Parasitic helminths and HIV-1 infection: the effect of immunomodulatory antigens
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Effects of helminths and *Mycobacterium tuberculosis* infection on HIV-1: a cellular immunological perspective

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Chapter 2
Abstract

Purpose of review: In many regions of the world, a high prevalence of HIV-1, helminthic and Mycobacterium tuberculosis (Mtb) infections can be found. Here, we summarize the types of immune responses induced and/or modulated by these pathogens and the consequences for HIV-1 disease.

Recent findings: Helminths predominantly induce strong T helper (Th) 2 responses which are downregulated in chronic disease. The anatomical niche populated by helminths plays a key factor in the effect these parasites have on HIV-1 transmission and subsequent replication. Gut-associated helminths have been found to increase HIV-1 transmission via the lesions they provide. In spite of this, the many immune modulatory molecules secreted by the parasites may inhibit or slow HIV-1 infection. In contrast, Mtb is mainly restricted to the lung and the Mtb-specific Th-cells induced are highly susceptible to HIV-1 infection and replication. Antigens from both pathogens have immunomodulatory activity that can skew cellular immune response in specific directions.

Summary: The effect of helminths and Mtb on modulating immune responses is varied and complex with both their location and phenotype potentially influencing HIV-1 disease. These pathogens have evolved a complex array of molecules which have the capacity to modulate immunity and preserve pathogen survival.

Keywords: Coinfection, HIV-1, immune modulation, Mycobacterium tuberculosis, Schistosoma mansoni
Introduction
Despite the recent advancements in medical research and treatments, HIV-1, *Mycobacterium tuberculosis* (Mtb) and helminths still infect millions of people worldwide each year causing severe morbidity and resultant mortality. HIV-1 is a relatively new pathogen on the scene of circulating infectious agents and has been introduced into a setting where we have evolved over millions of years to coexist with pathogens, such as helminthic parasites and pulmonary bacteria. Characteristic of these pathogens are their ability to evade and modulate the host’s immune responses, thereby preventing the host from clearing infections. Moreover, the induced immune responses mounted can be highly beneficial for the replication and survival of these pathogens. HIV-1 is foremost a disease of the immune system causing immunodeficiency and therefore co-infections with HIV-1 and other pathogens is a complex scenario. This review aims to provide an overview of the impact three separate pathogens have on the immune system summarizing the possible consequences helminths and Mtb have on HIV-1 transmission and infection.

Key points:
- T helper (Th) 1, Th2, Th17 and regulatory T-cells have differential activation phenotypes which can be associated with HIV-1 infection and replication.
- Helminthic parasites and *Mycobacterium tuberculosis* (Mtb) possess an array of antigens that have immunomodulatory activity which will have consequences for HIV-1 transmission and disease progression.
- Parasitic molecules which down-modulate CD4+ T-cell stimulation and activation can be utilized for the development of compounds with anti-HIV-1 activity.
- Mtb-specific CD4 cells are highly susceptible for HIV-1 infection and preferentially depleted early in coinfected individuals.

Cellular responses in HIV-1 infection
Without the aid of the innate immune system no solid immune response can be elicited against infecting pathogens, with dendritic cells (DCs) playing a pivotal role. Besides antigen presentation, the pathogen recognition receptors (PRRs) on their surface allow them to distinguish between different pathogens and elicit specific T-cell responses. With regards to the adaptive immune response, CD4+ T-helper cells (Th) are key players. This population was first believed to consist of two main subtypes, Th1 for intracellular pathogens and Th2 for extracellular pathogens. More recently a third subtype, Th17 CD4 cells [1], were recognized and are believed to play a role in bacterial and fungal infections, whereas Th1 and Th2 are now believed to be important in viral and parasitic infections, respectively. In practice, however, all cell
types are likely induced during an infection and it is the balance between them that will determine the disease outcome.

