In this chapter we will discuss the impact of the most prevalent human helminth diseases on pregnancy. In the first part we will summarize the results of studies on those helminth infections that may have clinically relevant influences on successful pregnancy, and on maternal as well as offspring health in general, and which may also alter offspring’s immune system development in response to auto- and heterologous antigens in pre- and postnatal life. In the second part we will discuss how maternal helminth infection may influence the function of the fetomaternal interface e.g. via alterations of the cellular composition or gene-expression of the placenta, breastfeeding e.g. by changing the composition of mother’s milk and subsequently the developing immune system of the offspring e.g. the hematopoietic system. The discussion aims to aid our understanding of possible mechanistic events initiated by maternal helminth infection that shape the offspring’s development and, thereby, opens up new perspectives that warrant further investigation.

Introduction and General Aspects (e.g. Impact on Maternal and Offspring Health Status)

Almost one-third of the world’s population is infected with at least one helminth. Infection during pregnancy therefore is frequent and prevalence rates are reported to vary from 10 to 70% in endemic areas where co-infection with multiple worm species is not an uncommon event [1–4]. In general, poor maternal health as a result of insufficient nutrition or anemia in particular is a major contributor to increased
maternal and infant mortality and low birth weight and it is as well an important public health problem in developing countries [5]. Helminth infections can cause and/or exacerbate both malnutrition and anemia [6] and are therefore likely to contribute to problems during and after pregnancy as low-birth weight itself is associated with an increased risk to develop diseases later in life (as reviewed in refs. [7, 8]). In addition, pregnant women are at higher risk for carrying higher burden of either a single or multiple helminths or other parasitic protozoan co-infections, with Plasmodium species being the most common with the strongest negative impact on maternal health [2, 9, 10]. Multiple studies addressed the effects of maternal anemia resulting from helminth infection on parameters of offspring health, such as incidence or extent of low-birth weight (LBW) or childhood anemia, but the results of these studies reveal an inconclusive overall picture. A recent review on the impact of hookworm infection on pronounced maternal anemia during pregnancy revealed that infection intensity was associated with lower hemoglobin levels. This led to the recommendation of deworming treatment together with iron supplementation during pregnancy [11]. Albendazole treatment during pregnancy led to improvement of maternal anemia and thereby possibly of infant birth weight and mortality in a region of Nepal with high hookworm prevalence [12]. However, no differences were found in birth outcomes in pregnant women treated with albendazole or praziquantel within a randomized controlled study in Uganda [9], even though it was suggested that women with heavy hookworm infection and subsequent anemia profited from the treatment. Results from a study in Peru showed that treatment with mebendazole had again no overall influence on the birth weight but only a reduction within the group of the very-low birth weight children [13]. Furthermore, in a large study comprising 696 pregnant women in coastal Kenya, birth weight remained uninfluenced by maternal helminth or plasmodium infection [14]. A cross-sectional study with 746 pregnant women conducted in Ghana found that helminth-Plasmodium falciparum co-infection significantly increased the risk of anemia, low birth weight and small-for-gestational-age infants when compared to helminth infections [15] only.

In addition to the impact on child health and development in general, maternal helminth infections also influence the course and intensity of other infections during pregnancy. In this context, HIV transmission from mother to child and placental malaria are two important examples especially in sub-Saharan Africa which could be influenced by maternal helminth infections. A retrospective study of pregnant Kenyan women in an area endemic for lymphatic filariasis, schistosomiasis, and geohelminthes showed an increased risk of mother-to-child transmission (MTCT) of HIV in women that were infected with one or more species of helminths. Increased MTCT risk correlated with enhanced IL-5 and IL-13 production by helminth-antigen stimulated cord blood cells indicating an activated phenotype of the lymphocytes [16]. Moreover, upregulation of HIV co-receptors such as CCR5 and CXCR4 on the surfaces of CD4+ T cells and monocytes was shown before in PBMCs of S. mansoni infected patients [17]. It remains to be demonstrated whether this also applies to fetal CD4+ T cells and whether thereby increased transmission could occur by enhanced viral uptake into these pre-activated fetal cells. A Peruvian study on
the HIV related HTLV (human T-cell leukemia virus) showed a positive association between maternal Strongyloides infection with HTLV-1 seropositivity in children of HTLV-1-infected mothers. Again, this indicates that maternal helminth infection might be an additional risk factor for virus transmission to CD4+ T cells [18].

