Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/40905 holds various files of this Leiden University dissertation.

Author: Ateba Ngoa, U.
Title: The effect of parasitic co-infections on immune responses in Gabon : particular emphasis on malaria and helminths
Issue Date: 2016-07-07

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).
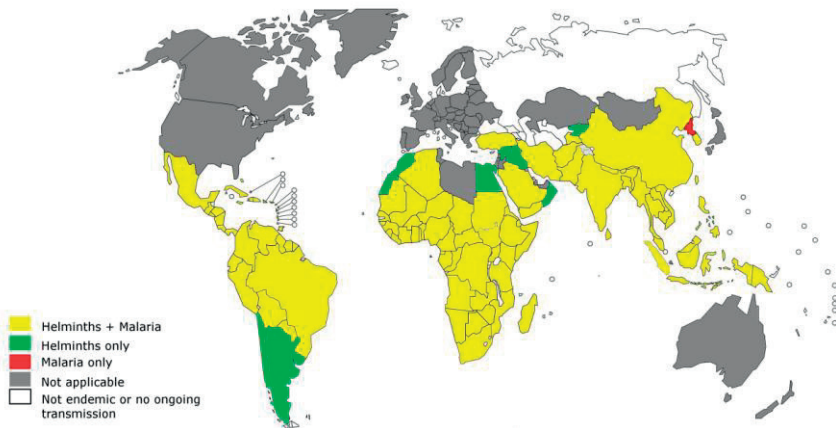

Figure 1: World map showing the geographical distribution of malaria and helminth infection or co-infection. Data on malaria are indicative of the percentage of population at risk in the world in 2013. These data were obtained from the WHO 2014 world malaria report. Data on helminths (lymphatic filariasis, 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

1

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).
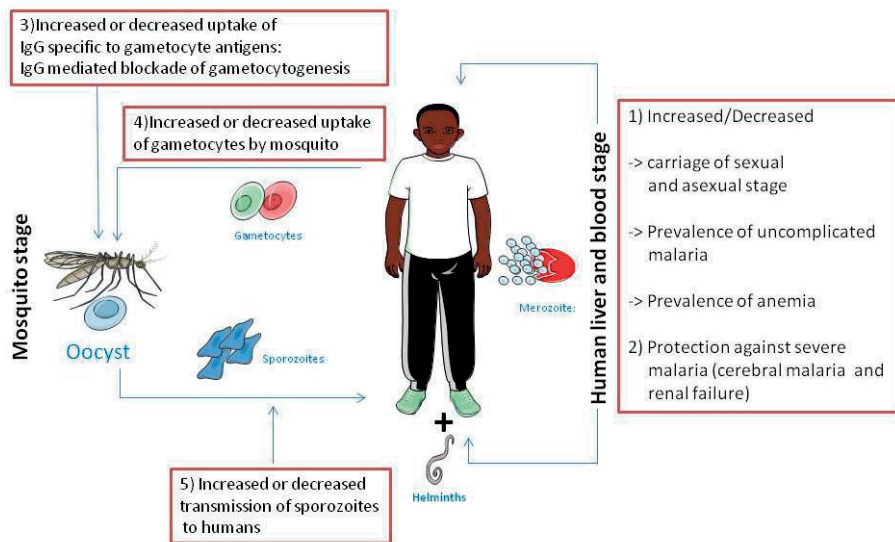

Figure 2: The possible influence of helminth infections on transmission and/or clinical outcomes of malaria parasites.

