37). In the study, antibodies to Pfs230 but not Pfs48/45 were positively associated with complement-mediated lysis of *P. falciparum* gametes *in vitro*. Interestingly they observed that Pfs230-induced gamete lysis was more pronounced in the presence of Pfs48/45.

Our study presented some limitations that need to be addressed. For example, a longitudinal study design would have been more appropriate, it will take into account the effect of seasonality on gametocytogenesis (38,39) and will allow the dynamic study of the association between helminths, *P. falciparum* sexual stage carriage and the immune response. Furthermore gametocyte carriage was determined by microscopy which has proven to be less sensitive than PCR to detect low parasite load (38). Finally functional assays such as Standard Membrane Feeding assays (SMFA) are instrumental to assess whether the decrease of sexual stage antibodies translate into an increased transmission of *P. falciparum* to the mosquito and should be included in future studies. Despite these limitations our study brings novel information on the complex interaction between helminths and malaria and generates a hypothesis that needs to be further tested.

Antibody responses to *P. falciparum* asexual stage antigens are not influenced by either *S. haematobium* or filarial infections

To date only few studies have assessed whether chronic helminth infections could influence carriage and antibody responses to asexual stages of *P. falciparum*. So far such studies have generated contradictory outcomes. This indicates the need for additional data and gives justification for the study described in chapter 4. This study was conducted in an area where malaria and helminths are highly endemic despite regular mass drug administration. It aimed at assessing the relationship between helminth infection, carriage of *P. falciparum* trophozoites and antibody responses to asexual stage antigens. Our first observation was that the asymptomatic carriage of *P. falciparum* was quite high (42% by microscopy and 75% by PCR) and was accompanied by a similarly high prevalence of *S. haematobium* (75%)

in the area. Moreover individuals infected with *S. haematobium* had a higher carriage of *P. falciparum* asexual forms as determined by microscopy. Interestingly this association disappeared when *P. falciparum* carriage was assessed by PCR. These findings suggest that schistosoma infected and uninfected individuals are equally susceptible to *P. falciparum* infection however higher parasite replication seems to be favoured in the presence of *S. haematobium*. Similar increase of microscopic carriage of asexual form of *P. falciparum* in helminth infected individuals have been reported elsewhere (40,41). However there are also studies where a negative association was reported (42,43). The difference in the studies might be due to confounding factors such as the characteristic of the study area, differences in study methodology, malaria seasonality, the type of helminths species assessed as well as the intensity and chronicity of helminth infections.

Despite the higher density of *P. falciparum* detected by microscopy in *S. haematobium* coinfected subjects we failed to link this to an impairment of the IgG response to asexual stage antigens (MSP1, AMA1 and GLURP). When considering published data, we noted that only one study have so far reported an increase in antibodies specific to asexual stage antigens in *S. haematobium* coinfected individuals (12). In this study, subjects free of schistosome infection who served as the comparator group to those with both schistosomes and malaria were selected from an area not endemic for helminths. This might have represented a bias since confounding factors such as differences in the living environment were not controlled for. Other population studies in which helminth infected and uninfected subjects were recruited in the same area showed a decrease of the IgG response to *P. falciparum* in helminths coinfected individuals (44) or even an absence of effect of helminths (45).

These differences reflect the complex interaction that exists between helminths and malaria parasite. It could indicate that in individuals living in affected areas the modulation induced by helminths on malaria specific humoral response might be confounded by the degree of endemicity as well as by past helminth infections. It is also possible that the modulatory effect of helminths that favour *P. falciparum* replication is exerted through different mechanisms. For example through T cells hypo responsiveness or possibly through metabolic changes. Future studies would need to consider both the humoral and

cellular immune response of the study participants. It would also be important to determine whether helminths are capable of favouring the switch from asymptomatic to symptomatic *P. falciparum* infection. This was not possible in this study considering its cross sectional design.

