Parasitic infections during pregnancy : birth outcomes and immunological changes

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CHAPTER 9

SUMMARIZING DISCUSSION

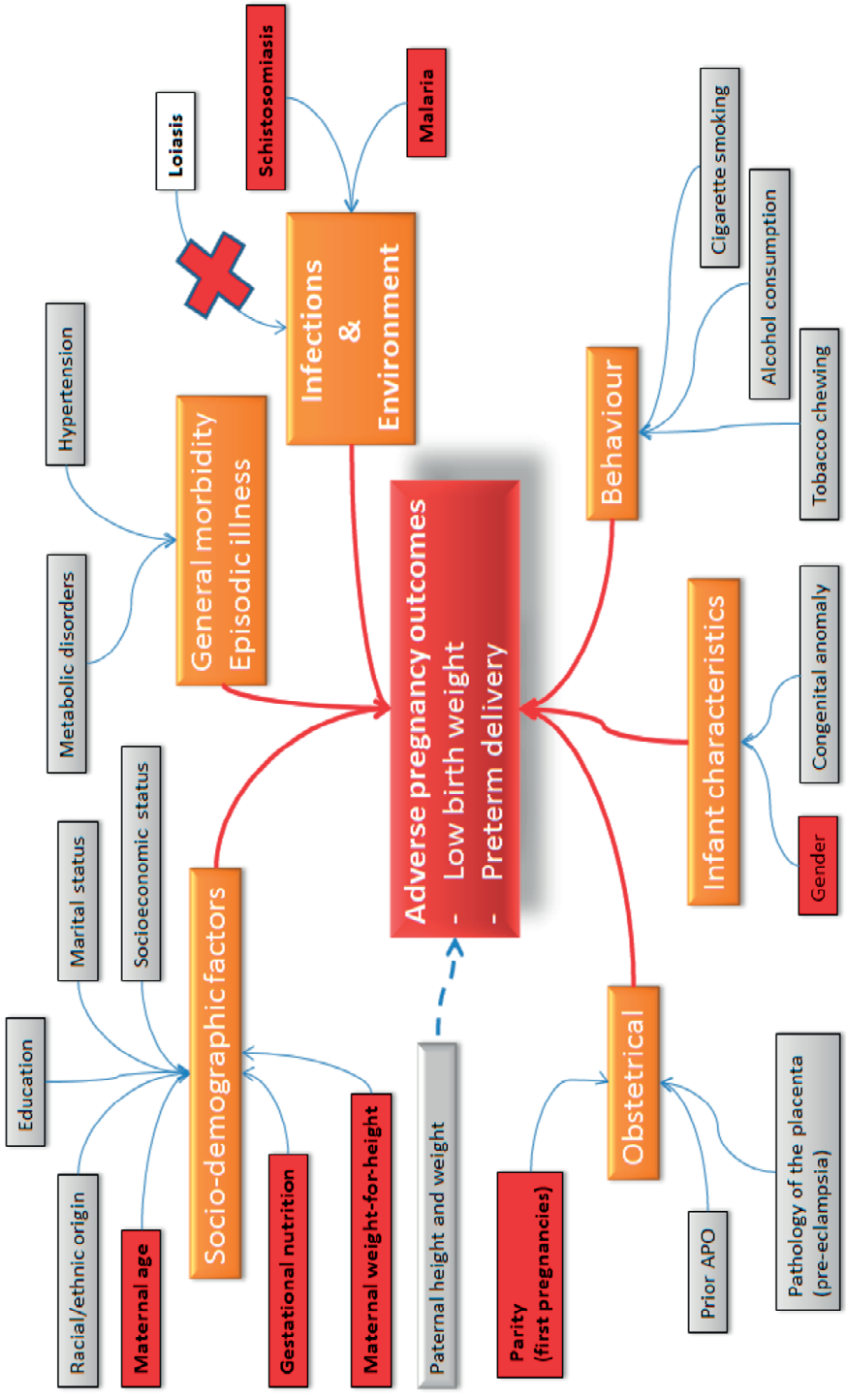

Figure 1: **Conceptual framework of risk factors of Adverse pregnancy outcome (APO)**
Risk factors of APO have been categorized (orange boxes). The red boxes are the risk factors evidenced throughout this thesis. The grey boxes represent known risk factors of APO which were not assessed in this thesis.The red cross shows loiasis was not found to be an independent risk factor in the analysis as it was expected.