In helminth endemic areas co-infection rates with malaria are high and well defined. There were reports of adverse birth outcomes in malaria and helminth co-infected women [15, 19]. Placental malaria (PM) can occur in up to 25% of pregnant women resulting in as many as 200,000 infant deaths annually. PM results in a global reduction of antibody transfer especially of IgG isotypes specific for RSV, Varicella, HSV and vaccine-associated antibodies against measles and tetanus toxoid [20−22]. The underlying mechanisms have yet to be elucidated but potentially include downregulation of FcγR expression in the inflamed placenta or competition between high titers of species-specific IgGs at the feto-maternal interface [23]. Since transplacental transmission of antibodies is predominantly limited to IgGs, possible effects during helminth infection on subsequent (helminth) infection and/or immunity could arise from transferred helminth-specific IgG4 antibodies which may block production of protective IgE in the offspring and from other maternal helminth-specific antibodies that might catch helminth antigens, before T and B cell priming arises in the offspring. It is currently unknown whether PM alters the transport of these helminth-specific antibodies or helminth antigens. Therefore, it will be interesting to investigate this in the future.

In the context of childhood immunization, several vaccination studies previously showed that children in Sub-Saharan Africa respond less well to vaccines against tuberculosis (BCG), typhoid fever, measles (as reviewed in ref. [24]). However, whether maternal helminth infections may have important implications on the child’s ability to generate a protective antibody response to a vaccine, this is still a matter of debate since clinical data vary. For example, no effects on the efficacy of hepatitis B immunization was observed in Egyptian infants born to schistosome infected mothers when anti-HBs titers were measured at 9 months of age [25]. Another study, again conducted in Egypt, revealed that the generation of protective levels of anti-HBs antibodies in offspring born to S. mansoni-HBsAg-positive mothers was significantly delayed and that the titers remained lower at all assessed timepoints after immunization, most strongly at 8 months after vaccination [26]. However, no group of S. mansoni positive mothers without chronic HBV infection or their offspring were included in the study to evaluate the course of titer development in this rural setting. By far the most informative study on the outcome of vaccine efficacy in infants born to worm-infected mothers was conducted recently in Uganda. Here, only infections with the filarial nematode Mansonella perstans during pregnancy were associated with changes in children’s responses to vaccination even though the mothers were exposed to a range of helminth infections. These included increased IL-10 production to mycobacterial antigens and tetanus toxoid, but unaltered IFN-γ, IL-5 and IL-13 levels [27]. In the same study group, effects of maternal deworming with albendazole and praziquantel on responses to BCG tetanus and measles were assessed, but no effects on vaccine efficacy were observed. However, albendazole treatment of hookworm infected mothers reduced IL-5 and
IL-13 production to tetanus toxoid indicating that hookworm infections may impair tetanus vaccine efficacy [28].

