Dissertation
zum Erwerb des Doctor of Philosophy (Ph.D.)
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

Doctoral Thesis for the awarding of a Doctor of Philosophy (Ph.D.)
at the Medical Faculty of
Ludwig-Maximilians-Universität, Munich

vorgelegt von
submitted by

MKUNDE SEITHY CHACHAGE

aus (Geburtsort)
born in (place of birth)

Dar es Salaam, Tanzania

am (Tag an dem die Dissertation abgeschlossen wurde)
submitted on (day of finalization of the thesis)

30th April 2013

Supervisors LMU:

Habilitated Supervisor	Prof. Dr. Hoelscher, Michael
Direct Supervisor	Dr. Geldmacher, Christof
3rd LMU Supervisor	Dr. Fröschl, Günter
4th LMU Supervisor	

Supervisor External:

Local Supervisor	Dr. Asli Bauer

Reviewing Experts:

1st Reviewer	
2nd Reviewer	

Dean: Prof. Dr. Dr. h. c. M. Reiser, FACR, FRCR

Date of Oral Defence: 19.09.2013

Immune system modulation by Infections with Helminths and HIV-1: Impact on pathogen-specific T cell responses, regulatory T cells and systemic Immune activation

Affidavit

Chachage, Mkunde

Surname, first name

Kaunda

Street

Mbeya

Zip code, town

Tanzania

Country

I hereby declare, that the submitted thesis entitled

Immune system modulation by Infections with Helminths and HIV-1:

Thesis Title

Impact on pathogen-specific T cell responses, regulatory T cells

Thesis Title (cont.)

and systemic Immune activation

Thesis Title (cont.)

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

The submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

I further declare that the electronic version of the submitted thesis is congruent with the printed version both in content and format.

Mbeya, 30th April, 2013

Place, Date

M. Chachage

Signature PhD Student

Dedication: To all the people of Mbeya who participated in this study

Abstract

Background: It has been hypothesized that helminth infections modify HIV susceptibility and disease progression by modifying the human immune system and thus might contribute to the high prevalence of HIV-1 in Africa.

Objective: To study immune system modulation of different helminth infections (*A. lumbricoides, Trichuris trichiura, Hookworms, S.haematobium* and *S. mansoni*) in relation to HIV-1 susceptibility and disease progression.

Methods: 381 adult volunteers from Mbeya-Tanzania were enrolled into the study. Helminth infections were diagnosed using the Kato Katz method. Participants were followed up at 3 months and 1 year after helminth treatment. Expression of regulatory (CD25, FoxP3, Tregs), memory (CD45RO, CD27) and activation markers (CCR5, HLA-DR/CD38) on T cells were studied *ex vivo* using polychromatic flow cytometry in fresh anticoagulated whole blood. HIV- and other pathogen-specific T cell responses were quantified in freshly isolated peripheral blood mononuclear cells using an Interferon gamma ELISPOT assay after stimulation with a peptide pool of HIV peptides or respective studied pathogen antigens. Results were analysed in relation to helminth and HIV infection status. HIV+ subjects on ART were excluded from analysis.

Results: Treg frequencies were increased especially in subjects infected with *T.trichiura* (p=0.008) but were also moderately high in relation to HIV infection (p=0.0472). Interestingly, a substantial fraction of Tregs (Median: 50%) expressed the HIV co-receptor CCR5, which potentially could support HIV entry into Tregs. Quantification of HIV-DNA copies in sorted CD4 T cells then demonstrated a 15 fold higher HIV infection rate in memory Tregs as compared to CD25-FoxP3- memory CD4 T cells (p=0.0032). All studied helminth species were associated with systemic immune modulation but only *T.trichiura* infection correlated with substantially increased expression of HLA-DR on T cells and increased density of CCR5 expression on memory CD4 T cells (P=0.02). HIV infection also correlated with immune activation and high proportion of CCR5/HLA-DR+ CD4 cells independent of helminth co-infection. Neither concurrent helminth infections nor their treatment had a significant effect on HIV- or other pathogen-specific T cell responses. However,

HIV infection alone correlated with depletion of specific T cell responses to pathogens such as *Mycobacterium tuberculosis* and Herpes Viruses, among others.

Conclusions: Helminth, especially *T.trichiura* infection correlated with increased systemic immune activation and might thus potentially contribute to increased susceptibility to HIV acquisition. Regulatory CD4 T cells are a frequent target of HIV infection *in vivo* and are preferentially infected compared to CD25-FoxP3- CD4 T memory cells.

Keywords: *HIV-1, Helminths, Pathogen-specific T cell responses, Regulatory T cells and T-cell immune activation*

Abbrevations

HIV- Human Immunodeficiency Virus

SIV-Simian Immunodeficiency Virus

MTB- *Mycobacterium Tuberculosis*

CMV- *Cytomegalovirus*

HSV-1-Human Simplex Virus

EBV-Epstein Barr virus

TH1 CD4- T helper 1 CD4 T cells

TH2 CD4- T helper 2 CD4 T cells

CTL- Cytotoxic T lymphocyte cells

TCR-T Cell Receptor

IFN-γ-Interferon gamma cytokine

TNFα- Tumour necrosis factor alpha cytokine

IL-2- Interleukin 2 cytokine

CD25-alpha chain of IL-2 receptor

PBMCs- Peripheral Blood Mononuclear Cells

CFP10- Culture Filtrate Protein 10

PPD- Purified Protein Derivative (or tuberculin)

EDTA- Ethylene-diamine-tetraacetic acid

CPDA- Citrate Phosphate Dextrose Adenine (Anticoagulant)

FBS- Foetal Bovine Serum

HEPES- Hydroxyethyl piperazineethanesulfonic acid (Buffer reagent)

R10 =RPMI/Glutamax medium supplemented with 10% FBS; 10 mM HEPES; 50Units Penicillin and 50 μg/ml of streptomycin (all Gibco, Invitrogen).

RT-Room Temperature

Table of Contents

List of Tables

List of Figures

1. Introduction

1.1. The Human Immunodeficiency Virus 1 (HIV-1) – General Introduction

HIV is a retrovirus belonging to a group of Lentiviruses. HIV eventually, causes an infection which can lead to acquired immune deficiency syndrome (AIDS) - a disease characterized by the failure of the immune system to control diverse opportunistic infections brought on by the progressive loss of CD4 T cells. Phylogenetic analysis of HIV sequences suggests that HIV might have been transmitted to humans as early as 1930s (Korber et al. 2000) even though it was defined as the causative agent of AIDS in 1983 (Barré-Sinoussi et al. 1983). Two types of HIV that are recognised as causative agents of AIDS in humans are HIV-1 and HIV-2. HIV-1 is diverse, virulent and has a wider distribution accounting for the global AIDS pandemic while HIV-2 is concentrated and has remained isolated in West Africa and countries with strong ties to such regions (Murphy et al. 2007).

By 2011, over 34 million people worldwide were estimated to be living with HIV, 69% of them living in sub-Saharan Africa (UNAIDS 2012). Tanzania is amongst sub-Saharan countries with high HIV prevalence (5.8%) whereby 1.6 million people, mostly adults between the age of 15-49 years, were estimated to be living with HIV by year the 2011 (UNAIDS 2012). Mbeya region is amongst the top three regions within the country with the highest burden of HIV infection (THMIS 2011).

HIV RNA genome has 3 structural genes: gag, env and pol that codes for the HIV core proteins. The gag gene encodes for the matrix (p17), capsid (p24), nucleocapsid (p7) and link (p6) proteins. The pol gene encodes for viral enzymes-reverse transcriptase, intergrase and protease while env encodes for the viral envelope glycoprotein-gp 160 which is cleaved into functional gp 120 and gp 41.In addition, the HIV-1 genome also contains genes encoding for the small accessory proteins (Nef, Vif, Rev, Tat, Vpu, Vpr) with regulatory functions. Figure 1- 1 shows an illustration of the virion structure (Murphy et al. 2007).

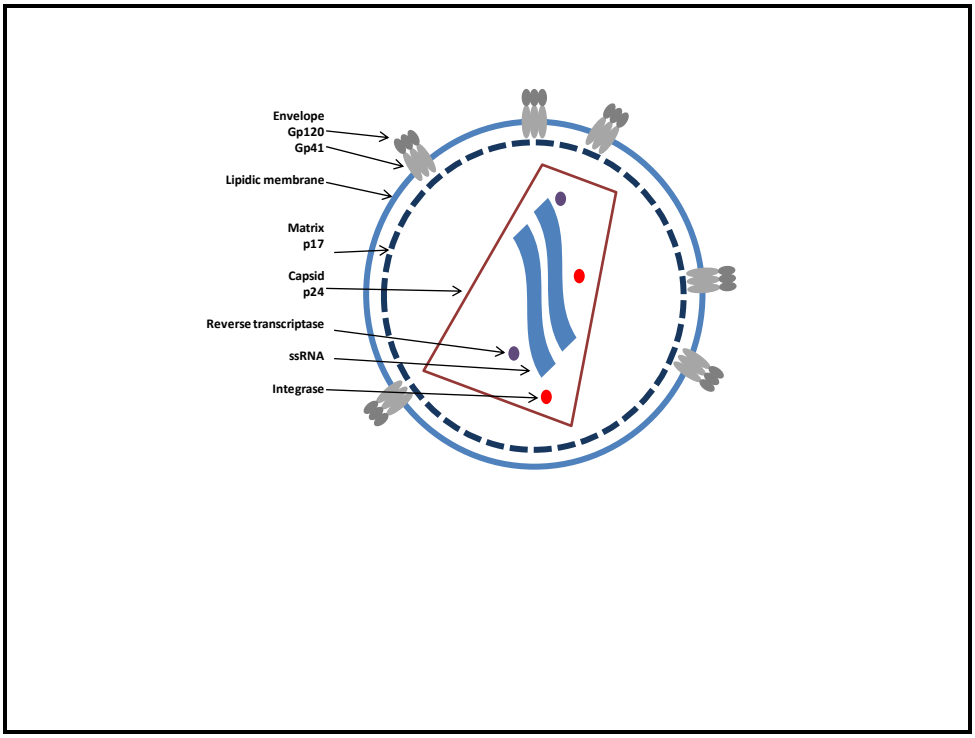


Figure 1- 1: Structure of the Human Immunodeficiency Virus (HIV) virion (schematic diagram by F. Nicoli).

HIV infection requires transmission of body fluids such as blood, semen or vaginal secretion from an infected to uninfected person (reviewed in (Murphy et al. 2007)). Sexual intercourse is the most common route of HIV infection worldwide where CD4 T cells but also Macrophages and Dendritic cells at the mucosa sites are targeted through receptor dependent mechanisms. HIV entry in the target cells is mediated through binding of HIV envelope's transmembrane glycoprotein gp 140 and gp 41 to CD4 receptor (Sattentau et al. 1988) and additional co-receptors (CCR5 or CXCR4)- mainly through the expression of CCR5 chemokine receptor (Deng et al. 1996; Liu et al. 1996). CCR5 expression is common on memory CD4 T cells in mucosal lymphoid tissues, the mucosa of the reproductive tract and intestine, the lungs and inflamed tissues (Brenchley et al. 2004; Picker et al. 2004; Qin et al. 1998) (also reviewed in (Geldmacher & Koup 2012)). Upon entry, HIV RNA is reverse transcribed into double-stranded DNA, which is subsequently integrated into a host chromosomal DNA (Figure 1- 2).

After integration of provirus, infected T cells can either establish a latent or a productive infection depending on their biological properties. T cell activation and

proliferation facilitate efficient HIV replication *in vivo* and *in vitro* (Geldmacher & Koup 2012; Zack et al. 1990; Zhang et al. 1999). Furthermore, memory activated CD4 T cells supports productive HIV infection *in vitro* (Schnittman et al. 1990) and *in vivo* (Brenchley et al. 2004). Within the memory CD4 T cells, HIV specific CD4 T cells are predominantly infected by HIV (Douek et al. 2002; Demoustier et al. 2002) at all stages of HIV infection (Douek et al. 2002). Preferential infection of HIV-specific CD4 T cells depletes the pool of these cells and is thought to contribute to HIV disease progression.

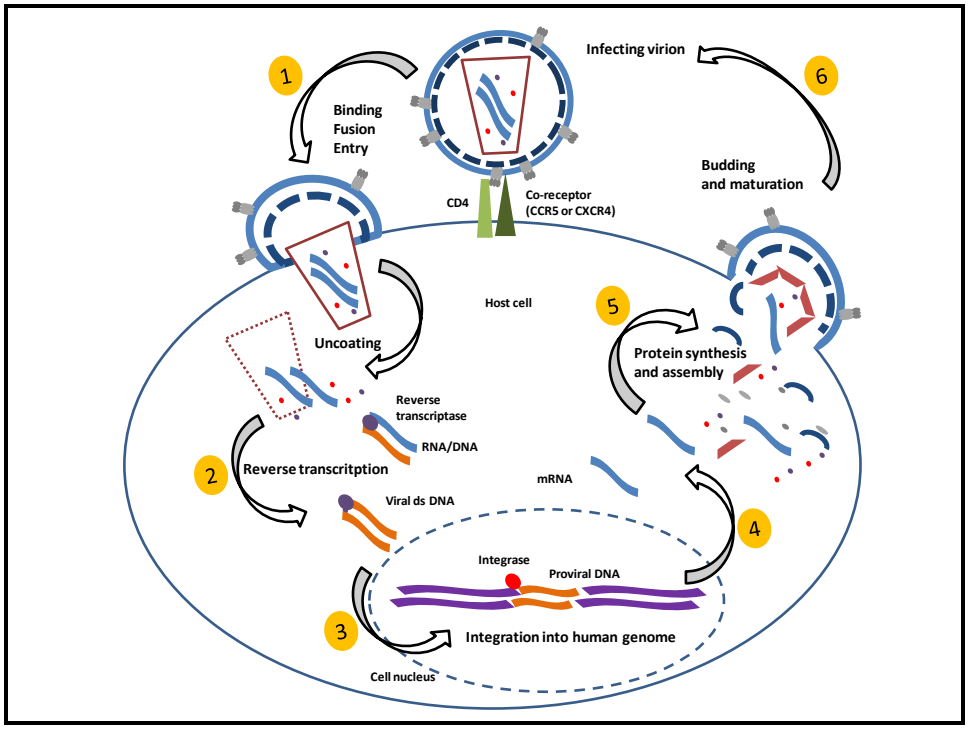


Figure 1- 2: HIV replication cycle. (1) Binding, fusion and entry of HIV into the host cell which is mediated by binding of HIV envelope to CD4 and co-receptor. **(2)** Reverse transcription of HIV single stranded RNA into a double stranded viral DNA. **(3)**Transport of viral DNA into the nucleus and integration of viral DNA in host chromosomal DNA. **(4)** Proviral gene expression of genomic viral RNA. **(5)**Virus self-assembly at the cellular membrane and viral budding. **(6)**Maturation of virion and infection of anew cell. (Schematic diagram by F. Nicoli).

1.1.1. HIV-specific Immune response

CD8 cytotoxic T cells have been well established as important cells in the virus control. In acute HIV infection, detectable CD8 T cell responses *in vitro* (Borrow et al. 1994) and *in vivo* (Koup et al. 1994) correlate with decline in plasma viremia. An inverse association of proportion of CD8 T cell responses and plasma viral load has also been demonstrated in individuals with chronic HIV infection (Ogg et al. 1998). Further evidence is provided in the Simian Immunodeficiency Virus (SIV)-rhesus macaques model whereby depletion of CD8 T cells in the blood of animals with chronic SIV infection led to a dramatic increase in plasma SIV load and CD4 T cell depletion (Jin et al. 1999; Schmitz et al. 1999). Importantly, the frequency of polyfunctional HIV-specific CD8 T cells is associated with slower disease progression as observed in HIV non-progressors (Betts et al. 2006). In 1995, it was shown that gag specific cytotoxic responses are associated with slow progression to AIDS (Rivière et al. 1995) and recently, it has been reported that CD8 T-cell recognition of multiple epitopes within specific Gag regions is associated with low steady-state viremia in subjects with chronic HIV-1 infection (Geldmacher et al. 2007; Kiepiela et al. 2007).

Although HIV-specific CD4 T cells are preferential targets for HIV infection (Douek et al. 2002), they also play a critical role in the defence against HIV. Typically, HIV and other intracellular pathogens are primarily controlled by the induction of CD4 T helper 1 (TH1) cells defined by their secretion of IFN-γ, TNFα and IL-2 cytokines (Murphy et al. 2007). IFN-γ secreting CD4 T helper 1 cells are involved in the classical activation of infected macrophages (Murphy et al. 2007). IFN-γ and TNFα are also considered as anti-viral cytokines, as their production by cyotoxic T cells (CTLs) upon activation through the encounter with the target cell presenting HIV antigens is directly linked to CTLs cytotoxic activity (Jassoy et al. 1993). Also, CD4 T cell proliferative activity in response to *in vitro* stimulation with HIV antigen results in IFN-γ production and is associated with a decline in plasma viremia in subjects with chronic HIV infection (Rosenberg et al. 1997; Eller et al. 2012). Of importance, IL-2 secreted by CD4 T helper cells is crucial for maintenance

of effective CTL response (Lichterfeld et al. 2004). The depletion of CD4 T cells during HIV therefore results in an immune devastation, which leaves infected individuals exposed to a wide range of opportunistic infections.

1.2. Helminths of public Health importance- General Introduction

Helminths comprise a group of nematode (round) and trematode (flat) parasitic worms which typically cause chronic infections in humans with the most devastation observed in developing countries especially within sub-Saharan Africa. The most common helminths of public health importance include: *Ascaris lubricoides*, *Trichuris trichiura*, Hookworm species (*Necator americanus* or *Ancylostoma duodenale*) and Schistosomes. Co-existence of humans and parasitic worms dates back to more than 1200BC, based on evidence from early written records and calcified egg worms from mummies (reviewed in (Cox 2002)). Human infection with these worms is usually through contact with their eggs or larvae (Bethony et al. 2006; WHO; CDC).

Infection with *A.lumbricoides* occurs by ingesting fertilized eggs which hatch to larvae after ingestion and penetrate to the intestinal mucosa. They then are carried via the portal (liver), then to the lungs where the larvae mature further. Thereafter, the larvae penetrate alveolar walls, ascend the bronchial tree to the throat before they are swallowed and re-enter the GIT. Upon reaching the small intestine, they develop into adult worms and produce eggs which are then passed through stool to the environment (Bethony et al. 2006; CDC; WHO).

Infection with *T.trichiura* is similar to *A.lumbricoides*, which also involves ingestion of developed eggs which hatch in the small intestine (jejunum) and infective released larvae then migrate to the colon (cecum) where they develop as adult worm. Adult worm burry their heads in the epithelium and female adult worms produce eggs which are passed with stool (Bethony et al. 2006; CDC; WHO).

Unlike *A.lumbricoides* and *T.trichiura*, Hookworm eggs hatch in the environment to release larvae which become infective 5-10 days after. The infective

larvae penetrate through the human skin and travels to the heart and lungs through vessels. They then penetrate into pulmonary alveoli, ascend the bronchial tree to pharynx, and are swallowed to enter the Gastro Intestinal Tract (GIT). When reaching the small intestine, larvae mature into adults. Adult worms live in the lumen of the small intestine, where they attach to the intestinal wall, feeding on red blood cells resulting in host blood loss (anaemia). Female adult worms produce eggs which are passed with stool (Bethony et al. 2006; CDC; WHO).

Schistosomes are a group of trematodes that cause schistosomiasis. There are three main species of schistosome infecting humans: *S.mansoni*, *S.haematobium* and *S.japonicum*. Of the three, only *S.mansoni* and *S.haematobium* are found in Africa while *S.japonicum* is geographically localized in the Far East. Schistosome life cycle involves 2 hosts, humans and specific snails which act as intermediate host. Eggs released in fresh water through faeces or urine hatch and release a stage of larva (*miracidia*) which swim and penetrate specific snail intermediate host. Within the intermediate host, *miracidia* undergo asexual production of sporocysts and production of *cercariae*- which are the infective form to humans. Upon release from the snail, the infective *cercariae* swim, penetrate the skin of the human host, and shed their forked tail, becoming another larval form called *schistosomulae*. *Schistosomulae* migrate through several tissues (including lungs and liver) and stages to their residence in the veins where they mature into adult female or male worms. Adult worms in humans reside in the mesenteric venules of the rectum or in venous plexus of urinary bladder. For instance, *S.mansoni* frequently reside in superior mesenteric veins draining the large intestine while *S.haematobium* most often occurs in the venous plexus of bladder, but it can also be found in the rectal venules. Adult worms pair in their host destination and live for a long time feeding on red blood cells. Female adult worms lay and deposit eggs in the blood stream and travel through the veins into the lumen of the intestine (*S.mansoni*) or urinary bladder (*S.haematobium*) and eggs are finally excreted with faeces or urine, respectively. However, a proportion of these eggs are trapped in the tissues such as intestine and liver portal system (for *S.mansoni*) or urinary bladder, ureter and other tissues of the pelvic organ (for *S.haematobium)*. The egg antigens then induce granuloma formation through chronic inflammation in the affected organs, which is the cause of schistosomiasis (CDC; WHO).

Infections with helminths are associated with serious morbidity especially in children. Morbidity is related to the intensity of helminth infection. People with light infections usually have no symptoms. Heavier infections can cause pathologic manifestations exerted either directly by helminths or by host immune and inflammatory responses triggered by helminth antigens (CDC; WHO).

1.2.1. Helminth-specific Immune response

Chronic infections with helminths are typical since helminths need time in their host to complete their development and transmission. Helminths have evolved in time and developed strategies to evade host immune reactions to ensure their long survival within the host and at the same time in most cases, controlling the amount of pathological damage they impart on the host. Unlike HIV, worm infections are controlled by the induction of modified CD4 T helper 2 (TH2) responses associated with regulatory mechanisms (reviewed in (Maizels & Yazdanbakhsh 2003)). This response is characterized by increased levels of IgE and eosinophils and by production of IL-4, IL-5, IL-13 by CD4 T helper 2 cells, accompanied by production of immune suppressive cytokines such as IL-10 and TGF-β, as demonstrated in murine models (Bancroft et al. 1998; McKenzie et al. 1998; Holland et al. 2000; Turner et al. 2011) and humans (P. J. Cooper et al., 2000; Jackson et al., 2004; Turner et al., 2003). However, in shifting the response towards TH2 (Cooper et al. 2000; Turner et al. 2011), helminths may modify TH1 cell responses to chronic infections such as HIV and TB and increase susceptibility to such infections (Borkow et al. 2001; Resende Co et al. 2007).

1.3. Modulation of pathogen-specific T cell responses by HIV-1 and/or Helminth (co) infections

Depletion of CD4 T cells during HIV infection usually leads to the failure of the immune system to control different opportunistic infections if left untreated. For example, latent infection with *Mycobacterium Tuberculosis* (MTB) is tightly controlled predominantly by IFN-γ secreting CD4-TH1 cells specific to MTB (Gallegos et al. 2008; Cooper et al. 1993) in healthy individuals; while in HIV

positive individuals, MTB-specific CD4 T cells are preferentially lost at an early stage of HIV infection (Geldmacher et al. 2010) and individuals are much more likely to develop active TB than HIV negative individuals, in regions where both pathogens co-circulate (WHO 2013).

Recently, infections with mostly *Strongyloides stercoralis* but also with *T.trichiura* and *A.lumbricoides* have been associated with reduction of IFN-γ and elevation of IL-10 cytokine levels in the supernatant after *in vitro* re-stimulation of whole blood of helminth infected individuals with MTB antigen (Resende Co et al. 2007). Furthermore, reduced efficacy to Bacillus Calmette-Guèrin (BCG) anti-tuberculosis vaccine has been reported in helminth infected mice (Elias et al. 2005) and humans, and its restoration after worm treatment (Elias et al. 2001). However, the influence of helminth infections on MTB-specific T cells in HIV-worm co-infected individuals has not been explored. It has also been shown that certain helminth infections can alter HIV and other pathogen-specific T cell responses towards a TH2 profile (Kamal et al., 2001; McElroy et al., 2005). Helminth infections might induce IL-10 expression in HIV-specific CD8 T cells (McElroy et al. 2005) and thus weaken anti-viral effector functions.

Cytomegalovirus (CMV), Herpes Simplex Virus (HSV), *Toxoplasma gondii*, Influenza and Epstein Barr virus (EBV) are common pathogens encountered by humans with a well controlled immune response (Murphy et al. 2007) but can cause AIDS related disease in highly progressed chronic HIV people (Lazenby, 2012; San-Andres et al, 2003). The progressive loss of CD4 T cells, particularly of pathogen-specific TH1 CD4 T cells and impaired functions of pathogen-specific CD8 T cells after HIV infection contributes to the failure of the immune system to control these opportunistic infections ((Komanduri et al. 1998), also reviewed in (Geldmacher & Koup 2012)) . Modulation of such pathogen-specific T cell responses by helminth infections has not been fully explored. Influence of *S.mansoni* on IFN-γ secreting CMV-specific T cells and on the expression of degranulation marker on T cells has been investigated before on *S. mansoni*-CMV co-infected individuals; and found no effect of *S.mansoni* infection on such cells (McElroy et al. 2005). To better understand the immune modulation by infections with different helminths, it is

necessary to also explore their immune modulation of specific T cell responses to different pathogens including HIV and MTB.

1.4. Regulatory T cells-General Introduction

Regulatory CD4 T cells (Tregs) have a high expression of CD25 (IL-2Ra) (Baecher-Allan et al. 2003; Seddiki et al. 2006; Hori et al. 2003), and co-express the transcription factor forkhead box P3 (FoxP3). Hence, both of these markers are typically used to identify and characterize Tregs. Tregs are essential for maintenance of self tolerance and they can suppress activation, proliferation and effector functions of a wide range of immune cells, including CD4 and CD8 T cells (reviewed in (Sakaguchi et al. 2008)).

Tregs were first described in 1995 in mice as CD4 T cells that express high levels of CD25. The depletion of CD25+ cells from CD4 T cells from mice lymphoid tissues led to an auto-immune condition and failure to regulate non-self antigens while the presence of such cells maintained self tolerance and down-regulated non-self antigens (Sakaguchi et al. 1995). Later on, CD4+CD25high Tregs where characterised in humans as comprising 1-2% of circulating CD4 T cells and >80% of these cells possessing a memory (CD45RO) phenotype (Ng et al. 2001; Baecher-Allan et al. 2001; Dieckmann et al. 2001). In 2003, FoxP3 was described as a key regulator for Treg development (Hori et al. 2003) after having observed *ex vivo* specific expression of this transcription factor in thymic CD4 T cells and periphery and tissue CD4+CD25high cells from mice (Fontenot et al. 2003; Hori et al. 2003) and later on in humans (Roncador et al. 2005). Expression of Foxp3 by Tregs interferes with the binding of transcription factors for T-cell growth cytokine- IL-2 at the IL-2 promoter, preventing transcriptional activation of IL-2 gene (Fontenot & Rudensky 2005).

Much of Treg activity has been demonstrated *in vitro* where they have been shown to be hyporesponsive to *in vitro* T-cell receptor (TCR) stimulation as well as suppress proliferation and effector functions of CD4+CD25- cells by inhibiting their IL-2 and IFN-γ production in *in vitro* co-cultures of Tregs and CD4+CD25- (Ng et al.

2001; Baecher-Allan et al. 2001; Hori et al. 2003; Fontenot et al. 2003). Tregs can be driven to expand after *in vitro* stimulation in the presence of IL-2 and still maintain their suppressive activity (Ng et al. 2001). Furthermore, CD4+CD25- cells can be induced *in vitro* to become Tregs (Hori et al. 2003; Roncador et al. 2005). IL-2 signalling through IL-2 receptor signalling is essential for maintenance of T cell homeostasis as demonstrated that mice deficient for IL-2 develop abnormal proliferation of lymphocyte and autoimmune manifestations (Sadlack et al. 1993; Schorle et al. 1991). Similarly, IL-2 signalling through IL-2 receptor alpha chain, CD25 ensures sustenance of sufficient Treg cell population necessary for maintenance of Treg homeostasis (Fontenot et al. 2005; Burchill et al. 2007).

1.4.1. Regulatory CD4 T cells during HIV or chronic Helminth infections

It is unclear whether the impact of Tregs in the context of HIV infection is beneficial or detrimental. FoxP3 expressing T cells tend to accumulate in lymphoid tissues of progressive HIV positive individuals (Nilsson et al. 2006; Andersson et al. 2005). On the other hand, frequency of CD25+FoxP3+CD4 T cells has been seen to be elevated in the periphery of individuals with chronic untreated HIV infection while the absolute count of these cells decline in the periphery (Angin et al. 2012; Presicce et al. 2011) and gut mucosa (Angin et al. 2012), where CD4 T cell depletion by HIV mostly occurs (Brenchley et al. 2004). Some scholars have argued that elevation of Tregs could either limit HIV replication by lowering HIV associated general immune activation (Card et al. 2009; Eggena et al. 2005) while others suggested that Tregs fuel HIV replication and disease progression by interfering with the ability of the HIV-specific immune cells to control HIV replication (Aandahl et al. 2004).

Circulating CD4+CD25+ Tregs of individuals with chronic HIV infection have been shown to suppress production of anti-viral IFN-γ and TNFα cytokines in HIV-specific CD8 and CD4 T cells *in vitro* (Aandahl et al. 2004). Furthermore, suppression of HIV Gag-specific cytolytic responses from CD8-CD25+ PBMCs has also been demonstrated *in vitro* (Kinter et al. 2007). Recently, Tregs have been reported to beneficially control HIV replication in conventional T cells *in vitro*

(Moreno-Fernandez et al. 2011). On the other hand, increased Tregs accumulation in tissues of HIV infected individuals is associated with disease progression (Cao et al. 2009; Suchard et al. 2010).

As mentioned above, CD4 TH2 responses accompanied by regulatory mechanisms control helminth infections. Infection of mice with *S.mansoni* is associated with increased level of CD4+FoxP3+ Tregs and TGF-β production as well as regulation of TH2 pathological responses caused by *S.mansoni* (Turner et al. 2011). High frequency of CD4+CD25+ Tregs in relation to *S. mansoni* (Watanabe et al. 2007) and Hookworm (Ricci et al. 2011) infections is also observed in humans which is reduced by almost 2-fold after treatment with praziquantel (Watanabe et al. 2007).

Although high levels of Tregs have been reported in either HIV or helminth infection alone, Tregs in the context of HIV-worm co-infection have not been studied. Because worm infections overlap with HIV infection in areas where both infections are prevalent, quantity of Tregs in co-infected individuals could have an impact on the course of HIV progression. It is thus important to determine the levels of Tregs in co-infected individuals and the effect of worm treatment in HIV disease progression.

1.4.2. Regulatory CD4 T cells as potential targets for HIV replication

HIV predominantly infects memory CD4 T cells (Schnittman et al. 1990; Brenchley et al. 2004; Dai et al. 2009) and its entry and transmission is linked to viral CCR5 tropism (Moore et al. 2004; Sattentau et al. 1988; Deng et al. 1996; Liu et al. 1996). It is also well established that CD4 T cell proliferation efficiently supports productive HIV infection *in vitro* (Chou et al. 1997; Zack et al. 1990). Particularly, IL-2 signaling which is required for antigen-specific T cell proliferation and differentiation (Chou et al. 1997), supports productive HIV infection and replication within CD4 T cells *in vitro* (Finberg et al. 1991; Ramilo et al. 1993; Chou et al. 1997; Goletti et al. 1996; Geldmacher et al. 2010). Moreover, expression of the IL2 receptor alpha chain (CD25) defines a CD4 T cell population that efficiently supports productive HIV infection in lymphoid tissue explants (Biancotto et al. 2008). Tregs

also express a higher proportion of HIV main coreceptor-CCR5 compared to memory CD4 T cells *in vivo* and their susceptibility to HIV infection has been demonstrated *in vitro* (Oswald-Richter et al. 2004). Furthermore, studies on homeostasis and differentiation status of Tregs have shown that > 80% of circulating Tregs in adults express the memory marker CD45RO (Booth et al. 2010; Antons et al. 2008) and that high frequencies of these memory Tregs (10-20%) are Ki67 positive and thus are actively cycling (Booth et al. 2010). Indeed the *in vivo* doubling time of memory T regs is only 8 days and thus 3-fold and 25-fold reduced to memory and naïve CD4 T cells, respectively (Vukmanovic-stejic et al. 2006).

The highly proliferative nature and high turn over of Tregs and the proposed mechanism of constant homeostatic replenishment of this cell subset by peripheral memory CD4 T cells and during antigen-specific CD4 T cell responses (Vukmanovic-Stejic et al. 2008; Vukmanovic-stejic et al. 2006) support the hypothesis that CD25+FoxP3+ CD4 T cells constitute a CD4 T cell subset that is highly susceptible to productive HIV infection *in vivo* and contribute to plasma viremia despite their relatively low frequencies.

Since helminth immunity is accompanied by the elevation of regulatory mechanisms, helminth induced Tregs are potential targets for HIV acquisition. Importantly, Treg infection by HIV could contribute to HIV disease progression in HIV-helminth co-infection. Hence, there is a need to further study and compare the expression of CCR5 on Tregs of HIV and worm (co)infected individuals and their susceptibility to HIV infection *in vivo*.