Summarizing Discussion

The accurate identification of pregnancies most at risk for poor birth outcomes is crucial to allow for targeted surveillance or preventive interventions to be instigated early in pregnancy and from birth. In this thesis, the epidemiology of adverse pregnancy outcomes including low birth weight and preterm delivery from singleton live births and their risk factors has been examined in four sub-Saharan African countries, then the complex dynamic interaction between the three major parasitic infections, malaria, schistosomiasis, and filariasis and pregnancy and its outcomes has been explored in an endemic rural area of Gabon (summary in Figure 1). Lastly, immunological assays have been performed to assess some immune mechanisms that characterise the interaction between human hosts and helminth parasites. Findings are a step towards our ultimate goal of providing novel preventive and curative interventions that are optimal for mother and child health in Africa.

Epidemiology of adverse pregnancy outcomes in sub-Saharan Africa

Our findings in chapter 2 show that the burden of adverse pregnancy outcomes, which include both preterm births and low birth weights, remains substantial in sub-Saharan Africa with overall incidences of 4% and 10%, respectively. These findings are in agreement with other data from the same region (1–3). With regards to low birth weight, a recent review reported estimates of its median incidence in sub-Saharan Africa to be 13.3% (IQR 9.9-16.4), whereas for prematurity it was 15.4% (IQR 10.6-19.1) (4), reflecting a discrepancy with our data for preterm birth. The match between our data about low birth weight and those of others may be explained by the excellent validity and precision of measuring low birth weight while in contrast measuring preterm birth requires a valid estimate of gestational age which is often difficult and varies between studies. In our study gestational age was estimated by Ballard score assessment. Important for our thesis it is the consistency of this measurement of preterm birth throughout all studies using the Ballard score.

From the risk factors assessments reported in chapter 2, after controlling for potential confounders, it transpired that independent risk factors in our study population were very young maternal age, first pregnancies, poor nutritional status including low pre-pregnancy weight-for-height and small stature of the mother. In this multi-country study, there was an important inter-country variability. For instance Gabon, our country of interest, had the highest incidence of low birth weight with 13% (95%CI 11-15%) followed by Benin with 11% (95%CI 9-13%), then Mozambique and Tanzania each with an incidence of 8% (95%CI 6-9%). There are disparities between countries for the distribution of risk factors. Young maternal age was more often seen in Gabon and

Mozambique, whereas *Plasmodium falciparum* infection was more common in Benin (12%) and Gabon (6%) compared to Mozambique (2%) and Tanzania (2%). This is interesting as in regions of high malaria transmission it is estimated that malaria infections cause about 19% of low birth weight deliveries (5). However, as malaria was more common in Benin than in Gabon, whereas more low birth weight infants were seen in Gabon, this would suggest that young maternal age, or additional unidentified risk factors other than malaria play an important role as risk factors.
It would be noteworthy to mention the prospective design of the study and the highly standardized data collection that was in place. Indeed participants were randomized and followed on in the context of a controlled trial that ensured high coverage of standard antenatal care including vitamin and micronutrient supplementations as well as insecticide treated nets as well as additional antenatal health care available and accessible for free to all participants. Despite this, the relatively poor outcomes in Gabon compared to other countries might indicate that some risk factors specific to Gabon still need to be explored in order to allow the full improvement and management of maternal and child health in the continuum of antenatal and postnatal care.

Loiasis and schistosomiasis infections and clinical outcomes of pregnancy
From the above observations and in the context of endemic parasitic infections in Gabon, it was interesting to assess the potential role played by major parasitic diseases such as malaria, schistosomiasis, and loiasis in the occurrence of adverse pregnancy outcomes in Gabon. Of relevance to the analysis is the fact that with the exception of malaria among the major parasitic infections in sub-Saharan Africa, the other parasitic infections lack adequate data to address their impact on pregnancy. Therefore, there is little known about filarial- and schistosome-specific morbidities that are experienced by pregnant and lactating mothers and their offspring.
To date there has been no systematic epidemiologic description of loiasis during pregnancy and its impact on mothers' and infants' health. The specific effect of loiasis on pregnancy and pregnancy outcomes is therefore not well understood. Moreover, its effect at the individual as well as at the community level has not been studied well. In addition, loiasis does not belong to the list of neglected tropical diseases to be controlled or eradicated in the worldwide campaigns. Loiasis was recently reviewed by Metzger and Mordmüller who raised the question whether *Loa loa* deserves to be neglected (6). In chapter 3, we observe in the population of pregnant women from Gabon that loiasis is common during pregnancy with more than 18% showing evidence of microfilariae in peripheral blood. This is expected to be a serious underestimation as there are many cryptic *L. loa* infections or "occult loiasis", term used for patients with no microfilariae in peripheral blood despite