P. falciparum **infection imposes a distinct signature on the cytokine and chemokine profiles, which is not impaired by a concurrent infection with** ***S. haematobium***

The innate and adaptive cellular immune responses play an important role in anti-malarial immunity (46). They serve to control the infection but at the same time can also be detrimental to the host by causing tissue damage (47). Several studies have been conducted in individuals naturally exposed to *Plasmodium sp.* to characterize their cytokine response when they are infected. These studies mainly focus on few cytokines and in addition their data analysis does not take into consideration the level of correlation that might exist among the measured cytokines. This is rather an important aspect given that from a functional perspective cytokines can have a synergistic (48,49) or antagonist effect (50). In chapter 6 we set out to assess the effect of *P. falciparum* and *S. haematobium* infections on the cytokine response of infected individuals. We assessed both the innate and adaptive immune responses using in vitro assays where cytokines and chemokines were measured after PBMC or whole blood stimulation with either SEB (to assess the adaptive immune response) or various TLRs ligands (to assess the innate immune response). Moreover instead of looking at individual cytokine s, we opted for "Principal Component Analysis" (PCA), which allows a simultaneous assessment of multiple variables that are then summarized into new synthetic variables or components containing most of the information in the dataset. An additional advantage of this PCA is that the synthetic components also take into consideration the magnitude of the correlation between the different cytokines/chemokines. What we observed when we compared the different principal components among the study groups is that; *P. falciparum* infection was associated with an increase of the general immune responsiveness of the study subjects. This increase was seen both for the innate and the adaptive immune system and was not impaired by a concurrent *S. haematobium* infection despite its ability to

decrease the pro-inflammatory immune response as observed in *S. haematobium* singly infected subjects. From an immunological point of view our finding might indicate that although schistosomes have the potential to modulate the immune response, the observed immune modulation is not sufficient to dampen the cell mediated immune response triggered by the presence of *P. falciparum* in the blood stream. It is possible that malaria parasites, seen as an acute infection by the host immune system, overcome the threshold set by the regulatory network induced by helminths. Another explanation for lack of an effect of helminth infections on cytokine response to malaria infection could be the epidemiological feature of the study area as well as on the characteristic of the study population. It is possible that the subjects that appear free of a particular helminth infection have been infected in the past or carry other helminths species capable of shaping the host immune system. Our study participants were recruited in an area where the prevalence of helminths has been shown to be consistently high (51) and although we did not assess whether they had past helminths infection, it is very likely that they were infected in the past. In this regard in chapter 5 we observed that regulatory T cells of new-borns from filaria infected mothers correlated negatively with the level of pro-inflammatory Th1 cells suggesting that regulatory T cells acquired functional capacity already before birth probably as a consequence of *in utero* exposure to helminths. If the in utero effect of helminth infections would be long-lived, these data would be in line with the notion that early life exposure to helminths might make it difficult to see any clear effect of current helminth infection on various immune responses.

Although poly-helminth infections are the norm in tropical and sub tropical areas (52,53), epidemiological studies where the effect of helminths on anti-malaria immunity was assessed have mainly focused on one or maximum two helminth species. This could be responsible for the disparities in outcomes. The influence of poly helminth infection as well as past helminth infections on the outcomes of the responses studied was further assessed in the meta-analysis describe in chapter 7. This meta-analysis indicates that there are currently 19 eligible studies conducted in endemic areas to assess the influence of helminths on immune responses of *P. falciparum* infected subjects. The results seem highly conflicting. However when these studies are pooled together within a meta-analysis, the emerging trend was that the cell

mediated immune response to *P. falciparum* was not affected by helminths in coinfected individuals. Interestingly when we stratified our assessment using a moderator analysis we noted that studies where stringent criteria were used, to control for the confounding effect of past exposure to helminth antigens, showed that in those who have no current or past exposure to helminths, there is significantly higher pro-inflammatory cytokines in malaria infected subjects (54,55). Additionally this moderator analysis indicated that poly-helminth infections with schistosomes and intestinal helminths resulted in a significant increase of regulatory cytokines (56). These findings support the results described in chapter 6 and further indicate that interaction between helminths and malaria is complex. It is indeed possible that the helminth induced modulation of their host immune system has the potential to interfere with their cell mediated immune response to *P. falciparum*. However in areas where the force of infection with helminths is high, this effect could be masked by past and/or current (poly) helminth infections.