HIV-1 targets all cells expressing its main receptor CD4 and one or both coreceptors CCR5 and CXCR4 [2]. Nevertheless, the surface expression levels of these coreceptors are not directly linked to the susceptibility of these cell types to HIV-1. In fact, even though the Th1 population has the highest surface expression of CCR5, these cells are found to be relatively resistant to HIV-1 [3-5**]. This was explained by their ability to produce RANTES, MIP-1α and MIP-1β, which are CCR5 agonist that will compete with HIV-1 for CCR5 binding [3]. A recent study by Gosselin and colleagues indicated that Th17 cells are highly permissive to both CCR5 using (R5) and CXCR4 using (X4) viruses whereas Th2 cells are relatively resistant to R5 and Th1 to both R5 and X4 viruses [5**]. Besides the Th-cells there are other important T-cell populations such as the regulatory T-cells (Tregs). Their susceptibility to HIV-1 varies and depends on both host and viral factors [6]. The role of this cell type in HIV-1 infection is still unclear. Some studies show that Tregs are lost during the infection, except in elite controllers which maintain normal Treg levels throughout their infection [7;8], however, other studies have described the opposite [9]. Much debate surrounds the significance of Th responses induced against HIV-1, with the presence of antibodies (Ab) suggesting a Th2 response, whilst HIV-1 specific CD8+ T-cells indicate a Th1 response [10;11]. The complexity with HIV-1 stems from the fact that stimulated CD4 Th responses are the cells being infected and subsequently eliminated.

**Host–Pathogen interactions**

Both host and pathogen have devised mechanisms to outsmart the other. For instance, host cells can express APOBEC3G, a cytidine deaminase which interferes with retrotransposition [12]. Despite the virus ability to produce Vif (a protein counteracting APOBEC3G), virions produced by T-cells with high APOBEC3G levels (Th1) are less infectious than virions produced in cells with lower APOBEC3G levels (Th2) [12]. On the other hand, the virus can use certain host properties to enhance its infectivity. For example, galectin-9 mediated stabilization of PDI (protein disulfide isomerase) activity at the cell surface of Th2 cells enhances HIV-1 infectivity by facilitating better viral entry [13*].

Previously galectin-1 has been associated with increased viral infectivity at this level via stabilizing virus attachment [14]. Furthermore, the gp120 Env protein of HIV-1 is able to bind several receptors besides CD4 which can promote trans-infection. In short, gp120 binds to dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN), mannose receptor (MR) and DC immunoreceptor (DCIR) after which the same virion
is presented to susceptible CD4 T cells \[15;16\]. In addition, binding to DC-SIGN alters Toll-like receptor (TLR) 3, 4, 5 and 8 signaling and induces transcription elongation of provirus in infected DCs \[17;18\] (Fig. 1A). Clearly there are numerous levels of host-pathogen interaction which can influence the cells susceptibility and the virions infectivity.

**Schistosoma mansoni** infection

Typically helminthic infections induce a Th2 response \[21\]. In case of intestinal helminths this will promote worm expulsion, while for lymph and blood-populating helminths the protective role is less obvious \[21-23\]. *Schistosoma mansoni*, a digenetic blood fluke, is one of the better studied parasites (Fig. 2A) and unlike most helminths, the adult worms evade immune detection by clever mechanisms such as molecular mimicry \[24\], whereas their eggs will induce typical Th2 responses \[21-23;25\]. Additionally, the eggs induce granuloma formation which is crucial for their migration to the intestinal lumen \[21;26\]. Once the eggs have reached the intestinal lumen or died the granuloma resolves leaving behind fibrotic plaques \[23\]. Since these plaques replace healthy tissue, this can eventually lead to clinical complications. The level of fibrosis is determined by a delicate balance of Th1 and Th2 cytokines \[25\]. Previous studies have associated elevated levels of TNF-α, IL-5, IL-10 and IL-13 (Th2-associated cytokines) to severe fibroses while high levels of INF-γ (a Th1-associated cytokine) result in little fibrosis \[25\]. The chronic phase of infection, typically 12 weeks following infection, is initiated (Fig. 2A) and is marked by a downregulation of the immune responses induced by the eggs. This leads to smaller granulomas, less IL-4 production and eosinophil recruitment \[23;27*\], however, the rate of egg secretion appears to be unaffected by this \[27*\].

The mechanisms involved in the induction and consecutively downregulation of the immune response by *S. mansoni* remain to be elucidated.