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).
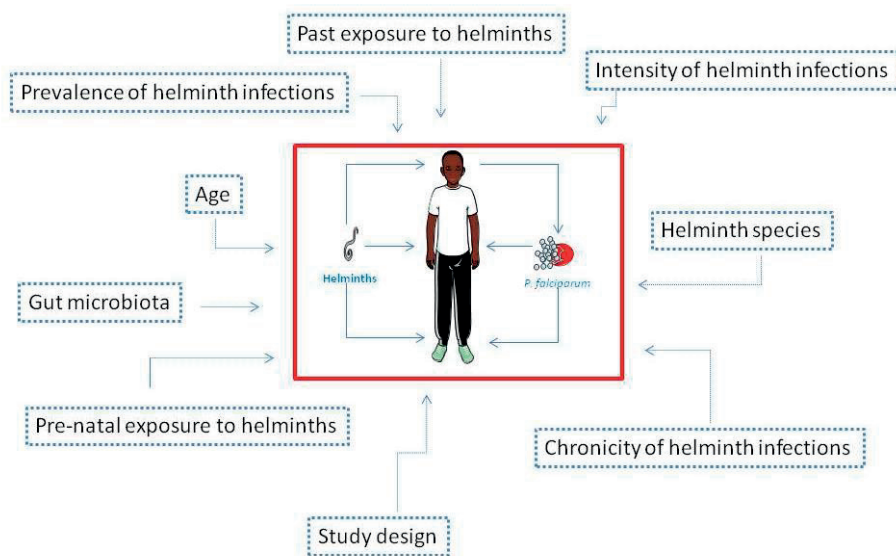

Figure 3: Infection with helminths or *P. falciparum* results in direct interaction between these parasites with the host immune system and metabolism. But co-infection can also result in direct or indirect interaction between these two parasites within the human host (depicted inside the red square). 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 *Heligosomoides polygirus* 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 *P. chabaudi* only (41). In contrast, a study by Karadjan and co-workers showed no effect of *Litosomoides sigmodontis* coinfection on the peak parasitemia of *P. chabaudi* whereas a decrease was observed for *Plasmodium yoelii* (42). Furthermore they did not report a difference in the level of IFN-γ or IL-10 between *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-γ was assessed (44). The meta-analysis confirmed a helminth-induced decrease of IFN-γ 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 *H. polygyrus* and *P. chabaudi* co-infection, DCs from co-infected host were less able to induce *in vitro* proliferation or IFN-γ production by CD4$^+$ T cells in response to malaria antigen stimulation when compared to mice infected with *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

1

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 $MSP1_{19}$ *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

1

Th1/pro inflammatory cytokines such as IFN-γ and TNF in helminths and malaria co-infected subjects. However, there are also studies that show no association between helminths and immune responses to *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 *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 *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.

I. Scope and aims of this thesis

The main objective of this thesis is to improve our understanding of helminth and *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 *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*

N°	Authors (year of publication)	Type of immunological assay performed	Type of stimuli used if cells were stimulated	Type of read out
1	Noone et al. (2013)	Plasma cytokine	NA	Plasma cytokines
2	Courtin et al.	Plasma cytokine	NA	Plasma cytokines
3	Diallo et al. (2010)	Whole blood culture	MSP1 antigens and *P. falciparum* schizont lysate	Cytokines measured in the cell culture supernatant
4	Dolo et al. (2012)	Plasma cytokine	NA	Plasma cytokine
5	Hartgers et al. (2009)	Whole blood culture	*P. falciparum* infected red bllod cells (iRBCs)	Cytokines measured in the cell culture supernatant
6	Lyke et al. (2006)	Serum cytokine	NA	Serum cytokine
7	Metenou et al. (2009)	Whole blood culture	iRBCs	Cytokines measured in cell culture supernatant
8	Metenou et al. (2011)	Whole blood culture	iRBCs	Intracellular cytokines
9	Muok et al. (2009)	Whole blood collected and stained withouth stimulation		T cells characterized by surface markers
10	Nmorsi (2009)	Plasma cytokines	NA	Plasma cytokines
11	Wilson et al. (2009)	Plasma cytokines	NA	Plasma cytokines
12	Lyke et al. (2012)	Surface staining of PBMC	NA	T cells characterized by surface markers
13	Metenou et al. (2012)	Whole blood culture Gene expression cDNA synthesis RT-PCR	iRBCs	RNA mDCs, pDCs
14	Panda et al. (2013)	Plasma cytokine Whole blood assay	NA	Plasma cytokine Regulatory T cells characterized by surface markers
15	Wammes et al. (2010)	Cell isolation, depletion and phenotyping Proliferation assay	iRBCs	T cells characterization T cells proliferation Cytokines in cell culture supernatatant
16	Wilson et al. (2008)	Whole blood culture	SEA, SWA and PHA	Cytokines in cell culture supernatatant
17	Hartgers et al. (2008)	Whole blood culture	iRBCs	Cytokines measured in the cell culture supernatant
18	Lyke et al. (2012)	PBMC culture Memory B cells assays ELISA Flow cytometry staining ELISPOT	SEA, SWAP, AMA1, MSP1	Antibodies, Memory B cells response to malaria or schistosoma antigens
19	Diallo et al. (2004)	Plasma cytokines	NA	Plasma cytokines

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.