evidence of infection as determined by clinical signs and/or ocular passage of adult worms. In highly endemic areas, occult loiasis has been reported to be the most common infection state (7,8). The diagnostic method for filarial infections, when depending on the detection of microfilariae, are very insensitive and we did not use any molecular techniques that have better sensitivity (9,10). Therefore the true prevalence of *L. loa* infection during pregnancy might be a lot more common than we have observed. Another point to consider is that the antimalarial treatment administered during pregnancy, may also have influenced the course of loiasis as has been shown for other helminthic infections (11). However, to date no effect of mefloquine or sulfadoxine-pyrimethamine has been demonstrated on filarial parasites.

From our findings, *L. loa* infection did not seem to be associated with an increased risk of adverse pregnancy outcomes. These findings are in line with the few previous reports. Firstly in 1963, two cases of asymptomatic *L. loa* filariasis in pregnant women of African origin in the United Kingdom were reported without any adverse birth outcome (12). Two further case reports similarly reported no evidence for adverse birth outcomes (13,14). Here we also reported the invasion of microfilariae into the intervillous space of the placenta of a subset of the women with detectable microfilaria in their peripheral circulation. There are no similar studies in the literature to compare our findings with. Interestingly, despite the invasion of the placenta, no inflammation, infarction, or chorioamnionitis was observed. These findings may suggest that circulating microfilariae are not pathogenic or demonstrate the ability for these parasites to evade successfully the host immune system and consequently maintain chronic infection (15). Indeed a study conducted in Gabon by Martinez and colleagues in 2009 showed that infections with *L. loa* are associated with increased frequencies of $CD4^{+}CD25^{hi}FOXP3^{+}$ T cells in humans and a decrease in frequency of Th17 cells and suppressed Th2 and Th1 responses which might prevent excessive inflammation in tissues affected by these parasites (unpublished data). Besides these mechanisms of T cell hyporesponsiveness probably mediated by a regulatory network mastered by Tregs, there has been evidence that circulating microfilariae affect also the immune responses by blocking complement activation and acquiring the host complement regulators H and C4b-binding protein in blood circulation (16). Concluding chapter 3, loiasis is definitely a common condition among pregnant women in rural settings in Gabon, however there is no evidence of association with adverse pregnancy outcomes. Given our findings regarding the effect of this parasite on the immune system, further studies might be needed to investigate the impact of loiasis on pregnancy itself and infant health after in utero exposure [Figure 2].

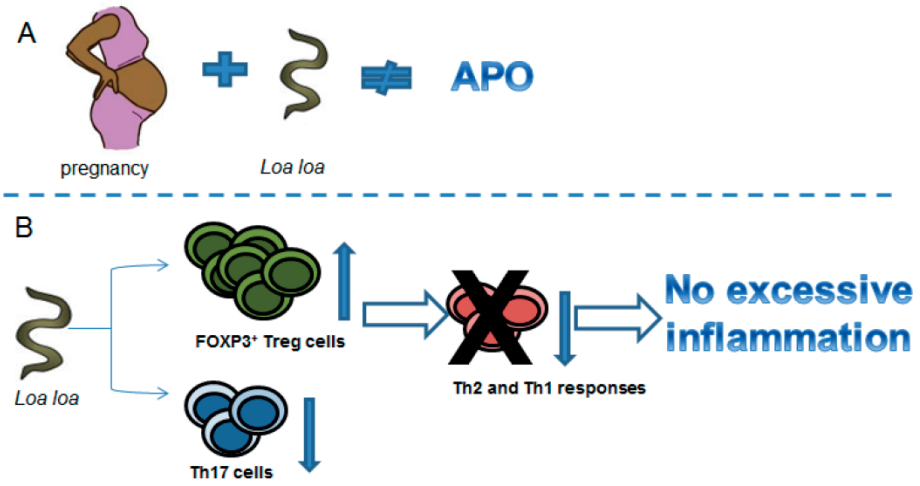

Figure 2: **Loiasis in pregnant women in Gabon**
Loiasis is a common condition among pregnant women in rural settings in Gabon (>18%); A. There was no evidence of association of loiasis with adverse pregnancy outcomes (APO); B. Infections with tissue dwelling filarial parasites, *Loa loa,* are associated with increased frequencies of $CD4^{+}CD25^{hi}FOXP3^{+}$ T cells in humans and a decrease in frequency of Th17 cells as well as a suppressed specific Th1 responses which might prevent excessive inflammation in tissues affected by the parasites.