**Host–Pathogen interactions**

The ability of helminths to evade the immune system on the one hand and exploit it for their own survival on the other suggests they manipulate the immune system at the cellular level. Since the eggs elicit a dominant immune response in *S. mansoni* infections, many studies have focused on the effect of soluble egg antigens (SEA) on cells of the immune system. Previous studies have defined SEA as a mixture of glycoconjugates that contain among others Lewis X (LeX) structures \[28\]. Via this glycosylation motive, SEA can interact with DC-SIGN and block DC-SIGN mediated HIV-1 trans-infection [unpublished data, E.E.I.M. Mouser]. Unlike gp120, which binds
DC-SIGN in a mannose dependent manner, LeX motifs binds in a fucose dependent manner and are unable to induce Raf-1 signaling [20] (Fig. 1B). SEA inhibits TLR3 and TLR2/4 induced maturation of monocyte derived DCs (moDCs). Both cytokine production (IL-6, IL-10, IL-12 and TNF-α) and surface marker expression (CD80, 83 and 86) is dampened in the presence of SEA [29]. Similarly the >50kD fraction of *Hymenolepis diminuta*, a rat tapeworm, also prevents lipopolysaccharide (LPS) induced cytokine production in a macrophage cell line [30]. Furthermore, SEA influences the Th response induced by LPS and poly I:C (a TLR3 ligand). Normally DCs exposed to LPS give rise to a Th1/Th2 mixture while DCs exposed to both LPS and SEA skew T cells towards Th2. Likewise, SEA is able to dampen the strong Th1 response induced by poly I:C [29]. SEA can also bind Dectin-2 and signaling via this receptor on bone marrow derived DC results in the activation of the Nlrp3 inflammasome and leads to production of IL-1β [31]. Again, SEA is no exception in having multiple effects, Likewise the >50kD fraction of *H. diminuta* has other properties such as preventing peripheral blood mononuclear cell proliferation [32]. Another well characterized parasitic glycoprotein is ES-62 from *Acanthocheilonema viteae*, a rodent filarial nematode. This glycoprotein is able to exert immune modulatory effects via its phosphorylcholine group [33;34].

*Mycobacterium tuberculosis* infection

Upon inhalation, the alveolar macrophages are among the first cells to be encountered by Mtb. Despite the capacity of macrophages to kill pathogens, Mtb can escape this fate. In fact, resting macrophages allow efficient replication whereas activated cells will either suppress or kill the bacteria [35]. Upon infection the alveolar macrophages will migrate back into the endothelium and induce a local immune response. This results in the recruitment of various cell types, including monocytes, macrophages, neutrophils and dendritic cells [36-38], all of which can be infected with HIV-1. These cells will form a granuloma that will enable Mtb to grow exponentially until T-cells are recruited to the site. Unlike with other pathogens, it takes approximately three weeks to induce a good T-cell response [37;39] (Fig. 2B) and since the number of bacteria in latency is directly correlated to the chance of disease progression [37], this delay is a major benefit for Mtb. Studies have shown that antigen presentation in the lung is not sufficient for inducing strong CD4 Th responses and for this to happen bacteria must enter the lymph node, more precisely a threshold of ~1500 cfu must be reached before Mtb specific Th responses are induced [40]. A recent study indicated that DCs are crucial for the migration of Mtb to the draining lymph node, however, when DCs themselves are infected their migration is delayed. This could be circumvented when DCs take up infected neutrophils [41*], nevertheless, the precise mechanisms Mtb
employs to cause the delay in CD4 Th onset remain to be determined.

Once formed, the Mtb specific T cells migrate to the lungs where they will surround the granuloma and drive the infection into latency via the secretion of TNF-α and IFN-γ [42;43]. Very rarely Mtb can be cleared; a potential reason being improper activation of the induced T cells due to the lack of antigen in the lung. For instance, Ag85B is a highly secreted immunodominant antigen (not required for bacterial survival) which can be found during acute infection, however, its production is downregulated.
in chronic infection [44*]. This results in a decreased percentage of IFN-γ secreting Ag85B specific T cells in the lung during progressive disease.