1.5. T cell activation

1.5.1. T cell activation and HIV disease progression

Untreated HIV infection leads to Acquired Immunodeficiency Syndrome (AIDS); a disease characterized by the failure of the immune system to control diverse opportunistic infections brought on by the progressive loss of CD4 T cells. People with chronic HIV infection display persistent immune activation (Mahalingam

et al. 1995; Sousa et al. 2002; Ascher & Sheppard 1988) which strongly predicts the decline of CD4 T cells and hence progression to AIDS (Brenchley et al., 2004; Hazenberg et al., 2003). Different activation markers on/in T cells predict the rate of HIV disease progression independent of viral load (Giorgi et al. 1999; Giorgi et al. 2002; Hazenberg et al. 2000; Hazenberg et al. 2003; Hunt et al. 2003; Liu et al. 1997; Sandler et al. 2011; Levacher et al. 1992). Most commonly studied are cell surface markers of activation, such as high levels of CD38 expression on CD8 T cells and the frequency of CD38+/HLA-DR+ co-expressing CD8 T cells (Giorgi et al. 1999; Levacher et al. 1992; Liu et al. 1997). Other activation markers include the HIV co-receptor CCR5 (Portales et al. 2012) and also a cell cycle marker Ki67 (Sachsenberg et al. 1998). Indeed, loss of CD4 T cells in chronic HIV infection is directly linked to an increase in proportion of cycling CD4 T cells (Sousa et al. 2002) for replenishment of the CD4 T cell pool. This high T cell turnover of CD4 T cells facilitates the infection of these cells by providing targets for HIV (Biancotto et al. 2008; Stevenson et al. 1990).

The etiology of systemic immune activation during HIV infection is not clear and most likely multi-factorial. Factors potentially contributing to activation include: persistent antigen-specific stimulation of T cells specific for HIV and other persistent pathogens, such as HHVs (Ascher & Sheppard 1988; Giorgi et al. 1999), translocation of microbes and microbial compounds, such as Lipopolysaccharide (LPS), that activate PAMP-receptors (Brenchley et al. 2006), chronically elevated levels of Type 1 Interferons (Bosinger et al. 2009; Jacquelin et al. 2009; Manches & Bhardwaj 2009) and probably helminth co-infections.

Indeed, increased systemic activation in CD8 (Kassu et al. 2003) and/or CD4 T cells (Eggena et al. 2005; Mkhize-Kwitshana et al. 2011) has been observed in HIV positive individuals co-infected with helminth and other pathogens. Moreover, increased immune activation in HIV-Helminth co-infection correlates with progression to AIDS as indicated by increased plasma HIV loads (Mkhize-Kwitshana et al. 2011; Eggena et al. 2005) and CD4 T cell decline (Eggena et al. 2005). However, the contribution of helminth infections in increasing systemic T cell activation during HIV-helminth co-infection is unclear due to a lack of well-controlled longitudinal studies. Treatment of helminth infections in co-infected

individuals has been shown to be insignificant in reducing systemic T cell activation (Kassu et al. 2003). Secor *et al.* also reported a decline in the density and frequency of CCR5 expression on CD4 T (and monocytes) cells after treatment of schistosome infection with albendazole but did not differentiate between HIV uninfected and infected individuals (Secor et al. 2003), limiting the interpretation of their results.

Furthermore, in HIV infected people, the effect of treatment for helminths on improving clinical HIV indicators is controversial. Although a positive influence of helminth treatment on CD4 T cells and plasma viral load decline has been reported (Kallestrup et al. 2005; Walson et al. 2008), some studies found no association (Brown et al. 2004) or a negative relation (Brown et al. 2005). Hence, factors associated with immune modulation by helminth (co)infections and the impact of treatment need to be investigated further. If anti-helminthic treatment does indeed reduce HIV progression, it would be a cost effective alternative to reduce HIV progression.

1.5.2. T cell activation and Susceptibility to HIV

In 1995, Bentwich *et al.* proposed that systemic immune activation associated with chronic helminth infection may be the driving force of HIV transmission in Africa (Bentwich et al. 1995) as such infections are common in Africa (reviewed in (Hotez et al. 2007)). Since then, several studies have linked systemic immune activation in African populations to helminth infection (Kalinkovich et al. 1998; Kalinkovich et al. 2001; Secor et al. 2003). A series of such studies was conducted in Israel with newly arrived Ethiopian migrants who were characterized by a high prevalence of helminth infections such as Schistosomes, Hookworm, *A.lumbricoides* or *T.trichiura*. Compared to Ethiopian migrants that had stayed in Israel for longer periods and had received standard anti-helminthic treatment upon arrival, HLA-DR expression on CD4 and CD8 T cells and lymphocyte apoptosis, was substantially higher in the new arrivals (Kalinkovich et al. 1998). Also, PBMCs of these immigrants were highly susceptible to *in vitro* infection with HIV, which correlated with the state of immune activation (Shapira-Nahor et al. 1998). Within a similar study population, the same group also reported higher CCR5 and CXCR4 expression

levels in Ethiopians, regardless of the length of their residence and thus also after anti-helminthic treatment (Kalinkovich et al. 2001). Contrary, a more recent study observed no changes in the T cell immune activation profile of HIV negative subjects between helminth infected with *T.trichiura* and/or *A. lumbricoides* and non-helminth infected groups except for a 2-fold increased frequency of CCR5 expression on CD4 T cells in helminth infected subjects (Mkhize-Kwitshana et al. 2011).

Low systemic immune activation is a correlate of protection against HIV infection (Card et al. 2009; Koning et al. 2005). This is demonstrated in recent human studies reporting that low immune activation in highly exposed HIV uninfected individuals contributes to their resistance to HIV infection (Koning et al. 2005; Bégaud et al. 2006). Koning *et al.* extensively showed that the blood of high risk seronegative men from the Amsterdam cohort had lower frequencies of co-expression of HLA-DR and CD38 on CD4 T cells, low cycling cells on T cells as defined by the expression of Ki67 nuclear antigen and low proportion of memory CD4 T cells expressing CCR5 in comparison to men who were seronegative at the time of analysis but later on became HIV positive (Koning et al. 2005). Similarly, Begaud *et al.* observed significantly lower expression of HLA-DR and CCR5 on CD4 T cells in exposed seronegative heterosexuals from a Central African cohort (Bégaud et al. 2006), suggesting a role of CD4 T cell immune activation in HIV susceptibility.

While these studies support a link between systemic immune activation and HIV susceptibility, lack of well-controlled longitudinal studies that clearly define helminth species-specific association markers of immune activation before and after treatment prohibits definite conclusion. It is not entirely clear, whether helminths are primarily a cause of systemic T cell activation or whether different helminths equally associate with it in populations from endemic areas of Africa. The present study therefore aimed to investigate the effect of infections with different helminth species and helminth eradication on the profile of T-cell immune activation.

1.6. Study Objectives

This study aimed to provide important insights into the complicated immunological interactions between different helminth infections and HIV infection.

The **primary** scientific objective was to study helminth associated modulation of immune system and the impact of deworming by assessing:

a) the influence of helminth infections on the quantity of IFN-γ producing CD4 and CD8 T cells specific for other pathogens.
b) whether helminth infections are associated with increased frequency and absolute numbers of different T cell subsets including regulatory (CD25+FoxP3+) CD4 T cells
c) whether helminth infections are associated with increased markers of T cell activation (HLA-DR, CD38) and HIV co-receptor (CCR5)

The **secondary** scientific objective was to study HIV infection rate of CD25+FoxP3+ CD4 T cells in comparison to other T-cells subsets *ex vivo*.

2. Materials and Methods

2.1. Study volunteers and Blood Processing

381 adult (18-50years) volunteers from 9 geographically distinct areas within the Mbeya region in South West Tanzania were enrolled into the Worm_HIV_Interaction_Study (WHIS) prospective cohort. Blood, urine and stool specimens were collected from each participant at baseline (W0), during the follow up at 1-3 months (W1) and one year (W3) after helminth treatment with Albendazole and Praziquantel. Stool and urine specimens were used for diagnosis of infections by 5 different helminth species (*T.trichiura*, *S.mansoni*, *S.haematobium*, *A.lumbricoides* and Hookworm species). Fresh stool specimens were used for Kato-Katz diagnosis of geohelminth (*T.trichiura*, *A.lumbricoides*, Hookworms) and *S. mansoni* infections. Briefly, two Kato-Katz thick smears (41.7 mg each) were prepared from each fresh stool. Kato-Katz slides were microscopically examined for helminth eggs by experienced technicians within one hour (for hookworm eggs) and within two days (for other helminth eggs) after slide preparation. *S.haemotobium* infection was diagnosed by microscopic examination of a filtered urine sample (20ml) for *S. haematobium* eggs. Helminth infection was defined as the presence of at least one worm egg in the two examined samples. HIV status was determined using HIV 1/2 STAT-PAK, (Chem-bio Diagnostics Systems) and positive results were confirmed using ELISA (Bio-Rad). Discrepancies between HIV 1/2 STAT-PAK and ELISA were resolved by Western Blot (MPD HIV Blot 2.2, MP Biomedicals). HIV positive study volunteers on antiretroviral therapy were excluded from analysis unless otherwise stated. 40ml of venous blood were drawn from each participant using anticoagulant tubes (CPDA, EDTA; BD Vacutainer). Absolute CD4 T cell counts were determined at each time point from anti-coagulated whole blood using the BD Multitest IMK kit (BD) according to manufacturer Instructions. Complete blood count (CBC) was also performed on whole blood of all subjects in order to analyse the levels of eosinophils in the study groups at each time point. Blood samples were processed within less than 6 hours of the blood draw at the NIMR-MMRC laboratories.

Frequencies of CD25+FoxP3+ CD4 T cells and surface CCR5 expression as well as frequencies of activation markers (HLA-DR, CD38 and CCR5) on T cells were determined on fresh anti-coagulated whole blood at each of the three time points. Also, Peripheral Blood Mononuclear Cells (PBMCs) isolated from fresh anti-coagulated whole blood at each of the three time points as described below. PBMCs were isolated using the Ficoll centrifugation method and Leucosep Tubes (Greiner Bio one) according to standard protocols from manufacturer (Greiner Bio one). Sufficient amount of PBMCs were used for characterization of pathogen-specific T-cell responses targeting HIV, MTB and Cytomegalovirus (CMV) among others by using Inteferon-γ (INF-γ) ELISPOT; and the rest of the PBMC were cryo-preserved for further analysis.

2.2. Quantification of IFN-γ secreting pathogen-specific T cell responses

2.2.1. Antigens

In vitro PBMC stimulation was performed with different antigens described herein. A pool of 15 HIV frequently recognized peptides representing Gag and Nef from isolates of subtype A, C and D (Elephants & Peptides, Germany), which have been previously shown to detect IFN-γ secreting HIV-specific T cell responses by 94% (Geldmacher et al. 2007) was used for PBMC stimulation at a concentration of 2µg/peptide/ml. Also p24 Gag TL9 peptide (Elephants & Peptides, Germany), which is presented by HLA-1 alelles B42 and B81 and frequently recognized by HIV infected individuals from Mbeya region (Geldmacher et al. 2007; Geldmacher et al. 2009) was used to screen HIV positive volunteers for IFN-γ secreting HIV-specific CD8 T cells targeting HIV TL9 Gag epitope at the concentration of 2µg/ml (Table 2-1 and Table 2- 2).

For detection of MTB-specific T cell responses in relation to HIV and helminths, Early Secreted Antigenic Target 6 (ESAT6) and Culture Filtrate Protein 10 (CFP10) (Lion Bioscience) and Purified Protein Derivative (PPD) tuberculin (Staten Serum Institute, Denmark) antigens were used. Also, CMV, Influenza, HSV-1, EBV

(all from New England Peptides) and *T.gondii* (Virion) were used for *in vitro* cell stimulation at different concentrations described in Table 2- 1.

Table 2- 1. Antigens used in the WHIS study

Pathogen	Antigen	Nature of Antigen	Concentration used
HIV	FRP-HIV*	Pool of 15mer peptides	2µg/peptide/ml
	TPQDLNTML (TL9)	Single optimal epitope	2µg/ml
MTB	ESAT6 & CFP10	Pool of recombinant proteins	20µg/ml
Mycobacteria	Tuberculin (PPD)	Purified Protein Derivative	10µg/ml
CMV	CMV	Whole inactivated	20µg/ml
	TPRTGGGAM (TM10)	Single optimal epitope	2µg/ml
Influenza-A	Influenza-A virus	Whole inactivated	20µg/ml
HSV-1	HSV-1	Whole inactivated	20µg/ml
EBV	EBV	Whole inactivated	20µg/ml
T.gondii	*T.gondii*	Whole inactivated	20µg/ml

*Detailed information of HIV-Frequently Recognised Peptides (HIV-FRP) described in Table 2- 2.

Table 2- 2. Description of HIV-Frequently Recognized Peptides (HIV-FRP) used in the WHIS study

Peptide #	Protein	Subtype	Sequence	Isolate
7	Gag	C	GKKHYMLKHIVWASR	Du422
20	Gag	C	SLYNTVATLYCVHEK	Du422
35	Gag	C	GQMVHQAISPRTLNA	Du422
36	Gag	D	QMVHQSLSPRTLNAW	98UG57143
45	Gag	C	TPQDLNTMLNTVGGH	Du422
45	Gag	A	TPQDLNMMLNIVGGH	90CF402
50	Gag	C	MLKDTINEEAAEWDR	Du422
53	Gag	C	WDRVHPVHAGPIAPG	Du422
73	Gag	C	PFRDYVDRFFKTLRA	Du422
76	Gag	C	LRAEQATQEVKNWMT	Du422
83	Gag	C	TILRALGPGATLEEM	Du422
89	Gag	S	VGGPSHKARVLAEAM	
18	Nef	C	PVRPQVPLRPMTYK	Du151
21	Nef	C	YKAAFDLSFFLKEK	Du151
29	Nef	C	WVYHTQGYFPDWQN	Du151
33	Nef	C	PGPGVRYPLTFGWC	Du151

2.2.2. IFN-γ ELISpot assays

Freshly isolated PBMCs from 171 out of 381 adult volunteers who were enrolled into the WHIS cohort were screened for different pathogen-specific T cell responses by *in vitro* overnight stimulation of 200,000 PBMCs/well with different antigens described above and in Table 2- 1 and Table 2- 2. Assays were performed as previously described elsewhere (Mashishi & Gray 2002) with few modifications. Briefly; Polyvinylidene difluoride plates (Millipore) were pre-wetted 4 times with 200µl of sterile PBS and coated with 50µl anti human-IFN-γ monoclonal antibody 1-D1k (Mabtech, Sweden) at a concentration of 5µg/ml in PBS overnight at 4°C. The plates were then manually washed 4 times with 200µl sterile PBS followed by blocking for a minimum of 30 minutes with R10 medium (Gibco, Invitrogen).

Freshly isolated PBMCs were re-suspended at 4 x 10^6 cells/ mL with R10 and 200,000 cells (=50µl) were then added per well. Thereafter, pre-aliquoted peptides and proteins were added to the wells. Peptides were added at a concentration of 2µg/ml while proteins (with the exception of PPD) were added at a final concentration of 20µg/ml in 50µl of R10. PPD was used at a final concentration of 10µg/ml in 50µl of R10. Then plates were incubated at 37°C in 4.5% CO2 for 20 hours. After 20 hours, plates were washed 5 times with 200 µl of PBS using an automated plate washer (Bio Tek), followed by an incubation with 100µl biotinylated anti-IFN-γ monoclonal 7-b6-1 antibody (Mabtech, Sweden) at a concentration of 1µg/ml in 0.5% FBS in PBS for 2 hours at room temperature in the dark. After that, 5 automated washes with 200 µl of PBS followed by one hour incubation with 100µl streptavidin - alkaline phosphatise conjugate (Mabtech, Sweden) at a concentration of 1µg/ml in 0.5% FBS in PBS were performed. Plates were automatically washed 5 times with PBS; finally plates were developed by adding 100µl BCIP/NBT substrate solution (Thermo Scientific). After 10 minutes, the reaction was stopped by rinsing the plates three times with de-ionised water. On the following day, the blue colored spots formed by IFN-γ -secreting cells were counted with an automated CTL ImmunoSpot plate reader (Cellular Technology Limited).

PHA (Sigma) at a concentration of 40µg/ml in R10 served as a positive control while wells with only PBMCs or R10 served as negative controls. Responses that

were three times the negative control and > 25 SFC/ million PBMC were considered as positive. Assay results were considered invalid/failed and hence excluded if the negative control wells had > 50 SFC/ million PBMC or if the positive control wells did not have > 1000 SFC/ million PBMC.

2.3. Characterization of CD25+FoxP3+CD4 T cells in fresh whole blood

Fresh anti-coagulated whole blood samples were incubated for 30 minutes using the following fluorochrome labelled monoclonal antibodies for cell surface staining (mABs); CD3-Pac Blue (BD), CD4 Per-CP Cy5.5 (eBioscience), CD25 PeCy7 (eBioscience), and CCR5 APC-Cy7 (BD). Red blood cells in samples were then lysed by incubating and washing samples twice for 10minutes with 1X cell lysis solution (BD). Cells were thereafter stained intracellularly with FoxP3 Alexa Fluor 647 (eBioscience) according to manufacturer's instructions. Briefly, intracellular staining with FoxP3 included: further permeabilization and fixation of cells with Fix/Perm solution for 40 minutes at 4°C, washing with PermWash buffer and intracellular staining with FoxP3 Alexa Fluor 647 (all from eBioscience) for 30 minutes at 4°C. Stained cells were then washed using PermWash buffer and finally fixed with 2% paraformaldehyde prior to acquisition. Acquisition was performed on FACS CANTO II (BD). Compensation was conducted with antibody capture beads (BD) stained separately with the individual antibodies used in the test samples. Flow cytometry data was analysed using BD FACSDiva software (version 6.1.3). The absolute Treg numbers in the peripheral blood was calculated from the total CD4 T cell counts and the percentage Tregs.

2.4. Quantification of cell associated HIV gag viral DNA from sorted CD45RO+CD25+FoxP3+ and CD45RO+CD25-FoxP3- CD4 T cells

2.4.1. Cell sorting

Cryopreserved PBMCs were thawed and washed twice in pre-warmed (37°C) complete media (RPMI plus 10% heat inactivated Fetal Bovine Serum (GIBCO) that was supplemented with Benzonase (5U/ml, Novagen). Surface staining was

performed with CD3-Pacific Blue, CD4 Per-CP Cy5.5, CD25 PeCy7 and CD45RO PE (BD) for 30 minutes in the dark at RT; intracellular staining was performed with FoxP3 Alexa Fluor 647 (eBioscience) and HELIOS FITC (BioLegend) as described above. Cell sorts were performed on a FACSAria cell sorter (BD) after gating on CD3+CD4+CD45RO+ cells into Treg populations (CD25+FoxP3+HELIOS+ and CD25+FoxP3+HELIOS-) and memory populations (CD25-FoxP3-HELIOS+ and CD25-FoxP3-HELIOS-) as shown in Figure 3- 15A. Between 293 and 750,000 fixed CD4 T cells from each of the four different populations were collected, depending on the number of PBMCs available from each individual. Cells were collected in FACS buffer consisting of PBS mixed with 0.5% Bovine Serum Albumin (BSA, Sigma), 2mM EDTA and 0.2% Sodium Azide at pH 7.45. Median of fixed cell count number collected for each population were as follows: CD25+FoxP3+HELIOS+ (Median: 9017 and IQR: 3931-14412); CD25+FoxP3+HELIOS- (Median: 4381 and IQR: 1579-9799); CD25-FoxP3-HELIOS+ (Median: 2646 and IQR: 1336-5644) and CD25-FoxP3-HELIOS- (Median: 185000 and IQR: 79000-315000). Sorted Cells were then centrifuged at 13000rpm for 3 minutes and the supernatant removed. Cell pellet was stored at −80°C until further analysis.

2.4.2. Quantification of cell associated HIV gag viral DNA

Quantification of cell associated HIV gag viral DNA was performed as previously described (Douek et al. 2002) with minor modifications. Sorted CD4 T cell subsets from 22 HIV+ subjects were lysed by adding 30 µl (0.1 mg/ml) of proteinase K (Roche) containing 10mM, pH8 Tris-Cl (Sigma) for 1 h at 56°C followed by Proteinase K inactivation step for 10 min at 95°C. Cell lysates were then used to quantify cell associated HIV DNA quantified by qPCR as previously described with some modifications (Geldmacher et al. 2010). Briefly, Gags primers and probe used were as follows: 783gag, forward, 5′-GAGAGAGATGGGTGCGAGAGCGTC-3′ (Tm>60), 895gag, reverse, 5′-CTKTCCAGCTCCCTGCTTGCCCA-3′ (Tm>60); FAM-labeled probe 844gagPr, 5′-ATTHGBTTAAGGCCAGGGGGA-ARGAAAMAAT-3′ and had been designed to optimally cover subtypes A and C prevalent in Mbeya Region (Geldmacher et al. 2010). To quantify the cell number in each reaction mix, copy number of the human prion gene (which is a single copy

gene) was also assessed by qPCR. Prion primers and probe sequences were as follows: Prion forward: 5´TGC TGG GAA GTG CCA TGA G; Prion reverse: 5´CGG TGC ATG TTT TCA CGA TAG; probe 5´FAM-CAT CAT ACA TTT CGG CAG TGA CTA TGA GGA CC **TAMRA** (Hoffmann et al. 2010). 5 µl of lysate was used in a total reaction volume of 25 µl containing 0.8 µM Gag primers or 0.4 µM Prion primers, 0.4 µM probe (all from ThermoFisher), a 0.2 mM concentration of each deoxynucleoside triphosphate (Applied Biosystems), 3.5 mM $MgCl_2$ and 0.65 U platinum *Taq* in the supplied buffer (Invitrogen). Standard curves were generated using HIV-1 gag gene (provided by Brenna Hill, Vaccine Research Center, NIH, Bethesda) and prion gene (provided by Dieter Hoffmann, Institute of Virology, Technische Universität München) encoding plasmids. Real time PCR was performed in a Bio-Rad cycler CFX96 (Bio-Rad): 5-min at 95°C, followed by 45 cycles (15 s at 95°C and 1 min at 60°C). Importantly, to assure comparability of the results, cell-associated gag DNA from the 4 different memory CD4 T cell subsets, which were sorted from the same patient specimen, were quantified simultaneously.

Cell associated Gag DNA in memory Tregs and CD25-FoxP3- memory CD4 T cells independent of Helios Expression was calculated as follows: ∑Gag DNA load (Helios+)+(Helios-) divided by ∑sorted cells (Helios+)+(Helios-).

2.5. Characterization of maturation and activation markers on CD4 and CD8 T cells in fresh whole blood

Fresh anti-coagulated whole blood samples were incubated for 10 minutes with CCR5 PECy7 followed by 30 minutes incubation using the following fluorochrome labelled monoclonal antibodies for cell surface staining (mABs); CD3-Pacific Blue (BD), CD4 Per-CP Cy5.5 (eBioscience), CD8 V500 or CD8 Amcyan, CD27 APC-H7, CD45RO APC, HLA-DR FITC and CD38 PE (all from BD). Stained cells were finally fixed with 2% paraformaldehyde prior to acquisition. Acquisition was performed on FACS CANTO II (BD). Compensation was conducted with antibody capture beads (BD) stained separately with the individual antibodies used in the test samples. Flow cytometry data was analysed using FlowJo (version 9.5.3; Tree Star Inc). Depending on the expression of CD27 and CD45RO markers on CD4 and CD8

T cells, T cell subsets were defined as follows: naïve (CD27+CD45RO-), "central-like" memory (CD27+CD45RO+), "effector-like" memory (CD27-CD45RO+) and "terminally differentiated" (CD27-CD45RO-) CD4 and CD8 T cells. In addition, total memory CD4 T cells were defined as the sum of central memory, effector memory and terminally differentiating CD4 T cells.

2.6. Statistical analysis.

Data analyses were performed using Prism version 4.0 software (GraphPad, Inc.). Comparisons of two groups were performed using the Mann-Whitney test. Comparisons of paired groups were performed using the Wilcoxon matched pairs test. Comparisons of study groups with respect to their responsiveness towards different antigens were performed using Fisher's exact test. For association analyses, Spearman rank correlation test or linear regression analysis was used. Differences were considered significant at P values of <0.05. Tests used for statistical analysis are described in the figure legends.

3. Results

3.1. Characterization of pathogen-specific T cell responses during infection with HIV-1, Helminth or HIV-Helminth co-infection

Helminth infections are controlled by the induction of modified CD4 T helper 2 (TH2) responses associated with regulatory mechanisms (reviewed in (Maizels & Yazdanbakhsh 2003)). However, in shifting the response towards TH2 (Cooper et al. 2000; Turner et al. 2011), helminths may modify TH1 cell responses to chronic infections such as HIV and TB and increase susceptibility and/or progression to such infections (Borkow et al. 2001; Resende Co et al. 2007). Modulation of specific T cell responses to different pathogens by helminth infections has not been fully explored. This study therefore examined the immune modulation of IFN-γ secreting specific T cell responses to HIV, MTB, Influenza, *T.gondii* and herpes viruses in relation to HIV and helminths.

3.1.1. Baseline characteristics of WHIS study participants

To determine the immune modulation of different pathogen-specific T cells, IFN-γ secretion in PBMCs of 171 out of 381 adult volunteers from the WHIS cohort was examined after *in vitro* overnight stimulation with different antigens described above in Materials and Methods. Baseline characteristics of study individuals analysed for different pathogen specific T cell responses are shown in Table 3- 1.

Table 3- 1. Baseline characteristics of study individuals analysed for pathogen-specific T cell responses (N=171)

	HIV negative							**HIV positive**						
	No Worms	All Worms	*T. trichiura*	*S.haema tobium*	*S. mansoni*	*A. lumbric oides*	Hookworm	No Worms	All Worms	*T. trichiura*	*S.haema tobium*	*S. mansoni*	*A. lumbrico ides*	Hookworm
Male:Female	7:8	47:62	11:16	2:1	12:16	6:12	16:16	5:10	12:20	2:4	-	0:4	4:2	6:6
Age (years)*	31(20.4 -42.3	31.6 (22.4-41.1)	31.6 (22.5-43.6)	28.1 (18-34.9)	27.1 (21.4-33.65)	32.1 (22.3-37.85)	37.95 (27.2-47.95)	37.3 (29.3-45.6)	35.7 (29.6-42.35)	37.6 (35.05-53.3)	-	37.4 (27.95-44.3)	31.95 (26.65-46.1)	33.1 (27.6-40.9)
Worm Load (egg count)*		108 (34.5-303)	72 (36-240)	1 (1-2)	72 (27-174)	1127 (213-2806)	120 (37.5-285)		75 (28.5-238.5)	42 (15-84)	-	22.5 (18-126)	258 (114-1170)	78 (42-243)
Single:multiple worm infection		69:40	12:15	1:2	26:2	12:6	17:15		19:13	1:5	-	3:1	5:1	6:6
CD4 count (cells/ul)*	832.9 (637-1082)	922.3 (748.2-1141)	977.1 (752.7-1201)	1295 (1146-1389)	940.5 (759.4-1159)	945.9 (836.2-1108)	829.7 (636.9-939.3)	400.2 (290.6-689.2)	507.4 (274.4-723.1)	383.4 (126.7-1033)	-	536.1 (255.4-805.3)	528.8 (286.1-604.9)	601.9 (431.6-955.7)
plasma VL (copies/ml)*								92150 (11530-265500)	86550 (2815-317500)	14890 (6615-48250)	-	59950 (9390-215000)	166500 (22345-405500)	8882 (548.5-112950)

***Values are given in median and (Inter quartile Range-IQR)**

3.1.2. Influence of HIV and Helminth infection on HIV-specific T cells

3.1.2.1. Cross-sectional analysis

To determine whether different helminth infections alter HIV specific T cell responses, we analysed the quantity of IFN-γ secreting T-cells specific for HIV in HIV positive individuals with or without helminth co-infection. The frequency of responders to HIV antigens and their median of detectable HIV-specific T cells in HIV positive subjects with or without helminth co-infection is shown in Table 3- 2.

Table 3- 2. Frequency of HIV positive subjects responding to HIV antigens

Antigen	Frequency of responders (%), Median, IQR (SFC/million PBMC)		
	All HIV+ study subjects (N=48)	HIV+W- (N=15)	HIV+W+ (N=33)
HIV-FRP	95.8%, Median: 1785, IQR: 643.8-4121	100%, Median: 1148, IQR: 455-4103	93.9%, Median: 2550, IQR: 705-4128
HIV-TL9	27.1%, Median: 290, IQR: 117.5-3175	20%, Median: 105, R: 77.5-222.5	30.3%, Median: 1311, IQR: 160-3871

Almost all (96%, N=48) HIV+ study volunteers responded to a pool of HIV-FRP antigen as measured by IFN-γ release ELISpot assay (Table 3- 2). Both HIV positive groups with or without helminth co-infection had similar frequencies of responders. Next, the magnitude of HIV-specific T cell responses against HIV-FRP was determined. No significant differences were observed in the quantity of HIV-FRP specific T cell responses between HIV positives with helminth co-infection (Median, 1943 SFC/10^6 PBMCs; IQR, 543.8-4121 SFC/10^6 PBMCs and those without co-infection (Median, 1148 SFC/10^6 PBMCs; IQR, 455-4103 SFC/10^6 PBMCs. P=0.5782. data not shown). Even when co-infected subjects were stratified by the helminth species, there was no apparent association of any helminth specie with the quantity of HIV-FRP T cell responses.

27% of HIV positive subjects responded to HIV-TL9 epitope (Table 3- 2). Helminth infections had no association with frequencies of responses to TL9. There was no significant increase in the magnitude of detectable T cell responses to TL9 between HIV-helminth co-infected TL9 responders (Median, 1311 SFC/10^6 PBMCs;

IQR, 160-3871 SFC/10^6 PBMCs) and responders with HIV infection alone (Median, 105 SFC/10^6 PBMCs; Range, 77.5-222.5 SFC/10^6 PBMCs. P=0.1119. data not shown).

A linear relationship was observed especially between HIV-TL9-specific T cells and total number CD4 T cells (Figure 3- 1A-B). However, no correlation was observed between either HIV-TL9- or HIV-FRP-specific T cells and plasma viral loads (Figure 3- 1C-D).

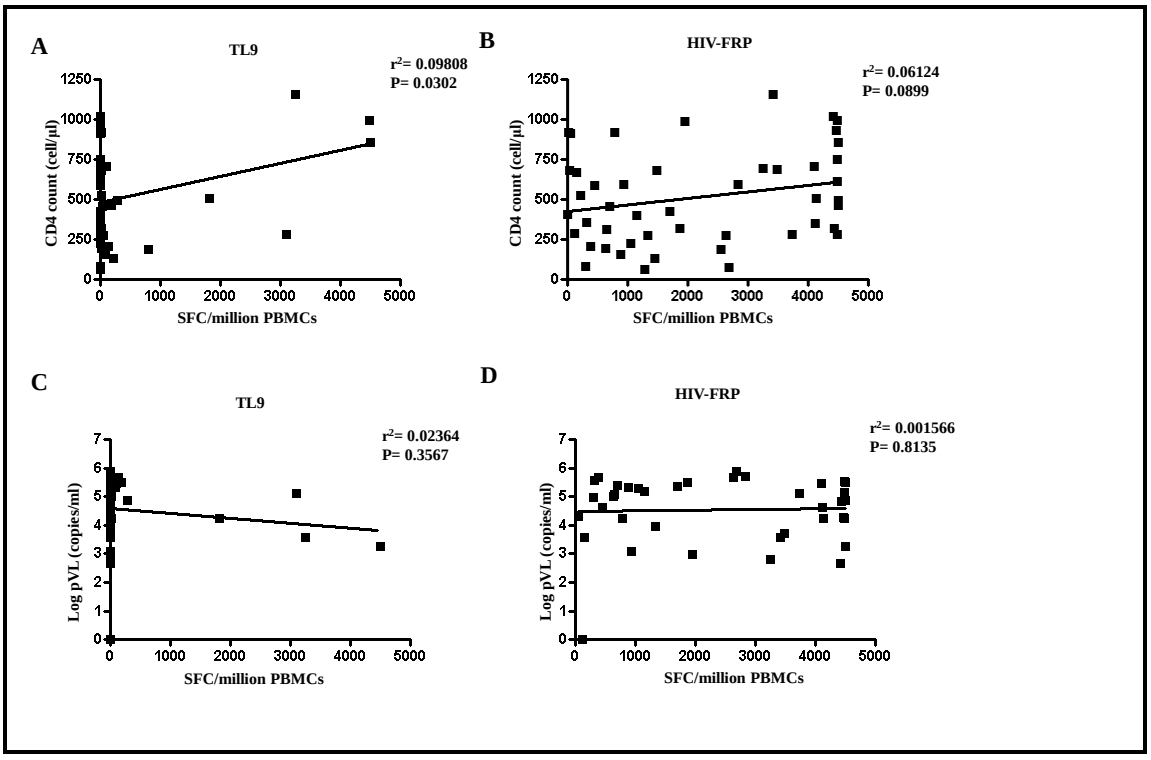


Figure 3- 1: Associations between HIV-specific T cell responses and HIV disease progression markers in HIV-1 infection. Shown is the linear regression analysis of CD4 T cell counts with either: (A) quantity of HIV TL9-specific T cells or (B) quantity of HIV FRP-specific T cells. Linear regression analysis between log of plasma viral loads and the quantity of either HIV TL9-specific T cells or HIV FRP-specific T cells is shown in (C) and (D) respectively.

