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

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Summarizing Discussion
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This chapter contains the summary of the thesis titled: "Parasitic helminths and HIV-1 infection: the effect of immunomodulatory antigens".

In our life-time we will encounter many pathogens and these infections may occur concomitantly. For one pathogen to influence another’s disease process there must be some cross over. In the case of HIV-1 and parasitic helminths the immune system forms such an overlap. Parasitic helminths possess numerous immunomodulatory properties that skew and dampen immune responses whilst HIV-1 aims to drive the immune system into a state of chronic activation. Although it seems highly likely for these pathogens to influence each other’s disease course, little is known about the effect of immunomodulatory antigens from parasitic helminths on HIV-1 infection. To gain more insight, we analyzed the effect of helminth products in a number of human HIV-1 in vitro model systems.

Throughout the course of this thesis three helminth products were analyzed for their effect on HIV-1; soluble egg antigen (SEA) derived from homogenized Schistosoma mansoni eggs, Brugia malayi antigen (BmA) derived from homogenized adult B. malayi worms and Excretory Secretory molecule 62 (ES-62) a purified molecule derived from adult Acanthocheilonema viteae (the phosphorylcholine group is responsible for the molecules activity). The main questions were:

1. Can helminth products interfere with HIV-1 cis- and/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?

1. Can helminth products interfere with HIV-1 cis- and/or trans-infection?

HIV-1 can target cells expressing its primary receptor CD4 and its main co-receptors CCR5/CXCR4. Nevertheless, CD4+ effector memory T-cells are considered to be the main target cells for productive infection, whereas DCs and macrophages are understood to serve as a reservoir. We therefore determined the effect of SEA, BmA and ES-62 on HIV-1 cis-infection of CD4+ T-cells (chapter 4 and 5). None of the helminth products was capable of altering viral outgrowth of either CCR5- or CXCR4-using HIV-1 in CD4+ T-cell blasts, determined by capsid p24 production. This indicates that SEA, BmA and ES-62 are unable to interfere with HIV-1 binding to CD4 or CCR5/CXCR4, nor with the subsequent steps, e.g. conformational changes in the viral envelope protein required for membrane fusion and cell entry.

Besides a reservoir function, DCs are also well known for their role in supporting
HIV-1 trans-infection. Here virions bind to C-type lectin receptors (CLRs) on the cell surface after which the DC presents the virus to a susceptible CD4⁺ T-cell. Trans-infection is potently mediated by DC-SIGN, a CLR that recognizes both mannosylated and fucosylated glycan structures found on a large array of pathogen- and host-derived glycoproteins including SEA and BmA but not ES-62 ⁶⁻¹². In capture/transfer assays where Raji DC-SIGN cells were pre-exposed to SEA or BmA, significantly less HIV-1 R5 and X4 viral outgrowth was detected in the Raji DC-SIGN-CD4⁺ T-cell co-cultures (chapter 4 and 5). Although both SEA and BmA interfere with HIV-1 capture, SEA was more potent. Whether this results from differences in the concentration, the number or binding strength of DC-SIGN binding component(s) remains to be elucidated. The capacity of SEA and BmA to block HIV-1 trans-infection was confirmed with immature DCs (iDCs). However, as these cells express higher levels of DC-SIGN, multiple other CLRs capable of mediating HIV-1 trans-infection and have a rapid receptor turnover rate, a higher concentration of SEA/BmA was required for inhibition. A more in-depth analysis revealed that kappa-5 is the likely DC-SIGN binding component of SEA (chapter 4).

In conclusion, of the three parasitic products examined none affected HIV-1 cis-infection whilst SEA and BmA inhibited HIV-1 trans-infection. As trans-infection has been postulated to be associated with HIV-1 transmission as well as early viral dissemination within lymph nodes both these parasite products will likely have the capacity to influence the early stages of HIV-1 disease.

2. Do T-cell responses induced under the influence of parasitic helminths have an altered susceptibility to HIV-1 infection?