**Host–Pathogen interactions**

*Mtb* expresses a large and complex array of antigens of which many are able to alter/skew pathogen induced immune responses. The unique wax-like structure covering *Mtb* is composed of, amongst others, glycolipids and long-chain fatty acids [45]. Lipoglycan mannose capped lipoarabinomannan (ManLAM) is an important virulence factor and abundantly expressed [45]. However, the effect ManLAM has on moDCs is still under debate. Nigou and colleagues have shown that moDCs exposed to LPS in combination with ManLAM produce less IL-12, which they suggest is mediated through MR signaling [46]. Pathak and colleagues have confirmed this finding, although in macrophages [47]. In contrast, Gringhuis and colleagues observe increased IL-12 secretion when costimulating moDCs with LPS and ManLAM which they demonstrate is mediated predominantly via DC-SIGN signaling [20] (Fig. 1A). In addition to IL-12, Gringhuis et al. observed an increased level of IL-6 and IL-10. Besides altered cytokine production, ManLAM has also been postulated to play a role in modulating apoptosis in macrophages thereby potentially evading immune responses [48]. Additional components of *Mtb*’s shell are phosphatidyl-myoo-inositol mannosides (PIMs), which have been shown to inhibit the responses of macrophages to LPS via both CD14 dependent and independent mechanisms [49]. Next to structural components, *Mtb* also secretes antigens such as early secreted antigen target protein 6 (ESAT-6) which can induce IL-6 and TGF-β production by DCs via TLR2 signalling hence inducing Th17 cells [50*]. Only recently, Th17 cells were shown to be involved in *Mtb* infection although their precise role is unclear, and recently reviewed [51*]. Another mechanism of immune evasion employed by *Mtb* is the secretion of immunological decoys such as the above mentioned Ag85B [44*]. We have provided some examples of the complex interactions between *MtB* and the immune system but more in-depth reviews can be found [48;52].

**Implications for HIV-1: location, location, location**

*Mtb* and helminths have specific anatomical niches within the host where they preferentially reside, whereas HIV-1 is considered a systemic infection. Nevertheless, HIV-1 transmission occurs at specific sites, usually mucosal tissues, and viral replication has also been shown to occur preferentially in lymph nodes and the gut. Inevitably there will be some kind of overlap with significant consequences for either the pathogens replication and/or which immune responses are induced.
The genital tract is the major site for HIV-1 transmission. Two recent studies describe the presence of Th17 cells (highly susceptible to HIV-1) in both the cervix and foreskin suggesting that these cells are initially targeted. However for transmission to occur HIV-1 must reach these cells and which can occur via lesions. A parasitic infection associated with an enhanced risk for HIV-1 infection is *Schistosoma haematobium*, the causative agent of urogenital schistosomiasis, which resides in the veins surrounding the bladder and female genital tract. Like the eggs of *S. mansoni* they induce granulomas to migrate through the tissue. This will leave lesions in the genital tract through which HIV-1 can enter the body. Additionally, the granulomas are localized foci of activated immune cells which can be targeted by HIV-1 hence contributing to successful transmission as recently shown in [57].

The gut is not only a site for HIV-1 transmission but the gut-associated lymphoid tissue (GALT) is regarded as the first and main site of HIV-1 replication, with up to
80% of all CD4+ T-cells being lost during acute infection [11;58;59]. In addition, the gut is home to many helminths which have the potential to influence HIV-1 susceptibility and disease course. An overview of epidemiological studies conducted on this matter is provided by Secor et al. within this issue as well as by Brown et al. [60].

A recent study by Chenine and colleagues actually demonstrated that rhesus macaques suffering from acute S. mansoni infection (with egg secretion) are more susceptible to infection with clade C R5 simian-human immunodeficiency virus (SHIV) upon *intra rectal* exposure than uninfected controls [61]. A supplementary study by Siddappa and colleagues indicated that this effect was not seen upon intravenous exposure to the virus [62], suggesting that the mucosal changes induced by S. mansoni cause the enhanced susceptibility to SHIV. It has also been demonstrated that mice with a chronic S. mansoni infection, recruit CD4+FoxP3+ Tregs expressing both CD103 and CCR5 to the site of infection, in this case the colon. These cells can dampen the egg induced Th2 responses [27*] and are also a potential target for HIV-1 [6]. Thus both during acute and chronic S. mansoni infection there is a population of HIV-1 susceptible cells in the colon which could potentially contribute to HIV-1 transmission as well as early virus replication.

The lymph nodes are also a major site for HIV-1 replication. Furthermore, the lymph nodes are the place where immune responses against other pathogens are induced. Therefore, depending on the type of immune response induced, the CD4+ T-cells might be more or less susceptible to HIV-1. For example, Mtb specific T-cells are highly susceptible to HIV-1 (see below) [63**] hence the localized lymph nodes to the lung may provide for an environment promoting HIV-1 replication. Other pathogens that may influence the CD4 cellular milieu in the lymph node are lymphatic filarial parasites. These actually live in our lymphatic system and thus can easily target the lymph nodes. A recent study on *Litomosoides sigmodontis* in mice confirms that the immune suppressive cells (nematode-elicited macrophages (NeMφ) F4/80+) recruited to the pleural cavity spread to the draining lymph node when the infection becomes patent [64].