3.1.2.2. Effect of worm treatment with praziquantel and albendazole on HIV-specific T cells

To investigate whether worm treatment has any effect on the quantity of IFN-γ secreting HIV-specific T cell responses, PBMCs from HIV positive study volunteers stimulated *in vitro* with a pool of HIV-FRP or TL9 at baseline (W0), 3 months (W1)

and a year (W3) after treatment were compared. Only volunteers who had no detectable worms post infection were analysed. There were no significant changes in the quantity of HIV-FRP specific T cells of HIV co-infected individuals 1-3months after helminth treatment. However, there was a significant decrease of these cells a year after deworming when compared to the baseline (baseline median, 2248 SFU/10^6 PBMCs; one year after treatment median, 1674 SFU/10^6 PBMCs. P=0.0406. Figure 3- 2A).

There was no apparent influence of worm treatment on the quantities of TL9-specific T cell responses observed in co-infected subjects1-3 months after treatment. However, a slight but non-significant decrease of HIV-TL9 specific T cells was observed in the study group a year after treatment (baseline median: 2469SFU/10^6 PBMCs, IQR: 210-4494 SFU/10^6 PBMCs; one year after treatment median: 1041 SFU/10^6 PBMCs, IQR: 151.3- 3270SFU/10^6 PBMCs. P=0.0625. Figure 3- 2B). Taken together, these results suggest that helminths have no dramatic influence on IFN-γ release HIV-specific CD8 T cell responses.

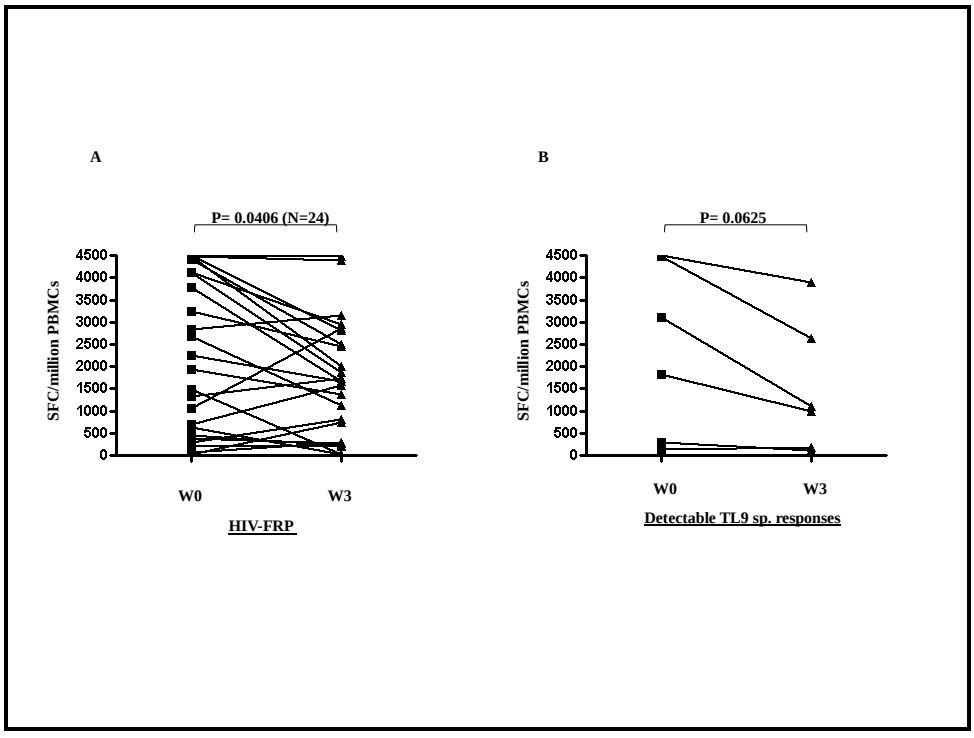


Figure 3- 2: Effect of worm treatment on the quantity of HIV-specific T cells in the peripheral blood of HIV-Helminth co-infected volunteers. Comparison of SFC/10^6 PBMCs responding to a pool of HIV-FRP and HIV-TL9 peptides before (W0) and one year (W3) after helminth treatment is shown in (A) and (B) respectively. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

3.1.3. Influence of HIV and helminth infections on other pathogen-specific T cells and effect worm treatment.

To determine whether helminth infections reduce the level of pathogen-specific T cell responses, defined by the capacity to secrete IFN-γ upon overnight *in vitro* restimulation, freshly isolated PBMC from WHIS study participants were restimulated with different pathogen-specific antigens at baseline (W0), approximately 3 months (W1) and a year (W3) after helminth treatment. Immune modulation of pathogen-specific T cell responses by HIV and helminths that were examined included specific immune responses against: MTB, CMV, INF-a, HSV, EBV and *T.gondii*.

3.1.3.1. Mycobacterium tuberculosis (MTB)-specific T cell responses

For detection of MTB- specific T cell responses, cells from WHIS volunteers with no signs of active TB were stimulated with tuberculin (PPD) and a pool of ESAT6 and CFP10 antigens. ESAT6 and CFP10 antigens which are more specific in detection of MTB-specific responses (Sester et al. 2006; Ulrichs et al. 1998; Lalvani et al. 2001; Chapman et al. 2002), are recognized by >50% HIV negative while PPD-a non specific Mycobacterium antigen, is recognized by 70% of HIV negative and positive individuals from Mbeya-Tanzania (Geldmacher et al. 2008).

PPD responses, which are typically CD4 T cell responses (Schuetz et al. 2012) were detected in 72% (N=171) of all HIV positive and HIV negative study volunteers (Table 3- 3). In line with previous findings (Geldmacher et al. 2008), HIV infection was associated with the decrease of responders to PPD by 46% ($P<0.0001$, Fisher's exact test, Table 3- 3). Both HIV positive groups with or without worm co-infection were less responsive to PPD in comparison to HIV negative, Worm negative control group ($P<0.0001$ for HIV+W- and $P=0.0034$ for HIV+W+, Fisher's exact test) with no significant influence of worm infection on these differences ($P=0.11$, Fisher's exact test, Table 3- 3). Of note, all of HIV positive, *S.mansoni* co-infected individuals responded to PPD (N=4; Median=188.8, IQR=46.25-378.8) and the difference in frequency of responders between this group and the HIV control group was significant ($P=0.009$, Fisher's exact test). However, the number of HIV+*S.mansoni*+ PPD responders was too few for a conclusive comparison.

Table 3- 3. Frequency of study subjects responding to *Mycobacterium tuberculosis* (MTB) antigens

Antigen	Frequency of responders (%), Median, IQR (SFC/million PBMC)						
	All study subjects (N=171)	HIV- (all) (N=123)	HIV+ (all) (N=48)	HIV-W- (N=15)	HIV-W+ (N=108)	HIV+W- (N=15)	HIV+W+ (N=33)
PPD	72.5%, Median: 275, IQR: 132.5- 630	85.3%, Median: 287.5, IQR: 145- 697.5	39.6%, Median: 158.8, IQR: 77.5- 337.5	93.3%, Median: 275, IQR: 100-760	84.3%, Median: 306.3, IQR: 145-695	20%, Median: 235, IQR: 76.2- 2428	48.5%, Median: 158.8, IQR: 77.5- 302.5
ESAT6/CFP10	37.4%, Median: 160, IQR: 55- 497.5	39%, Median: 160, IQR: 55- 557.5	33.3%, Median: 163.8, IQR: 60- 413.8	60%, Median: 130, IQR: 57.5- 556.3	36.1%, Median: 173.8, IQR: 48.7- 626.3	33.3%, Median: 172.5, IQR: 56.2- 2373	33.3%, Median: 167.5, IQR: 77.5-500

When the median magnitude of responses between all HIV positive volunteers (Median, 22.5 SFC/10^6 PBMCs; IQR, 2.5- 118.8 SFC/10^6 PBMCs) was compared with the HIV negative subjects (Median, 260 SFC/10^6 PBMCs; IQR, 95-630 SFC/10^6 PBMCs), chronic HIV infection was associated with a median loss of PPD-specific CD4 T cells ($P<0.0001$, Figure 3- 3A). A similar loss was observed when comparing the median magnitude of responses of HIV negative, worm negative control subjects with either HIV infected only (Median, 10 SFC/10^6 PBMCs; IQR, 2.5 - 57.5 SFC/10^6 PBMCs; $P= 0.0004$) or HIV positive, worm co-infected subjects (Median, 25 SFC/10^6 PBMCs; IQR, 6.25 -158.8 SFC/10^6 PBMCs; $P= 0.0001$) with no apparent influence of worm infection (Figure 3- 3B).

No median differences in the quantities of PPD-specific CD4 T cell responses could be found between HIV-helminth+ subjects (Median, 258.8 SFC/10^6 PBMCs; IQR, 95.0- 583.8 SFC/10^6 PBMCs) and the control group (Median, 275.0 SFU/10^6 PBMCs; IQR, 100.0-760.0 SFU/10^6 PBMCs. $P=0.5965$; Figure 3- 3B) even when the test group was stratified by the helminth species infected with. No linear correlation could be observed between PPD-specific CD4 T cells and total CD4 T cell count (Figure 3- 3C) or plasma viral loads (pVL).

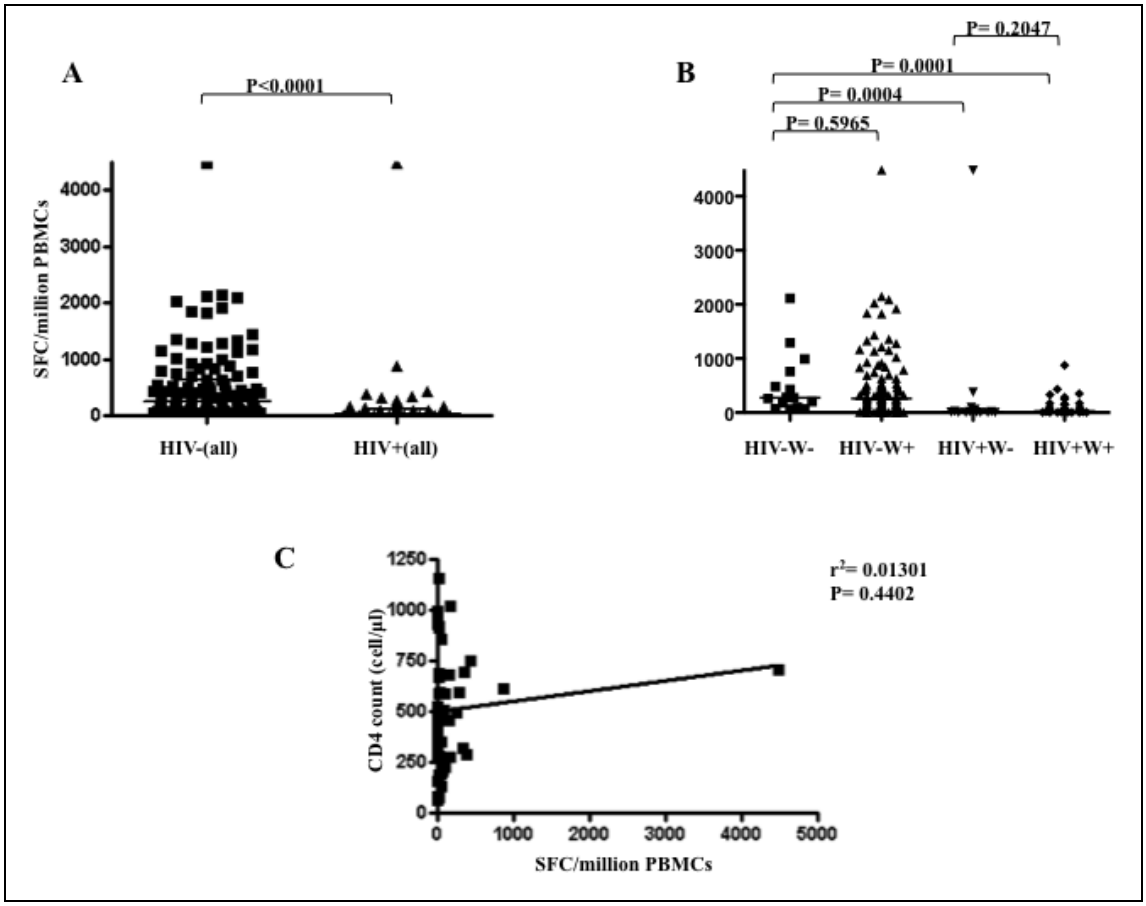


Figure 3- 3: Chronic HIV-1 infection and not helminth infection is associated with reduction of MTB-specific TH1 cell responses in peripheral blood. Shown are the medians of SFC/10^6 PBMCs responding to PPD in study volunteers stratified by HIV (A-B) and helminth infection status (B). A linear regression analysis of SFC/10^6 PBMCs responding to PPD and CD4 T cell counts is shown in (C). Statistical analysis was performed using Mann-Whitney test when comparing groups.

No influence of helminth treatment was observed on quantities of IFN-γ secreting, PPD-specific T cells of helminth infected and co-infected individuals ~3 months and a year post helminth treatment. A moderate increase of PPD-specific T cells was observed a year after helminth treatment of individuals infected with Hookworm from a median of 287.5 to 337.5 SFU/10^6 PBMCs (P= 0.0457. Figure 3-4A). However, the statistical power of this observation was lost when analysed only detectable quantities of PPD-specific T cells (P=0.0894. Figure 3- 4B).

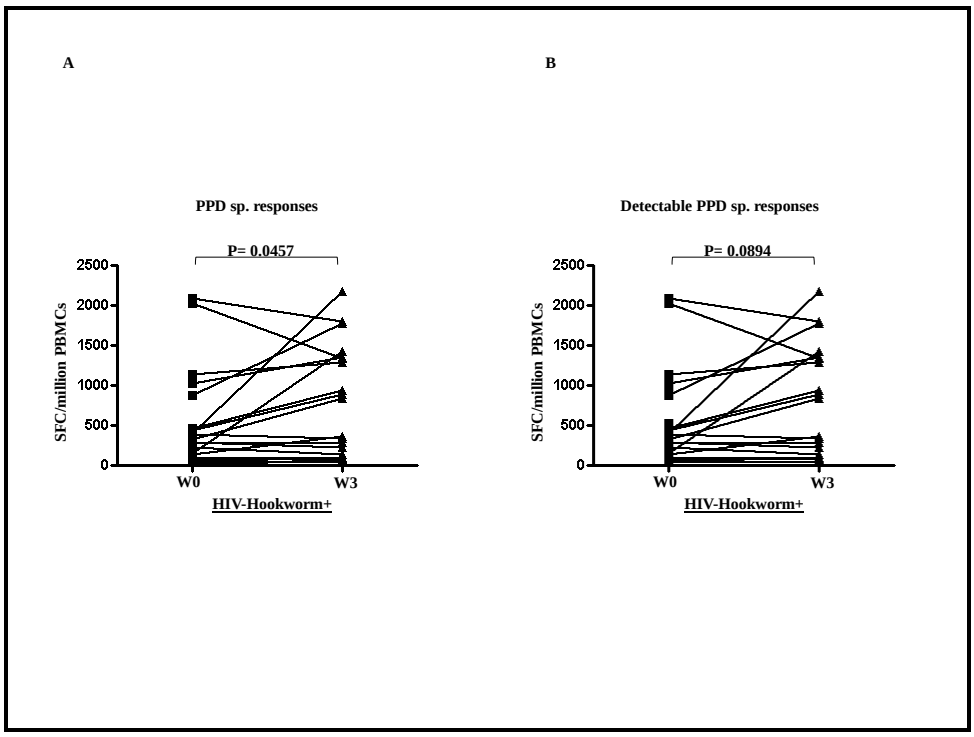


Figure 3- 4: Effect of worm treatment on the quantity of MTB-specific T cells in the peripheral blood of HIV negative, Hookworm infected volunteers. Comparison of SFC/10^6 PBMCs responding to PPD before (W0) and one year (W3) after helminth treatment for each HIV-Hookworm+ subject is shown in (A). Comparison of SFC/10^6 PBMCs of only PPD responders at W0 and W3 is shown in (B). Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

Overall, only 37% of the all the study subjects responded to the pool of ESAT6/CFP10 antigens (Table 3- 3). Similar percentages of responders were observed between HIV+ and HIV- subjects with no differences in the median quantities of detectable ESAT6/CFP10-specific T cells. Similarly, no differences in the magnitude of detectable responses to ESAT6/CFP10 were found between HIV-Worm+ and the control subjects even when stratified by their different helminth infection status. However, 1.7 fold more HIV negative controls responded to ESAT6/CFP-10 than worm infected subjects (Table 3- 3) to a non-significant level (P=0.0935, Fisher's exact test). Helminth treatment was not associated with an increase in the quantities of IFN-γ producing ESAT6/CFP10-specific T cells of helminth infected and co-infected individuals 1-3 months and a year after treatment.

3.1.3.2. Human Herpes virus-specific T-cell responses: CMV, HSV-1 and EBV

IFN-γ release by CMV-specific T cells from WHIS study volunteers were detected by stimulating PBMCs *in vitro* with whole inactivated CMV and a CMV_CM10 single optimal epitope (Currier et al. 2002) presented by HLA-I allele B7 to CD8 T cells. Whole inactivated CMV is recognized by almost all HIV negative and HIV positive individuals in Mbeya-Tanzania (Schuetz et al. 2012) while CMV_CM10 peptide is recognized by about 35% (Geldmacher, unpublished data). Similarly, almost all (98%) WHIS study volunteers responded to whole inactivated CMV while only 21% responded to CMV_CM10, with median detectable T cell response of 340 SFC/10^6 PBMC (Table 3- 4).

Table 3- 4. Frequency of study subjects responding to CMV, HSV-1 and EBV antigens

Antigen	Frequency of responders (%), Median, IQR (SFC/million PBMC)						
	All study subjects (N=171)	HIV-(all) (N=123)	HIV+ (all) (N=48)	HIV-W- (N=15)	HIV-W+ (N=108)	HIV+W- (N=15)	HIV+W+ (N=33)
CMV (whole inactivated)	98.2%, Median: 695, IQR: 257.5- 1835	99.2%, Median: 808.8, IQR: 313.8-2020	95.8%, Median: 498.8, IQR: 128.8-1979	100%, Median: 1018, IQR: 267.5-1558	99.1%, Median: 767.5, IQR: 317.5-2063	100%, Median: 565, IQR: 207.5-3975	93.9%, Median: 437.5, IQR: 122.5-1333
CMV_CM10	21%, Median: 340, IQR: 60- 1405	18.7%, Median: 352.5, IQR: 47.5-1543	27.1%, Median: 325, IQR: 71.2-1761	26.7%, Median: 1868, IQR: 873.8-2330	17.6%, Median: 327.5, IQR: 47.5-735	33.3%, Median: 460, IQR: 123.8-2759	24.2%, Median: 228.8, IQR: 71.2-872.5
HSV-1 (whole inactivated)	69%, Median: 88.7, IQR: 47.5- 172.5	78%, Median: 100, IQR: 47.5-185	45.8%, Median: 70, IQR: 45- 97.5	93.3%, Median: 85, IQR: 45- 125	75.9%, Median: 101.3, IQR: 48.7-187.5	46.7%, Median: 68.7, IQR: 51.2-146.3	45.5%, Median: 75, IQR: 42.5- 85
EBV (whole inactivated)	93.6%, Median: 2460, IQR: 968.8- 3981	97.6%, Median: 2421, IQR: 1154-4038	83.3%, Median: 2505, IQR: 605-3944	100%, Median: 2248, IQR: 1950-3578	97.2%, Median: 2460, IQR: 1093-4216	66.7%, Median: 3590, IQR: 605-4460	90.9%, Median: 2493, IQR: 565-3868

HIV infection was associated with a moderate decrease of the overall magnitude of whole inactivated CMV-specific T cell responses in HIV positive volunteers (Median, 423.8 SFC/10^6 PBMCs; IQR, 115- 1431 SFC/10^6 PBMCs) when compared with the HIV negative subjects (Median, 767.5 SFC/10^6 PBMCs; IQR, 310- 1978 SFC/10^6 PBMCs; P= 0.0419. Figure 3- 5A). There was no association of helminth infections on the quantities of IFN-γ secretion by CMV-specific T cells in helminth infected subjects with or without HIV co-infection. Within HIV+ infected volunteers,

a significant linear regression between CMV-specific T cells and total CD4 T cell count was observed (Figure 3- 5B). A significant negative association was observed between CMV-specific T-cells and pVL (Spearman r= -0.4032, P= 0.0121, data not shown).

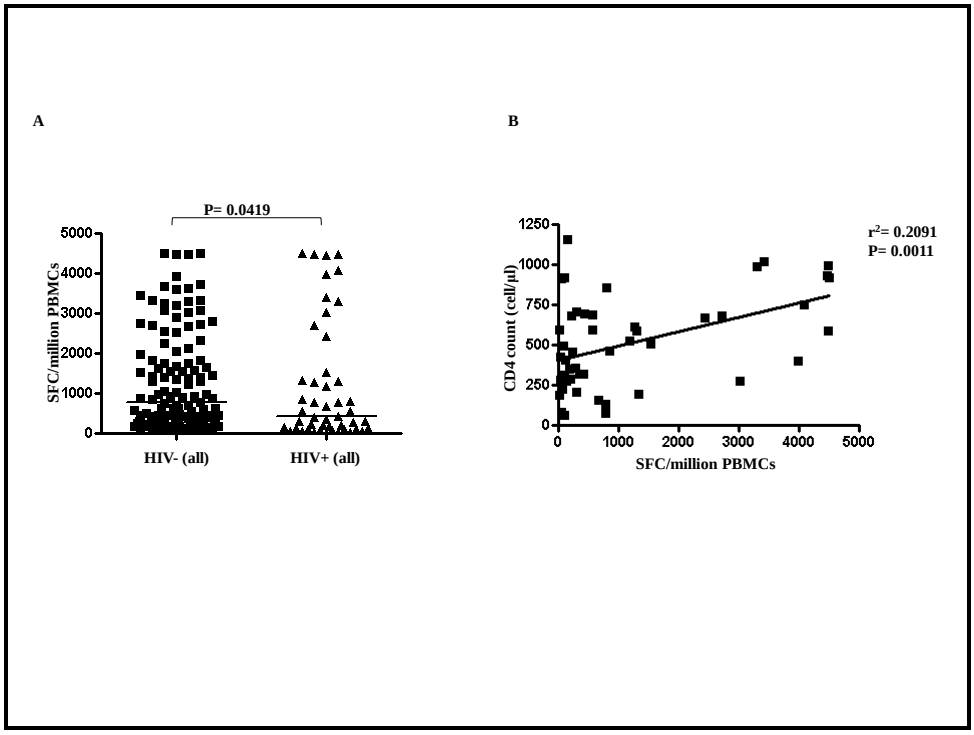


Figure 3- 5: Chronic HIV-1 infection is associated with a moderate decrease of CMV-specific T cell responses in peripheral blood. Shown are SFC/10^6 PBMCs responding to CMV antigen in study volunteers stratified by HIV infection status. A linear regression analysis of SFC/10^6 PBMCs responding to CMV and CD4 T cell counts is shown in (B). Statistical analysis was performed using Mann-Whitney test when comparing groups.

No changes in the quantities of IFN-γ producing, CMV-specific T cells could be observed in HIV negative worm infected subjects 3 months after worm treatment. However, a general significant decrease in CMV specific T cells from a median of 632.5 to 585.0 SFC/10^6 PBMCs was observed in this group a year post treatment (P= 0.0160, Figure 3- 6A). This decline was present even when analysis involved only detectable responses (P= 0.0361, Figure 3- 6B). Similarly, no significant changes in the quantities of CMV-specific T cells was observed 3 months post helminth treatment in HIV and worm co-infected subjects; but a significant decrease from a

median of 645.0 to 560.0 SFC/10^6 PBMCs was observed a year after helminth treatment (P= 0.019, Figure 3- 6C). This effect was true even when taking only detectable responses into account (P=0.0313, Figure 3- 6D).

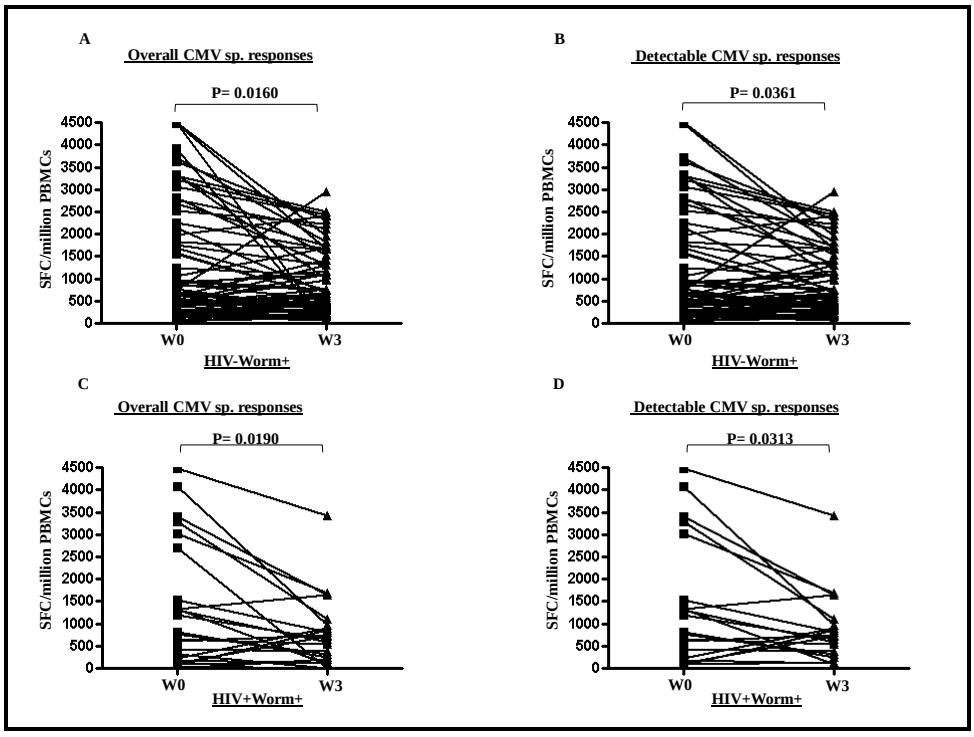


Figure 3- 6: Effect of worm treatment on the quantity of CMV-specific T cells in the peripheral blood of Helminth infected volunteers. Shown is the comparison of SFC/10^6 PBMCs responding to CMV before (W0) and one year (W3) after helminth treatment for each (A) HIV-Worm+ and (C) HIV+Worm+ subject. (B) and (D) compares SFC/10^6 PBMCs of only CMV responders at W0 and W3 for HIV-Worm+ and HIV+Worm+ subjects respectively. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

HIV negative controls responded 1.5 fold more to CMV_CM10 peptide than worm infected subjects (Table 3- 4) to a non-significant level (P= 0.4781, Fisher's exact test). Generally, neither HIV nor helminth infections were associated with the differences in the frequency and magnitude of responses to CMV_CM10 peptide. However, helminth infection was associated with a non-significant decrease in the quantities of detectable CMV_CM10-specific T cells when compared HIV-Worm+ (Median, 327.5 SFC/10^6 PBMCs; IQR, 47.5- 735 SFC/10^6 PBMCs) to HIV negative controls (Median, 1868 SFC/10^6 PBMCs; IQR, 873.8- 2330 SFC/10^6 PBMCs; P=

0.2901; Table 3- 4). Worm treatment had no influence on the quantities of IFN-γ secreting, CM10-specific CD8 T cells on worm infected and co-infected subjects.

While 78% (N=123) of HIV negative study volunteers responded to HSV-1 antigen, only about 46% (N=48) of HIV positive subjects had detectable responses against HSV-1 (P<0.0001, Fisher's exact test), showing an association of HIV infection with a decrease in frequency of detectable responses to HSV-1 (Table 3- 4). When comparing the overall magnitude of HIV+ subjects (Median, 22.5 SFC/10^6 PBMCs; IQR, 2.5- 68.75 SFC/10^6 PBMCs) with that of HIV- subjects (85 SFC/10^6 PBMCs; IQR, 32.5-157.5 SFC/10^6 PBMCs), HIV infection was associated with loss of IFN-γ secreting HSV-specific T cells by ~4 fold (P<0.0001. Figure 3- 7A). When compared with HIV-Worm- control subjects, a similar significant decline of median quantities of HSV-specific T cells was observed on HIV+Worm- subjects (Median, 40 SFC/10^6 PBMCs. P= 0.0327) and HIV+Worm+ co-infected individuals (Median, 20 SFC/10^6 PBMCs. P= 0.0002) with no significant effect of helminth infection on the specific immune response against HSV-1 (Figure 3- 7B). Helminth infections were not associated with a significant reduction of quantities of HSV-1-specific T cell responses, when comparing HIV negative subjects with or without helminth infections (Figure 3- 7B). There was a slight, non-significant linear association between HSV-1-specific T cells and total CD4 T cell count (Figure 3- 7C). No such association could be found when comparing HSV-1-specific T cells and pVL. However, a negative association was found between HSV-1-specific T cells and pVL (Spearman r= -0.3487, P= 0.0319, data not shown). Worm treatment had no influence on the quantities of IFN-γ secreting, HSV-1-specific T cells on worm infected and co-infected subjects.

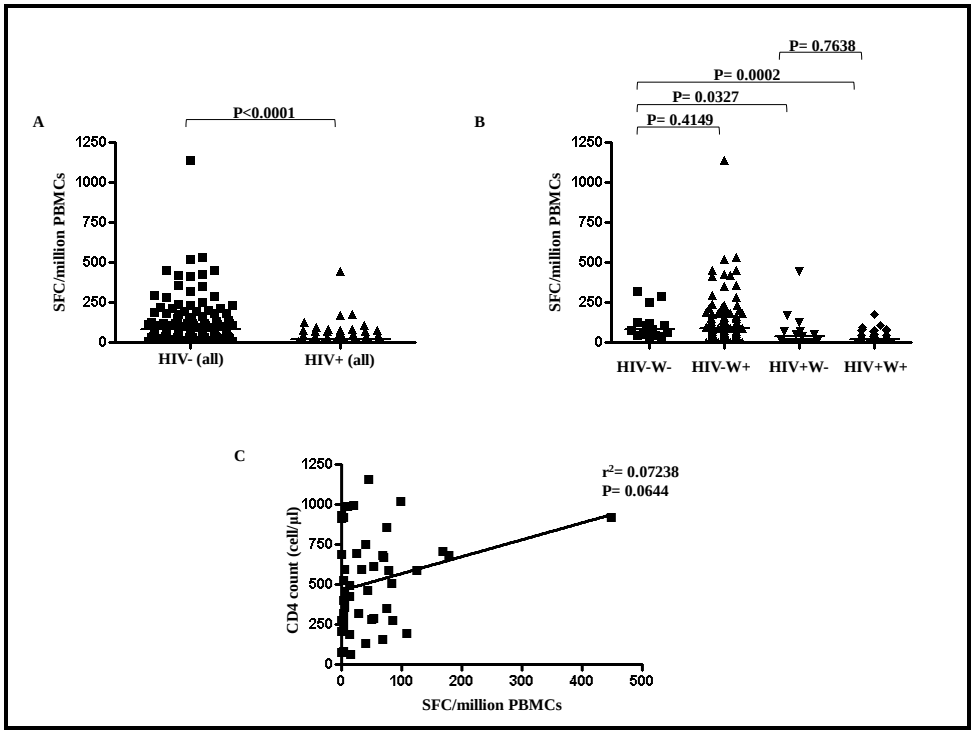


Figure 3- 7: Chronic HIV-1 infection and not helminth infection is associated with decreased HSV 1-specific T cell responses in peripheral blood. Shown are SFC/10^6 PBMCs responding to HSV-1 antigen in study volunteers stratified by HIV (A-B) and helminth infection status (B). A linear regression analysis of SFC/10^6 PBMCs responding to HSV-1 and absolute CD4 T cell counts is shown in (C). Statistical analysis was performed using Mann-Whitney test when comparing groups.

Over 93% of the HIV negative and HIV positive WHIS study volunteers responded to EBV antigen (Table 3- 4). Following a similar trend, HIV infection was also associated with a decrease in the frequency of detectable responses to EBV (P=0.0019. Fisher's exact test). However, HIV infection had no significant impact on the overall magnitude of EBV-specific T cell responses when compared to HIV negative (Median, 2358 SFC/10^6 PBMCs; IQR, 1063-4003 SFC/10^6 PBMCs) and HIV positive subjects (Median, 2048 SFC/10^6 PBMCs; IQR, 398.8- 3765 SFC/10^6 PBMCs P= 0.0979; data not shown). Similarly, helminth infections were not associated with changes in the quantities of EBV-specific T cells. Worm treatment had no influence on the quantities of IFN-γ producing EBV-specific T cells of worm infected and co-infected subjects.