Our findings in Chapter 4 show that urogenital schistosomiasis infection is also common among pregnant women in our rural Central African region, in line with previous reports (17). Here it can also be stated that the diagnostic method used might have not been sufficiently sensitive and therefore we have underestimated the burden of schistosomiasis in pregnancy. Thus, the prevalence of schistosomiasis might be higher than 9% in our population of pregnant women. The finding showing a significantly higher risk for delivering low birth weight infants in *S. haematobium* infected pregnant women compared to those free of infection is very important. This finding is consistent with experimental evidence from animal models and also with human case reports indicating adverse impact of schistosomiasis on delivery outcomes (18). Animal models indicate that both acute and chronic infections in rodents may directly result in decreased birth weight of the offspring (19,20). *S. haematobium* causes pathology in the urinary and genital tracts as a result of trapped eggs. Histology studies of urogenital schistosomiasis have shown that the tissue around both viable and dead eggs has increased vascularity and high density of macrophages, lymphocytes, foreign body giant cells, eosinophils, neutrophils, plasma cells, Langerhans cells, fibroblasts, and multinucleate histiocytes (21,22), and local lesions such as sandy patches as well as rubbery

papule have been reported (23). In order to determine the potential mechanistic link between maternal schistosomiasis and poor birth outcomes, some data have indicated that maternal schistosomiasis results in pro-inflammatory signature that is detectable in maternal and foetal compartments of the placenta and a subset of these responses are associated with decreased birth weight (24) [Figure 3]. Other proposed mechanisms of schistosomiasis-mediated adverse birth outcomes include anoxia due to pro-inflammatory cytokines, placental inflammation, and maternal iron deficiency due to chronic bleeding (18,25). Studies have demonstrated increased risk of anaemia associated with *S. mansoni* infection in pregnant women (26). In the study population assessed in chapter 4 approximately half of the participants were anaemic. However, there was no observed association between anaemia and urogenital schistosomiasis in the analysis. This finding may be explained by the multiplicity of causes of anaemia in this geographical setting including malaria, nutritional deficiencies, and pregnancy by itself.

Our findings in chapter 4 show that urogenital schistosomiasis is associated with increased risk of placental malaria infection in this study population. We found in chapter 4 that malaria infection was an independent risk factor of low birth weights, in line with previous reports (5). As both, schistosomiasis and malaria, affect disproportionally the “rural poor”, these two major parasitic infections are likely to have a combined deleterious effect on pregnant women in regions of co-endemicity. On the other hand, it should be noted that malaria and filarial infection in the study reported in chapter 3 did not show to be associated.

Assuming causal inference even though we are aware of the limitations associated with the observational design of the study, the finding that urogenital schistosomiasis affects birth outcomes whereas loiasis is not associated with adverse pregnancy outcomes, suggests that schistosomiasis might be pathogenic by the mechanisms discussed above, whereas *L. loa* infection can better evade the immune system.

In conclusion, from our data and from previous reports, it appears that maternal urogenital schistosomiasis is independently associated with decreased birth weight of the offspring while loiasis is not. These findings raise the issue of the necessity of a screening and treatment program for maternal schistosomiasis in order to prevent the deleterious consequences on pregnancy. Indeed, WHO recommends offering pregnant women treatment with praziquantel. However, two trials that have investigated the benefit of interventions against schistosomiasis during pregnancy on birth outcomes have shown no significant effect of the intervention on birthweight. One trial in the Philippines on *S. japonica* infected pregnant women treated at 12-16 weeks of gestation showed that praziquantel did not have a significant effect on birthweight or the prevalence of low birth weight and small for gestational age

(27). The other trial in Uganda that treated pregnant women infected with *S. mansoni* later in gestation (mean gestational age at enrolment 26.6 weeks) showed no effect of praziquantel on maternal anaemia or proportion of low birth weights (28). In the Ugandan study, this was true even when analyses were restricted to women found to be infected with *S. mansoni*. These results may be interpreted that treatment during gestation could be too late to have an effect and leaves room for considering preconception care of schistosomiasis as an alternative.

