Parasitic helminths and HIV-1 infection: the effect of immunomodulatory antigens

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General Introduction
The Immune System

Our immune system consists of many different cell types which can be divided into two different arms of the immune system (Fig. 1). The innate immune system comprises of Natural Killer (NK) cells, granulocytes (eosinophils, basophils and neutrophils), macrophages and dendritic cells (DCs) and act as the first line of defense. These cells respond very fast and in a general manner. Although no memory cells are induced, cells do acquire “inheritable” epigenetic changes that are responsible for a modified response to such a pathogen upon a second exposure. Besides the innate response there is the adaptive immune system consisting of CD4+ and CD8+ T-lymphocytes as well as antibody-producing B-lymphocytes. These cells elicit a very specific immune response and induce memory cells which can rapidly eliminate the pathogen upon a second exposure.

Despite the effectiveness of our immune system some pathogens including HIV-1, *Schistosoma mansoni* and *Brugia malayi* can persist and cause long lasting infections. In this introduction we focus on the immune cells that are affected and/or infected by these pathogens; dendritic cells, macrophages and CD4+ Th-cells.

![Figure 1. A schematic overview of the different branches of the immune system](image.png)

Antigen presenting cells

Antigen presenting cells (APCs) are cells of the innate immune system. Several cell types are classified as being APCs, of which myeloid dendritic cells (DCs) and macrophages are addressed in this thesis. Typically, the function of DCs is considered to be the activation of the adaptive immune system while the function of macrophages is the phagocytosis of pathogens and cell debris. *In vivo*, distinct classes of functionally different DCs and macrophages are recognized.
Myeloid Dendritic cells
DCs are found in an immature state (iDCs) spread throughout the body, predominantly at surfaces in contact with the outside environment to protect us from invading pathogens. DCs continuously scan their environment for signs of danger. To this end they express an array of pathogen recognition receptors (PRR) such as the Toll-like receptor (TLR) family and C-type lectins (CLRs) which have the capacity to sample their environment via endocytosis and phagocytosis. Dependent on their lineage and the environment, DCs can develop into subsets with distinct characteristics and functions. For instance, in the skin there are at least 3 subsets of DCs; the epidermal Langerhans cells (LC), the CD1a+ and CD14+ dermal dendritic cells. LCs form a unique subset of DC that express the CLR Langerin on their surface and possess Birbeck granules in their cytoplasm. Antigens, including HIV-1 particles, taken up by Langerin end up in the Birbeck granules where they are degraded, thereby LC protect the host from infections.

Once DCs encounter an infectious agent the combination of PRRs activated by the pathogen will determine the maturation profile of the DC. In general, DC maturation will reduce the cells capacity for endo- and phago-cytosis while antigen presentation via MHC-II molecules is upregulated. Additionally CCR7 is upregulated, allowing the DC to migrate to the lymph node where they will induce CD4+ T-cell responses (Fig 2A, B).

Macrophages
Similar to DCs, macrophages can be found throughout the body. For some time it was thought that there are two types of macrophages; the classically activated or M1 macrophages and the alternatively activated or M2 macrophages. Lately however, it has become clear that the programming of macrophages is relatively flexible and that there is a whole range of macrophages with intermediate phenotypes.

The M1 macrophages are pro-inflammatory macrophages that can be induced by interferon (IFN)-γ. They play a role in the defense against viruses, bacteria and protozoa and have been demonstrated to play a role in autoimmune diseases. Contrary, M2 macrophages are considered anti-inflammatory macrophages and are induced by interleukin (IL)-4 or IL-13. They have been associated with worm expulsion, anti-inflammatory effects and regulation of wound healing.

CD4+ effector memory T-lymphocytes
Dependent on the pathogen encountered in the periphery, DCs will mature and induce a specific Th-cell subset from naïve T-cells in lymph nodes (Fig 2A). There are three main Th-cell subsets; Th1, induced by DCs which encountered intracellular pathogens,
Th2, induced by multicellular parasites and Th17, which are induced by either fungi or bacteria. Development of a specific Th-cell subset dependents on the polarizing signal that the DCs provide.