Pregnancy is a controlled and protected state that primarily protects the embryo and fetus against potentially harmful immune responses to paternal antigens, while still allowing life-saving responses e.g. necessary to control potentially harmful infections [29]. The fetal immune system has developed T and B lymphocytes as well as antigen-presenting cells (APC) by the end of the first trimester, the latter being already capable of antigen specific priming of T cells [30]. However, the fetal immune system does appear to be restricted in the diversity of responses it can launch. Current studies indicate that immune differentiation favors regulatory and Th17 phenotypes rather than a Th1 phenotype [31]. Comparison of infants born in developed and developing countries also revealed differences in the proportions of peripheral immune cells and their responsiveness to TLR ligands when stimulated in vitro, for example by producing higher amounts of IL-10 when stimulated with a TLR2 ligand [32]. Furthermore, it appears that maternal helminth infection might have a major influence on the offspring’s immune responses to subsequent helminth infection i.e. to an “homologous” antigen exposure with filarial diseases having the most obvious effects observed so far (these will be discussed in the section “Filarial worms” below). Here, maternal transfer of parasite antigens was found to occur via the formation of antigen-antibody-FcγR immune-complexes in the placental-blood barrier or directly via breast feeding and subsequent enteric resorption. The potential effects of parasite antigens entering the offspring’s circulation during the developmental phase of the immune system are likely to be diverse: antigen presentation within the thymus may trigger clonal elimination of T cells, whereas extrathymical presentation in the secondary lymphoid organs in the absence of secondary costimulatory signals could favor either T cell anergy or, upon engagement of PD-L or CTLA-4, lead to induction of peripheral tolerance and regulatory T cells [33]. Furthermore, helminth antigens possess immunomodulatory properties which suppress innate immune responses to TLR ligands in vitro [34, 35] and induce de novo expansion of Treg cells in vitro and in vivo [36–38]. Reduced innate immune responses in PBMCs from children in helminth endemic areas may suggest that such immune regulation could occur as a result of maternal helminth infection [32]. Whether and how helminth antigens which are transported from infected mothers to the fetus could affect the offspring’s innate immune system reactivity in such a manner, is a challenging question and remains unanswered until now.

Helminths antigens interact differently with the maternal immune system potentially leading to a variety of modulations in the fetal immune system. The induction of adaptive immune responses activates maternal immune cells and thereby induces cytokine and immunoglobulin production as well as epigenetic modifications depending on the phase of infection. Transfer of immunological molecules and immune cells via the placenta and breast milk or epigenetic inheritance via the gametes could differentially shape the immune system development in the offspring and modulate the allergen susceptibility in postnatal life. (See Fig. 2.1) Adapted from [39].

In mice and humans, the fully developed placenta is composed of three major layers: The outer layer of the mouse placenta which mediates implantation and
Maternal Helminth Infections

invasion into the uterus is composed of trophoblast giant cells. The layer with analogous function in humans is composed of invasive extravillous cytotrophoblast cells. The function of the middle layer of the mouse placenta, the spongiotrophoblast, is largely unknown. However, some of the spongiotrophoblast cells can differentiate into trophoblast giant cells resembling the cytotrophoblast cell columns that anchor the villi of the human placenta. The labyrinth layer of the mouse placenta is comparable in function to the chorionic villi of the human placenta. In both the mouse and human placentas, the labyrinthine and villi, respectively, are covered by syncytiotrophoblasts that are in direct contact with maternal blood [91]. Adapted from [92].

Maternal Helminths in Mouse and Man: Geohelminthes, Filarial Worms, and Schistosomes