1

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.

REFERENCES

1.Brooker S, Clements AC, Hotez PJ, Hay SI, Tatem AJ, Bundy DA, et al. The co-distribution of Plasmodium falciparum and hookworm among African schoolchildren. Malar J. 2006 Nov 3;5(1):99.

2.Brooker SJ, Pullan RL, Gitonga CW, Ashton RA, Kolaczinski JH, Kabatereine NB, et al. Plasmodium-helminth coinfection and its sources of heterogeneity across East Africa. J Infect Dis. 2012 Mar 1;205(5):841–52.

3.WHO | World Malaria Report 2014 [Internet]. [cited 2015 Nov 17]. Available from: http://www.who.int/malaria/publications/world_malaria_report_2014/en/

4.Mulu A, Legesse M, Erko B, Belyhun Y, Nugussie D, Shimelis T, et al. Epidemiological and clinical correlates of malaria-helminth co-infections in Southern Ethiopia. Malar J. 2013;12:227.

5.Kepha S, Nuwaha F, Nikolay B, Gichuki P, Edwards T, Allen E, et al. Epidemiology of coinfection with soil transmitted helminths and Plasmodium falciparum among school children in Bumula District in western Kenya. Parasit Vectors. 2015;8(1):314.

6.Degarege A, Legesse M, Medhin G, Animut A, Erko B. Malaria and related outcomes in patients with intestinal helminths: a cross-sectional study. BMC Infect Dis. 2012;12:291.

7.Lyke KE, Dicko A, Dabo A, Sangare L, Kone A, Coulibaly D, et al. Association of Schistosoma haematobium infection with protection

1

against acute Plasmodium falciparum malaria in Malian children. Am J Trop Med Hyg. 2005 Dec;73(6):1124–30.

8. Lemaitre M, Watier L, Briand V, Garcia A, Le Hesran JY, Cot M. Coinfection with Plasmodium falciparum and Schistosoma haematobium: additional evidence of the protective effect of Schistosomiasis on malaria in Senegalese children. Am J Trop Med Hyg. 2014 Feb;90(2):329–34.

9. Nkuo-Akenji TK, Chi PC, Cho JF, Ndamukong KKJ, Sumbele I. Malaria and helminth co-infection in children living in a malaria endemic setting of mount Cameroon and predictors of anemia. J Parasitol. 2006 Dec;92(6):1191–5.

10. Kinung'hi SM, Magnussen P, Kaatano GM, Kishamawe C, Vennervald BJ. Malaria and helminth co-infections in school and preschool children: a cross-sectional study in Magu district, north-western Tanzania. PloS One. 2014;9(1):e86510.

11. Mulu A, Legesse M, Erko B, Belyhun Y, Nugussie D, Shimelis T, et al. Epidemiological and clinical correlates of malaria-helminth co-infections in Southern Ethiopia. Malar J. 2013;12:227.

12. Nacher M, Singhasivanon P, Yimsamran S, Manibunyong W, Thanyavanich N, Wuthisen R, et al. Intestinal helminth infections are associated with increased incidence of Plasmodium falciparum malaria in Thailand. J Parasitol. 2002 Feb;88(1):55–8.

13. Nacher M, Singhasivanon P, Silachamroon U, Treeprasertsuk S, Vannaphan S, Traore B, et al. Helminth infections are associated with protection from malaria-related acute renal failure and jaundice in Thailand. Am J Trop Med Hyg. 2001 Dec;65(6):834–6.

14. Nacher M, Singhasivanon P, Traore B, Dejvorakul S, Phumratanaprapin W, Looareesuwan S, et al. Short report: Hookworm infection is associated with decreased body temperature during mild Plasmodium falciparum malaria. Am J Trop Med Hyg. 2001 Aug;65(2):136–7.