The DCs supply naïve T-cells with various signals. The first signal consists of a pathogen-derived peptide presented in MHC-II molecules which are recognized by the T-cell receptor. This will ensure the antigen specificity of the immune response. Secondly, ligation of co-stimulatory molecules are required, for instance CD86/
CD80 on the DC interacting with CD28 on the T-cell. This signal will drive the clonal expansion of the Th-cells. In the absence of co-stimulation Th-cells become anergic which potentially results in induced tolerance. The third signal is crucial for the polarization of the Th-cell response and consists of cytokines and surface molecules expressed by the DCs. For Th1 cell induction either IL-12 or type 1 interferons (IFNs) or intracellular adhesion molecule-1 (ICAM-1) are required. Unlike for Th1 cells the exact mechanism behind Th2 cell induction is not entirely clear. Expression of OX40 ligand (OX40L) and monocytic chemotactic protein 1 (MCP-1) by the DCs have been associated with Th2 induction. Furthermore, cytokines such as IL-4, and IL-13 are capable of polarizing T-cell responses towards Th2 in vitro but they are not produced by DCs. The exact mechanism of Th17 polarization is also not completely understood and lies beyond the scope of this thesis. The DCs also provide the T-cells with a fourth piece of information, namely where they encountered these pathogens. This allows the T-cells to upregulate the appropriate receptors to migrate towards the site of infection. For example, gut derived DCs produce retinoic acid (RA). RA enhances the expression of integrin α4β7 and chemokine receptor CCR9 on T-cells which subsequently allow these T-cells to home to the gut.

Human Immunodeficiency Virus Type 1

HIV-1 originates from simian immunodeficiency virus (SIV), a non-human primate virus that has successfully been transmitted to humans on at least four occasions giving rise to four groups of HIV-1 namely M, N, O and P. Viruses belonging to group M are responsible for the major HIV-1 pandemic. They can be subdivided into 11 subtypes (A to K) and more than 51 subtype recombinant viruses all with a particular geographical distribution.

In principle HIV-1 viruses can infect any cell that expresses their receptor, CD4, and their co-receptor, CCR5 (R5 using viruses) or CXCR4 (X4 using viruses). HIV-1 can be transmitted via sexual intercourse, blood-to-blood contact and from mother-to-child. Interestingly, infection is usually established by one or a few CCR5 using virions. To this day it is not known what properties this founder virus possess that enable for its preferential transmission and why R5 viruses are predominately transmitted.

Infection

Receptive anal intercourse has the highest risk of HIV-1 transmission. For successful transmission to occur the virion must pass the intestinal wall, either through ruptures,
transcytosis or DC uptake (Fig. 3). Ruptures in the intestinal wall can occur during intercourse but transmission may also occur through lesions induced by co-infecting pathogens, such as *Schistosoma mansoni* (see below) \textsuperscript{21,22}. Once past this barrier, virions can directly infect susceptible cells via CD4 and co-receptor binding, known as *cis*-infection (Fig. 3). Asides from this direct route, much attention has been placed on studying HIV-1 transmission via *trans*-infection. DCs in the lamina propria can send dendrites into the gut lumen to sense the environment \textsuperscript{23}. Here HIV-1 virions can be captured by a C-type lectin receptor (CLR). Subsequently, the DC can actually pass the virion to a susceptible CD4\(^+\) T-cell (Fig. 3) \textsuperscript{24}. This route of infection is very efficient. One of the most studied CLRs involved in this process is dendritic cell-specific intracellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) \textsuperscript{25,26}. However,
since DC-SIGN is capable of binding a whole array of sugar motifs found on other pathogens as well as our own host glycoproteins, trans-infection is potentially easy to inhibit (see chapter 3 & 4) 27-30.