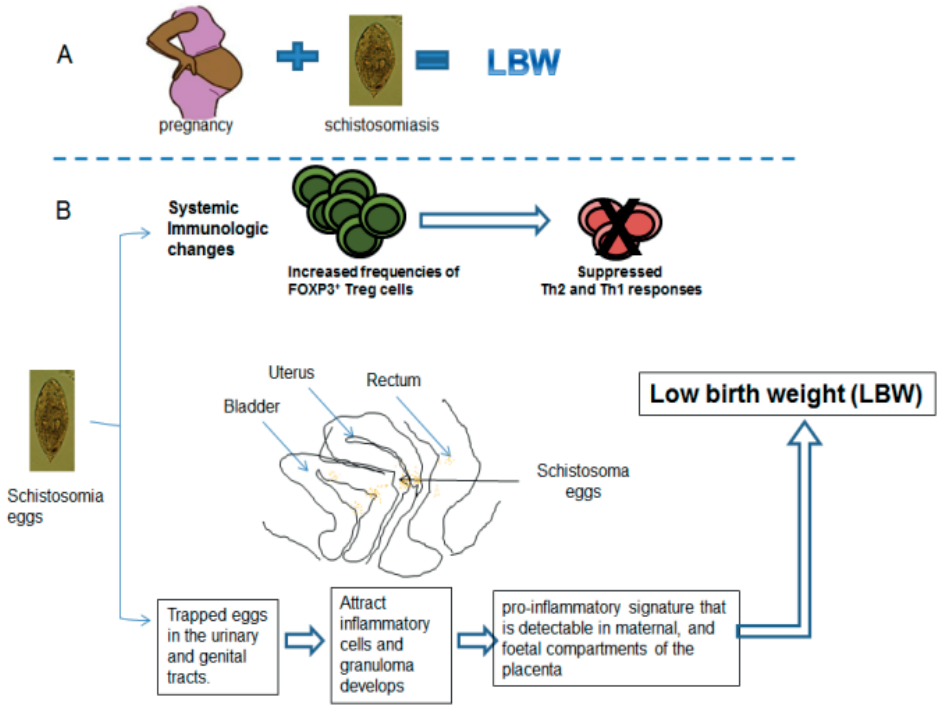

Figure 3: **Schistosomiasis during pregnancy**
A. *Schistosoma haematobium* infection is associated with Low Birth Weight (LBW); B. Schistosome infection induces immunological changes with increased frequencies of Tregs which in turn can down modulate Th1 and Th2 effector cells. In the urinary and genital tracts, pathology is caused by trapped eggs which find their way from the urinary system to the female genital region and form granulomas in the uterus, fallopian tube, and ovaries with possible complications during gestation.

With regards to helminth infections, although they are known to be important modulators of the immune system of their hosts, the helminth-induced mortality is low as compared to malaria and majority of infections with these parasites do not lead to noticeable immunopathology, however in some target

tissues, pathology can cause significant disabilities, for example elephantiasis resulting from lymphatic filariasis or liver granulomas formed around schistosome eggs. In our assessments, schistosomiasis has shown to be associated with increased risk of low birth weight babies while loiasis did not, while both helminth infections are described as down-regulating the immune responses of their hosts [Figure 2 and Figure 3]. These findings suggest that it is the local inflammation in the urinary and genital tissues caused by trapped eggs which extends to the maternal foetal interface that may be responsible of the adverse outcomes of pregnancy possibly related to schistosomiasis. Another aspect highlighted by these findings is that if there is immunopathology in a target organ this is not reflected in peripheral blood. Furthermore, the association observed between schistosomiasis and malaria may be seen as an added factor to increase the local inflammation of the maternal and foetal interface and lead to the observed increased rate of low birth weights.

Malaria in pregnancy and intervention strategies

Regarding malaria in pregnancy, there is strong evidence that shows pregnant women have increased susceptibility to malaria infection and show increased morbidity as well as mortality both in the mother and her offspring (5,29). Also our results in chapter 4 indicate that malaria parasitaemia was associated with an increased risk of low birth weight. The main pathogenic action of malaria in pregnancy is thought to be from the parasites sequestered in the intervillous space of the placenta and from the resulting local inflammation that depending on the stage of pregnancy would affect placentation or intrauterine growth of the foetus.