15. Panda M, Sahoo PK, Mohapatra AD, Dutta S kanti, Thatoi PK, Tripathy R, et al. Decreased prevalence of sepsis but not mild or severe P. falciparum malaria is associated with pre-existing filarial infection. Parasit Vectors. 2013;6:203.

16. Naing C, Whittaker MA, Nyunt-Wai V, Reid SA, Wong SF, Mak JW, et al. Malaria and soil-transmitted intestinal helminth co-infection and its effect on anemia: a meta-analysis. Trans R Soc Trop Med Hyg. 2013 Nov;107(11):672–83.

17. Bal MS, Mandal NN, DAS MK, Kar SK, Sarangi SS, Beuria MK. Transplacental transfer of filarial antigens from Wuchereria bancrofti-infected mothers to their offspring. Parasitology. 2010 Apr;137(4):669–73.

18. Pires ML, Ferreira RS, Araújo S, Albuquerque R, Remigio Neto J, Da Silva AS, et al. [Transplacental passage of Wuchereria bancrofti microfilariae in newborns of microfilaremic mothers]. Rev Inst Med Trop São Paulo. 1991 Apr;33(2):91–5.

19. Ndibazza J, Webb EL, Lule S, Mpairwe H, Akello M, Oduru G, et al. Associations between maternal helminth and malaria infections in pregnancy and clinical malaria in the offspring: a birth cohort in entebbe, Uganda. J Infect Dis. 2013 Dec 15;208(12):2007–16.

20. Metenou S, Babu S, Nutman TB. Impact of filarial infections on coincident intracellular pathogens: Mycobacterium tuberculosis and Plasmodium falciparum. Curr Opin HIV AIDS. 2012 May;7(3):231–8.

21. Perez-Mazliah D, Langhorne J. CD4 T-cell subsets in malaria: TH1/TH2 revisited. Front Immunol. 2014;5:671.

22. Stevenson MM, Riley EM. Innate immunity to malaria. Nat Rev Immunol. 2004 Mar;4(3):169–80.

23. Taylor-Robinson AW, Phillips RS. Functional characterization of protective CD4+ T-cell clones reactive to the murine malaria parasite Plasmodium chabaudi. Immunology. 1992 Sep;77(1):99–105.

1

24. Taylor-Robinson AW, Phillips RS. Reconstitution of B-cell-depleted mice with B cells restores Th2-type immune responses during Plasmodium chabaudi chabaudi infection. Infect Immun. 1996 Jan;64(1):366–70.

25. Perry JA, Rush A, Wilson RJ, Olver CS, Avery AC. Dendritic cells from malaria-infected mice are fully functional APC. J Immunol Baltim Md 1950. 2004 Jan 1;172(1):475–82.

26. Ing R, Segura M, Thawani N, Tam M, Stevenson MM. Interaction of mouse dendritic cells and malaria-infected erythrocytes: uptake, maturation, and antigen presentation. J Immunol Baltim Md 1950. 2006 Jan 1;176(1):441–50.

27. D'Ombrain MC, Hansen DS, Simpson KM, Schofield L. gammadelta-T cells expressing NK receptors predominate over NK cells and conventional T cells in the innate IFN-gamma response to Plasmodium falciparum malaria. Eur J Immunol. 2007 Jul;37(7):1864–73.

28. Nganou-Makamdop K, van Gemert G-J, Arens T, Hermsen CC, Sauerwein RW. Long Term Protection after Immunization with P. berghei Sporozoites Correlates with Sustained IFNγ Responses of Hepatic CD8+ Memory T Cells. PLoS ONE [Internet]. 2012 May 1 [cited 2015 Nov 18];7(5). Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3341355/

29. Schmidt NW, Podyminogin RL, Butler NS, Badovinac VP, Tucker BJ, Bahjat KS, et al. Memory CD8 T cell responses exceeding a large but definable threshold provide long-term immunity to malaria. Proc Natl Acad Sci U S A. 2008 Sep 16;105(37):14017–22.

30. Hanum P S, Hayano M, Kojima S. Cytokine and chemokine responses in a cerebral malaria-susceptible or -resistant strain of mice to Plasmodium berghei ANKA infection: early chemokine expression in the brain. Int Immunol. 2003 May;15(5):633–40.