Adding to the complexity of understanding how maternal helminth infections may influence pregnancy and the offspring’s development, it is necessary to consider the different helminth species separately from each other. It is becoming clear that different helminth species exert different effects. Therefore, this chapter aims to accumulate all available data on the main helminthic species studied so far in the context of pregnancy in order to highlight possible differences between these species.
An estimated 1.44 billion people are infected with at least one of the main geohelminthes namely Ascaris lumbricoides (causing ascariasis), Trichuris trichuria (causing trichuriasis), and the hookworms Ancylostoma duodenale and Necator americanus. The latter affects approximately 500 million people worldwide. In 2005 it was estimated that 25% of pregnant women in Subsaharan Africa were infected [11, 40, 41]. Anemia is a common clinical symptom of hookworm disease and, as mentioned above, this per se causes an increased risk for low-birth weight [11]. In the previously mentioned randomized trial in Uganda more than 2500 pregnant women were randomly treated with albendazole and praziquantel in the second and third trimester of pregnancy. Here, albendazole treatment of hookworm infected mothers resulted in reduced Th2 responses (as indicated by IL-5 and IL-13 quantification in supernatants from tetanus toxoid stimulated whole blood cells) in the infants following tetanus toxoid vaccination, while IFN-γ responses remained comparable to those from the control group. This also applied for immune responses against Mycobacterium tuberculosis as a measure of BCG immunization efficacy. In addition, no effect on tetanus vaccine efficacy as measured by immunoglobulin (Ig) G levels and no differences were observed in infection episodes with malaria or in incidence rates of diarrhea or pneumonia between children of treated and untreated mothers [28]. This indicates that maternal hookworm infection leads to rather subtle changes within the immune system of the offspring which might expand towards unrelated (non-worm) worm antigens. Whether these changes persist throughout life remains unknown. Regarding the geohelminth Ascaris lumbricoides, there were reports of higher frequencies of both IFN-γ+ and IL-4+ CD4+ T cells in A. lumbricoides antigen-stimulated cord blood from newborns of infected mothers indicating that maternal ascariasis also leads to sensitization of the infant immune system in a homologous manner [42]. However, no data are currently available on vaccine responses in children born to Ascaris-infected mothers. In a recent study conducted in Ecuador maternal geohelminth infection was associated with increased risk of geohelminth infection during early childhood. Interestingly, this also correlated to elevated levels of IL-10 in the cord blood indicating a possible regulatory immunological influence [43]. No information is available on the effects of the quite commonly observed maternal infection with Trichuris trichuria and on possible alterations within the offspring’s immune responses to autologous or heterologous antigens [44].

To summarize, the impact of maternal geohelminthes infections on the reactivity of the infants immune system has up to now been assessed in quite diverging manners (e.g. cord blood and stimulation and cytokines, antihelminthic treatment during pregnancy or vaccine efficacy in children) which hampers comparability and, thereby, the overall interpretation of the results. Furthermore, the group of geohelminthes are subsumed into one group, but as shown above, differences between single species definitely need to be taken into account as well as additive versus contrasting effects. Major new prospective studies such as the ECUAVIDA birth cohort study will directly assess the impact of geohelminth infection during the last
trimester of pregnancy and in early life (the first 2 years) on the developing immune responses to heterologous antigens. Therefore, vaccine immunity to common vaccines like hepatitis B, tetanus, and haemophilus influenza B during the first 5 years of life and the development of eczema, allergen skin reactivity, and asthma will be investigated in tropical Ecuador [45].

**Filarial Worms**

The most common filarial diseases are caused by *Wuchereria bancrofti* and *Onchocerca volvulus* with approximately 160 million people infected worldwide. Quite a few studies have addressed the effects of in utero sensitization to filarial antigens in the offspring. The overall consensus from the epidemiological as well as experimental studies is that maternal filarial infection significantly increases childhood susceptibility to subsequent filarial infections [46–49], with some studies reporting up to a 13-fold increase in risk of infection from filarial disease in the offspring [50, 51]. First epidemiological evidence for this interrelation was provided by the work of Lammie et al in Haiti showing a threefold increase in risk of developing filarial disease in children born to microfilaremic mothers [52]. Other field studies demonstrated materno-fetal transmission of *Wuchereria bancrofti* microfilariae [53] and, interestingly, *Onchocerca volvulus* microfilariae which are the immunologically relevant parasitic stage of the parasite were also detected in cord blood and placental tissue [46, 48, 53]. This *in utero* sensitization to *O. volvulus* antigens possibly resulted in heightened parasite specific Th1 and Th2 cytokine responsiveness by cord blood cells [54, 55]. In a Polynesian study, lymphocyte responsiveness as determined by IL-2, IL-5, IL-10 and GM-CSF production upon filarial antigen stimulation of offspring from mothers with Bancroftian disease was markedly decreased when compared to that of non-infected mothers. However, these responses were antigen specific and did not extend to non-parasitic antigens. Notably, this hypo-responsiveness to the parasite was shown to persist for as long as 19 years if the mother was microfilaremic at the time of delivery [56]. The results of these studies indicate that maternal transfer of parasites or their antigens released within the placenta might mechanistically be involved in how maternal filarial infections shape the offspring’s immune responses later in life possibly leading to hyporesponsiveness or tolerance.