There are preventive measures recommended by WHO and routinely implemented, which rely on the use of long lasting insecticide-treated nets (LLITNs), intermittent preventive treatment of malaria in pregnancy with sulphadoxine-pyrimethamine (IPTp-SP), and prompt diagnosis and effective treatment of malaria infections. In Chapter 5 we have evaluated mefloquine as an alternative antimalarial drug to SP for IPTp due to the concerns for its long term use for IPT raised by the spread of parasite resistance and the need of optimal health decision-making especially in resource-limited countries. No difference was found in the incidence of low birth weight between mefloquine and SP, suggesting both drugs were equally effective. However, it is possible that the intensive antenatal care strategies together with the decrease in malaria transmission in some study sites, during the study period, have decreased low birth weight attributable solely to malaria, compromising the statistical power to detect a difference between groups. Also, low birth weight being prone to multiple risk factors and confounders, as we have

demonstrated in previous chapters, particularly in chapter 2, may not be the suitable outcome to reflect the efficacy of malaria control strategies.
Interestingly, the prevalence of maternal parasitaemia and anaemia at delivery were significantly lower in women receiving mefloquine compared to SP recipients. The better performance of mefloquine over SP with regards to malaria and anaemia demonstrates an advantage of the former drug despite a poor safety profile. That performance of mefloquine is very interesting in that context of co-endemicity between malaria and schistosomiasis because mefloquine has previously been demonstrated to exert considerable activity against *S. mansoni* and *S. japonicum* in rodents (30–32). Recently the first exploratory trial evaluating mefloquine alone and in combination with artemisinin in schoolchildren infected with *S. haematobium* provided evidence for a clinically relevant effect in humans (33).
In Chapter 6, our data showed a marked reduction of egg excretion in pregnant women infected with *S. haematobium* when receiving mefloquine as intermittent preventive treatment against malaria, adding important information on the activity of mefloquine on one of the most important parasitic diseases co-endemic with malaria and infecting pregnant women. The beneficial consequences of that reduction in egg excretion and the associated granulomatous inflammation could be extended to include reduced exposure of the newborn to inflamed or bleeding vaginal mucosa – therefore potentially reducing exposure to vertically transmitted infections including HIV and hepatitis B virus. Another benefit for the women is the reduction of pathological consequences of chronic urogenital schistosomiasis including pelvic inflammatory disease (21,22,34,35). The latter point is relevant in the light of our findings described in Chapter 4 that maternal urogenital schistosomiasis was associated with increased risk of low birth weight babies. Therefore IPTp programs including mefloquine, if implemented may serve as a 2-pronged approach against two of the most important parasitic infections.

Immunological effects

The paradox of pregnancy is characterized by an immunologic tolerance to potentially foreign foetal antigens despite an apparently adequate maternal defence against infection. Changes are known to occur locally at the maternal-foetal interface but may also affect systemic immune responses. The influence of co-infections on immunological mother-child interaction is of particular interest as infections of the mother are known to influence susceptibility of the newborns to the same parasites. For example, children born to mothers with placental malaria have a higher susceptibility to malaria in early infancy and sensitization to filarial antigens in utero influences the subsequent development of the immune response and higher susceptibility to filarial infection (36). In addition, a study on BCG vaccination at birth showed that

after 2 to 10 years, the responses to mycobacterial proteins differed between children born to mothers with schistosomiasis or filariasis compared to children from non-infected mothers (37); while from the same prospective study, infants sensitized to schistosome or filarial antigens in utero had suppressed IFN-γ and increased IL-5 responses to mycobacterial antigens at one year (37). Similarly, a study by Elliot and colleagues indicated that maternal hookworm, and de-worming with albendazole during pregnancy, might influence the infant immune response following BCG vaccination at birth (38). However, the same authors later had results suggesting that maternal helminth infection may have little, if any, adverse effect on the outcome of infant immunization, and therefore are unlikely to explain poor vaccine efficacy in the tropics (39). A randomised, placebo-controlled trial from the same group showed that maternal anthelmintic treatment during pregnancy can have a small effect on an infant's response to tetanus immunisation, but has no effects, either beneficial or detrimental, on the occurrence of infectious diseases during infancy, infant mortality, or growth and anaemia outcomes at one year of age (40). These results questioned the expected benefits of routine antenatal anthelmintic treatment as previously advocated (41).

Most research groups in affluent countries have assessed the interaction between helminths and host's immune system and whether by modulation of bystander responses, helminths could influence the outcome of vaccinations or inflammatory diseases or conditions such as pregnancy, through studying animal models, travellers or experimentally infected humans, and only a few groups have taken these questions to areas where helminth infections are highly endemic.