3.1.3.3. Influenza- and *Toxoplasma gondii*-specific T cell responses

Influenza and *T.gondii* are also common pathogens that humans encounter and develop a controlled immunity against; but can cause AIDS related diseases in people with highly progressive chronic HIV infection (Murphy et al. 2007). Since worms have been reported to modulate pathogen-specific TH1 cell responses (Kamal et al. 2001), IFN-γ secretion in PBMCs of WHIS volunteers restimulated with respective antigens was examined at baseline, ~3 months and a year after worm treatment.

Table 3- 5 summarizes the frequency of responders to antigens and their median of detectable quantity of pathogen-specific T cells in HIV negative and HIV positive subjects with or without helminth infections.

Table 3- 5. Frequency of study subjects responding to Influenza and *T.gondii* antigens

Antigen	Frequency of responders (%), Median, IQR (SFC/million PBMC)						
	All study subjects (N=171)	HIV-(all) (N=123)	HIV+ (all) (N=48)	HIV-W- (N=15)	HIV-W+ (N=108)	HIV+W- (N=15)	HIV+W+ (N=33)
Influenza-a (whole inactivated)	86%, Median: 257.5, IQR: 132.5-467.5	96.7%, Median: 312.5, IQR: 166.3-527.5	58.3%, Median: 75, IQR: 55-192.5	100%, Median: 332.5, IQR: 292.5-707.5	96.3%, Median: 307.5, IQR: 153.8-517.5	73.3%, Median: 91.2, IQR: 58.7-223.8	51.5%, Median: 70, IQR: 55-175
T. gondii	39.2%, Median: 175, IQR: 87.5- 405	39%, Median: 205, IQR: 95- 405	73.3%, Median: 105, IQR: 41.25-207.5	46.7%, Median: 172.5, IQR: 57.5-432.5	38%, Median: 226.3, IQR: 107.5-405	33.3%, Median: 127.5, IQR: 50-641.3	42.4%, Median: 90, IQR: 40-217.5

147 out of 171 (86%) WHIS volunteers responded to Influenza antigen with median detectable IFN-γ responses of 257.5 SFC/10^6 PBMCs (Table 3- 5). Overall median quantities for Influenza-specific T cell responses were significantly depressed in HIV+ subjects (45.0 SFC/10^6 PBMCs) compared to HIV negative ones (307.5 SFC/10^6 PBMCs. P<0.0001. Figure 3- 8A). Similar significant decline was observed with HIV+Worm- (Median=75.0 SFC/10^6 PBMCs) and HIV+Worm+ subjects (Median= 30.0 SFC/10^6 PBMCs) when each group was compared to HIV-Worm- controls (P<0.0001 for both groups) with no significant influence of helminth infections on the magnitude of Influenza-specific responses (P value= 0.0808. Figure 3- 8B). Similar magnitude of Influenza responses were observed when compared HIV negative subjects with or without helminth infections (Figure 3- 8B). There was a

negative association between Influenza-a-specific T cells and pVL (Spearman r=-0.3527, P=0.0298) suggesting that increased pVL contributes to a decrease in Influenza-specific T cell responses (Figure 3- 8C). No linear relationship between Influenza-specific T cells and total numbers of CD4 T cells was found (Figure 3- 8D).

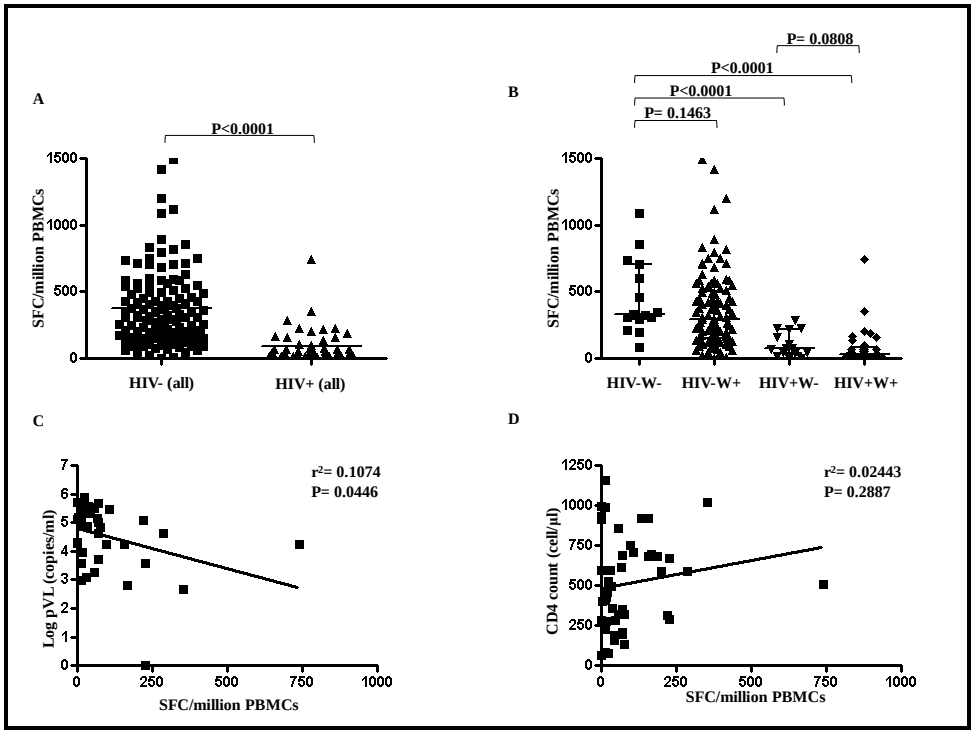


Figure 3- 8: Chronic HIV-1 infection and not helminth infection is associated with reduction of Influenza-a-specific T cell responses in peripheral blood. Shown are SFC/10^6 PBMCs responding to Influenza-a antigen in study volunteers stratified by HIV (A-B) and helminth infection status (B). (C) Shows a linear regression analysis of SFC/10^6 PBMCs responding to Influenza antigen and log of plasma viral loads. Linear regression analysis between SFC/10^6 PBMCs responding to Influenza antigen and absolute CD4 T cell counts is shown in (D). Statistical analysis was performed using Mann-Whitney test when comparing groups.

No effect of helminth treatment was observed regarding the quantities of influenza-specific T cells of infected and co-infected individuals 3 months and a year after treatment. However, within HIV negative *T.trichiura* infected subjects, a significant decrease in influenza-specific T cell response was observed 3 months after helminth treatment from a median of 397.5 to 217.5 SFU/10^6 PBMCs (P= 0.0024. Figure 3- 9) even though no helminth association was found on this study group at baseline. Of note, the observed decrease in response was not significant when only

detectable quantities of influenza-specific T cells were compared (P=0.0979, data not shown).

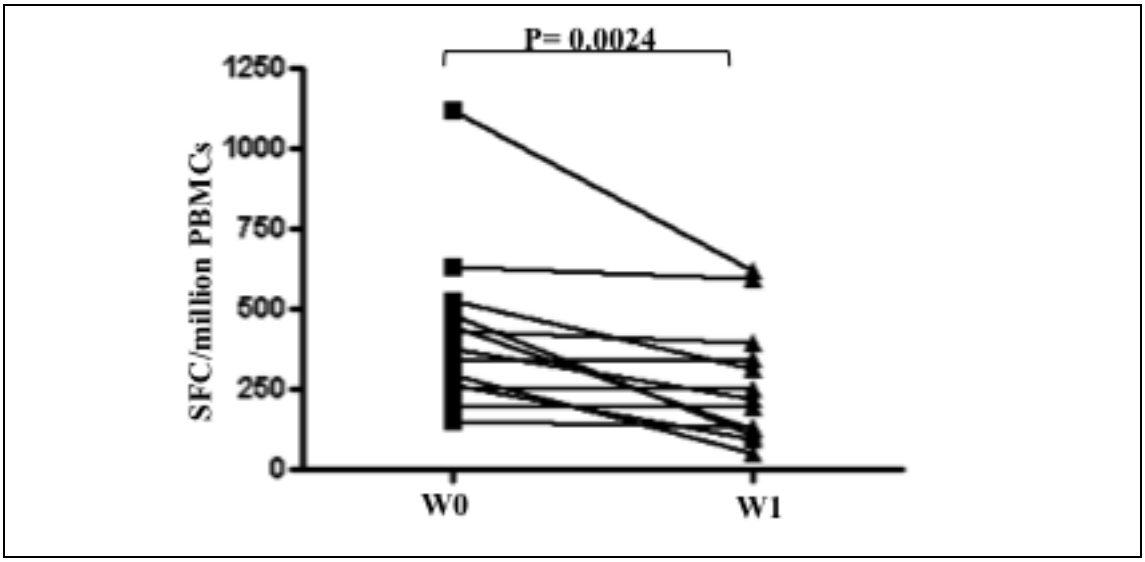


Figure 3- 9: Effect of worm treatment on the quantity of Influenza-specific T cells in the peripheral blood of HIV negative, *T.trichiura* infected volunteers. Comparison of SFC/10^6 PBMCs responding to Influenza-a before (W0) and ~3months (W1) after helminth treatment for each HIV-*T.trichiura*+ subject is shown. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

Only 39% of HIV positive and HIV negative WHIS volunteers responded to *T. gondii* antigen with median response of 175 SFC/10^6 PBMCs (

Table 3- 5). HIV infection was associated with a moderate loss of detectable quantities of *T.gondii*-specific T cells (Median: 205 SFC/10^6 PBMCs vs 105 SFC/10^6 PBMCs for HIV- and HIV+ respectively; P=0.0576; data not shown). Helminth infections were not associated with changes in the frequency and magnitude of detectable *T.gondii*-specific T cell responses. Worm treatment had no influence on the quantities of IFN-γ positive, *T.gondii*-specific T cells on worm infected and co-infected subjects.

Taken together, these results suggest that helminth infections have no impact on the quantities of IFN-γ secreting pathogen-specific T cells and the frequency of responders to different pathogens. However, HIV alone has a great impact on the quantity of most IFN-γ releasing pathogen-specific T cell responses and co-infection

with no apparent influence of helminth infections. In addition, worm treatment does not seem to have any detectable effect on the quantities of pathogen-specific T cells of volunteers as measured by IFN-γ release ELISpot assay up to a year after helminth treatment.

3.2. Characterization of regulatory T cells during HIV, Helminth or HIV-Helminth co-infection

Tregs are essential for maintenance of self tolerance and they can suppress activation, proliferation and effector functions of a wide range of immune cells, including CD4 and CD8 T cells (reviewed in (Sakaguchi et al. 2008)), that can impede clearance of chronic infections such as HIV (Aandahl et al. 2004; Kinter et al. 2007). This study therefore aimed to investigate whether helminth and HIV infections modulate the frequency and numbers of Tregs on volunteers with helminth, HIV or HIV-helminth co-infection. Of importance, the *ex vivo* expression of CCR5 on Tregs and their *ex vivo* cellular HIV infection in comparison to memory CD4 T cells was also investigated.

3.2.1. Comparative analysis of CD25+FoxP3+ CD4 regulatory T cell numbers and frequencies in relation to chronic infection with different helminth species

Helminth infections have often been associated with elevated levels of regulatory T cells (Maizels & Yazdanbakhsh 2003) therefore we first determined cross-sectionally whether different helminths infections are associated with changes in the frequency and absolute numbers of CD25+FoxP3+ CD4+ T cells (Tregs) in fresh anti-coagulated peripheral blood of HIV negative and positive individuals with helminth infections in comparison with helminth negative controls. Next, the effect of treating helminth infections on the numbers and proportion of circulating Tregs was assessed up to a year after worm treatment in HIV negative and positive volunteers. A representative dot plot and gating of CD25+FoxP3+ CD4 T cells is shown in Figure 3- 10A.

3.2.1.1. Cross-sectional analysis

In HIV negative subjects, a significant increase in absolute number of Tregs was only observed in *T.trichiura* (Median, 22.5cells/µl; IQR, 14.51- 29.7cells/µl) infected individuals when compared with the control group (Median, 15.78cells/µl; IQR, 9.89-23.39cells/µl; P= 0.0126; Figure 3- 10B). Also, changes in the frequency of Treg cells were only observed within the *T.trichiura* infected group. Treg frequencies were

increased within the HIV negative- *T.trichiura* infected group (Median, 2.8%; IQR, 1.8-3.25%) when compared to the HIV-Worm- control group (Median, 1.9%; IQR, 1.4-2.45%; P=0.0082; Figure 3- 10C). A slight but non-significant increase in frequency of Tregs was also observed in volunteers infected with *A.lumbricoides* (Median, 2.3%; IQR, 1.4-2.9%; P=0.2642; Figure 3- 10C).

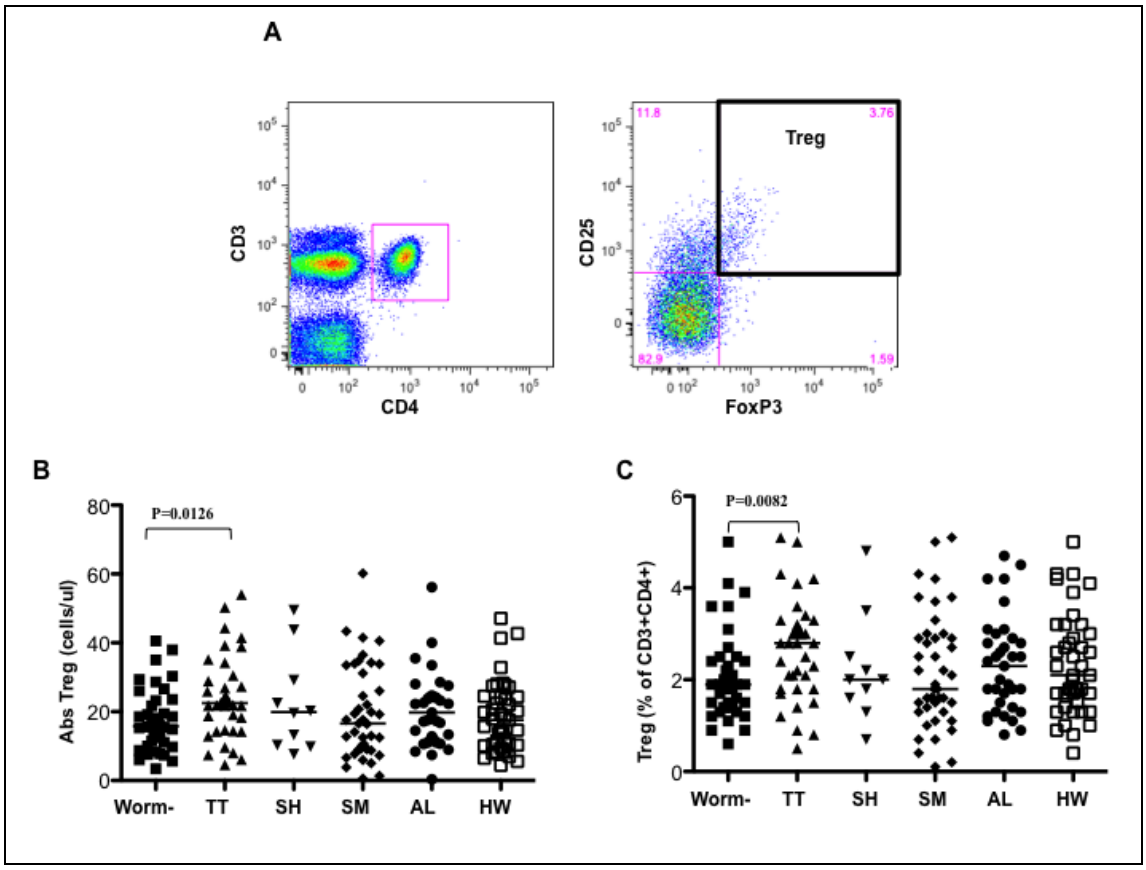


Figure 3- 10: Absolute numbers and frequency of CD25+ FoxP3+ regulatory T cells in the peripheral blood in relation to chronic Helminth infections. Representative dot plots and gating strategy for the detection of regulatory T cells through CD25 and FoxP3 expression on $CD3^{+}CD4^{+}$ T cells are shown in (A). The absolute number (B) and frequency (C) of Tregs are shown on the y-axis. The worm infection status is indicated on the x-axis stratified into worm negative individuals or those infected with TT (*Trichuris trichiura*), SH (*Schistosoma haematobium*), SM (*Schistosoma mansoni*), AL (*Ascaris lumbricoides*) or HW (Hookworm). Statistical analysis was performed using Mann-Whitney test for comparing groups.

In HIV positive individuals, the median of absolute Tregs of HIV co-infected and single infected individuals were 9.864cells/µl and 10.14 cells/µl respectively with no apparent influence of helminth co-infection on the number of Tregs (P=0.9082, data not shown). A similar frequency of Tregs was also observed between

HIV+Worm- (Median, 2.7%; IQR, 1.35-4.2%) and HIV+Worm+ co-infected individuals (Median, 2.35%; IQR, 1.5-4.350%; P=0.8452, data not shown).

3.2.1.2. Effect of treatment with praziquantel and albendazole on CD25+FoxP3+ CD4 regulatory T cells in HIV negative and HIV positive individuals

Reduction of Treg levels after anti-helminth treatment has been reported in individuals infected with *S.mansoni* (Watanabe et al. 2007). To therefore investigate the effect of helminth treatment in changing the absolute Treg numbers and frequencies, absolute counts and proportion of Tregs from helminth infected individuals were determined and compared before and up to one year after worm treatment. Only the individuals that were worm free post treatment were analysed. No significant changes were observed within the HIV-Worm+ nor HIV+Worm infected group 1-3 months after worm treatment (Table 3- 6).

However, Treg numbers were highly decreased in HIV-W+ subjects a year after de-worming from a median of 20.66 cells/µl to 12.22 cells/µl (P<0.0001; table XX). HIV-*T.trichiura*+ subjects showed a decrease in median Treg numbers from 22.67cells/µl to 13.07cells/µl one year post worm treatment (P<0.0001; Table 3- 6). Similarly, HIV-*S.mansoni*+ and HIV-*A.lumbricoides*+ subjects demonstrated a reduction in median Treg numbers a year post treatment (P=0.0082 and 0.0215 respectively; Table 3- 6). Lower T reg numbers were also observed in treated HIV-Hookworm+ subjects (median, 12.87cells/µl than at baseline (median, 19.75; P= 0.0024; Table 3- 6). A moderate decrease in Treg counts a year after treatment was observed with the control subjects who also received anti-helminthic treatment from a median of 15.51 cells/µl (IQR: 7.96- 20.91 cells/µl) to 8.923 cells/µl (4.479- 13.73 cells/µl; P= 0.0266; Table 3- 6). However, the median change of Treg counts a year post treatment between helminth infected and control groups were insignificant with the exception of HIV-Hookworm+ group (Table 3- 6).

Within HIV positive infected volunteers, only volunteers who were treated for Hookworms showed a decrease in median Treg numbers from 14.61cells/µl to 5.22cells/µl one year post worm treatment (P=0.0004; Table 3- 6). Minor changes in

Treg counts were also observed with the control group (P=0.0322; Table 3- 6) after treatment, with no substantial differences between this group and helminth infected subjects (Table 3- 6).

Table 3- 6. CD25+FoxP3+ regulatory CD4 T cell counts in HIV negative and positive volunteers before and after anti helminthic treatment

	HIV NEGATIVE VOLUNTEERS						
Status	WormNeg	Trichuris	S.h	S.m	Ascaris	Hookworm	All Worm
Treg counts (cells/ul)							
N (pairs)	26	19	6	30	24	30	110
Median (IQR) Baseline	15.55 (9.503-20.4)	23.16 (14.62-27.32)	14.92 (9.305-27.79)	15.28 (9.088-31.84)	17.48 (11.25-24.01)	19.58 (13.86-24.33)	18.83 (10.76-24.52)
Median (IQR) 3months after treatment	15.74 (7.626-18.79)	21.71 (16.35-29.37)	13.86 (12.2-17.44)	20.56 (10.64-35.56)	16.3 (10.9-21.7)	17.16 (13.6-24.72)	17.57 (11.57-27.06)
P (Baseline vs 3months after treatment)*	0.3219	0.4566	0.6875	0.2848	0.9658	0.8855	0.4552
P(Infected vs. controls)**		0.3888	0.9423	0.1806	0.4092	0.5058	0.3914
N (pairs)	13	21	7	24	15	30	97
Median (IQR) Baseline	15.51 (7.96-20.91)	22.67 (18.73-26.6)	20.26 (9.864-43.74)	16.5 (9.697-31.28)	24.1 (12.85-28.02)	19.75 (11.37-25.15)	20.66 (12.8-27.37)
Median (IQR) 1year after treatment	8.923 (4.479-13.73)	13.07 (8.726-17.61)	11.45 (9.54-15.39)	10.94 (8.569-18.48)	11.05 (8.663-15.33)	12.87 (7.226-21.14)	12.26 (8.556-17.95)
P (Baseline vs 1year after treatment)*	**0.0266**	**< 0.0001**	**0.0313**	**0.0082**	**0.0215**	**0.0023**	**< 0.0001**
P(Infected vs. controls)**		0.8825	0.8459	0.1961	0.2724	**0.0126**	0.1204

	HIV POSITIVE VOLUNTEERS						
Status	WormNeg	Trichuris	S.h	S.m	Ascaris	Hookworm	All Worm
Treg counts (cells/ul)							
N (pairs)	8	3	3	1	4	13	24
Median (IQR) Baseline	8.394 (2.494-15.9)	17.66 (10.88-30.12)	7.589 (4.255-13.06)	22.57551	10.47 (6.052-27.2)	14.61 (7.384-25.45)	13.78 (6.897-21.53)
Median (IQR) 3months after treatment	4.956 (1.765-9.248)	14.47 (1.244-39.29)	11.94 (2.972-19.65)	16.45837	7.614 (4.922-14.71)	9.868 (6.699-16.22)	10.34 (6.382-16.68)
P (Baseline vs 3months after treatment)*	0.25	1	1		0.625	0.2439	0.2246
P(Infected vs. controls)**		0.6303	0.3758		1	0.5618	0.5
N (pairs)	11	4	4	3	3	15	28
Median (IQR) Baseline	7.743 (5.868-14.87)	20.87 (7.567-28.61)	5.922 (2.714-11.69)	22.58 (4.71-23.9)	6.666 (5.847-14.27)	14.61 (3.871-19.38)	12.79 (4.369-21.78)
Median (IQR) 1year after treatment	3.498 (1.712-10.01)	5.539 (3.225-11.53)	2.897 (1.293-6.146)	9.867 (6.888-124)	2.93 (2.23-4.747)	5.223 (3.328-8.189)	4.994 (2.981-8.057)
P (Baseline vs 1year after treatment)*	**0.0322**	0.125	0.125	0.75	0.25	**0.0004**	**0.0004**
P(Infected vs. controls)**		0.5684	0.1946	0.1719	0.2652	0.9215	0.5511

*P values between baseline and 1-3 months follow up performed using the Wilcoxon-matched pairs test

**P values in median change with time between helminth infected and non-infected controls using the Mann-Whitney test

The influence of worm treatment on the frequencies of Tregs was determined next by employing the same analysis strategy used with absolute Treg numbers comparison. Again, no changes were observed within the HIV-Worm+ nor HIV+Worm infected group 1-3 months after worm treatment (Table 3- 7). A general decrease was however observed in the frequency of Tregs from 2.3% to 1.4% a year after treatment (P<0.0001; Table 3- 7). HIV-*T.trichiura*+ subjects had a decrease in median Treg frequencies from 2.8% to1.4% a year post worm treatment (P<0.0001; Table 3- 7). Lower Treg frequencies were also observed in HIV negative subjects treated for *S.mansoni*, *A.lumbricoides* and Hookworm infections a year after de-worming (all P<0.05; Table 3- 7). However, the difference between visits were not significant in all helminth infected groups when compared to subjects in the control group, who also a showed a moderate decrease in the frequency of Tregs a year after treatment from a median of 1.6% (IQR: 1.35-2.15%) to 1% (0.75-1.45%; P=0.003; Table 3- 7).

Worm treatment had an influence on the frequency of Tregs within HIV+Worm+ coinfected individuals (medians, 2.1% at baseline and 1.3% a year after treatment; P=0.0012; Table 3- 7). Again, this change was particularly observed with HIV+Hookworm+ subjects who had a median decrease of Treg frequency from 2.3% to 1.3% a year after worm treatment (P=0.017) with no substantial differences with the control group (P=0.876; Table 3- 7).

Table 3- 7. Frequency of CD25+FoxP3+ regulatory CD4 T cells in HIV negative and positive volunteers before and after anti helminthic treatment

	HIV NEGATIVE VOLUNTEERS						
Status	WormNeg	Trichuris	S.h	S.m	Ascaris	Hookworm	All Worm
% Treg							
N (pairs)	26	19	6	30	24	30	109
Median (IQR) Baseline	1.85 (1.375-2.225)	2.8 (2.1-3)	1.9 (1.15-2.525)	1.6 (1.3-2.9)	1.85 (1.3-2.575)	2.2 (1.7-2.925)	2.1 (1.5-2.9)
Median (IQR) 3months after treatment	1.75 (1.375-2.525)	2.3 (1.5-3.5)	1.65 (1.475-2)	2.1 (1.3-3.425)	1.75 (1.45-2.9)	2.35 (1.8-2.9)	2 (1.5-3.15)
P (Baseline vs 3months after treatment)*	0.9772	0.7273	0.7498	0.2061	0.5577	0.3173	0.4973
P(Infected vs. controls)**		0.3199	0.9574	**0.0731**	0.1783	0.3722	0.306
N (pairs)	17	22	8	26	15	30	101
Median (IQR) Baseline	1.6 (1.35-2.15)	2.8 (2.025-3.025)	2.1 (1.425-3.25)	1.8 (1.25-2.9)	2.5 (1.5-3.1)	2.45 (1.625-3.2)	2.3 (1.6-3)
Median (IQR) 1year after treatment	1 (0.75-1.45)	1.4 (0.9-1.75)	1.2 (0.975-2.425)	1.45 (1.075-1.725)	1.2 (0.7-1.7)	1.6 (0.9-1.95)	1.4 (0.9-1.85)
P (Baseline vs 1year after treatment)*	**0.0032**	**< 0.0001**	0.1829	**0.0077**	**0.0353**	**0.0004**	**< 0.0001**
P(Infected vs. controls)**		0.2111	0.6091	0.8425	0.7972	0.6771	0.9608

	HIV POSITIVE VOLUNTEERS						
Status	WormNeg	Trichuris	S.h	S.m	Ascaris	Hookworm	All Worm
% Treg							
N (pairs)	9	3	3	1	5	13	25
Median (IQR) Baseline	2.7 (1.35-3.75)	1.7 (1.6-6.8)	1.2 (0.6-3.6)	4.7	2.2 (1.45-6.5)	2.7 (1.9-5.05)	2.4 (1.65-5.05)
Median (IQR) 3months after treatment	1.4 (1.05-2.3)	1.9 (1-8.1)	2.6 (0.7-3.8)	1.9	2 (1.1-2.3)	2.8 (1.95-3.7)	2.1 (1.7-3.5)
P (Baseline vs 3months after treatment)*	0.4258	0.75	0.25		0.1875	0.7796	0.501
P(Infected vs. controls)**		0.8382	0.1265		0.2772	0.2142	0.174
N (pairs)	11	4	4	3	4	15	30
Median (IQR) Baseline	2.8 (1.1-4.4)	4.25 (1.7-7.775)	0.95 (0.625-3)	3.1 (1.5-4.7)	2 (1.275-5.875)	2.3 (1.8-3.5)	2.1 (1.575-3.875)
Median (IQR) 1year after treatment	1.2 (0.6-2.2)	1.95 (0.625-3.35)	0.65 (0.325-2.475)	2.4 (1.3-15.8)	0.85 (0.7-1.75)	1.3 (0.8-1.8)	1.3 (0.775-2.325)
P (Baseline vs 1year after treatment)*	**0.0231**	0.125	**0.0975**	0.75	0.125	**0.017**	**0.0012**
P(Infected vs. controls)**		0.2143	**0.0502**	0.3324	0.7983	0.876	0.6681

*P values between baseline and 1-3 months follow up performed using the Wilcoxon-matched pairs test

**P values in median change with time between helminth infected and non-infected controls using the Mann-Whitney test

3.2.2. Expression of HIV-co receptor, CCR5 on CD25+FoxP3+ CD4 regulatory T cells in relation to chronic infection with different helminth species

In vitro and *in vivo* expression of HIV co receptor, CCR5 on Tregs has been demonstrated before (Moreno-Fernandez et al. 2009; Dunham et al. 2008; Oswald-Richter et al. 2004), suggesting Tregs as potential targets for HIV. In order to determine, whether Tregs could be potential substrates for direct HIV infection, *in vivo* expression of HIV co-receptor CCR5 on circulating Tregs of the study subjects was analysed. Fresh anti-coagulated whole blood was used to achieve maximum sensitivity for CCR5 detection. A large fraction of about 50% of Tregs expressed CCR5 regardless of their helminth status, suggesting that Tregs are potential cellular targets for HIV infection (Figure 3- 11).

Following this observation, immune modulation of CCR5 on Tregs by different helminth species was analysed to determine whether helminth infections and co-infection had any effect on the expression of CCR5 on circulating Tregs, which could potentially influence HIV susceptibility or disease progression. No helminth specie was particularly associated with changes in the CCR5 expression on Tregs within HIV negative subjects (Figure 3- 11B). There were also no significant differences in the frequency of Tregs that expressed CCR5 in people with HIV infection alone compared to HIV+Helminth+ co-infected subjects (data not shown).

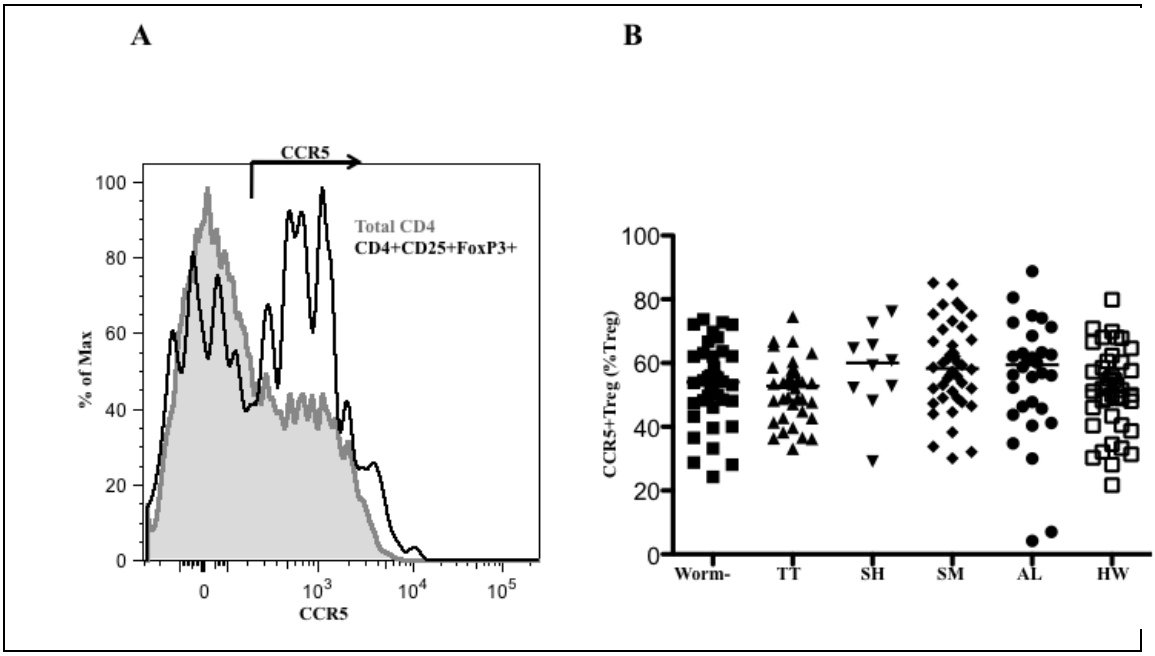


Figure 3- 11: ***In vivo*** **HIV-co receptor (CCR5) expression on regulatory T cells**. Shown in (A) is a representative histogram analysis for CCR5 expression on total CD4 T cells (grey) and CD25+ Foxp3+ CD4 T cells (black). For maximum staining sensitivity, fresh anticoagulated whole blood was used to determine CCR5 expression on CD4 T cells. The frequency of CCR5+Tregs (B) is shown on the y-axis. The worm infection status is indicated on the x-axis stratified into worm negative individuals or those infected with TT (*Trichuris trichiura*), SH (*Schistosoma haematobium*), SM (*Schistosoma mansoni*), AL (*Ascaris lumbricoides*) or HW (Hookworm). Statistical analysis was performed using Mann-Whitney test for comparing groups.

3.2.3. Effect of treatment with praziquantel and albendazole on the frequency of CCR5 on CD25+FoxP3+ CD4 regulatory T cells

A slight increase on the frequency of CCR5+Tregs was observed in HIV-*T.trichiura*+ subjects 1-3 months after worm treatment (Median, 53.7% at baseline and 55.2% post treatment; P= 0.0549; Figure 3- 12A) but even more pronounced a year post treatment from a median of 50.5% to 60.1% (P= 0.0005; Figure 3- 12B). Of note, changes in the frequency of CCR5+Tregs were higher in individual treated for *T.trichiura* infection compared to control subjects, who showed no differences in the frequency of CCR5+Tregs up to a year after treatment (Table 3- 8).