Once inside the cell the viral capsid is uncoated and the 9kb RNA genome is reverse transcribed into DNA, which will enter the nucleus and integrate into the host genome 31. This makes it impossible to eliminate HIV-1 without killing the cell. All elements required for the assembly of new virions are expressed from the integrated viral DNA 31, 32. To this end the full length HIV-1 RNA transcripts are spliced, most HIV-1 genomes have 4 5’ splice donor and 8 3’ splice acceptors sites which result in a range of RNA transcripts required for the translation of all viral proteins and proper HIV-1 replication 33. However, HIV-1 transcription is paused by the TAR stem-loop structure (situated at the 5’ end of the transcript). Positive transcription elongation factor b (P-TEFb) is required for transcription of full length HIV-1 RNA 34, 35. P-TEFb is efficiently recruited by the viral protein Tat but since Tat is not packaged into virions the initial round of transcription of the integrated HIV-1 DNA depends on cellular factors 36, 37. Recent studies have shown that NF-κB is one of these factors 38, 39. Failure to overcome the transcriptional pause by TAR will induce transcriptional latency. Following all proteins being translated new virion particles are assembled at the cell membrane before budding and release (Fig. 4).

**Figure 4. A schematic overview of the HIV-1 replication cycle.** First, HIV-1 will bind its receptor, CD4, and co-receptor, CCR5/CXCR4 (1) after which the virus membrane fuses with the cell membrane (2). Subsequently, the HIV-1 core is unpackaged and the RNA genome is reverse transcribed into double stranded (ds) DNA (3). The dsDNA is escorted into the nucleus where it will integrate into the host’s DNA genome via homologous recombination (5). Once incorporated into the human genome, mRNA will be transcribed which due to splicing can encode for all the required proteins necessary to form new virions (6). These mRNAs are translated to proteins by ribosomes in the cytosol (7). After all proteins are formed two full length RNA genomes are exported. The new viral particles are formed at the cell membrane (8) and via a process called budding, new virus particles are released from the infected cells (9).
HIV-1 and the immune system
HIV-1 primarily infects CD4$^+$ effector memory T-cells, Th-cells. There are three main types of helper cells namely Th1, Th2 and Th17. Each subset expresses its own set of cytokines, chemokines and surface receptors. Remarkably, CCR5 expression is not directly linked to HIV-1 susceptibility. For instance, Th1 cells express high levels of CCR5 but also produce high levels of its natural ligands, MIP-1$\alpha$, MIP-1$\beta$ and RANTES thereby limiting the susceptibility of these cells to HIV-1 R5 infection $^{40-42}$. Recently, Mycobacterium tuberculosis specific CD4$^+$ T-cells producing high levels of IL-2 and low levels of MIP-1$\beta$ have been associated with an increased susceptibility for HIV-1 infection while Cytomegalovirus specific cells with the opposite profile are less infectious $^{43}$. These results indicate that many factors contribute to the infection of T-cells.

HIV-1 infection is not limited to CD4$^+$ T-cells, HIV-1 can target all cells expressing CD4 and CCR5/CXCR4 which include antigen expressing cells (APCs) such as DCs and macrophages. In fact, macrophages and DCs are among the first cells HIV-1 encounters and infects upon transmission. Since both macrophages and DCs live relatively long, are insensitive to the cytopathic effects of HIV-1 and support latent infection, these cells are thought to contribute to the persistent nature of HIV-1 infection $^{44,45}$.

Helminths
There are many parasitic helminths which can be transmitted via several routes including vectors, contact with contaminated soil or water and ingestion of contaminated food $^{46}$. For millions of years helminths have co-evolved with the human host and they acquired the capacity to evade, skew and dampen human immune responses. The characteristic Th2 cell activation induced by helminths is not limited to helminth specific immune responses but also skews immune responses to other pathogens towards a Th2 phenotype $^{47}$. Additionally, helminths induce regulatory T-/B-cells, NK cells and suppressive macrophages which contribute to the dampening of immune responses. Again, these cells have an effect on all immune responses and can therefore have a beneficial effect on the outcome and/or onset of autoimmune diseases and allergies $^{48}$. Although helminths have been around for millions of years, the improved sanitation, housing conditions and access to clean water have eliminated nearly all helminths in the western world $^{46}$. In poorer regions of the world helminths are still very common.
and due to their impact on the immune system may influence the HIV-1 epidemic in these regions.