The type of immunological assays used in epidemiological studies might influence outcomes

When we conducted the meta-analysis described in chapter 7 we noticed that our current knowledge of malaria and helminth co-infection was based on studies that used different immunological assays. For example some studies report on circulating plasma or serum cytokines (25,26,57,58) whereas others measure cytokines after stimulation of whole blood (27,59–62) or PBMC (63) either in supernatants or intra-cellularly. In addition the type of stimuli used when cells are stimulated can also be different between studies. These differences may contribute to the heterogeneity of the results observed in different studies. Indeed when performing a moderator analysis aiming to understand the variation in study outcomes we noted that the Th17 response was higher in helminth and malaria co-infected individuals when this cytokine was measured by flow cytometry after intracellular staining (27). Moreover studies where cells were stimulated with PHA (64) or *P. falciparum*-infected red blood cells (iRBC) (27,59,61), were more likely to report a higher Th2 or anti-inflammatory response in helminths and malaria co-infected individuals compared to those with malaria only. These observations underline the

importance of statistical method such as meta-analysis to account for the heterogeneity in study design when interpreting results from population based studies. Moreover they also indicate the need for standard operating procedures to harmonize the protocols used in field studies.

Key points on what this thesis adds to our knowledge

Regulatory T cells of a newborn from helminth-infected mother can suppress the development of effector T cells probably as a result of *in-utero* exposure to helminths.

Helminths can alter B cell profile and function but in particular lead to expansion of atypical and double negative B cells.

Helminths can influence antibody response to *P. falciparum* sexual stage antigens.

Individuals chronically infected with helminths do not have an impaired IgG response to *P. falciparum* asexual stage antigens.

P. falciparum leads to a general increase of the innate and adaptive immune response in infected individuals.

The innate and adaptive cytokine responses of individuals infected with *P. falciparum* is not altered by a concurrent *S. haematobium* infection.

The absence of an effect of helminths on the cellular immune response of individuals with malaria is confirmed by a meta-analysis of all the studies available to date.

Past infection with helminths or current poly-helminth infections can mask the effect of a helminth on *P. falciparum* immune response.

There is substantial degree of heterogeneity in studies that assess the association between helminths and malaria. Standardized and uniform protocols are needed to be able to answer whether helminth infections affect immune responses to *P. falciparum*.

Direction for future research

Helminths and malaria transmission

Indirect evidence suggests a role for helminths in sustaining malaria transmission. However to date very few studies have addressed this question. In this thesis we showed that *S. haematobium* was able to decrease antibody responses to *P. falciparum* sexual antigens. This might lead to an overall impairment of the transmission blocking immunity. Whether this finding could result in a significant increase of malaria transmission in a given area would need to be addressed in future studies. These studies might also need to use approaches such as Standard Membrane Feeding Assays (SMFA) to assess the transmission blocking activity of these antibodies or ecological studies to determine whether malaria incidence, or asymptomatic carriage of sexual and asexual stage *P. falciparum* correlate with the prevalence/burden of helminths in the population.

Helminths and malaria vaccine

Malaria vaccines bear a lot of promise for the eradication or at least the control of malaria in affected areas. To date more than 150 malaria vaccine candidate are or were undergoing clinical evaluation (65) and only one candidate vaccine, the RTS'S has reached a phase III where it was shown to confer modest protection against malaria (66). Data are still scarce as to whether helminths could impair or augment the response to these candidate vaccines in endemic populations. A study recently showed that the immunogenicity of the GMZ2-4 blood stage malaria vaccine candidate was significantly reduced in 1 to 5 years old children who were infected with *Trichuris trichiura* (67). Similar studies need to be repeated. If this effect is confirmed, understanding the mechanism of this interaction could be of importance in the design of malaria vaccine and immunization program. A particular attention should also be given to malaria transmission blocking vaccine candidates such as Pfs48/45 antigen given that the antibody response elicited has been shown to be impaired by helminths.