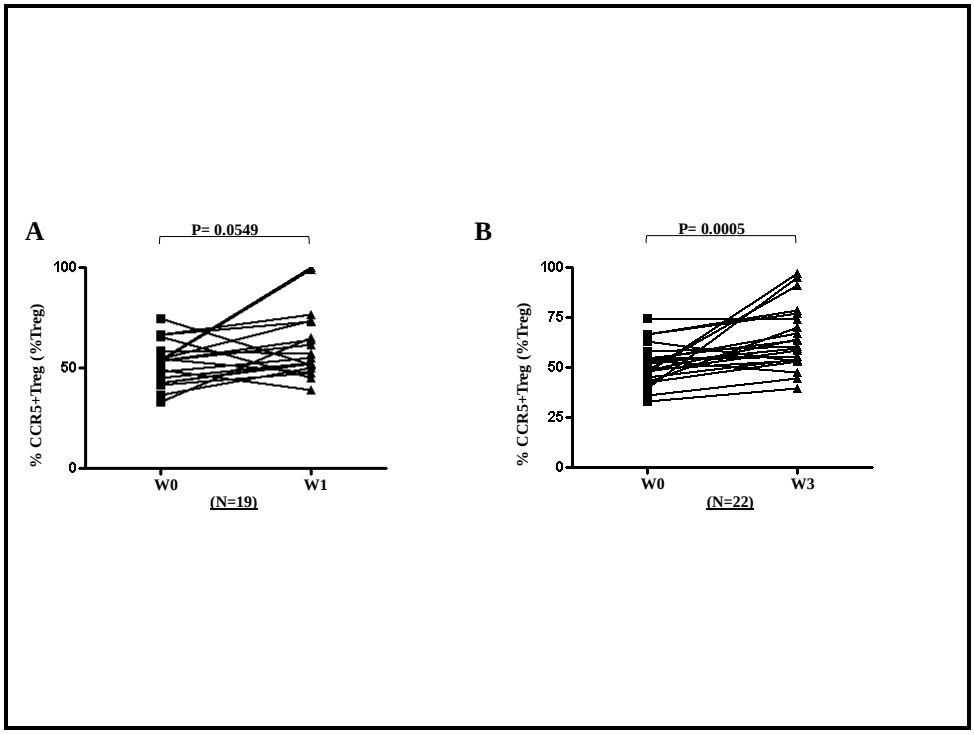


Figure 3- 12: Effect of helminth treatment on frequencies of CCR5 expression on regulatory CD4 T cells in the peripheral blood of *T.trichiura* infected volunteers. Comparison of frequencies of CCR5 expression on regulatory CD4 T before (W0) and up to 3 months (W1) after helminth treatment is shown in (A); while comparison of frequencies of CCR5 expression on regulatory CD4 T before (W0) and one year (W3) after helminth treatment are shown in (B). Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

In contrast, frequency of CCR5+Tregs of *S.mansoni* infected volunteers decreased from 58.30% to 53.6% 1-3 months after worm treatment (P= 0.0093; Table 3- 8). This decline was also observed a year after treatment (Median, 58% at baseline

and 52.2% a year post treatment; P= 0.0186; Table 3- 8). Also a decrease in frequency of CCR5+Tregs from a median of 58.8% to 47% was observed in HIV-*A.lumbricoides*+ subjects 1-3 months post treatment (P=0.0235; Table 3- 8); but no change was observed a year post treatment in this group. No changes in the frequencies of CCR5+Tregs of HIV-Hookworm+ subjects were observed up to 3 months after worm treatment, but an increase from 50.55% to 57.80% could be seen a year after treatment (P=0.0221; Table 3- 8). Compared to worm negative control subjects, who showed no effect of worm treatment, the median change in frequency of CCR5+Tregs post treatment were very minor and insignificant for *S.mansoni*, *A.lumbricoides* and Hookworm infected subjects (Table 3- 8).

There was no observed influence of worm treatment on the frequency of CCR5 expressing Tregs on HIV positive co-infected subjects. A slight increase in the median frequencies of CCR5+Tregs was however observed with HIV+Hookworm+ subjects a year after worm treatment (from 46.4% to 49.4%; Table 3- 8). This change differed significantly to the control group (P=0.0394; Table 3- 8).

Table 3- 8. Frequency of CCR5+ regulatory CD4 T cells in HIV negative and positive volunteers before and after anti helminthic treatment

	HIV NEGATIVE VOLUNTEERS						
Status	WormNeg	Trichuris	S.h	S.m	Ascaris	Hookworm	All Worm
CCR5+Treg+ (% of Treg)							
N (pairs)	26	19	6	30	23	30	108
Median (IQR) Baseline	55.6 (49.3-64.45)	53.7 (42.8-58.6)	65.1 (52.88-73.48)	58.3 (50.93-71.85)	58.8 (45.7-63.4)	51.3 (40.48-60.35)	55.4 (46.13-65.28)
Median (IQR) 3months after treatment	50.95 (43.7-60.5)	55.2 (50.2-73)	56.4 (48.28-65.43)	53.6 (38.63-60.4)	47 (36.4-61.5)	50.1 (45.1-55.8)	51.75 (44.4-61.48)
P (Baseline vs 3months after treatment)*	0.134	**0.0559**	0.4375	**0.0093**	**0.0235**	0.9827	**0.0749**
P(Infected vs. controls)**		**0.0812**	1	0.8348	0.2034	0.3339	0.2135
N (pairs)	17	22	8	25	12	28	95
Median (IQR) Baseline	50 (42.8-58.7)	50.5 (44.38-55.6)	60 (52.35-70.85)	58 (48.35-64.3)	58.1 (45.88-61.28)	50.55 (41.23-60.45)	53.4 (46.5-61.4)
Median (IQR) 1year after treatment	49 (42.3-65.1)	60.1 (53.43-75.05)	55.6 (40.08-67.85)	52.2 (43.95-56.6)	51.6 (43.28-54.7)	57.8 (50.58-61.98)	55.3 (47.8-61.8)
P (Baseline vs 1year after treatment)*	0.9622	**0.0005**	0.7422	**0.0186**	0.4238	**0.0221**	0.2234
P(Infected vs. controls)**		**0.0571**	0.2088	0.6242	0.6335	**0.0663**	0.2066

	HIV POSITIVE VOLUNTEERS						
Status	WormNeg	Trichuris	S.h	S.m	Ascaris	Hookworm	All Worm
CCR5+Treg+ (% of Treg)							
N (pairs)	9	3	3	1	5	13	25
Median (IQR) Baseline	52.1 (34.1-63.3)	62.8 (60.1-85.2)	60.1 (53.2-74.2)	45.2	51.6 (27.95-54.15)	38.7 (35.8-55)	52.3 (38.5-59.5)
Median (IQR) 3months after treatment	50 (36.2-65.7)	65.5 (51.6-65.7)	48 (29.1-56.6)	76.8	54.7 (45.55-59.3)	42.4 (34.1-54.05)	51.1 (37.9-59.3)
P (Baseline vs 3months after treatment)*	0.8203	0.5	0.25		0.625	0.7354	0.8717
P(Infected vs. controls)**		0.4623	1	0.1384	**0.0445**	0.3046	0.1984
N (pairs)	8	4	4	3	4	15	30
Median (IQR) Baseline	62.4 (41.85-70.48)	66.7 (53.5-81.55)	56.65 (29.95-70.68)	45.2 (41.1-69.2)	52.2 (48-54.83)	46.4 (35.4-58.9)	51.95 (38.3-61.7)
Median (IQR) 1year after treatment	50.85 (40.88-56.93)	54.25 (40.28-77.98)	51.2 (27.5-68.53)	47.6 (40.2-59.6)	63.9 (54.75-73.65)	49.4 (37-62.9)	52.65 (42.45-64.48)
P (Baseline vs 1year after treatment)*	0.4609	0.375	0.125	1	0.125	0.4212	0.517
P(Infected vs. controls)**		0.353	0.1651	**0.0999**	0.1152	**0.0394**	**0.0943**

*P values between baseline and 1-3 months follow up performed using the Wilcoxon-matched pairs test

**P values in median change with time between helminth infected and non-infected controls using the Mann-Whitney test

3.2.4. Characterization of CD25+FoxP3+ CD4 regulatory T cells in relation to chronic HIV-1 infection

Increase in frequency of circulating CD25+FoxP3+CD4 T cells and decline in absolute count of such cells in subjects with chronic untreated HIV infection has been reported before (Angin et al. 2012; Presicce et al. 2011). This study also investigated the changes in the frequency and absolute numbers of circulating Tregs in relation to chronic HIV-1 infection.

3.2.4.1. Absolute numbers and frequency of CD25+FoxP3+ CD4 regulatory T cells

Treg numbers were decreased in HIV+ subjects (Median, 10.00cells/µl; IQR, 4.826-17.83cells/µl) compared to HIV- subjects (Median, 18.01cells/µl; IQR, 10.76 - 24.67cells/µl; P<0.0001; Figure 3- 13A). Similarly, HIV infection was associated with a significant decrease in absolute Tregs irrespective of helminth infection status (Figure 3- 13B). The median of absolute Tregs of HIV co-infected and single infected individuals were 9.864cells/µl and 10.14cells/µl respectively compared to HIV negative control group which was 15.78cells/µl (P=0.0079 and P=0.0295 respectively).

Generally, a moderate increase in Treg frequencies was observed in HIV infected people (Median, 2.4%; IQR, 1.5-4.2%) when compared to HIV negative ones (Median, 2.05%; IQR, 1.45-2.95%; P=0.0472; Figure 3- 13C). No significant increase of Treg percentages in the HIV+Worm- (Median, 2.7%; IQR, 1.35-4.2%) individuals was detected when compared to the HIV-Worm- subjects (P=0.1612; Figure 3- 13D). However, there was a moderate increase of frequency of Treg in the HIV+Worm+ co-infected individuals (Median, 2.35%; IQR, 1.5-4.350%; P=0.0478). Nonetheless, co-infection could not be attributed to this observation as no significant differences in the percentage levels of Tregs were found between the HIV co-infected and non co-infected group (Figure 3- 13D). Within HIV+ subjects there was a linear, positive association between absolute Treg numbers and CD4 T cell counts ($p < 0.0001$; $r^2 = 0.2405$; Figure 3- 13E), suggesting that the observed depletion of Tregs is closely linked to the loss of CD4 T cells.

Also, since Tregs have been described to suppress HIV-specific T cell responses (Aandahl et al. 2004), an association between Treg numbers and HIV specific CD8 T cell responses was analysed. HIV responses were determined in HIV positive subjects by IFN-γ ELISpot analyses after stimulation of PBMCs with a pool of frequently recognized HIV specific peptides as described in the previous chapter. No association between Treg numbers and the magnitude of HIV-FRP-specific CD8 T cell responses was found (data not shown).

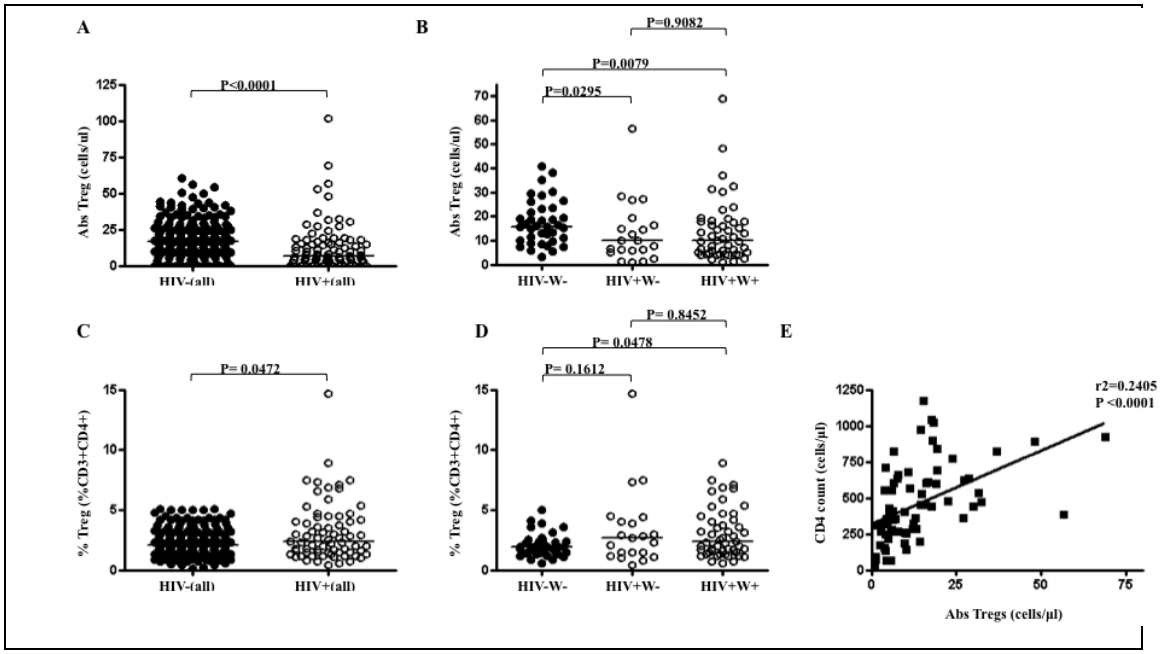


Figure 3- 13: Absolute numbers and frequency of CD25+ FoxP3+ regulatory T cells in the peripheral blood in relation to chronic HIV-1 infection. Regulatory CD4 T absolute numbers were compared between: all HIV+ vs all HIV- irrespective of their helminth infection in (A) and HIV-Worm- vs HIV+ stratified by worm status in (B). Frequency of regulatory CD4 T was compared between: all HIV+ vs all HIV- irrespective of their helminth infection in (C) and HIV-Worm- vs HIV+ stratified by worm status in (D). A linear regression analysis of absolute CD4 counts and regulatory CD4 T cell counts is shown in (E). Statistical analysis was performed using Mann-Whitney test when comparing groups.

3.2.4.2. Expression of HIV-coreceptor, CCR5 on CD25+FoxP3+ CD4 regulatory T cells

In vivo expression of HIV co-receptor CCR5 on circulating Tregs of the study subjects was analysed in relation to HIV-1 infection status as described above. When

HIV+ individuals were analysed independent of their helminth infection, a moderate decrease in the frequency of CCR5+ Tregs was observed in association with HIV infection (median: 49.25% for HIV+ compared to 54.5% for HIV-, p= 0.009, Figure 3- 14).

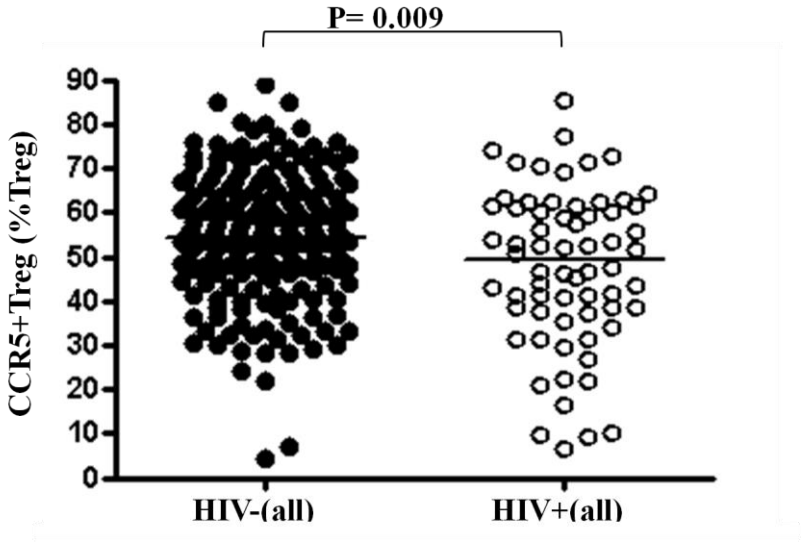


Figure 3- 14: *In vivo* HIV-co receptor (CCR5) expression on regulatory T cells in relation to chronic HIV-1 infection. The frequencies of CCR5+ expressing Tregs are compared between HIV negative and HIV positive subjects irrespective of their worm infection status. Statistical analysis was performed using Mann-Whitney test.

3.2.4.3. Comparison of *ex vivo* HIV proviral DNA load in memory CD25+FoxP3+ CD4 regulatory T cells and memory CD25-FoxP3- CD4 T cells

Because these results demonstrated that high frequencies of CD25+ FoxP3+ CD4 Tregs express CCR5, it is plausible that Tregs potentially support CCR5 mediated viral entry in HIV infected subjects. Furthermore, memory Tregs are highly proliferative *in vivo* in HIV positive subjects (unpublished data from Osei Kuffour *et al.*), which should support productive HIV infection of this specific cell subset *in vivo*. Therefore, we next determined HIV infection rates of memory CD4 T cells and memory Tregs *ex vivo*. Four different subsets of CD45RO+ memory CD4 T cells characterized by their Helios, CD25 and FoxP3 expression were sorted (Figure 3-15A) from PBMCs of 22 HIV+ subjects and HIV gag DNA within the different sorted

subsets was quantified. Helios, a Ikaros transcriptional factor family member, was included in this study because it was originally proposed to be a marker that distinguished thymic derived from peripheral induced Tregs (Sugimoto et al. 2006; Thornton et al. 2010; Getnet et al. 2010). However, this notion has been contested more recently (Akimova et al. 2011). *In vitro*, Helios expression is up-regulated after/during cell division in regulatory and effector T cells (Akimova et al. 2011) and recent data suggests that Helios is a key negative regulator of IL2 expression and proliferation in Tregs (Baine et al. 2012).

Similar to HIV negative subjects (Booth et al. 2010), a large majority of CD25+FoxP3+ T regs expressed the memory marker CD45R0 in HIV+ subjects (median, 87.30%; IQR, 71.5%-93.7%), and most of these expressed Helios (median, 76.30%; IQR, 67.75%-84.75%). In contrast only a minor fraction of CD25-FoxP3- memory CD4 T expressed Helios (median, 1.65%; IQR, 1.15%-2.75%). Irrespective of their Helios expression, HIV gag DNA copies could detected in >80% of both sorted memory CD25+FoxP3+ Treg populations and CD25-FoxP3- memory CD4 T cell populations, confirming that Tregs are indeed frequent targets of HIV *ex vivo*.

Figure 3- 15B shows the HIV gag DNA load detected within memory CD4 T cells and memory Tregs. A 15-fold higher median gag DNA load was detected in memory Tregs as compared to CD25-FoxP3- memory CD4 T cells ($\sum$Helios^{+}Helios-; 16072 versus 1074 copies/10^6 cells, p=0.0032). From 16 subjects plasma viral load (pVL) data were obtained for determining whether cell associated DNA Gag load detected in the two memory CD4 T cell populations contribute to pVL. There was a linear correlation between cell associated DNA gag in memory CD25-FoxP3- memory CD4 T cells and pVL (p=0.03, r^2=0.31, Figure 3- 15C). However, no such linear correlation could be detected in memory Tregs (p=0.28, r^2=0.08, data not shown).

Figure 3- 15D shows the HIV gag DNA load within memory Tregs CD25-FoxP3- memory CD4 T cells further delineated by Helios expression. Gag DNA loads in FoxP3+CD25+ Helios- memory Tregs (119-fold increased, median: 18407 copies/10^6 cells; IQR: 1556- 106067 copies/10^6 cells; P= 0.0068), FoxP3-CD25-Helios+ memory CD4 T cells (104-fold increased, median: 16096 copies/10^6 cells;

IQR: 837.9 – 47903 copies/10^6 cells, P= 0.0289) and FoxP3+CD25+ Helios+ memory Tregs (26-fold increased, median: 4106 copies/10^6 cells; IQR: 0- 44612 copies/10^6 cells; P= 0.0705) were much higher compared to FoxP3-CD25- Helios- memory CD4 T cells (median: 154.4 copies/10^6 cells; IQR: 0- 10241 copies/10^6 cells), which constitute the largest of these memory CD4 T cell populations.

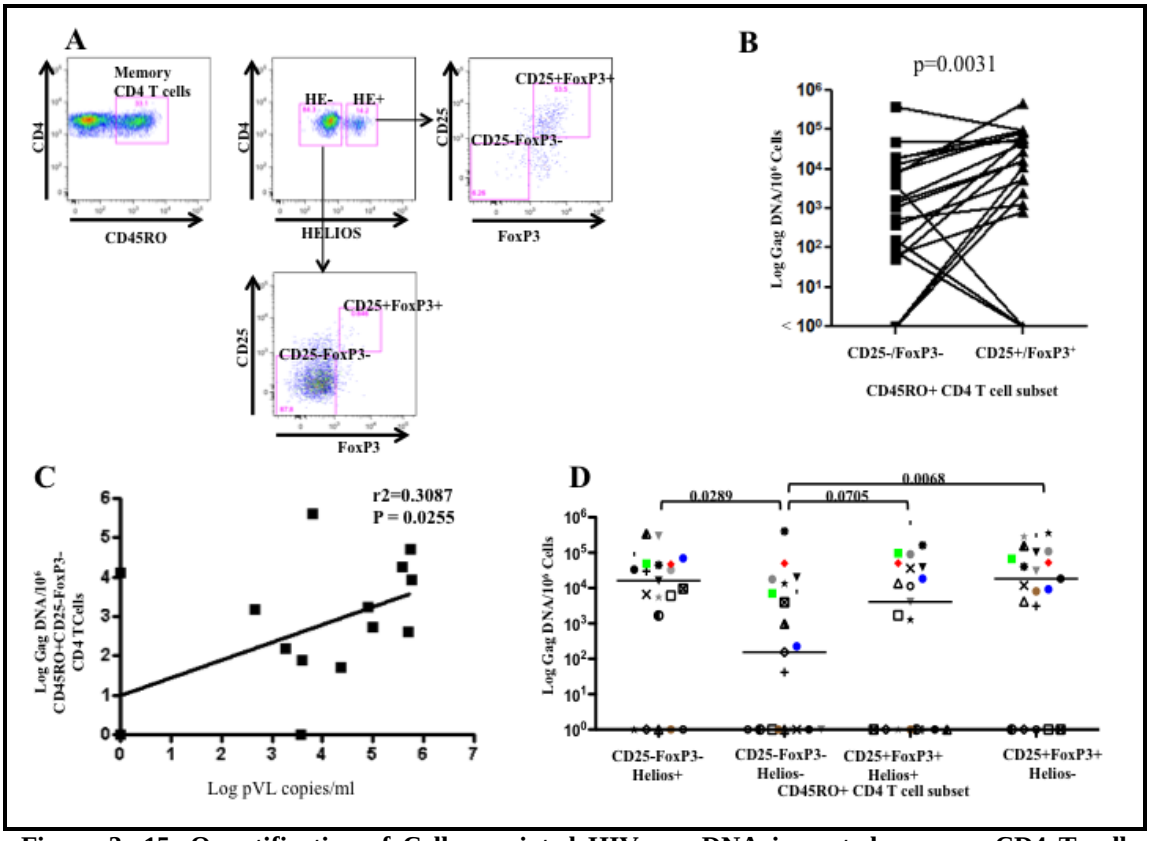


Figure 3- 15: Quantification of Cell associated HIV gag DNA in sorted memory CD4 T cell subsets. Gating/sorting strategy used to sort different memory (CD45RO+) CD4 T cell populations delineated by Helios, CD25 and FoxP3 expression (A). The number of gag copies/10^6cells detected in $CD25^-/FoxP3^-$ and $CD25^+/FoxP3^+$ memory CD4 T cells from 21 different subjects is shown in (B). A linear regression analysis of log number of gag copies/10^6cells detected in $CD25^-/FoxP3^-$ and log plasma viral load copies (pVL) is shown in (C). The number of gag copies/10^6cells detected in these memory cell subsets further delineated by Helios expression is shown in (D). Gag DNA within different CD4 T cell populations of the same subject was quantified during the same RT-PCR run. The statistical analysis was performed using the Wilcoxon-rank-matched pairs test.

3.3. Characterization of maturation and activation markers on CD4 and CD8 T cells during HIV, Helminth or HIV-Helminth co-infection

It was proposed 18 years ago that chronic immune activation brought about by chronic infections such as helminths may be the driving force of HIV in Africa (Bentwich et al. 1995) as such infections are common in Africa. However, data regarding the influence of different helminth (co)infections on systemic immune activation is scarce and discrepant. This study therefore aimed to investigate the effect of worms and de-worming on the profile of T-cell subsets and T-cell immune activation in relation to HIV susceptibility and disease progression.

3.3.1. Characterization of CD4 and CD8 T cell subsets relation to chronic infection with different helminth species

3.3.1.1. Cross-sectional

To assess the role of helminth infections on modulating T-cell subsets, frequency of expression of maturation markers, CD27 and CD45R0 on CD4 and CD8 T cells was compared between HIV negative and HIV positive individuals, with or without helminth infections to determine the distribution of T cell subsets in relation to chronic helminth infection. A representative dot plot and gating of T cell subsets is shown in Figure 3- 16.

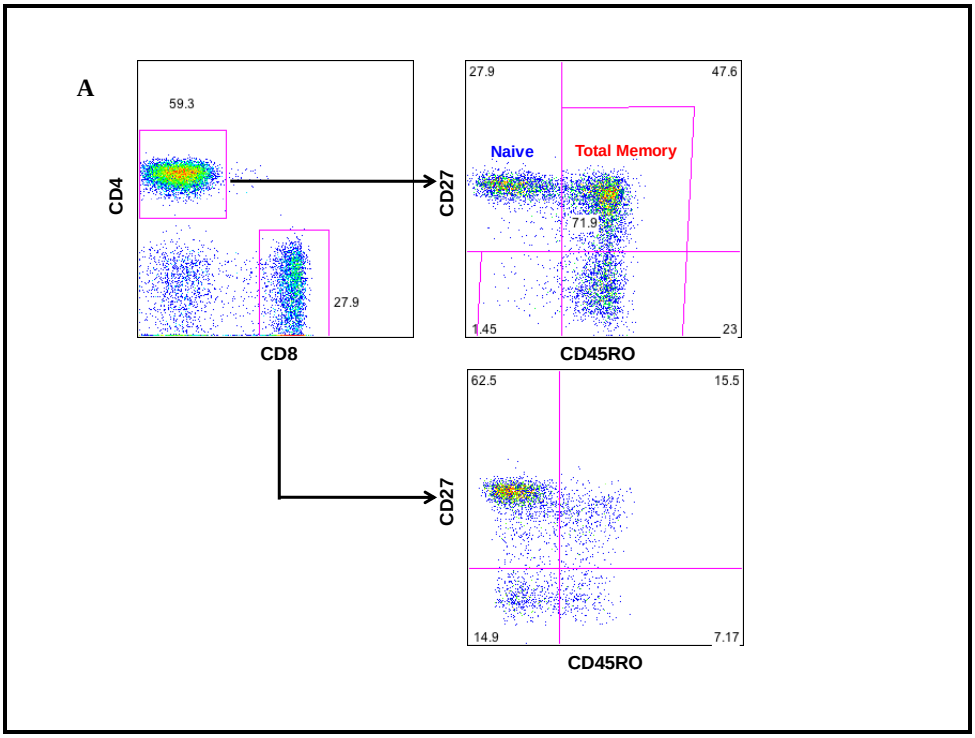


Figure 3- 16: Frequencies of expression of T-maturation markers on CD4 and CD8 T cells. Shown is the gating strategy for the expression maturation markers (CD27+ and CD45RO+) on CD4 and CD8 T cells of a representative subject.

In HIV negative volunteers, similar frequencies of naïve (CD27+CD45RO-), "central-like" memory (CD27+CD45RO+), "effector-like" memory (CD27-CD45RO+) and "terminally differentiated" (CD27-CD45RO-) CD4 and CD8 T cells were observed between helminth infected and non-helminth infected individuals (Table 3- 9). However, a trend towards increased frequency of terminally differentiated CD4 T cells was seen in relation to helminth infection (P= 0.0531; Table 3- 9); mainly contributed by *T.trichiura* and *S.haematobium* infection (Figure 3- 17A).

Table 3- 9. Expression of maturation markers on CD4 and CD8 T cells of HIV negative individuals in relation to chronic infection with different helminth species

	HIV negative		
	No helminth	All Helminth+	P value
N (%CD4)	**43**	**181**	
%CD4+Naive	38.1% (24.6-49.2%)	38.4% (27.8-47.15%)	0.9622
%CD4+Central Memory	40.2% (32.1-48.3%)	38.1% (31.85-43.45%)	0.2118
%CD4+Effector Memory	19.7% (12.5-23.2%)	19.5% (14.7-25.25%)	0.5852
%CD4+Terminally differentiated	1.58% (0.88-2.7%)	2.17% (1.235-4.165%)	**0.0531**
%CD4+Total Memory	62.15% (50.81-75.43%)	61.57% (52.75-72.12%)	0.9812
N (%CD8)	**43**	**180**	
%CD8+Naive	40.6% (32.8-52.1%)	41.95% (29.75-54.45%)	0.9068
%CD8+Central Memory	15.2% (9.45-22.6%)	15.2% (10.85-21.4%)	0.7763
%CD8+Effector Memory	9.63% (5.38-13.6%)	8.78% (4.81-14.4%)	0.8456
%CD8+Terminally differentiated	27.5% (20.9-36.3%)	29.15% (19-38.4%)	0.9622

Infection with *T.trichiura* was associated with a trend towards a slight decreased frequency of central memory CD4 T cells (Median: 37.75%; IQR: 31.1- 41.3%) compared to control individuals (Median: 40.2%; IQR: 32.1-48.3%. P= 0.0943. Figure 3- 17B). In contrast, a non significant increase in effector memory CD4 T cells was observed in *T.trichiura* infected volunteers (Median: 21.05%; IQR: 17.85- 29.05%) in comparison to helminth negative volunteers (Median: 19.7; IQR: 12.5- 23.2%. P= 0.0531. Figure 3- 17C).

Infection with *S.haematobium* was also associated with 1.25 less median frequencies of central memory CD4 T cells (Median: 32.05%; IQR: 28.75- 36.65%. P= 0.0158. Figure 3- 17B) compared to controls. Higher frequency of naïve CD8 T cells was observed in *S.haematobium* infected people (Median: 52.25%; IQR: 41.88- 61.05%) compared to controls (Median: 40.6%; IQR: 32.8-52.1%. P=0.035. Figure 3- 17D). On the other hand, there was a trend towards a decreased frequency of terminally differentiated CD8 T cells (Median: 22%; IQR: 15.3- 29.05%) compared to control individuals (Median: 27.5%; IQR: 20.9- 36.3%; P= 0.0989; data not shown).

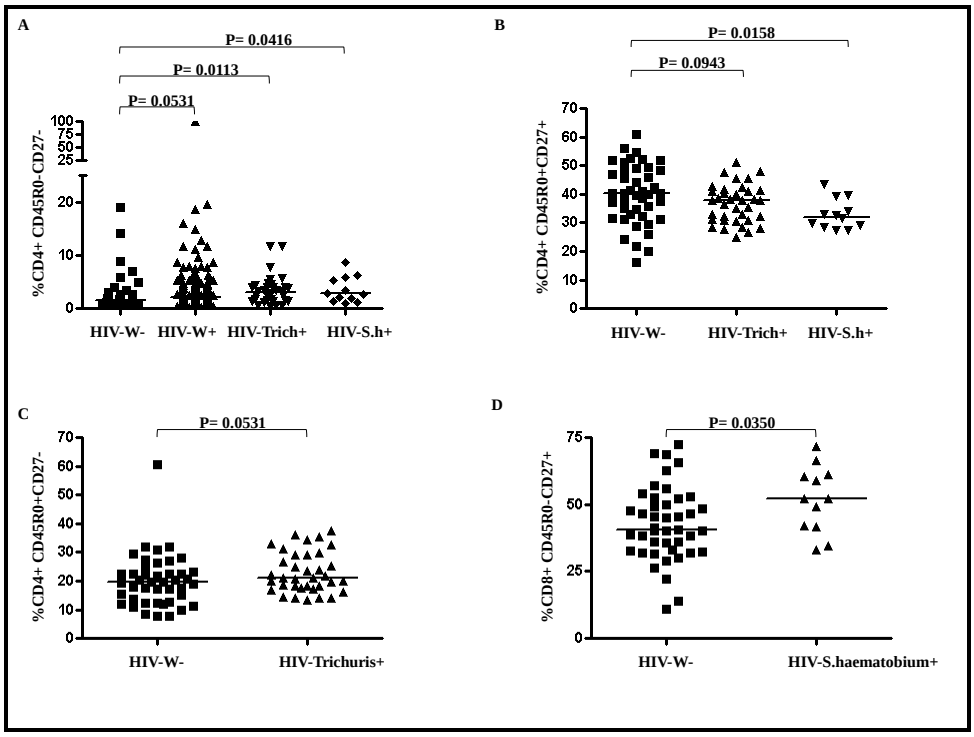


Figure 3- 17: Frequency of T-cell subsets in relation to *T.trichiura* and *S.haematobium* infections. The frequencies of CD45RO-CD27-(**A**), CD45RO+CD27+**(B),** CD45RO+CD27-**(C)** and CD45RO+CD27-**(D)** on CD4 **(A-C)** and CD8 **(C)** T cells is shown between the control and worm infected subjects. Statistical analysis was performed using Mann-Whitney test for comparing groups.