A number of studies have suggested that Th2 cells were more susceptible to HIV-1 infection than Th1 cells ¹³⁻¹⁷. Although this hypothesis is no longer supported ⁵, ¹⁸, parasitic helminth infections are still considered a risk factor in HIV-1 infection and transmission due to their Th2 skewing properties. Epidemiological studies addressing the effect of helminths on HIV-1 infection and transmission have reported contradictory findings ¹⁹. Hence, to shed more light on the matter, we set up an in vitro model to study the HIV-1 susceptibility of Th-cells induced by DCs matured under either Th1/Th2 (Tmix), Th1 or Th2 promoting conditions in absence (unexposed DCs) or presence (exposed DCs) of helminth products.

Strikingly, Th2 cells induced by SEA exposed DCs (matured in Th2 promoting conditions) harbored significantly less HIV-1 R5 infection compared to Th2 cells induced by unexposed DCs (chapter 4). This reduced level of infection could not be correlated with a downmodulation of CCR5 surface expression. Additionally, except for a reduced level of IFN-γ and MIP-1β producing T-cells associated with SEA’s capacity
to skew towards a more pronounced Th2 phenotype, no changes in the cytokine/chemokine profile of these T-cells was observed. In Tmix cell cultures the level of HIV-1 R5 infection was slightly reduced (not statistically significant) when DCs were exposed to SEA during maturation. This slight reduction motivated us to examine whether more pronounced Th2 skewing could induce protection of Tmix cell cultures for HIV-1 R5. Exposing DCs to increasing concentrations of recombinant omega-1 (rω-1), the Th2 driving component of SEA\textsuperscript{20-22}, during maturation resulted in Tmix cell cultures with significantly reduced HIV-1 R5 infection levels. Although we did not find more pronounced Th2 skewing based on the cytokine/chemokine profile of the cells we did observe a modest downmodulation of CCR5 which potentially contributes to the overall reduction in HIV-1 R5 infection levels. The mechanism(s) responsible for the reduction of R5 but not X4 infection remains to be elucidated. Potentially, not only Th2 but also regulatory T-cells could be induced in the presence of SEA and rω-1, which have been shown to be less susceptible to R5 but not X4 HIV-1 infection\textsuperscript{23-25}.

In addition to the reduced levels of infection when SEA or rω-1 was added to maturing DCs, we found that Th1 cell cultures tend (p=0.09) to have lower levels of HIV-1 R5 infection than Tmix cell cultures induced by unexposed DCs. This was not observed for Th1 cell cultures induced by SEA exposed DCs and can potentially be explained by the level of MIP-1β. MIP-1β is a chemokine associated with Th1 cell responses\textsuperscript{26}. Indeed, the highest percentage of MIP-1β producing cells was found in Th1 cell cultures induced by unexposed DCs which was significantly reduced in Th1 cell cultures induced by SEA exposed DCs. As MIP-1β is the natural ligand for CCR5 it can compete with HIV-1 for binding when present at sufficient concentrations. This seems to be the case in Th1 cell cultures induced by unexposed DCs. However, in vivo MIP-1β may diffuse rapidly, making it unlikely that MIP-1β can reach such high concentrations in vivo, suggesting that this may be an in vitro artifact. The finding that Tmix cell cultures have higher percentages of MIP-1β\textsuperscript{+} cells than Th2 cell cultures, though the level of HIV-1 infection is similar, supports the notion that MIP-1β only interferes with infection when high levels are present.

Unlike SEA, BmA and ES-62 were unable to alter HIV-1 susceptibility of Th-cells induced by exposed DCs (chapter 5). Furthermore, DCs exposed to BmA or ES-62 did not skew the induced Th-cell responses towards a more pronounced Th2 phenotype. This does not necessarily indicate that B. malayi is unable to skew induced Th-cell responses or alter HIV-1 susceptibility of Th-cells, but does suggest that the antigens from adult worms and the phosphorylcholine (PC) group are not involved in such mechanisms.

In conclusion, of the helminth products analyzed only SEA and rω-1 were capable of altering the HIV-1 susceptibility of Th-cells. This effect was limited to CCR5 using HIV-1. However, as R5 viruses are the types predominantly transmitted the presence
of SEA may have major consequences on HIV-1 transmission and disease progression in individuals infected with *S. mansoni*.

### 3. Can helminth products affect HIV-1 transcription in antigen presenting cells?

Antigen presenting cells are among the first cells HIV-1 encounters upon entering the human body. These cells can act as a viral reservoir due to their insensitivity to HIV-1’s cytotoxic effects, their relatively long lifespan and ability to induce viral latency \(^3\,4\,27\).