Beyond helminths: Rural VS urban differences

Rapid urbanization is taking place in Africa. In western countries an increase of urbanization index has been linked with a decrease of biodiversity and emergence of inflammatory diseases (68). Such an alteration of the biodiversity might be reflected in differences in immune responses of subjects from different areas. This was recently shown by Mbow *et al.* in a study where they compare the immune profile of subjects living in rural Africa to those of individuals from urban Africa and Europe (69). What they observed was a rural to urban gradient marked by a higher Th1 and Th2 and markedly a Th2/Th1 ratio in subjects from rural Africa by comparison to those from urban Africa and Europe. Similar rural and urban differences were also observed between individuals from urban Africa and Europe and when memory T cells subsets were assessed (69). In a different study Smolen *et al.* showed that innate cytokine response of 2 years old children measured after stimulation of whole blood cells with TLR ligands were similar between children from North America, South America and Europe despite obvious differences in their living environment (70). Yet children from South Africa presented a different profile. Difference in innate response between African and Europeans children was reported in a study by Labuda et al. (71). How geographical differences in immune response of rural and urban dwellers can affect response to malaria parasites or how this affects interaction between helminths and malaria parasites is not yet known. Filling this gap in our knowledge will be of particular importance in the future, since according to the United Nation nearly 50% of African population will be leaving in urban cities by 2035.

Beyond helminths: Microbiome and malaria

Additional effort should be invested in assessing the relationship between *P. falciparum* and the microbiome of their host, particularly the gut microbiota. A recent study indicated that the gut microbiome could protect their human host against *P. falciparum* through antibody responses against the Galα1-3Galβ1-4GlcNAc-R glycan (also known as alpha-gal) (72). This xeno-glycan has been shown to be expressed both by bacteria of the gut flora and by *Plasmodium sp.* parasite. In this study the protective effect of the microbiome was shown to be

mediated through the production of anti-alpha-gal antibodies capable of blocking *Plasmodium sp*. growth in vitro through complement activation and to interfere with the invasion of hepatocytes by the parasite (72). It is possible that additional xeno-glycans commonly expressed by gut bacteria and by *Plasmodium sp.* are able to generate such an anti-malarial immunity. Their identification and the characterization of the mechanism by which they are able to modulate anti-malaria immunity could open the door to a new era of glycan based anti-malaria vaccines. Whether the composition of the microbiome could influence the immunogenicity and efficacy of malaria vaccine candidates would also need to be addressed. This has been shown for influenza vaccine in mice (73). Moreover the interaction of helminths with malaria might need to take the microbome into account as it has been shown that helminths can influence the gut microbiome (74).

Age might matter

Studies that describe the epidemiology of helminths and malaria coinfection have mainly focused on school aged children and adults to some extent. There is currently an important gap in our knowledge on co-infection in pre-school age children. However there are data suggesting that this group is also affected. In a exploratory studies where the burden of helminths was assessed in 1 to 5 years old children in Gabon we found a prevalence of 29% of intestinal helminths (*Ascaris lumbricoides, Trichuris trichiura*, *Necator Americanus*) and 7% of *S. haematobium* (unpublished data*)*. It is likely that helminths have a more potent effect on anti-malaria immunity in this age group.

Standard operating procedure for immuno-epidemiological studies

In this thesis we were able to show that the differences in immunological assays set up to study the effect of helminths on immune responses to *Plasmodium spp.* infection influence the direction of the study outcome. Given the importance of immuno-epidemiological studies, there is a need to harmonize the protocols used or at least to define some strict guidelines.

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