This data collectively shows that changes in the frequency of different T cell subsets were mainly associated with *T.trichiura* and *S.haematobium* while *A.lumbricoides*, Hookworm and *S.mansoni* showed no such association.

In HIV positive volunteers, similar frequencies of all studied CD4 and CD8 T cell subsets could be observed except for a 1.5-fold significant increase in terminally differentiated CD8 T cells in helminth infected volunteers in comparison to volunteers in the control group (Table 3- 10).

Table 3- 10. Expression of maturation markers on CD4 and CD8 T cells of HIV positive individuals in relation to chronic infection with different helminth species

	HIV positive		
	No helminth	All Helminth+	P value
N (%CD4)	**26**	**51**	
%CD4+Naive	35.05% (22.45-49.65%)	35.7% (20.4-51.1%)	0.7753
%CD4+Central Memory	37% (28.6-46.7%)	35.1% (25.8-41.3%)	0.2839
%CD4+Effector Memory	19% (12.8-26%)	21.6% (14.2-30.1%)	0.3432
%CD4+Terminally differentiated	1.925% (0.79-2.98%)	2.29% (0.79-4.38%)	0.6644
%CD4+Total Memory	65.01% (50.43-77.42%)	64.28% (48.89-79.49%)	0.7671
N (%CD8)	**26**	**53**	
%CD8+Naive	24.45% (19.8-32.6%)	20.5% (13.65-32%)	0.1358
%CD8+Central Memory	22.45% (17.8-37.2%)	20% (14.55-27.75%)	0.0994
%CD8+Effector Memory	14.8% (8.33-21.4%)	15.8% (10.55-20.75%)	0.8185
%CD8+Terminally differentiated	26.9% (17.75-40.85%)	40.6% (28.45-49.45%)	**0.0068**

Co-infection with *T.trichiura* was associated with a moderate increase in the frequency of effector memory CD4 T cells (Median: 26.85%; IQR: 20.85-40.3%) compared to the control HIV+ group (Median: 19%; IQR: 12.8-26%. P=0.0423; Figure 3- 18A). Similarly, HIV+*T.trichiura*+ volunteers had a higher frequency of terminally differentiated CD8 T cells (Median: 43%; IQR: 38.3-48.15%) than non-infected HIV+ volunteers (Median: 26.9%; IQR: 17.75-40.85%. P= 0.0091; Figure 3-18 B).

In comparison to non-infected HIV+ volunteers, HIV+*S.haematobium* infected individuals also demonstrated an increase in the frequency of terminally differentiated CD8 T cells (Median: 44.15%; IQR: 39- 50.6%; P= 0.0153; Figure 3-18C).

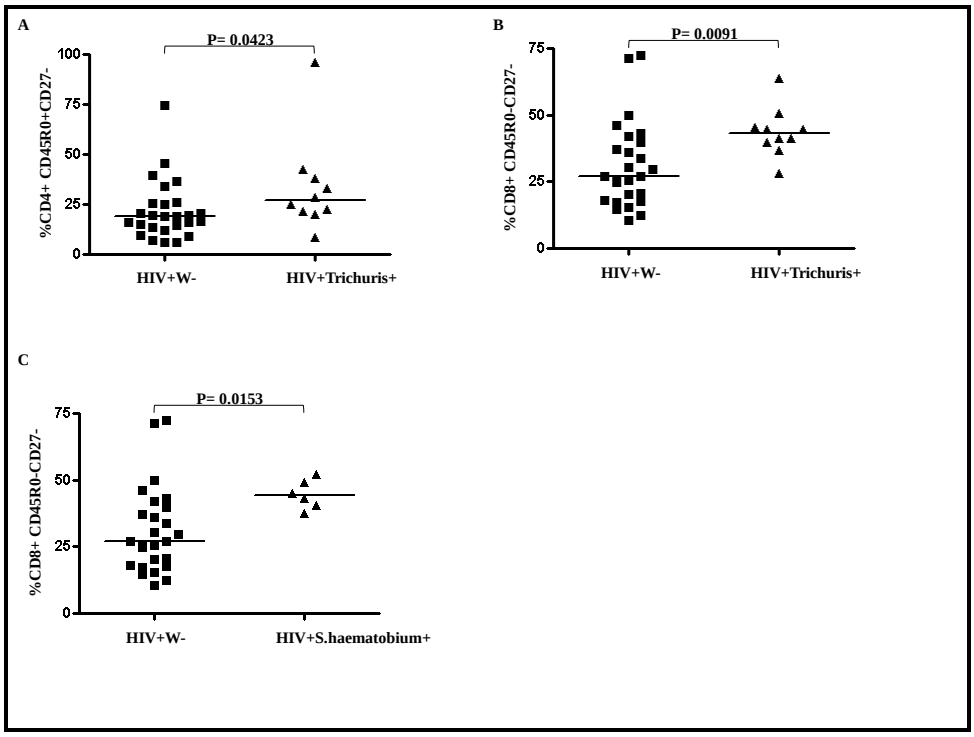


Figure 3- 18: Frequency of T-cell subsets in relation to HIV-1 co-infection with *T.trichiura* or *S. haematobium*. The frequencies of CD45RO+CD27-(**A**), CD45RO-CD27-**(B-C),** on CD4 **(A)** and CD8 **(B-C)** T cells is shown between the control and HIV+worm co-infected subjects. Statistical analysis was performed using Mann-Whitney test for comparing groups.

3.3.1.2. Effect of treatment with praziquantel and albendazole on T cell subsets in HIV negative and HIV positive individuals

To investigate the effect of treating helminths on the profiles of CD4 and CD8 T cell subsets, frequencies of different T cell subsets from helminth infected individuals who are either HIV negative or HIV positive were determined and compared before and up to one year after worm treatment. Only the individuals with no detectable worms post treatment were analysed. For logistic reasons, all study groups including controls were de-wormed. Since the effect of treatment on the frequency of T cell subsets was also observed with the control subjects in some cases, the effect of de-worming on test groups was only considered significant if changes observed in the test groups was significantly different compared to the changes observed in the control groups.

In HIV negative individuals, a minor decrease in naïve (Table 3- 11). Changes in the frequency of naïve CD4 T cells were also observed in the control group 3 months after anti helminthic treatment (Median at baseline: 34.8% (23.15-47.85%) vs Median 3 months post treatment: 40.7% (25.6-52.85%), P=0.1039, data not shown). This change differed significantly to the one observed in the worm infected group (P=0.0118). A slight increase in effector memory CD4 T cells was also observed up to 3 months after helminth treatment (Table 3- 11) but this change did not differ significantly when compared to the change observed in the control group (P=0.8318), which showed no effect of treatment (0.3497). A decrease in the median frequencies of central memory CD4 T cells was particularly observed in the worm infected group a year after treating for worms (P=0.0047, Table 3- 11). A decrease in the frequency of central memory CD4 T cells was also observed within non-infected control subjects a year post treatment, from a median of 38.7% to 32.5% (P=0.0004, data not shown). Compared to controls, changes observed in the worm infected group following treatment were significant (P=0.0101, data not shown). Also an increase in terminally differentiated CD4 T cells was seen in HIV negative individuals a year post-treatment with no substantial differences between changes observed in this group compared to changes observed in the control group (P=0.2118). A slight decline in the median frequencies of central memory and terminally differentiated CD8 T cells as well as an increased frequency of effector memory CD8 T cells was also seen in HIV negative individuals a year post treatment (Table 3- 11). When compared to non-helminth infected control subjects who also showed a significant effect of treatment, the observed median differences in the frequency of central memory and terminally differentiated CD8 T cells were minor but significant (P=0.0327 and 0.0174 respectively, data not shown). The median differences in the frequency of effector memory CD8 T cells which were observed in the worm infected group were insignificant when compared to control subjects (P=0.8661, data not shown), which showed no effect of treatment.

Table 3- 11. Expression of maturation markers on CD4 and CD8 T cells at before and after de-worming on HIV negative individuals

	%CD4+Naive	%CD4+Central Memory	%CD4+Effector Memory	%CD4+Terminally differentiated	%CD4+Total Memory	%CD8+Naive	%CD8+Central Memory	%CD8+Effector Memory	%CD8+Terminally differentiated
HIV- (N^a=133)						**HIV-(N^a=132)**			
With infection at baseline	38.6(27.75-47.55)	37.7 (31.75-43.05)	18.6 (14.35-25.85)	2.42 (1.3-4.315)	61.45 (52.41-72.41)	40.95 (29.1-54.3)	14.2 (10.3-19.95)	8.825 (5.145-15.25)	30.65 (19.1- 41.3)
3 months after treatment	37.2 (26.65-45)	37.5 (32.9-44.25)	19.2 (15.1-28.8)	2.26 (1.25-4.925)	62.67 (54.89-73.36)	39.8 (27.8-53.55)	14.55 (10.45-21.7)	9.24 (5.84-15.25)	28.6 (19.25- 44.9)
P value*	**0.0067**	0.4407	**0.0142**	0.8314	**0.0079**	0.3309	0.4719	0.1818	0.7012
HIV-(N^b=115)									
With infection at baseline	40.2 (29.8-48.8)	37.2 (31.1-42.6)	18.4 (14.2-24.5)	2.42 (1.23-4.15)	59.67 (51.05-70.2)	44.2 (32.7-57.6)	14.5 (10.4-21.3)	8.365 (4.78-13.4)	28.4 (17.6-40.1)
1 year after treatment	40.9 (31.8-46.9)	35.9 (30.6-41.3)	20.1 (16.3-25.9)	2.94 (1.37-5.95)	59.84 (52.97-68.25)	45.1 (30.2-55.4)	14.1 (9.47-19.3)	8.8 (5.24-12.95)	28.4 (18.5-37.8)
P value*	0.5956	**0.0047**	**0.0236**	**0.0002**	0.5472	0.2557	**0.0106**	0.1161	**0.0271***

*P values between baseline and follow up visits performed using the Wilcoxon-matched pairs test

[a] Number of baseline vs 1-3 months after helminth treatment pairs

[b] Number of baseline vs a year after helminth treatment pairs

Although cross-sectional analysis showed no association of *S.mansoni* infection with changes in proportion of T-cell subsets, frequency of naïve CD4 T cells slightly decreased in *S.mansoni* infected subjects up to 3 months after treatment from a median of 41.3% (IQR: 33.6- 48.45%) to 40.5% (IQR: 31.2- 47.15%; P= 0.0161; data not shown). The observed median difference in this group was significant when compared to the control group (P=0.0123, data not shown). Similar non-significant decrease was seen a year after treatment. Also, a small increase in frequency of effector memory CD4 T cells was observed in the same group up to 3 months post treatment from a median of 18.4% (IQR: 11.85- 23.7 %) to 18% (IQR: 14.95- 25.6 %; P= 0.0032; Figure 3- 19A) whereas there was no change observed in the non-helminth control group (Median 20.3% to 19.8%, P=0.3497, data not shown). differences in the frequency of effector memory CD4 T cells were insignificant between *S.mansoni* infected group and controls (P=0.3314, data not shown). In addition, *S.mansoni* infected volunteers had slightly more frequency of total memory CD4 T cells up to 3 months after treatment (Median: 59.48%; IQR: 52.82- 68.84%) than at baseline (Median: 59.25%; IQR: 51.55- 66.32%; P= 0.0166. Figure 3- 19B). This change was significant when compared to the control group (P=0.0250, data not shown). An effect of treatment on the proportion of total memory CD4 T cells was also seen a year after treatment but to a non-significant level (Figure 3- 19C).

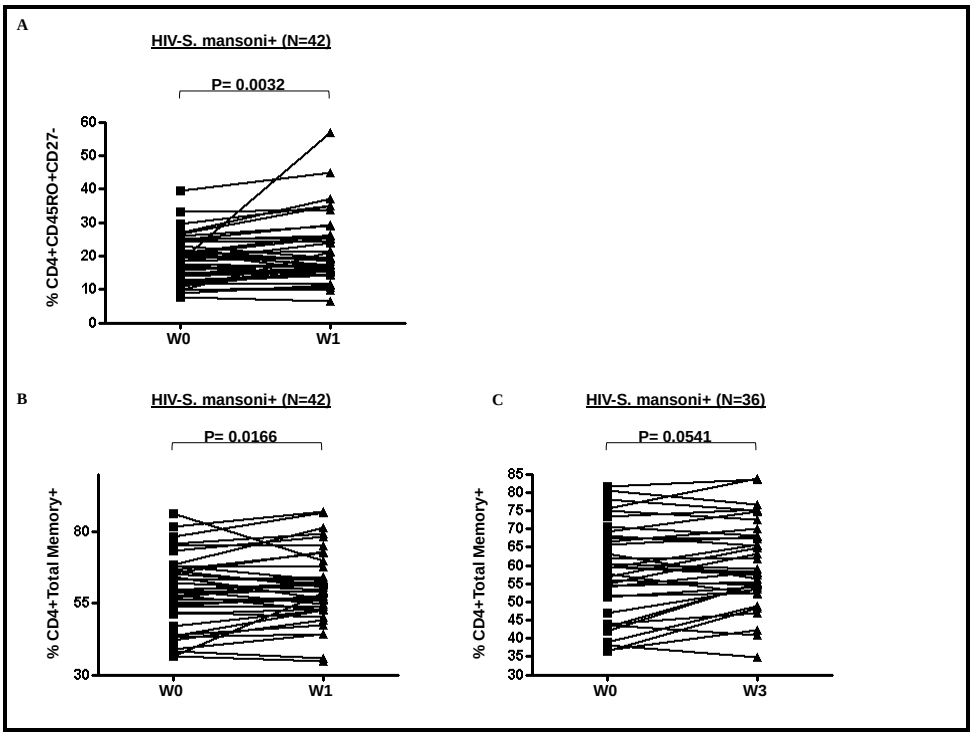


Figure 3- 19: Effect of helminth treatment on frequency of T-cell subsets in the peripheral blood of *S.mansoni* infected volunteers. Comparison of percentage CD45RO+CD27- on CD4 T cells before (W0) and 1-3 months (W1) after helminth treatment are shown in (A). (B) Shows a comparison of percentage CD4+ Total memory at W0 and W1, while (C) shows the comparison of percentage CD4+Total memory at W0 and a year after helminth treatment (W3). Total memory was defined by the expression of CD27 and CD45RO as described in the method chapter. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

Also, cross-sectional analysis showed no association of Hookworm infection with changes in proportion of T-cell subsets. However, Hookworm treatment led to a non-significant decrease in frequency of naïve CD8 T cells from a median of 47.8% (IQR: 32.55- 57.25%) to 41.7% (IQR: 28.55- 54.25%; P= 0.059) especially a year post treatment (data not shown). The observed median difference was insignificant when compared to the control group. In contrast, Hookworm treatment led to a non-significant increase in frequency of effector memory CD8 T cells from a median of 7.655% (IQR: 3.87- 17.80%) to 12.8% (IQR: 5.48- 18.05%; P= 0.0737) up to 3 months post treatment (data not shown) where as controls decreased from a median of 10.1% to 8.58% (P=0.211, data not shown). Of note, this treatment-induced change was significant when compared to the control group (P= 0.0248, data not shown).

In general, helminth treatment had a moderate impact on the frequency of different T/cell subsets. Compared to non-helminth controls, the treatment induced differences that were observed in different T-cell subsets were very minor.

In HIV positive individuals, no significant changes in the frequencies of CD4 and CD8 T cell subsets was observed up o 3 months post treatment, except for a moderate increase in central memory CD8 T cells (Table 3- 12), which was insignificant when compared to the changes observed in the non-helminth controls (P=0.1131, data not shown), which showed no effect of treatment. However, a significant increase in frequency of terminally differentiated CD4 and CD8 T cells was seen a year after treatment (Table 3- 12), but these changes were insignificant when compared to control groups (P=0.1255 and 0.1026 respectively), which showed no effect of treatment (data not shown). A significant decrease in the frequency of naïve CD8 T cells was also observed in this group a year after treating helminth infections (Table 3- 12) whereas non-helminth infected control subjects showed a decrease from a median of 22.45% to 16.95% (P=0.0161, data not shown). Compared to controls, the median differences observed in the worm infected group were insignificant (P=0.6018, data not shown).

Table 3- 12. Expression of maturation markers on CD4 and CD8 T cells at before and after deworming on HIV positive individuals

	%CD4+Naive	%CD4+Central Memory	%CD4+Effector Memory	%CD4+Terminally differentiated	%CD4+Total Memory	%CD8+Naive	%CD8+Central Memory	%CD8+Effector Memory	%CD8+Terminally differentiated
HIV+(N[a]=39)									
With infection at baseline	37.2 (25.7- 53.4)	34.9 (26.8- 39.6)	19.1 (12.4- 28.7)	2.01 (0.79- 4.17)	62.85 (46.77- 74.33)	23.1 (15.1- 33.3)	19.7 (13.5- 23.9)	13.9 (8.6- 20.3)	40.5 (28.3- 50.1)
3 months after treatment	32.7 (21.2- 49.8)	35.7 (30- 44.1)	19.8 (13.2- 30.1)	2.33 (0.85- 4.550)	67.7 (49.86- 79.1)	21.2 (16.4- 30)	21.1 (13.9- 28.5)	14.8 (10.6- 20.1)	38 (28.4- 49.1)
P value*	0.0964	0.0815	0.1958	0.5325	0.0978	0.2389	**0.0382**	0.3825	0.4663
HIV+(N[b]=39)									
With infection at baseline	37.2 (25.7- 53.4)	35.2 (25.8- 42)	19.2 (11.9- 25.9)	2.29 (0.73- 4.17)	62.85 (46.77- 74.33)	21 (14.5- 32.3)	19.5 (13.5- 23.1)	14.8 (10.1- 20.6)	40.5 (30.6- 50.3)
1 year after treatment	32 (22.9- 44.6)	32.7 (24.6- 41.5)	22.8 (17.1- 30.1)	4.11 (1.81- 11.3)	67.5 (55.4- 77.14)	17.5 (12.5- 24.5)	14.1 (12.2- 20.3)	15.6 (10.1- 22.1)	49.3 (32.4- 55)
P value*	0.2875	0.4534	**0.0205**	**0.0044**	0.3102	**0.0077**	**0.0531**	0.2567	**0.0091**

*P values between baseline and 1-3 months follow up performed using the Wilcoxon-matched pairs test

[a] Number of baseline vs 1-3 months after helminth treatment pairs

[b] Number of baseline vs a year after helminth treatment pairs

The number of HIV+*T.trichiura* infected group pairs was too small to make a comparative analysis of the effect of treatment on T cell subsets.

Treatment of *S.haematobium* infected volunteers (N=5) seemed to decrease frequency of naïve, but increase frequency of terminally differentiated CD4 and CD8 T cells a year after treatment (P= 0.0625 for all). Frequency of naïve CD4 T cells decreased from a median of 40.3% (IQR: 26.95-51.25%) to 32% (IQR: 20.64-38.95 %) but where insignificant compared to non-helminth infected controls (P=0.1023, data not shown). The decrease in frequency of naïve CD8 T cells was from 20.5% (IQR: 18.7-26.9%) to17.4% (IQR: 13.3-20.4%; data not shown) where as controls showed a median decrease from 22.45% to 16.95% (P=0.0161, data not shown). Compared to controls, the differences in the frequency of naïve CD8 T cells were insignificant (0.7919, data not shown) In contrast, frequency of terminally differentiated CD4 T cells increased by about 4-fold, from a median of 2.82% (IQR: 1.89- 8.39%) to 11.2% (IQR: 3.77- 15.45%; Figure 3- 20A). these changes observed were minor when compared to the control group (P=0.0512, data not shown) which showed no effect of worm treatment. Median frequencies of terminally differentiated CD8 T cells were increased from 43.3% (IQR: 39- 48.55%) to 55.1% (IQR: 51.4-63.3%; Figure 3- 20B) where as within the control group, the median increase was from 23.2% to 27.1% (P=0.7334, data not shown). These treatment induced changes which were observed within the worm infected group were significant when compared to controls (P=0.0307, data not shown). *S.haematobium* infected volunteers also had slightly higher frequency of total memory CD4 T cells a year post treatment (Median: 67.5%; IQR: 61.42- 79.51%) than at baseline (Median: 60.29%; IQR: 48.94-72.76%; P=0.0625. Figure 3- 20C) which were insignificant when compared to controls (P=0.4850, data not shown). A 1.5-fold decline in the frequency of central memory CD8 T cells from a median of 21.3% (IQR: 17.35-22.95%) to 13.9% (IQR: 10.34-16.7%) was observed as well in this group, one year post treatment (Figure 3-20D) which were also insignificant when compared to controls.

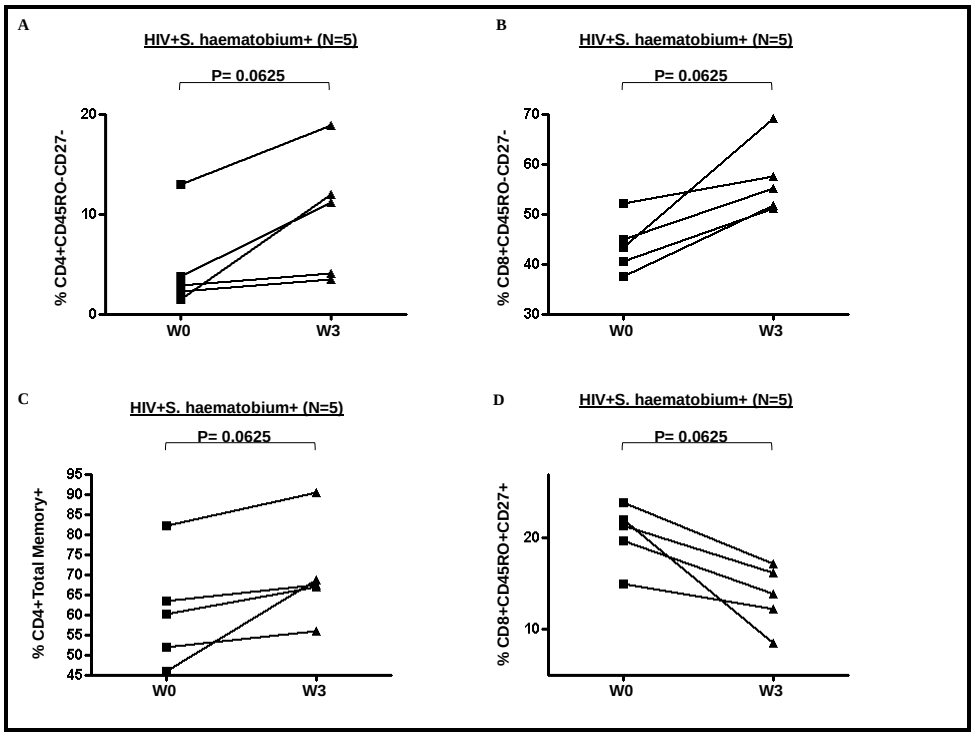


Figure 3- 20: Effect of helminth treatment on the frequency of T-cell subsets in the peripheral blood of HIV+ *S.haematobium* co-infected volunteers. Shows comparison of percentage CD4+CD45RO-CD27- (A), CD8+CD45RO-CD27- (B), CD4+Total memory (C) and CD8+CD45RO+CD27+ (D) before (W0) and a year (W3) after helminth treatment. Total memory was defined by the expression of CD27 and CD45RO as described in the method chapter 2.5. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

Although cross-sectional analysis showed no association of Hookworm co-infection with changes in proportion of T-cell subsets, HIV+Hookworm infected volunteers demonstrated a moderate decrease in frequency of naïve CD4 T cells only up to 3 months after treatment from a median of 30.8% (IQR: 23.1- 55.45%) to 28.5% (IQR: 6.84- 49.45%; P= 0.0479; Figure 3- 21A). However, the statistical power was lost a year after treatment (P= 0.7148, data not shown). The observed changes were insignificant when compared to the control groups (data not shown). Similarly, a significant decrease in the frequency of naïve CD8 T cells from a median of 21.1% (IQR: 10.13- 37.75%) to 16.1 % (IQR: 6.79- 22.7%; P= 0.0052) was observed in the same group especially a year after treating helminth infections (Figure 3- 21B) which was also insignificant when compared to the control group (P=0.4253). Treatment of Hookworm infection was associated with increased frequency of terminally differentiated CD4 and CD8 T cells a year after treatment. Frequency of terminally differentiated CD4 T cells increased by 3-fold, from a median of 1.99% (IQR: 0.475-

9.24%) to 6.025% (IQR: 2.12- 16.1%; P= 0.058; Figure 3- 21C). this change was insignificant when compared to the control group (P=0.1427, data not shown). Median frequencies of terminally differentiated CD8 T cells increased from 31.25% (IQR: 24.6- 55%) to 49.65% (IQR: 35.9- 58.95%; P= 0.0245; Figure 3- 21D). this treatment induced change was significant when compared to the control group (P=0.0448)

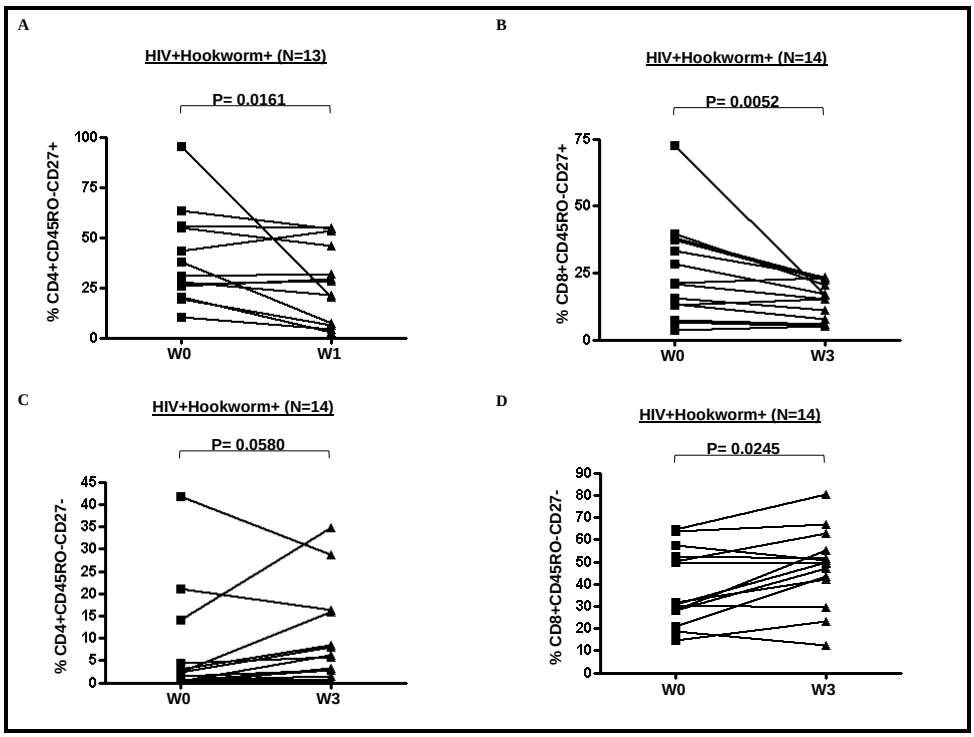


Figure 3- 21: Effect of helminth treatment on the frequency of T-cell subsets in the peripheral blood of HIV+Hookworm co-infected volunteers. Comparison of percentage CD4+CD45RO-CD27+ (A), before (W0) and 1-3 months (W1) after helminth treatment is shown in (A). Frequency of CD8+CD45RO-CD27+ (B), CD4+CD45RO-CD27- (C) and CD8+CD45RO-CD27- (D) is also shown at W0 and and a year (W3) after helminth treatment. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

Collectively, this data shows that helminth treatment has a moderate influence in the frequency of different T-cell subsets studied in relation to HIV and helminth co-infections. Compared to non-helminth controls, the treatment induced differences that were observed in different T-cell subsets were very minor.

3.3.2. Characterization of CD4 and CD8 T cell subsets in relation to chronic HIV-1 infection

HIV infection independent of helminth infection showed a significant effect on the frequency of central memory CD4 T cells and frequencies of all studied CD8 T cell subsets (Figure 3- 22A-B). A decline in the frequency of naïve CD8 T cells was observed within HIV positives with (Median: 20.45%; IQR: 13.65-32%) and without (Median: 22.8%; IQR: 19.8-31.65%) helminth infection when compared to the HIV negative non-helminth infected group (Median: 40.6%; IQR: 32.8-52.1%; P value for both <0.0001), with no apparent influence of helminth infection (P=0.1358; data not shown). In contrast, when compared with HIV-Helminth- control group (Median: 15.2%; IQR: 9.45-22.6%), higher frequency of central memory CD8 T cells were observed in HIV+ volunteers without (Median: 22.4%; IQR: 17.8-37.2%; P=0.0032) or with (Median: 19.85%; IQR: 14.55-27.4%; P=0.0417) helminth infection with no significant influence of helminth infection (P= 0.0994; data not shown). Similarly, HIV+ volunteers with or without helminth co-infection had higher frequencies of effector memory CD8 T cells (Median: 15.75% and 14.9% respectively) compared to HIV negative controls (Median: 9.63%; P values=0.0009 and 0.0058 respectively) with no significant influence of helminth infection (P=0.987; data not shown). However, HIV infection alone was not associated with changes in the frequency of terminally differentiated CD8 T cells, but helminth co-infection was (Figure 3- 22C).

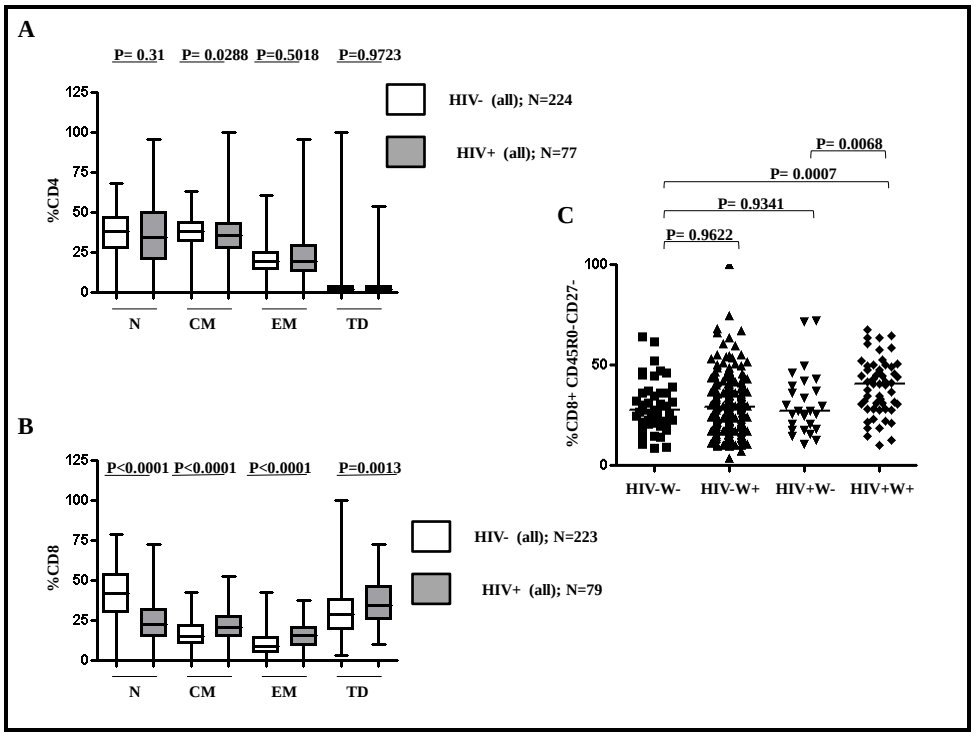


Figure 3- 22: Frequency of T-cell subsets in relation to HIV-1 infection. Median frequency of CD4 (A) and CD8 (B) T-cell subsets of HIV+ (grey) and HIV- volunteers. N, Naïve (CD45RO-CD27+); CM, Central memory like (CD45RO+CD27+); EM, Effector memory like (CD45RO+CD27-) and TD, Terminally differentiated (CD45RO-CD27-). (C) Shows the frequency of CD45RO-CD27- on CD8 T cells in relation to HIV and Helminth infections. Statistical analysis was performed using Mann-Whitney test for comparing groups.

In general, this data shows that HIV infection alone has a significant impact on T-cell subsets particularly on the CD8 T cell compartment. However, changes in the frequency of terminally differentiated CD8 T cells are even higher with helminth co-infections compared to HIV infection alone, showing an influence of helminth co-infection on modulating this T-cell compartment.

3.3.3. Expression of activation markers on CD4 and CD8 T cells in relation to chronic infection with different helminth species

3.3.3.1. Cross-sectional analysis

To examine whether helminth infections play a role in the modulation of the immune activation status, a comparison of the expression of immune activation markers on CD4 and CD8 T cells between HIV negative and HIV positive subjects with and without helminth infections was done. A representative dot plot and gating of activated CD4 and CD8 T cells is shown in Figure 3- 23A.