For this study we chose to work with *Schistosoma mansoni* and *Brugia malayi* as they invade humans, reside at sites of importance in HIV-1 infection and give rise to long lasting chronic infections.

**Schistosoma mansoni**

*Schistosoma mansoni* is a trematode blood fluke that has a snail from the *Biomphalaria* genus as its intermediate host. The necessity of this intermediate host restricts *S. mansoni* infection to regions where this snail can be found; Africa, the Middle East, the Caribbean, Brazil, Venezuela and Suriname. Infection mainly occurs in rural areas when people bath in fresh water lakes or work in close contact with water, including fishermen.

*Life cycle (Fig. 5)*

*S. mansoni* eggs are secreted by humans with their feces and will hatch upon coming into contact with fresh water. The miracidia released by the eggs infect their host, a snail. Inside the snail miracidia multiply asexually before developing into multicellular sporocysts which later develop into cercarial larvae. This asexual multiplication allows a snail infected with one miracidium to produce thousands of cercariae a day for months. The cercariae are secreted by the snail and search for their human host which they enter by penetrating the skin. In humans the cercariae migrate via the blood to the lungs and then into the portal vein where they transform into young worms (schistosomulae). In the portal vein these worms mature and mate after which they migrate to the mesenteric veins where they lay their eggs. An adult worm pair can lay up to 300 eggs a day which elicit strong immune responses to facilitate their migration from the blood vessel to the gut lumen. Most *S. mansoni* eggs enter the gut lumen at the large bowel or the rectum.

*Symptoms, complications and treatment*

Individuals living in endemic areas don’t typically develop symptoms during the acute phase of infection. Individuals from outside endemic areas can develop a temporary rash at the site where the cercariae penetrated the skin. Additionally, several weeks after infection acute schistosomiasis or Katayama fever can develop. This is a systemic reaction to the migrating schistosomulae which is characterized by symptoms such as fever, fatigue, myalgia and eosinophilia. At a later stage abdominal symptoms like diarrhea can occur. Most patients spontaneously recover 2 to 10 weeks after initial disease onset.
During the chronic phase of infection (onset of egg production) it are the eggs that induce a strong granulomatous immune response to facilitate their migration. Successful migration results in superficial bleeding, micro-ulcerations etc. in the gut causing a loss of appetite, abdominal pain and (bloody) diarrhea. Not all eggs will make it to the gut lumen, some get swept away in the bloodstream and get lodged in the liver. Here the granulomas are progressively replaced by fibrotic deposits, slowly destroying the liver. The severity of the symptoms depends on the intensity of the infection and the persons immune response.

*S. mansoni* can be treated efficiently with Praziquantel. This, however, does not affect the eggs or immature worms thus follow up treatment is advised. Additionally, it does not protect the individual form re-infections, therefore individuals living in endemic regions should be treated with Praziquantel on a regular basis.

**S. mansoni and the immune system**
The adult worms evade immune detection whereas the eggs induce a strong immune response to facilitate their migration to the gut lumen. One of the best studied antigen mixtures of *S. mansoni* is soluble egg antigen (SEA), the water soluble components derived from homogenized eggs. This mixture contains many glycosylated proteins which are able to bind the C-type lectin receptors DC-SIGN, mannose receptor (MR)