*S. mansoni* eggs induce granuloma’s consisting of alternatively activated macrophages (M2 cells), eosinophils, CD4\(^+\) and CD8\(^+\) T-cells which facilitate the egg’s migration to the gut lumen \(^28\). Hence the lesions in the gut wall formed by the eggs are surrounded by these cells. In chapter 4 we already demonstrated that CD4\(^+\) Th-cells induced by DCs matured in the presence of SEA have an reduced susceptibility to HIV-1 R5 infection. Additionally we set up a small pilot experiment to determine whether SEA has the potential to alter HIV-1 infection in macrophages. As components in SEA are known to bind and signal through the pathogen recognition receptors, we analyzed the effect of SEA on HIV-1 transcription. The level of unspliced (us) and multiple spliced (ms) HIV-1 RNA transcripts were compared between unexposed macrophages and those exposed to 25μg/ml SEA 2h prior and during HIV-1 R5 infection (SF162, TCID\(_{50}\) 1000). Strikingly, the level of usRNA was 45 fold lower and msRNA 440 fold lower in M2 cells exposed to SEA compared to unexposed cells 72h after infection (data not shown). In a second donor the level of HIV-1 usRNA was reduced by 9 fold and msRNA by 24 fold in SEA exposed M2 cells. Similarly, HIV-1 R5 RNA transcription was reduced in classically activated macrophages (M1 cells) from two donors (6 and 25 fold less usRNA and 6 and 21 fold less msRNA 48h after infection; data not shown). Although the data is preliminary, it does indicate that SEA has the potential to limit HIV-1 R5 viral outgrowth in macrophages.

There are several mechanisms that can potentially explain the above finding. It has been shown that signaling via DC-SIGN with a mannosylated ligand results in enhanced viral RNA transcription in iDCs \(^29\). Macrophages do not express DC-SIGN (data not shown) but signaling via other pathogen recognition receptors such as the mannose receptor (MR) could result in similar downstream modifications which reduce viral RNA transcription. Another possibility is that omega-1, one of SEA’s main components known to possess RNase activity after entering the cell via the MR, degrades HIV-1 RNA upon entering the cell or after transcription \(^20\). It is also feasible that SEA can modulate expression levels of HIV-1 restriction factors known to be expressed in macrophages such as APOBEC3G or miRNA-198 \(^30\,32\).

In conclusion, our data suggests that SEA may have the capacity to reduce HIV-1
R5 viral outgrowth in macrophages which would imply that despite *S. mansoni* eggs create lesions, the cells surrounding these lesions are less susceptible to HIV-1 R5 infection. Additional studies are required to confirm this data and to address the underlying mechanism(s) responsible.

We have not yet analyzed whether BmA or ES-62 can alter the early stages of HIV-1 infection in antigen presenting cells. In chapter 5 we have demonstrated that both BmA and ES-62 are unable to mature iDCs or alter LPS induced maturation (measured by cell surface marker expression and cytokine production) although BmA is capable of binding DC-SIGN, suggesting it may alter HIV-1 transcription in iDCs.

**Our findings in an *in vivo* context**

Utilizing relevant *in vitro* model systems we have been able to study the modulating effects that helminth products have on HIV-1 infection. However, for these effects to take place *in vivo* there must be an overlap in terms of helminth antigen location, the modulated immune cells and HIV-1.

*S. mansoni* eggs are secreted by adult worm pairs, which reside in the mesenteric veins. The eggs migrate to the gut lumen where they typically enter at the large bowel or rectum. This implies that SEA is abundantly present within the gut wall including the gut-associated lymphoid tissue (GALT). Irrespective of the route of transmission, HIV-1 rapidly disseminates through the body and especially affects the GALT. Here approximately 80% of the CD4+ T-cells are lost within the first two weeks of infection. Thereby, SEA residing within this tissue may be beneficial as it can inhibit *trans*-infection, reduce transcription and alter DC maturation to induce CD4+ T-cells less susceptible for CCR5 using HIV-1.