31. Nie CQ, Bernard NJ, Schofield L, Hansen DS. CD4+ CD25+ regulatory T cells suppress CD4+ T-cell function and inhibit the development of Plasmodium berghei-specific TH1 responses involved

in cerebral malaria pathogenesis. Infect Immun. 2007 May;75(5):2275–82.

32.Schmidt KE, Schumak B, Specht S, Dubben B, Limmer A, Hoerauf A. Induction of pro-inflammatory mediators in Plasmodium berghei infected BALB/c mice breaks blood-brain-barrier and leads to cerebral malaria in an IL-12 dependent manner. Microbes Infect Inst Pasteur. 2011 Sep;13(10):828–36.

33.van Riet E, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections induce immunomodulation: consequences and mechanisms. Immunobiology. 2007;212(6):475–90.

34.Everts B, Smits HH, Hokke CH, Yazdanbakhsh M. Helminths and dendritic cells: sensing and regulating via pattern recognition receptors, Th2 and Treg responses. Eur J Immunol. 2010 Jun;40(6):1525–37.

35.Everts B, Adegnika AA, Kruize YCM, Smits HH, Kremsner PG, Yazdanbakhsh M. Functional impairment of human myeloid dendritic cells during Schistosoma haematobium infection. PLoS Negl Trop Dis. 2010;4(4):e667.

36.Everts B, Perona-Wright G, Smits HH, Hokke CH, van der Ham AJ, Fitzsimmons CM, et al. Omega-1, a glycoprotein secreted by Schistosoma mansoni eggs, drives Th2 responses. J Exp Med. 2009 Aug 3;206(8):1673–80.

37.Vella AT, Pearce EJ. CD4+ Th2 response induced by Schistosoma mansoni eggs develops rapidly, through an early, transient, Th0-like stage. J Immunol Baltim Md 1950. 1992 Apr 1;148(7):2283–90.

38.Wammes LJ, Hamid F, Wiria AE, Wibowo H, Sartono E, Maizels RM, et al. Regulatory T cells in human lymphatic filariasis: stronger functional activity in microfilaremics. PLoS Negl Trop Dis. 2012;6(5):e1655.

39.Wammes LJ, Hamid F, Wiria AE, de Gier B, Sartono E, Maizels RM, et al. Regulatory T cells in human geohelminth infection suppress immune responses to BCG and Plasmodium falciparum. Eur J Immunol. 2010 Feb;40(2):437–42.

1

40. Taylor MD, LeGoff L, Harris A, Malone E, Allen JE, Maizels RM. Removal of regulatory T cell activity reverses hyporesponsiveness and leads to filarial parasite clearance in vivo. J Immunol Baltim Md 1950. 2005 Apr 15;174(8):4924–33.

41. Su Z, Segura M, Morgan K, Loredo-Osti JC, Stevenson MM. Impairment of protective immunity to blood-stage malaria by concurrent nematode infection. Infect Immun. 2005 Jun;73(6):3531–9.

42. Karadjian G, Berrebi D, Dogna N, Vallarino-Lhermitte N, Bain O, Landau I, et al. Co-infection restrains Litomosoides sigmodontis filarial load and plasmodial P. yoelii but not P. chabaudi parasitaemia in mice. Parasite Paris Fr. 2014;21:16.

43. Knowles SCL. The effect of helminth co-infection on malaria in mice: a meta-analysis. Int J Parasitol. 2011 Aug 15;41(10):1041–51.

44. Graham AL. Ecological rules governing helminth-microparasite coinfection. Proc Natl Acad Sci U S A. 2008 Jan 15;105(2):566–70.

45. Wang M, Cao Y, Luo E, Zhang Y, Guo Y. Pre-existing Schistosoma japonicum infection alters the immune response to Plasmodium berghei infection in C57BL/6 mice. Malar J. 2013;12:322.

46. Aksoy E, Zouain CS, Vanhoutte F, Fontaine J, Pavelka N, Thieblemont N, et al. Double-stranded RNAs from the helminth parasite Schistosoma activate TLR3 in dendritic cells. J Biol Chem. 2005 Jan 7;280(1):277–83.