In Chapter 7, we have conducted an exploratory study designed to assess the effect of maternal filarial infection on the neonatal T helper (Th) cells that are known to be involved in malaria driven immune responses (Th1 and Th17), using transcription factors that are now considered as hallmarks of T helper cells polarization. To this end we measured the percentage of $CD4^+$T cells and subsets of $CD4^+$T cells expressing Tbet, RORγt and FOXP3 in cord blood mononuclear cells (CBMCs) collected from offspring of loiasis infected and uninfected mothers. We did not find a significant effect of maternal filarial infection on the percentage of $Tbet^+$, $ROR\gamma t^+$, $CD25^{hi}FOXP3^+$ $CD4^+$T cells, nor on the level of expression of these transcription factors. This is in contrast to reports describing an expansion of $CD25^{hi}FOXP3^+$ $CD4^+$T cells in infected individuals (42). One possible explanation is that cord blood T cells are at different stages of differentiation than adult T cells. Treg cells possess a T cell receptor (TCR) repertoire as broad as $CD25^-$ T cells (43) and it is known that Tregs become suppressive after stimulation via the TCR. Once activated, they supress in an antigen non-specific manner (44). In newborns, most of the T cells are naïve (90% of CD3+ cells express CD45RA)(45), and are thus thought

not to have encountered an antigen. Therefore, either the regulatory T cells with suppressive activity are within the antigen experienced 10% or the cells are antigen experienced but not yet showing the memory CD45RO marker. Next set of studies should use markers for further characterization of these cells in cord blood. In any case, there was a negative association between $CD4^{+}CD25^{hi}FOXP3^{+}$T cells and $CD4^{+}Tbet^{+}$ as well as $CD4^{+}ROR\gamma t^{+}$ T cells in the infected group. These results suggest that filarial infection during pregnancy leads to an expansion of functionally active regulatory T cells that keep Th1 and Th17 in check [Figure 2]. Our finding is in line with a previous report, not in neonates, but in older subjects, that found similar frequencies of regulatory T cells between a group of geo-helminth infected and uninfected control group, but the suppressive activity was significantly pronounced in the geo-helminthes infected group (46).

This raises the question of how *in utero* exposure to maternal helminth infections may affect offspring health and development and more broadly with the concept of exposome which is composed of every exposure to which an individual is subjected from conception to death (47). The human foetus and infant can respond to unbalanced nutrition and other adverse influences by changing their developmental and growth trajectories (48,49). Some epidemiological studies have shown that early life events such as *in utero* exposure to an environment play an important role in determining the risk for common cardio-metabolic diseases in adulthood (50). In particular, intrauterine growth restriction is associated with a substantially greater incidence of adult hypertension, insulin resistance/type 2 diabetes and cardiovascular diseases deaths (51). These effects extend across the normal range of birth weights and are not confined to those born very small or premature. Although the mechanisms are unclear, animal studies suggest a role for epigenetic processes (51), and emerging human data indicate altered DNA (cytosine) methylation in neonates in association with intrauterine growth restriction and in babies born to obese mothers (50,52). Reports showing altered DNA suggest that such epigenetic changes may persist into adult life (53). The data on the role of epigenetic modification on early life exposures to infectious agents are emerging from the so called “farm studies” where children born on traditional farms with heavy exposure to microorganisms show altered DNA methylation patterns compared to non-farm newborns (54).

In the study reported in chapter 8 we provide evidence that human schistosomiasis is associated with significant increases of frequencies of $CD4^{+}CD25^{hi}FOXP3^{+}$ regulatory T cells that play an important role in controlling Th1 and Th2 responses. The study was conducted in school age children, paving the way for studies in cord blood. In our study, these Tregs showed to exert a suppressive effect on both proliferation and cytokine production. The

depletion of Tregs showed a restauration of the immune responses by improved proliferation and cytokine responses. Studies of the immunology of pregnancy have shown evidence of an increase of Tregs during pregnancy, and that the expansion of the Treg population is of importance for the allogenic foetus to evade immune attack from the mother (55). With the view to the potency and wide-ranging involvement of Treg cells in immune homeostasis and prevention of disease pathology, our finding that human schistosomiasis is associated with increased frequencies of Tregs raises the question whether helminth infections should be considered beneficial for pregnancy outcome. In chapter 4 however, schistosomiasis was rather associated with increased risks of low birth weight [Figure 3].