*Figure 5. A schematic overview of S. mansoni’s life cycle. S. mansoni eggs are secreted by the human host via their feces. In fresh water the eggs release miracidia which invade their intermediate host, a snail. In the snail several life stages are passaged resulting in the release of cercariae. These cercariae invade humans by penetrating their skin, they migrate to the portal vein and become adult worms (not shown). After finding their mate the worms migrate to the mesenteric veins where they lay eggs. These eggs induce strong immune responses which facilitate their migration to the gut lumen. From the gut lumen the eggs are secreted with the feces.*
and macrophage galactose type-lectin (MGL)\textsuperscript{51-53}. Interacting with these receptors on DCs renders these cells in a semi-mature state, with no cytokine production or upregulation of co-stimulatory molecules while antigen processing is similar to LPS matured DCs\textsuperscript{54, 55}. Additionally, SEA exposed DCs will have an altered response to TLR ligands and will skew subsequent T-cell outgrowths towards a Th2 phenotype (more information can be found in chapter 4)\textsuperscript{56}. Recently, the three main components of SEA were identified namely: omega-1, kappa-5 and IPSE/α-1. Each component was shown to have its own unique effect on the immune system. Omega-1, a member of the T2 RNase family, can drive Th2 skewing via DCs whereas kappa-5 is the main target for the IgE antibody response and IPSE/α-1 can induce IL-4 production in basophils\textsuperscript{57-61}.

To conclude, \textit{S. mansoni} eggs are capable of altering the responses of our immune cells which may also have implications for HIV-1 infection of these cells.

\textbf{Brugia malayi \& Acanthocheilonema viteae}

There are three closely related nematodes that cause human lymphatic filariosis, \textit{Wuchereria bancrofti}, \textit{Brugia malayi} and \textit{Brugia timori}. \textit{W. bancrofti} is responsible for 90\% of the human infections, however, the lack of an animal model supporting \textit{W. bancrofti} infection makes this parasite very difficult to study. Hence, most studies focus on the second most widely spread filarial nematode, \textit{B. malayi}, which can be found in South East Asia\textsuperscript{62}.

\textit{Acanthocheilonema viteae} is a rodent filarial nematode which secretes ES-62. This molecule has been studied extensively and has major implications for the rodent immune system (see below & chapter 5)\textsuperscript{63-65}. Since \textit{B. malayi} secretes a homologous molecule we studied the effects of ES-62 in parallel with BmA (homogenized \textit{B. malayi} adult worm) to determine their effects on the human immune system.

\textit{B. malayi}'s life cycle (Fig. 6)\textsuperscript{66, 67}

The intermediate host of \textit{B. malayi} is the mosquito. An infected mosquito has mature L3 larvae in its proboscis, which are secreted on the skin next to the puncture site when the mosquito takes a blood meal. The larvae penetrate the skin, migrate to the lymphatic vessels and home towards the lymph nodes where they transform into L4 larvae and subsequently into adult worms. In male hosts, the adult worms have a preference for the lymphatics surrounding the spermatic cord. For reproductive infection to occur a male and a female worm must find each other which requires the host to be infected with filarial larvae on at least two separate occasions. Once the adult worms have mated, the female releases live progeny, microfilariae (mf), which relocate to the peripheral blood vessels waiting to be taken up by a mosquito. In
the mosquito the mf migrate from the midgut via the hemocoel to the flight muscles where they transform into L2 and subsequently L3 larvae. Mature L3 larvae migrate to the proboscis and the life cycle is complete. Although *B. malayi*’s life cycle is highly complex the parasite does successfully spread. This is likely explained by the long life span of both the adult worm (5-12 years) and their offspring (300 days), which enhances the chance of the life cycle being completed.

**Symptoms, complications and treatment**

In endemic areas individuals demonstrate a wide range of symptoms, from asymptomatic to severe acute and chronic manifestations. The most common symptoms in the acute phase are fever, chills and lymphadenitis (swollen lymph nodes). Additionally, patients can suffer from episodes of adenolymphangitis (fever, inflamed lymph vessels and nodes) which causes long term damage to the lymphatic system. The symptoms are the result of the immune system fighting off the invading larvae as well as the failure to efficiently fight off bacterial skin infections due to the damaged lymphatic system. One of the major characteristics of chronic lymphatic filariasis is the blockage of a lymph vessel or node resulting in elephantiasis (swollen limb). Although most infections are asymptomatic, the lymphatic system of the patient may become damaged, causing subsequent problems.