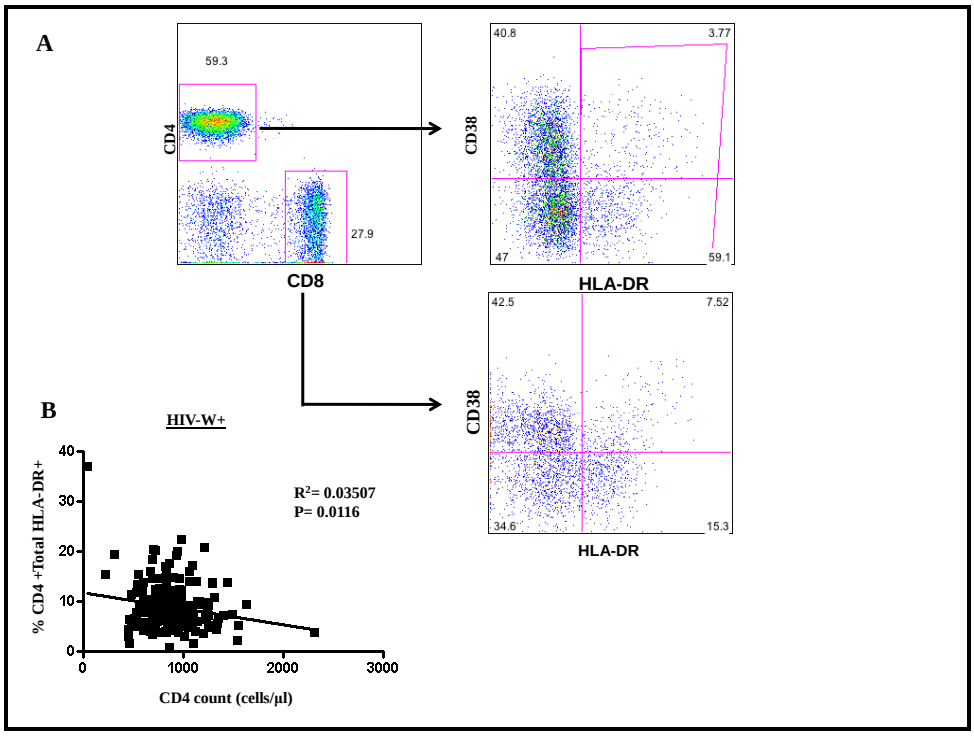


Figure 3- 23: Frequencies of expression of T-activation markers on CD4 and CD8 T cells. Shown in (A) is the gating strategy for the expression activation markers (HLA-DR+ and CD38+) on CD4 and CD8 T cells of a representative subject. A linear regression analysis of absolute CD4 counts and frequency of total HLA-DR expression on CD4 T cells is shown in (B).

Taken together as a group, helminth infected subjects had only moderately and mostly insignificant increased frequencies of HLA-DR$^+$ and/or CD38$^+$ CD4 and CD8 T cells when compared to non-infected subjects (Table 3- 13). Nonetheless, in

subjects with helminth infection the median proportion of HLA-DR$^+$/CD38$^+$ CD4 and CD8 T cells was significantly elevated from 2.16% versus 2.63% (%CD4, P=0.01) and 5.31% versus 7.1% (%CD8, p=0.04). Each of the studied helminth species modulated the percentages of HLA-DR and/or CD38 molecules on CD4 and/or CD8 T cells as described herein.

Within HIV-Helminth+ individuals, there was a linear, negative association between frequency of HLA-DR+CD4 expressing T cells and CD4 T cell counts (P= 0.0116; r^2= 0.035; Figure 3- 23B), suggesting that the observed elevated CD4 T cell activation is closely linked to loss of CD4 T cells.

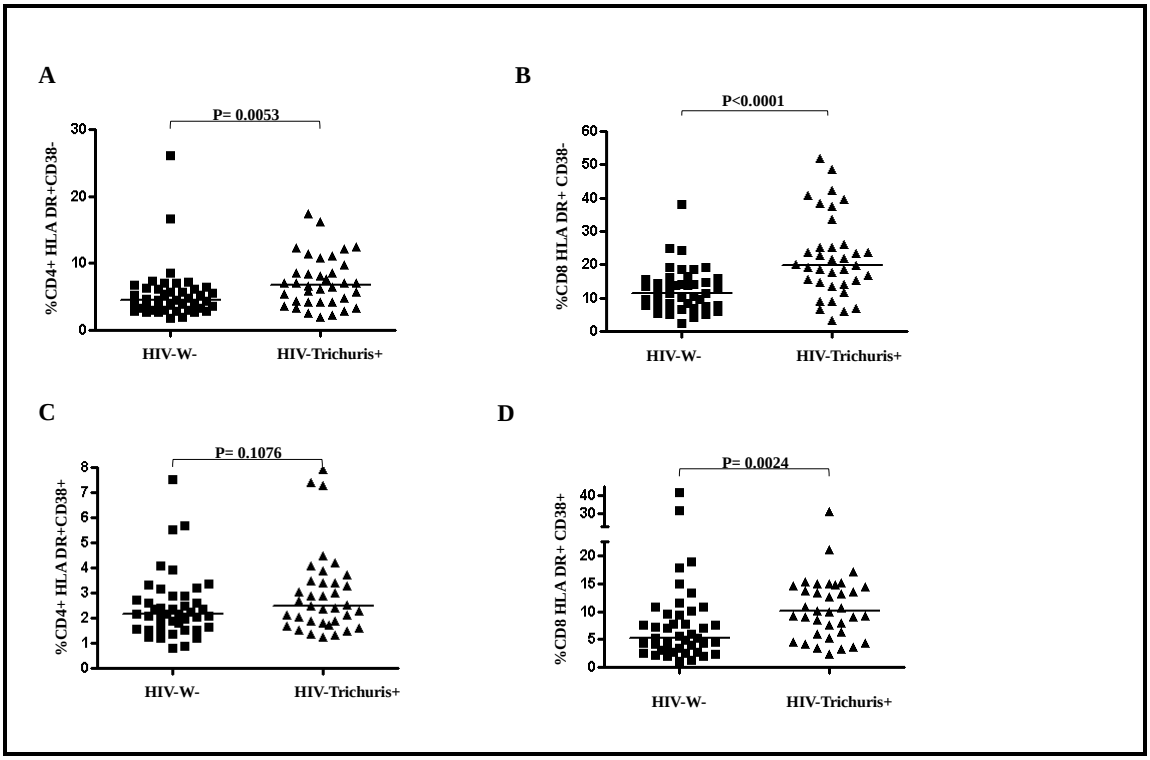
Table 3- 13. Expression of activation markers on CD4 and CD8 T cells in relation to chronic infection with different helminth species on HIV negative individuals

	HIV negative		
	No helminth	All Helminth+	P value
N (%CD4)	**43**	**181**	
%CD4+HLA-DR-CD38+	41% (30-47.9%)	44.2% (34.2-53.8%)	0.1929
%CD4+HLA-DR+CD38+	2.16% (1.55-2.87%)	2.63% (1.895-3.745%)	**0.0125**
%CD4+HLA-DR+CD38-	4.47% (3.06-6.24%)	5.12% (3.575-7.13%)	0.1752
%CD4+ Total HLA-DR+	7.01% (5.25-8.94%)	7.79% (5.89-11.23%)	**0.0741**
%CD4+ Total CD38+	43.16% (34.21-50.24%)	47.2% (37.74-57.01%)	**0.0976**
N (%CD8)	**43**	**180**	
%CD8+HLA-DR-CD38+	22.2% (13.8-33.6%)	27.15% (15.45-38.5%)	0.1284
%CD8+HLA-DR+CD38+	5.31% (3.06-9.56%)	7.095% (4.315-13%)	**0.0434**
%CD8+HLA-DR+CD38-	11.5% (7.42-15.6%)	11.55 (6.805-18.75%)	0.8436
%CD8+ Total HLA-DR+	18.62% (12.37-24.38%)	21.64% (12.21-31.4%)	0.2813
%CD8+ Total CD38+	29.61% (21.97-40.72%)	36.4% (24.52-51.36%)	**0.0388**

Percentages of HLA-DR+CD38- CD4 and CD8 T cells were markedly higher in the peripheral blood of *T.trichiura* infected volunteers (Figure 3- 24A-B). Tendency towards an increase in frequency of HLA-DR+CD38+ was also observed on CD4 T cells (1.2-fold, 2.5% vs 2.2%, P=0.1076); and even more significantly on CD8 T cells of individuals in the same group (1.9-fold, 10.0% vs 5.31%, P=0.0002, Figure 3- 24C-D). When the expression of HLA-DR was assessed independent of CD38, higher frequencies of CD4 and CD8 T cells expressing HLA-DR molecule was

observed in *T.trichiura* infected subjects compared to non-helminth infected individuals (9.5% vs 7.0%, P=0.0058 and 31.5% vs 18.6%, P<0.0001 respectively. Figure 3- 24E-F). A trend towards increased frequency of CD8 T cells expressing CD38 was also observed when the expression of CD38 was analysed independent of HLA-DR. (P=0.1023; data not shown).

There was a linear, positive association between frequency of HLA-DR+CD4 expressing T cells and frequency of Tregs (P= 0.0012; r^2= 0.2763; Figure 3- 24G) in HIV-*T.trichiura* infected subjects, suggesting that the observed elevation of Tregs is a function of increased frequency of CD4 T cell activation. A positive correlation was also observed between frequency of HLA-DR+CD4 T cells and Tregs count (P= 0.0649; Spearman r= 0.3154; data not shown), but a non-significant linear relation (Figure 3- 24H) within the same group.



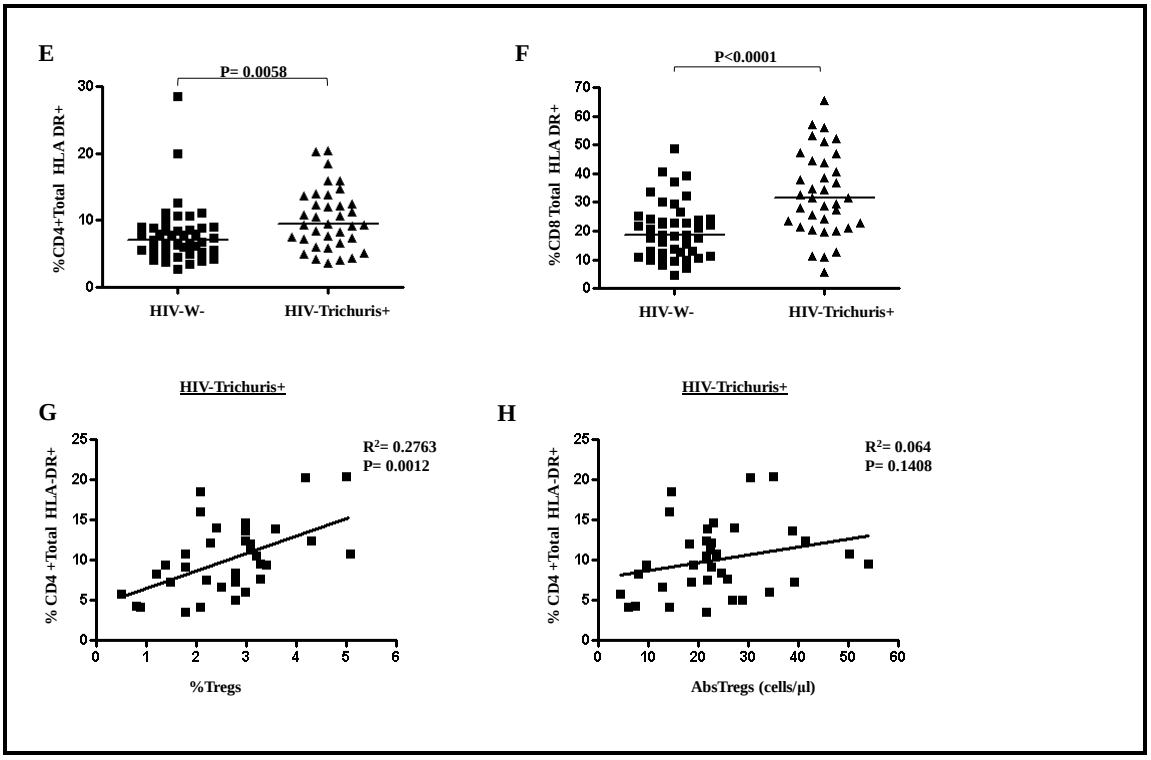


Figure 3- 24: Frequency of T-cell activation in relation to *T.trichiura* infection. The frequencies of CD4+HLA-DR+CD38-(A), CD8+HLA-DR+CD38- (B), CD4+HLA-DR+CD38+ (C), CD8+HLA-DR+CD38+(D), CD4+ Total HLA-DR+ (E) and CD8+ Total HLA-DR+ (F) T cells is shown between the control and *T. trichiura* infected subjects. Linear regression analysis of the frequency of total HLA-DR expression on CD4 and frequency (G) or numbers (H) of Treg cells is shown. Statistical analysis was performed using Mann-Whitney test for comparing groups.

Infection with *S.mansoni* seemed to increase the frequency of HLA-DR-CD38+ on CD4 and CD8 T cells of infected individuals, although not to a significant level. Median frequency of HLA-DR-CD38+ on CD4 T cells of HIV negatives with *S.mansoni* infection was 48% (IQR: 34.8- 55.4%) when compared to non-helminth infected individuals (Median: 41%; IQR: 30- 47.9%; P= 0.0686; data not shown) while that of CD8 T cells was 27.9% (IQR: 16.35- 38.85%) compared to control group (Median: 22.2%; IQR: 13.8- 33.6%; P= 0.0901; data not shown). Percentages of CD4 and CD8 expressing HLA-DR+CD38+ molecules were moderately higher in this group than the controls (P= 0.03 for both; Figure 3- 25A-B). Similarly, higher percentages of CD4 and CD8 T cells expressing CD38, independent of HLA-DR were also observed within *S.mansoni* infected group (Figure 3- 25C-D).

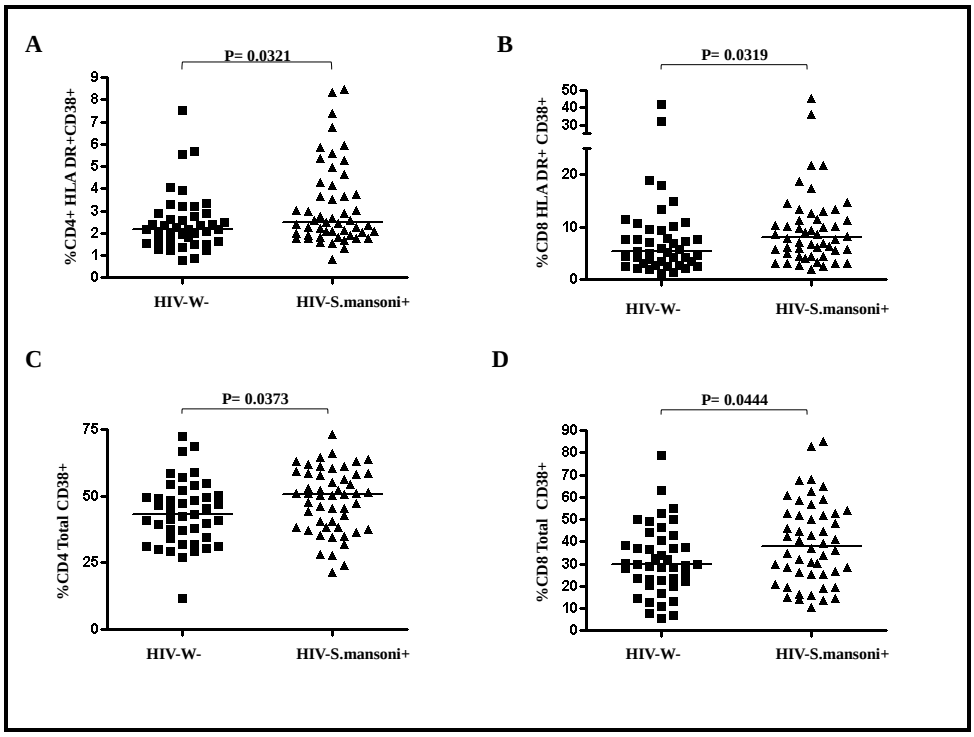


Figure 3- 25: Frequency of T-cell activation in relation to *S.mansoni* infection. The frequencies of CD4+HLA-DR+CD38+(A), CD8+HLA-DR+CD38+ (B), CD4+ Total CD38+ (C) and CD8+ Total CD38+(D) T cells is shown between the control and *S.mansoni* infected subjects. Statistical analysis was performed using Mann-Whitney test for comparing groups.

Likewise, infection with *S.haematobium* was associated with a moderate increase of frequency of HLA-DR-CD38+ expression but only on CD8 T cells (Median: 32.55%; IQR: 21.25- 46.15%) when compared to non-helminth infected individuals (Median: 22.2%; IQR: 13.8- 33.6%; P= 0.0416; Figure 3- 26A). A trend of higher frequency of CD8+CD38 expressing T cells was also observed when analysing the expression of CD38 independent of HLA-DR (Figure 3- 26B).

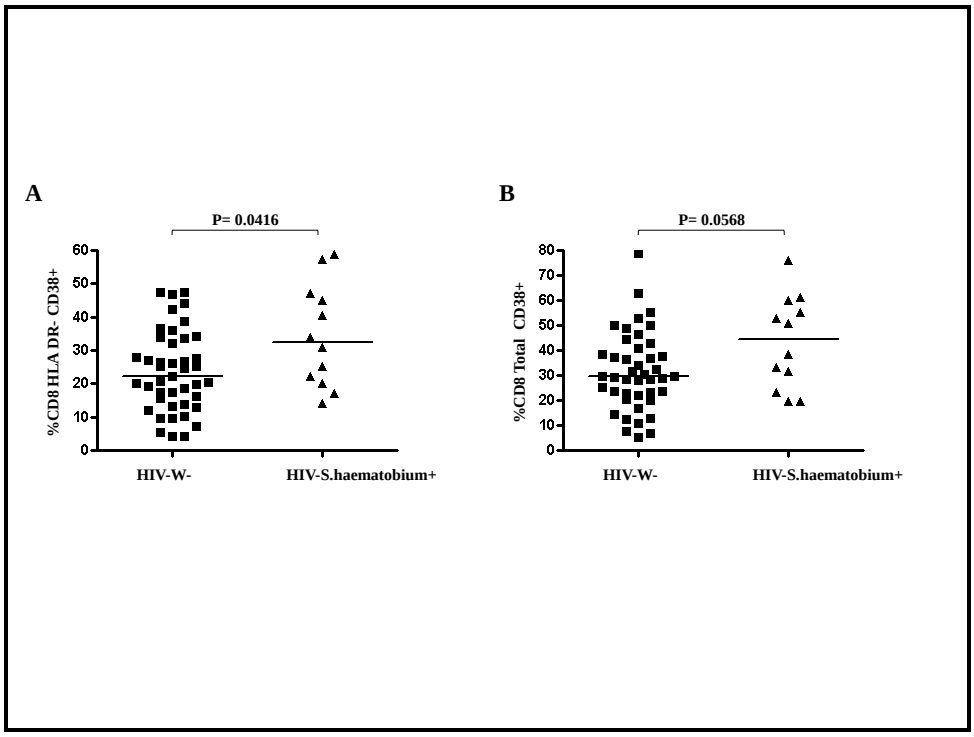


Figure 3- 26: Frequency of T-cell activation in relation to *S.haematobium* infection. The frequencies of HLA-DR-CD38+ (A) and Total CD38+ (D) on CD8T cells is shown between the control and *S.haematobium* infected subjects. Statistical analysis was performed using Mann-Whitney test for comparing groups.

Percentages of CD4 and CD8 T cells expressing HLA-DR+CD38+ were higher in the peripheral blood of *A.lumbricoides* infected volunteers compared to controls (Figure 3- 27A-B). When compared to non-infected controls, median frequencies of HLA-DR^+CD38^+ CD4 T cells were significantly elevated in subjects infected with *A.lumbricoides* by 1.5-fold (3.3% vs 2.2%, P=0.0003) while their median frequencies of HLA-DR^+CD38^+ CD8 T cells were significantly increased by1.2-fold (6.52% vs 5.31%, P=0.0413). Increase in frequency of CD4 T cells expressing HLA-DR independent of CD38 was also observed in *A.lumbricoides* infected subjects (Median: 8.915%; IQR: 6.47- 12.48%) when compared to non-infected subjects (Median: 7.01%; IQR: 5.25- 8.94%; P= 0.0165;Figure 3- 27C). Percentages of CD8 T cells expressing HLA-DR independent of CD38 seemed to be higher as well, but not to a significant level (P=0.1296; data not shown). Also, a trend towards increased frequency of CD8 T cells expressing CD38 independent of HLA-DR was observed in this group (Median: 37.52%; IQR: 25.21- 48.69%) when

compared to control subjects (Median: 29.61%; IQR: 21.97- 40.72%; P= 0.0566;Figure 3- 27D).

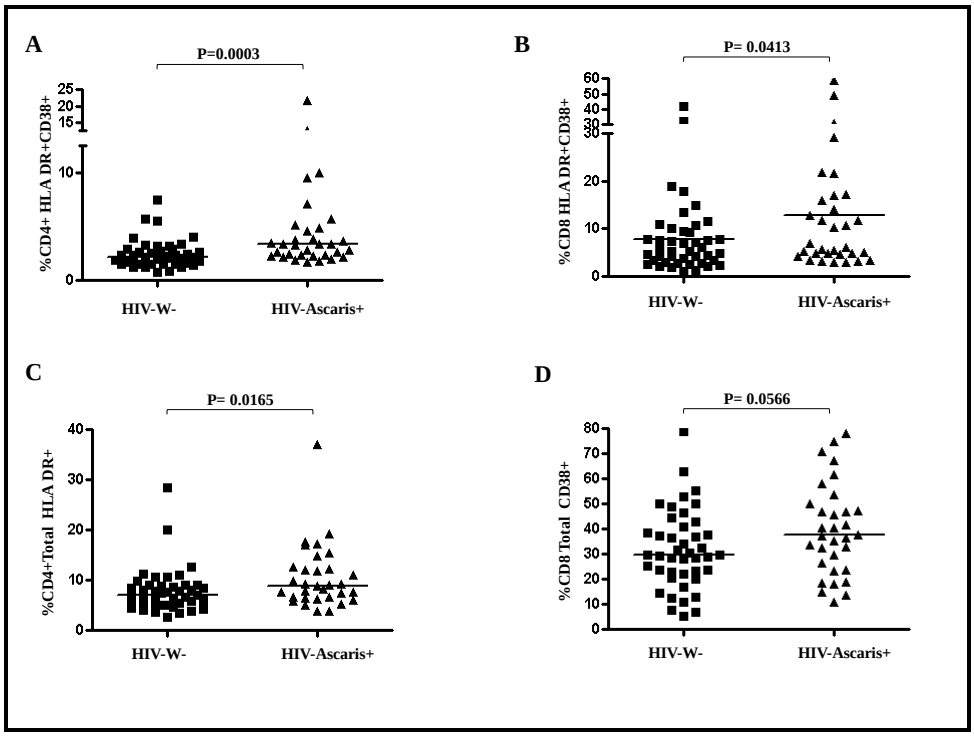


Figure 3- 27: Frequency of T-cell activation in relation to *A.lumbricoides* infection. The frequencies of CD4+HLA-DR+CD38+(A), CD8+HLA-DR+CD38+ (B), CD4+ Total HLA-DR+ (C) and CD8+ Total CD38+(D) T cells are shown between the control and *A.lumbricoides* infected subjects. Statistical analysis was performed using Mann-Whitney test for comparing groups.

To the contrary, Hookworm infected volunteers had lower median frequencies of CD8 T cells expressing HLA-DR+CD38- (Median: 7.545%; IQR: 4.25-12.6%) compared to control subjects (Median: 11.5%; IQR: 7.42-15.6%; P=0.0119;Figure 3-28A). Lower median frequencies of CD8 T cells expressing HLA-DR molecule independent of CD38 was also observed in HIV-Hookworm+ individuals (Median: 12.46%; IQR: 8.89-18.2%) than in non-helminth infected individuals (Median: 18.62%; IQR: 12.37-24.38%; P=0.0283; Figure 3- 28B). This suggests a down-regulation of the activation parameter HLA-DR on CD8 T cells in relation to Hookworm infection.

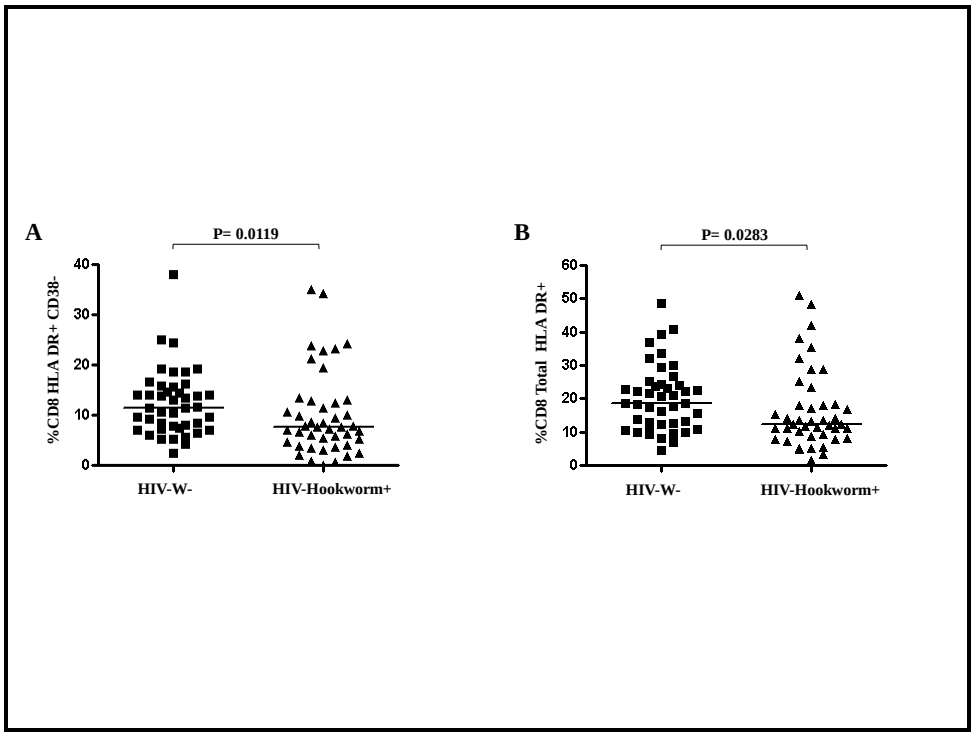


Figure 3- 28: Frequency of T-cell activation in relation to Hookworm infection. The frequencies of HLA-DR+CD38- (A) and Total HLA-DR+ (B) on CD8 T cells is shown between the control and Hookworm infected subjects. Statistical analysis was performed using Mann-Whitney test for comparing groups.

In HIV positive volunteers, helminth infections in general had no apparent influence on the frequency of expression of immune activation parameters on T cells, except for up-regulation of percentages of CD8 T cells expressing HLA-DR+CD38- and HLA-DR independent of CD38 (Table 3- 14).

Table 3- 14. Expression of activation markers on CD4 and CD8 T cells at in relation to chronic infection with different helminth species on HIV positive individuals

	HIV positive		
	No helminth	All Helminth+	P value
N (%CD4)	**26**	**51**	
%CD4+HLA-DR-CD38+	41.15% (30.95-52.85%)	42.9% (33.1-48.7%)	0.6627
%CD4+HLA-DR+CD38+	6.945% (2.79-14.65%)	9.81% (4.99-14.7%)	0.1664
%CD4+HLA-DR+CD38-	7.47% (4.235-11.9%)	8.72% (5.35-15.2%)	0.234
%CD4+ Total HLA-DR+	16.8% (7.815-26.88%)	20.43% (10.64-31.56%)	0.1536
%CD4+ Total CD38+	49.04% (44.55-66.05%)	51.7% (42.25-63.59%)	0.9871
N (%CD8)	**26**	**53**	
%CD8+HLA-DR-CD38+	21.1% (14-31.25%)	20.2% (13.15-26.35%)	0.5679
%CD8+HLA-DR+CD38+	24.5% (10.33-36.8%)	28.15% (15-41%)	0.2626
%CD8+HLA-DR+CD38-	11.7% (6.675-18.95%)	16.1% (9.325-24.85%)	**0.03**
%CD8+ Total HLA-DR+	35.53% (26.83-50.72%)	50.15% (35.05-60.38%)	**0.0269**
%CD8+ Total CD38+	42.8% (30.3-70.55%)	48.17% (32.46-71.5%)	0.5716

However, co-infection with mainly *T.trichiura* influenced the expression of immune parameters HLA-DR and CD38 on both CD4 and CD8 T cells. Frequencies of CD4 and CD8 T cells expressing HLA-DR+CD38+ were higher in the peripheral blood of *T.trichiura* co-infected volunteers compared to controls (Figure 3- 29A-B). A trend towards an increase in frequency of CD4 T cells expressing HLA-DR+CD38- was also observed in this group (Median: 15.45%; IQR: 6.185- 19.3%) compared to subjects with HIV infection alone (Median: 7.47%; IQR: 4.235- 11.9%; P=0.0805; data not shown). Furthermore, HIV+ *T.trichiura*+ subjects had a significant increase in frequency of CD8 T cells expressing HLA-DR independent of CD38 (Median: 61.87%; IQR: 47.15-71.6%) when compared to non-infected subjects (Median: 35.53%; IQR: 26.83-50.72%; P=0.0042;Figure 3- 29C). Percentages of CD8 T cells expressing CD38 independent of HLA-DR seemed to be higher as well, but not to a significant level (Figure 3- 29D). Altogether, this suggests that chronic *T.trichiura* co-infection in chronically HIV infected people contributes to immune activation of CD4

and CD8 T cells which might contribute to providing more CD4 targets for HIV and acceleration of HIV disease progression respectively.

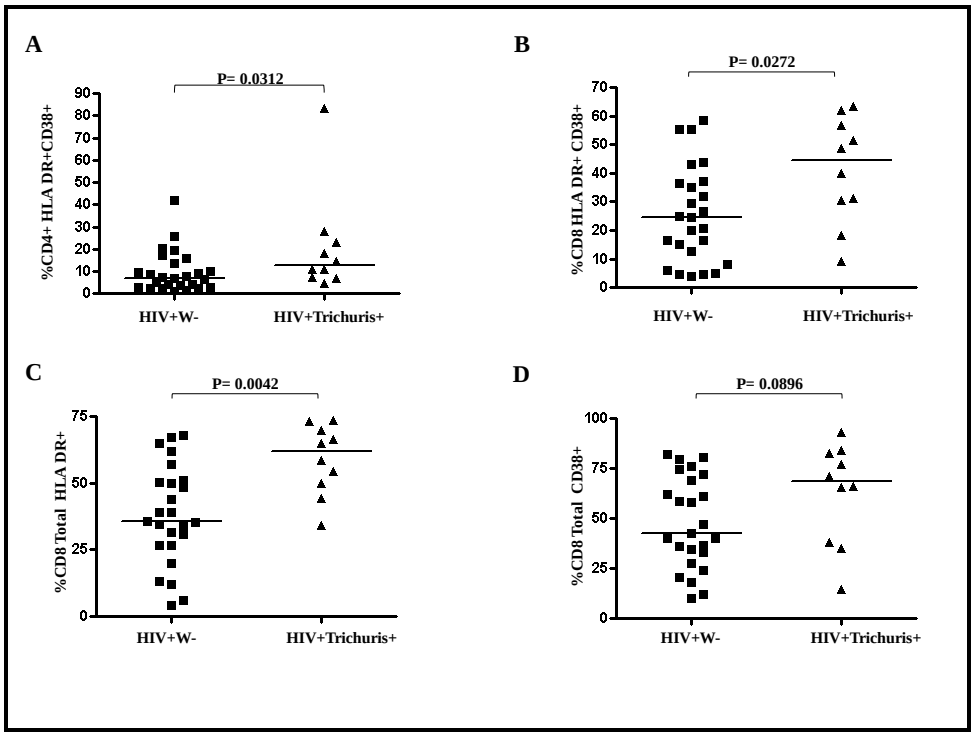


Figure 3- 29: Frequency of T-cell activation in relation to HIV-1 and *T.trichiura* co-infection. The frequencies of CD4+HLA-DR+CD38+(A), CD8+HLA-DR+CD38+ (B), CD8+ Total HLA-DR+ (C) and CD8+ Total CD38+(D) T cells is shown between the HIV+Worm- and HIV+*T.trichiura* infected subjects. Statistical analysis was performed using Mann-Whitney test for comparing groups.

Up-regulation of the frequency of HLA-DR+CD38- expression on CD8 T cells was also significantly observed on *S.haematobium* (N=6; Median: 22.1% P=0.0261) and non-significantly on Hookworm infected subjects (N=18; Median: 19.55%; P=0.0667) compared to HIV+W- subjects (N=25; Median: 11.7%; data not shown).

Figure 3- 30A-D shows a linear regression analysis between frequency of immune activation markers (HLA-DR and CD38) on T cells and CD4 T cell counts within HIV positive, helminth infected individuals. Linear regression analysis showed a negative association between frequency of total HLA-DR+CD4+ T cells and CD4 T cell counts (P= 0.0017; r^2= 0.1845; Figure 3- 30A), and that the frequency of total CD38+CD8 T cells seems to be linked to a decline in CD4 T cell counts (P= 0.0706;

r^2= 0.064; Figure 3- 30D), suggesting the role of immune activation contributed by HIV and Helminth co-infection on HIV disease progression.