Addressing the underlying mechanisms of antigen sensitization experimentally, Hague and Capron in 1982, using the microfilaric rodent nematode *Acanthocheilonema vitae* (also called *Dipetalonema viteae*) in infected rats, demonstrated elegantly that exposure to parasitic antigens and even transplacental infection of the fetus during pregnancy leads to a state of immune tolerance in the offspring rather than immunity (infective larvae reached maturity in the “sensitized” offspring which is normally not observed) [57]. Similarly, helminth antigen transfer from mother to offspring was observed using this rodent nematode in mice [58]. However, analysis of *Brughia pahangi* infection in jirds, yet another filarial model, failed to demonstrate effects of maternal infection on offspring infection in terms
of worm burden, lymphatic lesion formation, or antigen-specific inflammatory responses despite reduced B cell responses in the infected progeny [59]. In helminth infection of humans, B cell responses were found to be influenced however in a rather contrasting manner: King et al. clearly demonstrated B cell sensitization in cord blood samples of newborns from helminth infected mothers since production of IgE and IgG by fetal cord blood cells was present upon stimulation in vitro with schistosome and filarial antigens [60].

In summary, the studies on maternal filarial infections clearly demonstrate that sensitization to filarial antigens in utero does occur which could result in hyporesponsiveness to filarial antigens in the offspring via mechanisms that potentially involve early B cell priming despite T cell hyporesponsiveness, a conundrum yet to be solved.

**Schistosomiasis**

Infection with *Schistosoma species*, blood-dwelling trematodes (flukes), that affect more than 250 million people worldwide in 74 tropical and subtropical countries was shown to alter ongoing immune responses to homologous and heterologous antigens in humans and mice (reviewed in [61–64]). In contrast to other human trematode species like *Opistorchis felineus* or *Clonorchis sinensis*, it is the most frequently studied trematode. For example, infection with *S. mansoni* attenuates clinical manifestations of asthma. It is inversely associated with a positive skin prick test to aeroallergens [65] and shows to reduce Th1-responses to tetanus-toxoid in infected adults [66]. An estimated 40 million women of child-bearing age are infected in endemic areas. *In utero* sensitization to schistosome antigens was detected in offspring from *S. mansoni* and *S. haematobium* infected mothers demonstrated by immediate and delayed skin responses to parasite antigens [67] or proliferation [68] and cytokine responses [48] of cord-blood mononuclear cells stimulated with schistosome-antigens. Antigens from *S. mansoni* eggs were detected in cord blood, breast milk as well as in urine of infants from infected mothers up to 28 days after delivery [69]. Raised levels of pro-inflammatory cytokines such as TNF-α and IL-1β were found in maternal and cord blood samples, as well as in situ in the placentas of women infected with *S. japonicum* during pregnancy [70]. Placental transfer of maternally-derived SEA was also reported in human pregnancies suggesting a role of immunomodulatory components of the antigen in maternal cell activation and infiltration at the fetomaternal interface [69]. Indeed, helminth infection during pregnancy was shown to modulate the offspring’s immune system in response to worm antigens [48] as well as unrelated vaccine antigens (e.g. BCG or tetanus toxoid) [71, 72].