Lung, Mtb is mainly localized within this site and early during the HIV-1 infection, immune control of Mtb is lost. Geldmacher and colleagues demonstrated that Mtb specific CD4 T cells from the periphery with the cytokine/chemokine profile high in IL-2 and low MIP-1β, were more susceptible to HIV-1 infection *in vivo* than CD4 T cells with the reversed profile [63**]. A recent study by Jambo et al. could not confirm a significant decrease in peripheral Mtb specific T cells found by Geldmacher in HIV-1 infected individuals but did find a significant reduction of Mtb specific bronchoalveolar T cells [65*]. These data clearly confirm that being HIV-1 positive increases the risk of Mtb reactivation [66]. Fortunately, this risk can be reduced by antiretroviral therapy, (reviewed by [67]). Although, HIV-1 targets Mtb specific CD4 T cells, the lung is neither
a site of transmission nor a main site of HIV-1 replication; hence it seems unlikely that an Mtb-infected individual has an increased chance of contracting HIV-1.

**Conclusions**

We emphasize the Th responses induced by three pathogens (HIV-1, schistosoma and Mtb) and described the modulatory effects these have on immune stimulation. We have described pathogen specific molecules which possess immunomodulatory activity and which can likely influence HIV-1 transmission and replication. It has been postulated that the high rate of HIV-1 infections in sub-Saharan Africa could be linked to the high prevalence of helminthic infections [68]. Deciphering copathogen interactions will provide a better understanding of the detrimental and/or beneficial effects. It should be possible to exploit the immune-dampening strategies utilized by various pathogens to modulate HIV-1 replication.

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References

Papers of particular interest, published within the annual period of review, have been highlighted as:
* of special interest
** of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 286-287)


** The authors utilize blood from HIV-1 positive and negative individuals to demonstrate differences in HIV-1 susceptibility of Th1, Th2 Th17 and Th1Th17 cells. They conclude that the Th17 and Th1Th17 cells are preferentially infected with HIV-1.


* This manuscript describes a new mechanism whereby host factor Galectin-9 can modulate the HIV-1 infectivity of Th2 cells. In addition, they show that this same mechanism can control cellular migration.


* Here the authors show that specific regulatory CD4 cells are recruited to the colon in mice with a chronic S. mansoni infection.


* It is shown here that when DCs take up Mtb infected neutrophils they migrate faster to the draining lymph node than DCs that directly take up Mtb. This indicates the complex interactions Mtb has with various cells of the immune system which influences cellular activation in lymph nodes.


* Here the authors show that Mtb specific CD4 Th1 polarized cells in mice are sub-optimally activated at the site of infection. Hence they suggest increasing effector T cell activation by providing one or more epitope peptides as a successful strategy in treating Mtb.


* The authors show that there are differences between immune responses mounted against Bacillus Calmette-Guérin (BCG), the vaccination strain of Mtb and H37Rv, a natural strain of Mtb. These differences are mediated via ESAT-6 which is absent in the BCG strain. The authors claim that this molecule is important in inducing Th17 responses which are pivotal in maintaining protective immunity.


* This review describes the role of IL-17 and Th17 cells in the control of Mtb infection.


** This manuscript describes the phenotypes of cells residing in cervical mucosal tissue and their susceptibility to HIV-1 infection. They demonstrate that the susceptible cells are depleted in HIV-1 positive women, indicating selective infection and elimination of cells at mucosal surfaces.


** As described above in [53], the authors show that CD4 cells present within the foreskin carry a phenotype associated with higher susceptibility to HIV-1 infection, again reinforcing the idea that cells types residing at mucosal surfaces can play a role in HIV-1 transmission.


** Here the authors demonstrate the preferential infection of CD4 T cells raised against Mtb, which express high levels of IL-2 and low levels of MIP-1β versus cytomegalovirus-specific CD4 cells which possess the reverse profile. These results indicate that the specific phenotype of induced CD4 cells varies between pathogens and determines which cells are lost early in HIV-1 disease.


* The authors describe that Bronchoalveolar CD4 cells specific for Mtb but not S. pneumoniae are depleted in HIV-1 positive versus negative individuals.