47. Su Z, Segura M, Morgan K, Loredo-Osti JC, Stevenson MM. Impairment of protective immunity to blood-stage malaria by concurrent nematode infection. Infect Immun. 2005 Jun;73(6):3531–9.

48. Taylor JJ, Krawczyk CM, Mohrs M, Pearce EJ. Th2 cell hyporesponsiveness during chronic murine schistosomiasis is cell intrinsic and linked to GRAIL expression. J Clin Invest. 2009 Apr;119(4):1019–28.

49. van der Vlugt LEPM, Zinsou JF, Ozir-Fazalalikhan A, Kremsner PG, Yazdanbakhsh M, Adegnika AA, et al. Interleukin 10 (IL-10)-

producing CD1dhi regulatory B cells from Schistosoma haematobium-infected individuals induce IL-10-positive T cells and suppress effector T-cell cytokines. J Infect Dis. 2014 Oct 15;210(8):1207–16.

50. Langhorne J, Cross C, Seixas E, Li C, von der Weid T. A role for B cells in the development of T cell helper function in a malaria infection in mice. Proc Natl Acad Sci U S A. 1998 Feb 17;95(4):1730–4.

51. Bouchery T, Kyle R, Ronchese F, Le Gros G. The Differentiation of CD4+ T-Helper Cell Subsets in the Context of Helminth Parasite Infection. Front Immunol [Internet]. 2014 Oct 15 [cited 2015 Nov 18];5. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4197778/

52. van Riet E, Hartgers FC, Yazdanbakhsh M. Chronic helminth infections induce immunomodulation: consequences and mechanisms. Immunobiology. 2007;212(6):475–90.

53. Green TJ, Kreier JP. Demonstration of the role of cytophilic antibody in resistance to malaria parasites (Plasmodium berghei) in rats. Infect Immun. 1978 Jan;19(1):138–45.

54. Bouharoun-Tayoun H, Druilhe P. Plasmodium falciparum malaria: evidence for an isotype imbalance which may be responsible for delayed acquisition of protective immunity. Infect Immun. 1992 Apr;60(4):1473–81.

55. Lund FE, Garvy BA, Randall TD, Harris DP. Regulatory roles for cytokine-producing B cells in infection and autoimmune disease. Curr Dir Autoimmun. 2005;8:25–54.

56. Salazar-Castañon VH, Legorreta-Herrera M, Rodriguez-Sosa M. Helminth Parasites Alter Protection against Plasmodium Infection. BioMed Res Int [Internet]. 2014 [cited 2015 Jul 20];2014. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4170705/

57. Anosike JC, Onwuliri CO, Abanobi OC. A case of probable transplacental transmission of Wuchereria bancrofti microfilariae. Appl Parasitol. 1994 Nov;35(4):294–6.

1

58.Davis SM, Worrell CM, Wiegand RE, Odero KO, Suchdev PS, Ruth LJ, et al. Soil-Transmitted Helminths in Pre-School-Aged and School-Aged Children in an Urban Slum: A Cross-Sectional Study of Prevalence, Distribution, and Associated Exposures. Am J Trop Med Hyg. 2014 Nov 5;91(5):1002–10.

59.Belyhun Y, Medhin G, Amberbir A, Erko B, Hanlon C, Alem A, et al. Prevalence and risk factors for soil-transmitted helminth infection in mothers and their infants in Butajira, Ethiopia: a population based study. BMC Public Health. 2010;10:21.

60.Pearce EJ, M Kane C, Sun J, J Taylor J, McKee AS, Cervi L. Th2 response polarization during infection with the helminth parasite Schistosoma mansoni. Immunol Rev. 2004 Oct;201:117–26.

61.Caldas IR, Correa-Oliveira R, Colosimo E, Carvalho OS, Massara CL, Colley DG, et al. Susceptibility and resistance to Schistosoma mansoni reinfection: parallel cellular and isotypic immunologic assessment. Am J Trop Med Hyg. 2000 Jan;62(1):57–64.

62.Demeure CE, Rihet P, Abel L, Ouattara M, Bourgois A, Dessein AJ. Resistance to Schistosoma mansoni in humans: influence of the IgE/IgG4 balance and IgG2 in immunity to reinfection after chemotherapy. J Infect Dis. 1993 Oct;168(4):1000–8.