However, the effects of maternal *S. mansoni* infection on the immune responses to a heterologous antigen (i.e. Hepatitis B vaccine) in the offspring are inconclusive. For example and as mentioned in the introduction, Hepatitis B vaccine efficacy in infants born to schistosome infected mothers in Egypt was shown to be either re-
duced [26, 73] or unaffected [25]. In addition to direct effects of infection, maternal antihelminthic treatment with praziquantel was associated with increased risk of infantile eczema and, interestingly within the same study, an overall effect of albendazole treatment on reducing childhood eczema was observed [74, 75].

Experimentally, schistosomiasis comprises an excellent natural infection model to study the role of maternal infection during pregnancy on the offspring’s immune system development, since in contrast to the filarial models and other trematodes the human pathogen species infect rodents and develop egg-producing stages to mature. In mice, infection with *S. mansoni* during pregnancy and breastfeeding modulates the immunity against homologous antigen in postnatal infection leading to a reduction in granuloma size and number in the offspring. These findings were associated with early sensitization through transfer of circulating schistosomal antigens and maternal parasite-specific antibodies via the placenta and breast milk [69, 76, 77].

Summing up, maternal schistosome infections could lead to in utero sensitization since circulating antigens were detected in the placenta as well as the cord blood. However, the effects towards homologous (schistosomes) as well as heterologous (e.g. vaccines) antigens still need to be evaluated in detail and further epidemiological as well as experimental studies are necessary to address these interactions in detail.

### The Fetomaternal Interface as an Immunological Bottleneck

Identification of mechanisms that underlie maternal influences on fetal immune imprinting as a result of chronic maternal helminth infection is challenging, but necessary in order to identify potential intervention strategies. To gain further insight alterations at the fetomaternal interface have to be considered, since this represents on the one hand a border between mother and fetus but also a communication platform where considerable crosstalk takes place as we will describe below.

Human and murine placentas share considerable molecular and cellular features (see Fig. 2.2). The fully developed placenta in both is composed of three major layers: first the outer maternal layer which includes decidual cells of the uterus as well as the maternal vasculature that transports blood to and from the implantation site; secondly a middle “junctional” region which attaches the fetal placenta to the uterus and contains fetal trophoblast cells that invade the uterine wall and maternal vessels; and finally an inner layer which is composed of highly branched villi that are designed for efficient nutrient exchange called the *villous tree* in humans and the labyrinth in mice [78]. In humans, the chorionic villi, a continuous layer of multinucleated syncytiotrophoblasts (SynT) that line the intervillous space bathe in maternal blood from decidual spiral arterioles. In mice, maternal decidual spiral arterioles perfuse blood sinuses in the spongiotrophoblast (SpT) layer to reach the labyrinth and the trophoblast giant cells (TGCs), such as invasive cytrotrophoblasts.
(iCTBs), anchor the placenta to the uterus and invade the spiral arterioles. During murine pregnancies, maternal blood is in direct contact with a layer of mononuclear trophoblasts which is surrounded by a bilayer of SynTs that are in close proximity to fetal capillaries [79]. These structural and morphological features are optimized for fetomaternal exchange and immunological crosstalk. The balance of innate and adaptive immune responses at the maternal-fetal interface promotes survival of the semi-allogeneic embryo and, at the same time, allows effective immune responses to protect the mother from environmental pathogens [80]. This is achieved and/or results in a wide range of mechanisms including antibody transfer of certain isotypes, transfer of immune complexes, immune cell stimulation, transfer of cytokines, and other soluble molecules via the placental membrane which affects the offspring’s as well as the mother’s immune system. For example, the trophoblast, as an important component of the placenta, was shown to recognize and respond to microorganisms and their products through the expression of TLRs, thereby affecting the local cytokine milieu even though they cannot present antigens in a classical manner like APCs [81–83]. Placental inflammation for example during malaria has extensive consequences for the offspring and was shown to be associated with intrauterine growth restriction [84]. In the case of helminths such as schistosomes, soluble egg antigens that comprise a multitude of glycoproteins and glycolipids and that are released into the system throughout the disease, could reach the placenta and cause placental inflammation e.g. by activating circulating memory T cells that...
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