The treatment strategy is to prevent the parasite from spreading and to alleviate the symptoms of already infected individuals. There are several drugs available that have a limited effect on the adult worm, but that efficiently kill the mf and prevent production of new mf. Large scale treatment of individuals in endemic regions have been found to effectively reduce the transmission rate of lymphatic filariasis. More information can be found in the thesis of Shakya.

**Figure 6. A schematic overview of *B. malayi*’s life cycle.** A mosquito containing L3 larvae drops these on the skin when taking a blood meal. From here the larvae migrate to the lymphatic system where they mature into adult worms. After mating the female releases microfilariae which find their way to the peripheral blood. Here they wait to be taken up by a mosquito to complete their life cycle.
B. malayi, A. viteae and the immune system

Despite the presence of animal models for B. malayi, not much research has been performed with antigens derived from this parasite. One study indicates that the adult worms are responsible for Th2 skewing whilst another study suggests that the microfilariae are responsible. In contrast, the purified protein ES-62 has been studied extensively. ES-62 has been shown to alter DC and macrophage responses both in vivo and in vitro, induce Th2 immune responses and to have a beneficial effect on autoimmune diseases including lupus and arthritis. Nevertheless, all these studies were undertaken in rodents or using rodent derived cells. The effect of ES-62 on human cells is largely unknown (for more information see chapter 5). The active component of ES-62 is the phosphorylcholine (PC) group which is also found on components derived from B. malayi worms, indicating that the human immune system will be affected by ES-62 and other PC-containing components. Hence, ES-62 and BmA are both thought to alter human immune responses with potential consequences for HIV-1 infection and replication.

Scope and Outline of the Thesis

Both helminths and HIV-1 have major implications for their host due to the longevity of the infection and the extensive effect these pathogens have on our immune system. The complex pathogen interactions in co-infected individuals make it difficult to determine the effect one pathogen has on the other, which is reflected by the contradictions reported in epidemiological studies. It is generally accepted that the helminth infection usually precedes the HIV-1 infection assuming individuals live in endemic areas and HIV-1 is transmitted sexually. Consequently, in this thesis we studied HIV-1 infection in cells of the human immune system that were pre-exposed to specific helminth antigen mixtures in comparison to unexposed cells.

Our goal was to answer the following three questions:

1. Can helminth products interfere with HIV-1 cis- or trans-infection?
2. Do T-cell responses induced under the influence of parasite antigens have an altered susceptibility to HIV-1 infection?
3. Can helminth products affect HIV-1 transcription in antigen presenting cells?

In chapter 1 a general introduction is provided, highlighting the different cells of the immune system involved in HIV-1 and/or helminth infection. Additionally, HIV-1 and
the parasitic helminths used throughout this thesis are introduced. Both helminths and HIV-1 act on the immune system which is reviewed in chapter 2 as well as their potential influence on each other. Furthermore, in this chapter the impact of Mycobacterium tuberculosis on the immune system is discussed and its potential consequences for HIV-1 infection.

From the literature it was apparent that colorectal mucus (CM) had not been studied for the capacity to interfere with HIV-1 infection or replication. Since S. mansoni eggs are present in the large bowel and rectum both S. mansoni and CM can potentially influence HIV-1 capture by DCs and the induction of immune responses. Hence in chapter 3 we determined whether colorectal mucus could alter HIV-1 transmission and in chapter 4 the ability of soluble egg antigen (SEA) derived from homogenized S. mansoni eggs to affect HIV-1 infection was studied. In this chapter HIV-1 cis- and trans-infection were addressed as well as the HIV-1 susceptibility of T-cells induced by DCs exposed to SEA. In a similar fashion the ability of Brugia malayi antigen (BmA) derived from homogenized adult B. malayi worms and ES-62, a purified molecule from Acanthocheilonema viteae, to affect HIV-1 infection was determined in chapter 5. Finally, the results of the previous chapters are summarized and discussed in chapter 6.
References
6. Valladeau, J. *et al.* Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 12, 71-81 (2000).