63.Hagan P, Blumenthal UJ, Dunn D, Simpson AJ, Wilkins HA. Human IgE, IgG4 and resistance to reinfection with Schistosoma haematobium. Nature. 1991 Jan 17;349(6306):243–5.

64. Vereecken K, Naus CWA, Polman K, Scott JT, Diop M, Gryseels B, et al. Associations between specific antibody responses and resistance to reinfection in a Senegalese population recently exposed to Schistosoma mansoni. Trop Med Int Health TM IH. 2007 Mar;12(3):431–44.

65.Yazdanbakhsh M, Sacks DL. Why does immunity to parasites take so long to develop? Nat Rev Immunol. 2010 Feb;10(2):80–1.

66.Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. -

PubMed - NCBI [Internet]. [cited 2015 Dec 22]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18625747/

67.Clonal expansion of immunoglobulin M+CD27+ B cells in HCV-associated mixed cryoglobulinemia. - PubMed - NCBI [Internet]. [cited 2015 Dec 22]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17942751/

68.Diallo TO, Remoue F, Gaayeb L, Schacht A-M, Charrier N, De Clerck D, et al. Schistosomiasis coinfection in children influences acquired immune response against Plasmodium falciparum malaria antigens. PloS One. 2010;5(9):e12764.

69.Lyke KE, Wang A, Dabo A, Arama C, Daou M, Diarra I, et al. Antigen-specific B memory cell responses to Plasmodium falciparum malaria antigens and Schistosoma haematobium antigens in co-infected Malian children. PloS One. 2012;7(6):e37868.

70.Mutapi F, Roussilhon C, Mduluza T, Druilhe P. Anti-malaria humoral responses in children exposed to Plasmodium falciparum and Schistosoma haematobium. Mem Inst Oswaldo Cruz. 2007 Jun;102(3):405–9.

71.Nacher M, Singhasivanon P, Silachamroon U, Treeprasertsu S, Krudsood S, Gay F, et al. Association of helminth infections with increased gametocyte carriage during mild falciparum malaria in Thailand. Am J Trop Med Hyg. 2001 Nov;65(5):644–7.

72.Sangweme DT, Midzi N, Zinyowera-Mutapuri S, Mduluza T, Diener-West M, Kumar N. Impact of schistosome infection on Plasmodium falciparum Malariometric indices and immune correlates in school age children in Burma Valley, Zimbabwe. PLoS Negl Trop Dis. 2010;4(11):e882.

73.Noone C, Parkinson M, Dowling DJ, Aldridge A, Kirwan P, Molloy SF, et al. Plasma cytokines, chemokines and cellular immune responses in pre-school Nigerian children infected with Plasmodium falciparum. Malar J. 2013;12:5.

1

74.Nmorsi O, Isaac C, Ukwandu N, Ekundayo A, Ekozien M. Schistosoma haematobium and Plasmodium falciparum co-infection with protection against Plasmodium falciparum malaria in Nigerian children. Asian Pacific journal of tropical medicine. :16–20.

75.Metenou S, Dembele B, Konate S, Dolo H, Coulibaly YI, Diallo AA, et al. Filarial infection suppresses malaria-specific multifunctional Th1 and Th17 responses in malaria and filarial coinfections. J Immunol Baltim Md 1950. 2011 Apr 15;186(8):4725–33.

76.Diallo TO, Remoue F, Schacht AM, Charrier N, Dompnier J-P, Pillet S, et al. Schistosomiasis co-infection in humans influences inflammatory markers in uncomplicated Plasmodium falciparum malaria. Parasite Immunol. 2004 Sep;26(8-9):365–9.

77. Dolo H, Coulibaly YI, Dembele B, Konate S, Coulibaly SY, Doumbia SS, et al. Filariasis attenuates anemia and proinflammatory responses associated with clinical malaria: a matched prospective study in children and young adults. PLoS Negl Trop Dis. 2012;6(11):e1890.

78.Wilson S, Jones FM, Mwatha JK, Kimani G, Booth M, Kariuki HC, et al. Hepatosplenomegaly associated with chronic malaria exposure: evidence for a pro-inflammatory mechanism exacerbated by schistosomiasis. Parasite Immunol. 2009 Feb;31(2):64–71.