From our different findings parasitic infections are common in gestating women and can result in immunological and physiological changes. Some of these changes might influence fecundity, affecting conception and pregnancy. Blackwell and colleagues in a recent study demonstrated that different species of helminth are associated with contrasting effects on fecundity. Infection with roundworm (*Ascaris lumbricoides*) was associated with earlier first births and shortened inter birth intervals, whereas infection with hookworm was associated with delayed first pregnancy and extended inter birth intervals (56). It would be interesting and important to assess the effects of helminths on human fertility in Gabon.

Summary

In summary the work in this thesis indicates that low birth weight including preterm birth and intrauterine growth retardation, remains important in sub-Saharan Africa and particularly highly prevalent in Gabon. Among the risk factors of low birth weight in sub-Saharan Africa are very young maternal age, first pregnancy, poor gestational nutrition and small stature of the mother.
In Gabon, besides malaria, the other two major parasitic infections namely urogenital schistosomiasis and the filarial infection *L. loa*, are common in pregnant women. Maternal schistosomiasis like malaria showed to be associated with higher proportions of low birth weight babies, while loiasis was not.
In the context of long lasting insecticide treated bed nets, mefloquine as an alternative preventive treatment, despite showing no difference with sulphadoxine – pyrimethamine in preventing low birth weight, was however more effective in preventing malaria infection and anaemia. In the same context, it was observed that mefloquine administered for the prevention of malaria was effective against concomitant urogenital schistosomiasis, suggesting that mefloquine could seriously be considered as an alternative to praziquantel in the case of pregnancy. It could serve as a combined intervention for both malaria and schistosomiasis during pregnancy.
We have demonstrated that maternal infection with *L. loa* was associated with expansion in the neonatal cord blood of functionally activate Tregs that kept Th1 and Th17 immune responses in check, providing some insights on the impact of *in utero* exposure on the offspring's development and health.
Lastly we have explored the shaping of the immune responses by human schistosomiasis and the role played by regulatory T cells in the modulation of these immune responses. Despite expansion of regulatory T cells during loiasis and schistosomiasis, there is no effect or even an adverse effect, respectively, on pregnancy outcome, raising the question regarding the clinical impact of these immunological changes.

Future directions

One major limitation of our studies was the observational design. More intervention studies are necessary to further determine whether getting rid of parasites either by screening, prevention or treatment strategies would improve the outcome of pregnancies in endemic areas. Equally important are planning of studies that encompass health education directed at preventing pregnancy at a very young age, assess the outcomes of such preventions and identify additional risk factors that are specific to geographical areas.

The evidence that environmental factors during critical periods of pre- and immediate postnatal mammalian development can influence metabolism and the immune system, which in turn can modulate pathogenesis and disease susceptibility is interesting and should be studied. A relatively new area is the interaction between the immune system, metabolism and microbiota. The complex communities of microorganisms, termed “microbiota”, inhabit the body mucosa and surfaces and have a highly coevolved relationship with the immune system (57). Although many of these microbes carry out functions that are critical for the host physiology, they nevertheless can pose a threat of breach with ensuing pathologies. The mammalian immune system plays an essential role in maintaining homeostasis with resident microbial communities, and therefore resident bacteria profoundly shape mammalian immunity (57). The question how pregnancy affects the microbiota and whether any modulation of the immune system is through the alternation in the microbial community needs to be addressed. Fecal transplantation is proving an interesting treatment for a number of inflammatory conditions (58).

During the past decade there has been an explosion in the application of large scale, high-throughput “Omics” technologies (such as genomics, epigenomics, proteomics, and metabolomics) to biomedical research. These technologies coupled to powerful bioinformatics tools, are expected to continue to revolutionize the field of biology and medical research (59). Understanding the contribution of genetic and environmental variation in health outcomes of humans is essential for design of novel preventive and curative measures. The results from such studies can be clinically applied to improve patient care. The accurate prediction of pregnancies most at risk of poor birth outcomes is crucial to allow for targeted surveillance or preventive interventions to be instigated early in pregnancy and from birth onwards. The “Omics” technologies offer the potential for discovery of novel biomarkers for poor pregnancy outcome but need to be introduced into studies in the tropics where the problem is daunting. This would require well trained personnel, adequate infrastructure and proper study designs that are sufficiently powered to truly make a difference.

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