*S. manoni* eggs create lesions in the gut wall where they enter the lumen. This may increase the risk of HIV-1 transmission in individuals participating in receptive anal intercourse. Such an enhanced risk has been demonstrated for lesions in the female genital tract created by *Schistosoma haematobium* eggs. On the other hand, the cells in the granuloma surrounding the lesions are potentially less sensitive to HIV-1 R5 infection and HIV-1 *trans*-infection is being inhibited. Because of these multiple mechanisms, with sometimes opposing effects, it is difficult to determine whether helminth infections can influence HIV-1 transmission and/or disease. These complexities may help explain the contradictory findings reported in epidemiological studies addressing the clinical effects of co-infections.

Host factors residing at sites of helminth egg expulsion need to be taken into consideration. These factors may influence HIV-1 transmission mechanisms or the induction of localized immune responses. In this thesis we demonstrated that colorectal mucus (CM) does not alter HIV-1 *cis*-infection of CD4+ T-cells but does
possess the capacity to block HIV-1 \textit{trans}-infection (chapter 3). Through biochemical analysis of CM’s DC-SIGN binding fractions we identified the component responsible as human lactoferrin. CM from different individuals varied greatly for their DC-SIGN binding capacity, which was correlated with the levels of lactoferrin expressed in the sample. More in-depth analysis revealed that lactoferrin is likely in complex with its receptor intelectin-1 and that the C-terminal fraction of lactoferrin is involved in DC-SIGN binding. Thus, both SEA and CM can hinder HIV-1 transmission through receptive anal intercourse by blocking \textit{trans}-infection.

It remains unclear if \textit{B. malayi} infection can affect HIV-1 infection. Adult \textit{B. malayi} worms reside preferentially in the afferent lymphatics close to the lymph nodes \textsuperscript{39, 40}. Hence, products secreted by these parasites will directly enter lymph nodes where it can interfere with the induction of adaptive immune responses. DCs present in lymph nodes are in close proximity with CD4\(^+\) T-cells, indicating that BmA has the potential to limit \textit{trans}-infection within this compartment. However, asides from the fact that BmA can block HIV-1 \textit{trans}-infection we did not identify any immunomodulatory properties of BmA or ES-62. Our data does not imply that these worms have no effect on HIV-1 transmission or disease progression, but does suggest that the adult worm and the phosphorylcholine-containing molecules studied are not involved. For future studies it would be pertinent to focus on the microfilariae offspring which circulate in the peripheral blood vessels.

\textbf{Conclusions & future perspectives}

Infections with parasitic helminths and HIV-1 have major implications for their host, due to the longevity of the infection as well as the extensive damage these pathogens cause to the immune system. Epidemiological studies attempting to unravel the effects parasitic helminths have on HIV-1 infection have resulted in contradictory findings. Moreover, in helminth infections the worm load and how a person’s immune system responds can vary between individuals. It is these complexities that motivated us to focus on studying specific helminth products and their effect on HIV-1 infection in simplified in vitro model systems using human cells. This has allowed us to determine a number of immunomodulatory properties that can associate with alterations to HIV-1 infection and/or replication.

Based on our findings, there is no indication that high HIV-1 prevalence in areas endemic for helminths results from individuals being more vulnerable to HIV-1 infection due to immune alterations induced by these parasites. If anything, the immunomodulatory properties of SEA appear to protect cells from HIV-1 infection through a number of mechanisms. Although this is only one aspect of \textit{S. mansoni} infection, it does imply that a patient’s parasite status can influence HIV-1
transmission and disease progression. When conducting HIV-1 vaccine or treatment trials a patient’s helminth profile may therefore need to be taken into consideration. Furthermore, the immunomodulatory properties of parasitic helminths have been acquired as a result of co-evolution with the human immune system over millions of years. Therefore, studying these properties may result in the development of new strategies aimed at curtailing HIV-1 transmission or disease progression. For instance, one of the difficulties with HIV-1 vaccines is that the induced immune cells can be targeted by HIV-1. Hence, the addition of a molecule mimicking the protective effects observed with SEA may be highly beneficial in vaccine recipients. The potential of helminthic products in treatment of diseases is not limited to HIV-1 infection. There are indications that parasitic helminths can impose a beneficial influence on autoimmune diseases as well as allergies, emphasizing the importance of helminth studies. Furthermore, the knowledge we can obtain from studying helminthic infections, specifically with regards to mechanisms controlling and directing immune responses, will be invaluable and is worth pursuing.
References