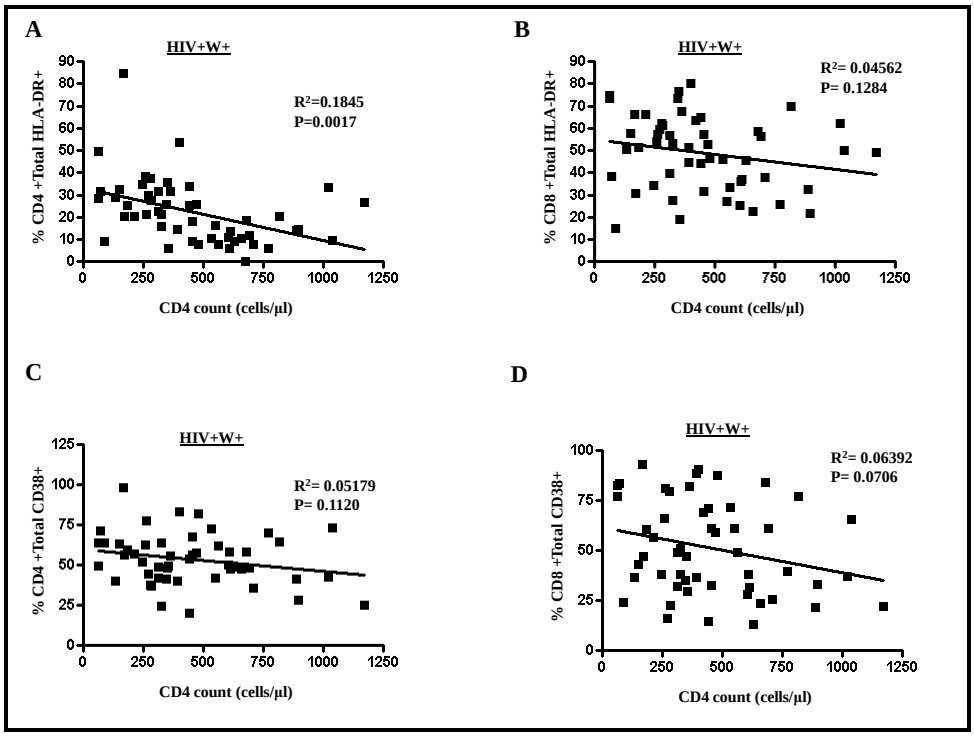


Figure 3- 30: Relationship between frequency of T-cell activation and CD4 T cell count. Linear regression analysis between CD4 T cell counts and %Total HLA-DR (A-B) or % Total CD38 (C-D) on CD4 and CD8 T cells is shown in HIV+Worm+ volunteers.

3.3.3.2. Effect of treatment with praziquantel and albendazole on the expression of activation markers on CD4 and CD8 T cells in HIV negative and HIV positive individuals

Data on the influence of anti-helminthic treatment on immune activation in worm infected individuals is still lacking. Treatment of intestinal helminth infections has been shown to reduce immune activation in HIV negative but not in HIV infected individuals (Kassu et al. 2003). In this study, the effect of treating helminths on the profiles of CD4 and CD8 T cells activation status was investigated by assessing the frequencies of HLA-DR and/or CD38 expression on T cells from helminth infected individuals who are either HIV negative or HIV positive, before and up to one year after worm treatment. Only the individuals with no detectable worms post treatment were analysed.

In general, less frequencies of activated CD4 and CD8 T cells were observed in helminth infected HIV negative subjects particularly a year post treatment (Table 3- 15). However, only very minor changes were observed in HLA-DR and or CD38 expression on CD4 and CD8 T cells with no substantial differences between helminth infected and the control group. For example, treatment with albendazole and praziquantel was associated with decreased median frequencies of total HLA-DR expression on CD4 (7.78% to 7.03%; P=0.0001) and CD8 T cells (21.6% to 17.16%; P<0.0001) a year after treatment, with no significant differences between these changes in median frequencies to that of non-infected individuals who also showed a moderate significant reduction in the median frequencies of HLA-DR+ T cells a year after treatment (Table 3- 15).

Table 3- 15. Expression of activation markers on CD4 and CD8 T cells at before and after de-worming of HIV negative individuals

Status	WormNeg	Trichuris	S.h	S.m	Ascaris	Hookworm	All Worm
%CD4 HLA-DR+CD38+							
N (pairs)	33	22	7	42	25	32	128
Median (IQR) Baseline	2.17 (1.575-2.875)	2.445 (1.668-3.425)	1.92 (1.69-4.68)	2.425 (1.865-4.723)	3.37 (2.25-4.21)	2.76 (2.118-3.688)	2.665 (1.9-3.815)
Median (IQR) 3months after treatment	1.98 (1.695-2.68)	2.21 (1.708-2.773)	1.66 (1.22-5.04)	2.575 (1.843-3.42)	2.45 (2.05-3.17)	2.31 (1.805-3.328)	2.415 (1.855-3.328)
P (Baseline vs 3months after treatment)*	0.2823	0.1727	0.375	**0.0172**	**0.09**	0.6808	**0.0043**
P(Infected vs. controls)**		0.8975	0.6057	0.3873	0.3149	0.8337	0.569
N (pairs)	25	21	11	36	17	25	110
Median (IQR) Baseline	2.38 (1.615-3.26)	2.49 (1.85-3.57)	1.73 (1.57-3.86)	2.455 (1.933-3.67)	2.8 (2.18-4.86)	2.59 (1.745-3.115)	2.475 (1.843-3.55)
Median (IQR) 1year after treatment	1.94 (1.28-2.305)	2.36 (1.55-3.12)	1.8 (0.89-2.89)	2.13 (1.465-3.23)	2.63 (1.605-4.305)	2.05 (1.52-2.735)	2.13 (1.515-3.145)
P (Baseline vs 1year after treatment)*	**0.0047**	**0.0199**	**0.0244**	**0.0112**	0.185	0.2878	**< 0.0001**
P(Infected vs. controls)**		0.5589	0.7572	0.5624	0.8981	0.2003	0.4027
%CD8 HLA-DR+CD38+							
N (pairs)	33	22	7	41	25	32	127
Median (IQR) Baseline	5.31 (2.88-10.5)	9.59 (5.103-14.7)	7.39 (2.68-16.7)	7.91 (4.975-12.05)	6.05 (4.81-16.45)	5.535 (3.445-8.893)	7.03 (4.34-13.3)
Median (IQR) 3months after treatment	5.52 (3.805-8.62)	7.55 (4.418-12.25)	8.59 (2.63-17.6)	5.59 (4.05-10.5)	6.4 (3.4-9.42)	5.725 (4.345-7.58)	6.16 (3.93-10.5)
P (Baseline vs 3months after treatment)*	0.4111	**0.0023**	0.4688	**0.0413**	**0.0803**	0.6946	**0.0063**
P(Infected vs. controls)**		0.2036	0.2936	0.3331	0.2148	0.4084	0.5284
N (pairs)	25	21	11	36	17	25	110
Median (IQR) Baseline	6.03 (3.63-11.2)	9.22 (5.825-11.75)	6.39 (3.05-16.4)	8.07 (5.158-12.55)	10.8 (3.8-17.15)	4.45 (3.39-7.405)	7.275 (4.328-12.48)
Median (IQR) 1year after treatment	3.91 (2.465-5.375)	5.46 (3.6-10.2)	3.23 (1.82-11.1)	7.745 (3.028-11.75)	7.99 (3.09-11.35)	4.91 (3.22-7.05)	5.86 (3.13-10.2)
P (Baseline vs 1year after treatment)*	**0.0011**	**0.0049**	**0.0039**	0.2018	**0.0373**	0.6092	**0.0001**
P(Infected vs. controls)**		0.9297	0.6307	**0.0585**	0.6446	**0.0478**	0.1408
%CD4 HLA-DR+CD38-							
N (pairs)	33	22	7	42	25	32	128
Median (IQR) Baseline	4.51 (3.215-5.97)	6.02 (4.023-10.9)	4.35 (3.06-6.07)	4.315 (3.165-5.835)	5.45 (3.62-7.77)	4.605 (2.988-6.723)	4.765 (3.415-6.833)
Median (IQR) 3months after treatment	4.44 (3.475-5.905)	5.51 (4.075-7.43)	4.59 (2.5-5.99)	4.875 (3.788-7.558)	4.7 (4.005-6.960)	4.49 (3.313-7.253)	4.745 (3.875-7.04)
P (Baseline vs 3months after treatment)*	0.7681	**0.0441**	0.5781	**0.0005**	0.502	0.8737	0.4586
P(Infected vs. controls)**		**0.0956**	0.8727	**0.0033**	0.7775	1	0.5148
N (pairs)	25	21	11	36	17	25	110
Median (IQR) Baseline	4.31 (2.95-6.21)	7 (4.195-9.185)	4.94 (3.06-6.14)	4.56 (3.303-5.93)	5.68 (3.23-7.24)	4.6 (3.03-6.355)	4.855 (3.543-7.003)
Median (IQR) 1year after treatment	4.52 (3.47-5.54)	4.84 (3.595-7.795)	4.26 (1.53-5.6)	3.975 (3.168-5.795)	4.47 (3.83-5.175)	4.33 (3.42-6.27)	4.38 (3.4-5.95)
P (Baseline vs 1year after treatment)*	0.2012	**0.002**	**0.0029**	0.1356	0.185	0.7672	**0.0008**
P(Infected vs. controls)**		0.12	0.2164	0.8546	0.8077	0.6003	0.5615
%CD8 HLA-DR+CD38-							
N (pairs)	33	22	7	41	25	32	127
Median (IQR) Baseline	11.6 (7.58-15.1)	19.7 (13.58-28.13)	6.55 (5.69-11.6)	11 (6.595-14.45)	12 (8.89-16.75)	7.845 (4.873-17.9)	11.3 (6.63-17.7)
Median (IQR) 3months after treatment	12.4 (8-16.55)	15.2 (11.02-25.5)	7.47 (4.85-14.7)	9.15 (6.035-12.9)	11.9 (8.13-17.3)	9.565 (6.17-14.3)	11 (6.65-16.1)
P (Baseline vs 3months after treatment)*	0.8442	**0.0495**	1	0.9638	0.8296	0.9627	0.337
P(Infected vs. controls)**		0.0922	1	0.8236	0.6153	0.9738	0.5161
N (pairs)	25	21	11	36	17	25	110
Median (IQR) Baseline	10.7 (7.3-15.75)	19.3 (14.55-24.6)	10.3 (5.69-18.9)	11.15 (7.193-15.7)	12 (8.89-23.95)	7.26 (5.025-10.32)	11.3 (6.965-18.68)
Median (IQR) 1year after treatment	10.1 (6.22-15.55)	15.7 (7.945-21.45)	7.55 (3.43-13)	9.19 (4.895-14.35)	7.78 (3.925-19.35`0	6.6 (4.52-14.5)	9.44 (5.095-17.2)
P (Baseline vs 1year after treatment)*	**0.0803**	**0.0018**	**0.0098**	**0.0448**	0.1698	0.5272	**< 0.0001**
P(Infected vs. controls)**		0.3319	0.5365	0.9357	0.9591	0.3721	0.9661
%CD4 Total HLA-DR+							
N (pairs)	33	22	7	42	25	32	128
Median (IQR) Baseline	7.45(5.38-8.745)	9.555 (6.005-13.69)	6.08 (4.98-10.75)	7.145 (5.125-10.13)	8.83 (6.55-12.1)	7.58 (5.665-10.19)	7.735 (5.83-11.7)
Median (IQR) 3months after treatment	6.88 (5.06-8.73)	7.645 (6.718-11.6)	6.45 (3.97-9.8)	7.36 (5.505-10.38)	7.29 (6.34-11.85)	7.715 (5.655-9.928)	7.455 (6.07-10.6)
P (Baseline vs 3months after treatment)*	0.2313	0.1353	1	0.2822	**0.0851**	0.9329	0.4201
P(Infected vs. controls)**		0.3671	0.9716	0.1511	0.2683	0.5288	0.8
N (pairs)	25	21	11	36	17	25	110
Median (IQR) Baseline	7.81 (5.29-9.625)	9.37 (6.995-12.42)	7.02 (4.98-10)	7.35 (5.403-9.855)	9 (5.765-11.6)	6.88 (4.92-9.46)	7.775 (5.668-10.58)
Median (IQR) 1year after treatment	6.74 (4.525-7.66)	7.3 (6.43-10.44)	7.18 (2.71-7.57)	6.975 (5.063-8.868)	6.93 (5.435-9.655)	6.89 (4.98-8.675)	7.035 (5.068-9.38)
P (Baseline vs 1year after treatment)*	**0.0106**	**0.0037**	**0.0051**	**0.032**	0.2366	0.6865	**0.0001**
P(Infected vs. controls)**		0.6672	0.9179	0.5526	0.6817	0.2687	0.5788
%CD8 Total HLA-DR+							
N (pairs)	33	22	7	41	25	31	127
Median (IQR) Baseline	20.71 (12.94-24.67)	33.54 (20.74-45.15)	22.95 (8.09-24.29)	20.71 (11.31-26.72)	24.3 (13.04-33.28)	13.61 (10.15-28.75)	20.92 (11.76-29)
Median (IQR) 3months after treatment	19.43 (14.18-26.72)	26.86 (18.88-37.43)	23.29 (7.07-27.21)	14.89 (9.94-27.55)	18.87 (12.57-28.85)	16.01 (10.87-24.66)	19.7 (11.63-27.6)
P (Baseline vs 3months after treatment)*	0.1334	**0.0025**	1	0.134	**0.0465**	0.9453	**0.0048**
P(Infected vs. controls)**		0.1009	0.2547	0.6834	**0.0976**	0.2649	0.5992
N (pairs)	25	21	11	36	17	25	110
Median (IQR) Baseline	18.62 (13.12-28.4)	29.3 (21.87-39.3)	22.95 (10.74-26.89)	20.42 (13.84-26.35)	25.49 (11.99-39.9)	11.71 (9.1-16.75)	21.16 (11.75-29.83)
Median (IQR) 1year after treatment	13.59 (10.22-20.3)	23.99 (13.92-29.05)	16.83 (4.76-20.43)	17.14 (10.87-26.29)	17.57 (8.805-35.25)	12.22 (8.08-22.78)	17.16 (9.47-26.97)
P (Baseline vs 1year after treatment)*	**0.004**	**0.0002**	**0.001**	**0.0146**	**0.0138**	0.5184	**< 0.0001**
P(Infected vs. controls)**		0.8255	0.7573	0.1099	0.8176	**0.0825**	0.2399
%CD4 Total CD38+							
N (pairs)	33	22	7	42	25	32	128
Median (IQR) Baseline	40.91 (31.85-53.04)	41.02 (33.65-57.42)	48.98 (33.69-67.03)	51.37 (42.37-59.65)	47.2 (36.95-59.68)	46.55 (34.82-55.01)	48.54 (36.63-58.21)
Median (IQR) 3months after treatment	42.02 (33.8-54.38)	43.2 (32.24-55.7)	42.16 (32.93-66.61)	49.8 (41.56-56.42)	49.63 (35.43-58.06)	46.55 (31.01-57.43)	47.69 (36.94-56.26)
P (Baseline vs 3months after treatment)*	0.6681	0.8202	0.1094	0.1041	0.9571	**0.098**	**0.0445**
P(Infected vs. controls)**		0.6244	0.5217	0.2882	0.9	0.2761	0.5163
N (pairs)	25	21	11	36	17	25	110
Median (IQR) Baseline	46.48 (36.07-54.41)	49.3 (35-57.65)	48.98 (33.69-63.67)	50.96 (40.46-58.44)	48.36 (41.98-59.68)	46.04 (38.89-56.77)	49.14 (38.99-58.31)
Median (IQR) 1year after treatment	44.17 (30.15-52.06)	46.79 (36.87-55.03)	45.7 (38.5-61.98)	47.08 (41.27-57.18)	47.4 (39.38-57.28)	38.28 (30.65-50.41)	45.29 (37.12-55.19)
P (Baseline vs 1year after treatment)*	**0.0238**	0.509	0.2402	0.1236	0.2977	**0.0001**	**< 0.0001**
P(Infected vs. controls)**		0.1027	0.4922	0.2877	0.489	0.4849	0.3693
%CD8 Total CD38+							
N (pairs)	33	22	7	41	25	32	127
Median (IQR) Baseline	29.61 (18.43-39.56)	30.8 (25.11-44.62)	50.8 (19.81-61.4)	39 (25.75-53.55)	36.6 (25.21-50.49)	31.42 (24.17-47.72)	35.8 (25.2-51.8)
Median (IQR) 3months after treatment	29.04 (16.66-41.43)	32.07 (23-45.56)	48.6 (19.8-70.6)	35.6 (20.54-47.26)	34.86 (19.29-44.78)	27.59 (17.13-40.7)	34.43 (20.49-46.32)
P (Baseline vs 3months after treatment)*	0.7342	0.1117	0.6875	0.2542	0.3327	0.1099	**0.0106**
P(Infected vs. controls)**		0.6207	0.9448	0.5336	0.6399	0.2328	0.3833
N (pairs)	25	21	11	36	17	25	110
Median (IQR) Baseline	29.78 (21.79-47.81)	42.3 (27.26-53.85)	38.39 (23.35-60.08)	40.78 (20.78-52.75)	40.4 (29.58-51.8)	36.19 (26.52-50.83)	40.38 (25.73-52.64)
Median (IQR) 1year after treatment	20.48 (15.42-29.64)	31.97 (22.63-41.77)	32.5 (18.83-44.98)	39.22 (23.33-53.99)	31.17 (23.44-40.89)	24.96 (20.9-38.74)	32.24 (21.94-45.15)
P (Baseline vs 1year after treatment)*	**0.0003**	**0.006**	**0.001**	0.9436	0.1556	**0.0123**	**0.0001**
P(Infected vs. controls)**		0.2607	0.5594	**0.0031**	0.8376	0.187	**0.0536**

*P values between baseline and 1-3 months follow up performed using the Wilcoxon-matched pairs test

**P values in median change with time between helminth infected and non infected controls using the Mann-Whitney test

Effective treatment of *T.trichiura* infection in HIV negative individuals was associated with decreased frequency of HLA-DR+CD38- expression on both CD4 and CD8 T cells especially a year after treating for helminths. Percentages of CD4+ HLA-DR+CD38- declined from a median of 7% (IQR: 4.195- 9.185%) to 4.84% (IQR: 3.595- 7.795%) a year after treatment (P= 0.002; Table 3- 15), while frequency of HLA-DR+CD38- CD8 T cells decreased from 19.3% (IQR: 14.55- 24.6%) to 15.7% (IQR: 7.945- 21.45%; P= 0.0018; Table 3- 15). No decline was observed in the helminth negative group (Table 3- 15). Compared to the control group, the treatment induced change in the median frequencies of HLA-DR+CD38- CD4 and CD8 T cells was insignificant in *T.trichiura* infected subjects (P=0.5615 and 0.9661 respectively, Table 3- 15).

Only very minor changes in HLA-DR expression on CD4 T cells could be detected 3 months after helminth treatment with no substantial differences between *T.trichiura* infected and the control group. The difference between visits for this group was from a median of 9.56% HLA-DR^+ CD4 T cells to a median of 7.65% (p=0.1353), but this change did not differ significantly to the control group (p=0.3671). Median frequencies of HLA-DR^+ CD8 T cells decreased substantially in *T.trichiura* infected people (33.54% to 26.86%, p=0.0025). Similar decrease in HLA-DR^+ CD8 T cell frequencies was observed in the control group (20.71% to 19.43, p=0.1334). Compared to the control group, the decrease in HLA-DR expression was pronounced, but still insignificant in *T.trichiura* (p=0.1009) infected subjects. Similarly, percentages of total HLA-DR expression on CD4 T cells moderately declined from a median of 9.37% (IQR: 6.995- 12.42%) to 7.3% (IQR: 6.43- 10.44%) a year after treatment (P= 0.0037; Figure 3- 31A), while that of CD8 T cells moderately dropped from 29.3% (IQR: 21.87- 39.3%) to 23.99% (IQR: 13.92- 29.05%; P= 0.0002; Figure 3- 31B) with no substantial differences between *T.trichiura* infected and the control group (Table 3- 15). Changes in the frequencies of HLA-DR^+CD38^+ and total CD38+ T cells were similar to HLA-DR^+ T cells with very minor differences between these changes and that observed in the control group (Table 3- 15).

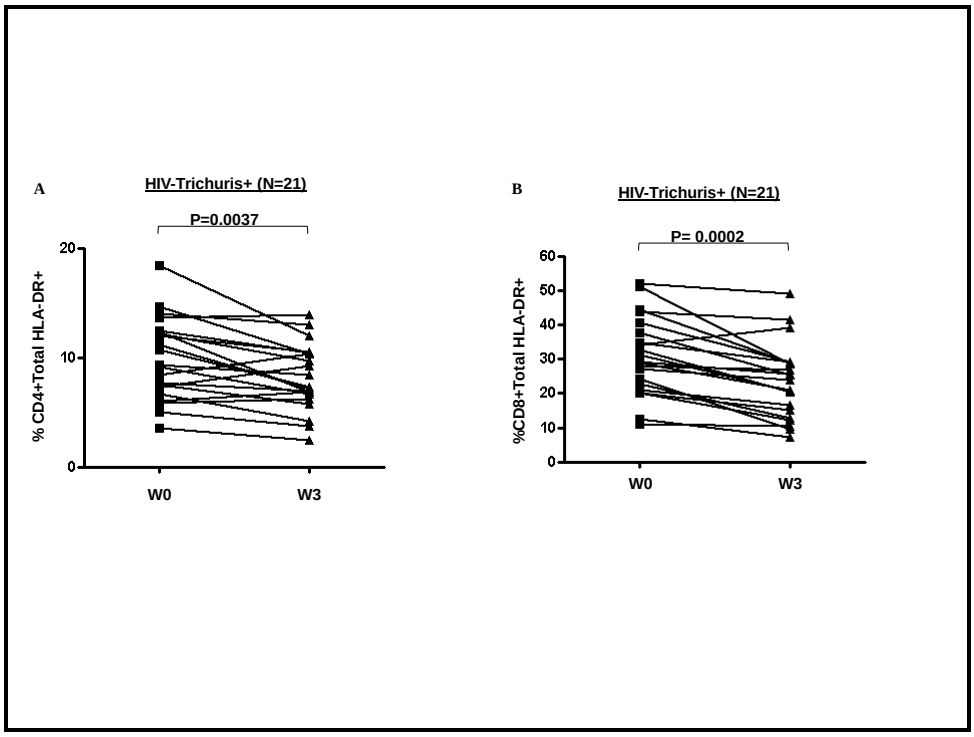


Figure 3- 31: Effect of helminth treatment on frequency of T-cell activation in the peripheral blood of *T.trichiura* infected volunteers. Comparison of percentage total HLA-DR+ on CD4 (A) and CD8 (B) T cells before (W0) and a year (W3) after helminth treatment is shown. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

A.lumbricoides infected subjects showed also decrease in the frequency of activated T cells following treatment. Particularly, median frequency of total HLA-DR+ CD8 T cells declined from 24.3% to 18.87% up to 3 months post treatment (P= 0.0465; Table 3- 15). This decline was also observed a year post helminth treatment (P= 0.0138; Table 3- 15). Compared to the control group, the decreased frequency of HLA-DR+ CD8 T cells 3 months after anti helminthic treatment was minor and insignificant in *A.lumbricoides* (P=0.0976) infected subjects (Table 3- 15). The decreased frequency of HLA-DR+ CD8 T cells a year after helminth treatment was also insignificant in *A.lumbricoides* (P=0.0976) infected subjects when compared to control who also showed a decline in the frequency of HLA-DR+ CD8 T cells after treatment (P=0.004, Table 3- 15).

For *S.mansoni* infected volunteers, median frequency of CD8 T cells expressing HLA-DR+CD38+ slightly declined from 7.91% (IQR: 4.975- 12.05%) to 5.59% (IQR: 4.05- 10.5%) up to 3 months post treatment (P= 0.0418; Table 3- 15);

but this treatment effect was not observed a year post treatment (P= 0.1993; Table 3- 15). However, compared to the control group, the decreased HLA-DR+CD38+ CD8 T cells was 3 months after anti helminthic treatment was insignificant in *S.mansoni* (p=0.3331) infected subjects (Table 3- 15). A slight but insignificant increase in the median frequency of HLA-DR-CD38+ CD8 T cells was observed a year after anti-helminthic treatment (27.9% to 28.25%, P=0.8259, data not shown). This median change was however significant (P=0.0143) compared to the median change observed within the control group (24.6% to 19.3%, P=0.0014, data not shown). Similarly, frequency of CD4+HLA-DR+CD38- T cells were slightly increased from 4.315% (IQR: 3.15- 5.85%) to 4.875% (IQR: 3.745- 7.665%) up to 3 months (but not a year) post treatment (P=0.0005; Table 3- 15). Similar median frequencies were observed between visits within the non-helminth infected controls (P=0.7681, Table 3- 15). Of note, the observed median changes in the frequency of HLA-DR+CD38- CD4 T cells within *S.mansoni* infected group was significant compared to the control group (P=0.0033, Table 3- 15). A decline in the frequency of CD8 T cells expressing HLA-DR+CD38- was observed from a median of 11.15% (IQR: 7.325- 15.2%) to 9.19% (IQR: 4.9- 13.9%) a year post treatment (P= 0.0446) with no substantial differences between the median changes in *S.mansoni* and the control group (Table 3- 15). Even though increased frequency of CD38 expression on T cells independent of HLA-DR was observed in association with *S.mansoni* infection (Figure 3- 25D), no treatment effect was observed on reducing the frequency of these cells up to a year after treatment (Table 3- 15).

Effective treatment of *S.haematobium* was unexpectedly associated with a slight decrease in frequency of HLA-DR marker on CD4 and CD8 T cells one year after treating for helminths. The frequency of HLA-DR+CD38- CD4 T cells declined from 4.94% (IQR: 3.06- 6.14%) to 4.26% (IQR: 1.53- 5.6%; P= 0.0029; Figure 3- 32A), while that of CD8 T cells was from 10.3% (IQR: 5.69- 18.9%) to 7.55% (IQR: 3.43- 13%; P= 0.0098; Figure 3- 32B). The control group showed no changes in median frequencies of HLA-DR+CD38+ T cells. Compared to the control group, the decrease in HLA-DR+CD38- expression was insignificant in *S.haematobium* infected subjects (Table 3- 15). Decreased frequency of total HLA-DR on both CD4 and CD8 T cells observed a year after treatment as illustrated on Figure 3- 32C-D; which was

also insignificant compared to the median changes in the non-helminth controls (Table 3- 15).

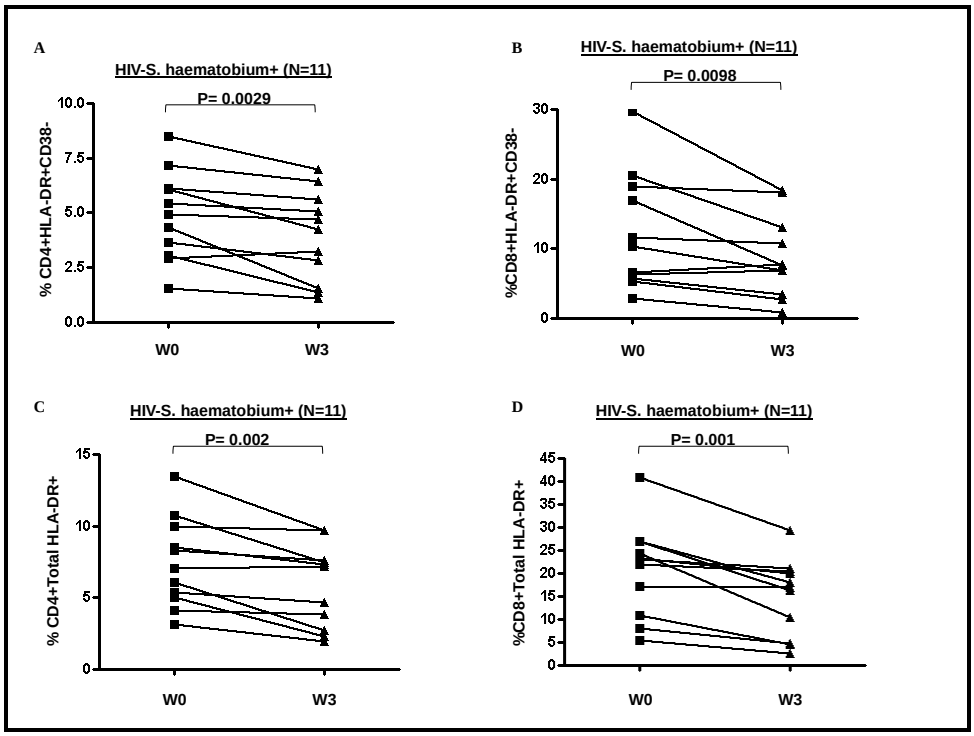


Figure 3- 32: Effect of helminth treatment on frequency of T-cell activation in the peripheral blood of *S.haematobium* infected volunteers. Comparison of percentage HLA-DR+CD38- (A-B) andTotal HLA-DR+ (C-D) on CD4 and CD8 T cells before (W0) and a year (W3) after helminth treatment is shown. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

Even though only down-regulation of frequency expression of either HLA-DR+CD38- or HLA-DR alone, independent of CD38 on CD8 T cells was observed in relation to Hookworm infection (Figure 3- 28A-B), no effect of treatment on increase of such cells could be observed up to a year post treatment (Table 3- 15). Instead, decreased frequency expression of either HLA-DR-CD38+ or CD38 alone, independent of HLA-DR on T cells was seen as a result of treatment in Hookworm infected subjects. Percentage of HLA-DR-CD38+ on CD4 T cells declined from a median of 43.9% (IQR: 36.15- 53.2%) to 36.7% (IQR: 29- 48.8%) a year after treatment (P= 0.0003; data not shown); while that of CD8 T cells dropped from 28% (IQR: 20.45- 40.65%) to 19.5% (IQR: 16.3- 32.15%; P= 0.0002; data not shown). The frequency of total CD38 expression on CD4 T cells also decreased a year after

de-worming from a median of 46.04% (IQR: 38.89- 56.77%) to 38.28% (IQR: 30.65-50.41%; P= 0.0001; Table 3- 15). Again, these changes were very minor and insignificant compared to median changes observed in the respective control groups (Table 3- 15).

Positive effect of treatment on the frequency of expression of immune activation parameters was also observed in HIV-helminth co-infected volunteers (Table 3- 16).

Table 3- 16. Expression of activation markers on CD4 and CD8 T cells at before and after deworming on HIV positive individuals

Status	WormNeg	Trichuris	S.h	S.m	Ascaris	Hookworm	All Worm
%CD4 HLA-DR+CD38+							
N (pairs)	16	5	5	5	5	13	33
Median (IQR) Baseline	8.15 (4.408-15.28)	14.7 (7.855-20.75)	8.45 (2.865-14.65)	10.7 (3.145-11.85)	6.18 (3.68-13.5)	9.09 (4.805-16.2)	9.25 (4.6-14.15)
Median (IQR) 3months after treatment	8.08 (5.245-15.2)	14.3 (8.56-23.15)	7.68 (3.6-18.9)	13.1 (8.84-21.1)	5.51 (3.515-14.95)	12.2 (5.295-32.15)	13.1 (5.385-21.1)
P (Baseline vs 3months after treatment)*	0.816	0.625	0.1875	0.125	1	**0.0327**	**0.0025**
P(Infected vs. controls)**		0.6497	0.3423	**0.0523**	0.9671	**0.0794**	**0.0768**
N (pairs)	12	4	5	6	4	14	33
Median (IQR) Baseline	6.235 (3.735-15.28)	12.8 (6.258-17.4)	8.45 (2.865-14.65)	8.495 (2.708-11.33)	5.055 (3.555-13.1)	9.17 (5.473-14.68)	9.09 (4.625-13.25)
Median (IQR) 1year after treatment	7.345 (4.59-11.8)	10.08 (3.393-17.65)	10.2 (1.67-16.65)	12.3 (3.643-32.63)	5.07 (1.495-11.24)	8.215 (4.955-14.85)	9.62 (3.405-13.5)
P (Baseline vs 1year after treatment)*	0.6772	0.25	0.8125	0.3125	0.375	0.9032	1
P(Infected vs. controls)**		0.3027	0.5621	0.2815	0.5853	0.8977	0.8675
%CD8 HLA-DR+CD38+							
N (pairs)	16	5	5	5	5	13	33
Median (IQR) Baseline	29.35 (13.6-41.7)	39.9 (24.5-50.15)	15.6 (8.8-45)	25.7 (18.95-43.05)	31.9 (17.39-43)	22.4 (12.85-37.75)	25.7 (16.15-42.05)
Median (IQR) 3months after treatment	22.4 (13.55-33.58)	38 (26.05-47.75)	22.2 (13.2-39.35)	54.6 (28.65-58.15)	26.8 (9.555-40.15)	33.3 (19.5-46.7)	33.3 (20.35-47.75)
P (Baseline vs 3months after treatment)*	0.3388	0.625	1	0.3125	0.3125	**0.0574**	0.211
P(Infected vs. controls)**		0.8364	0.4826	0.2648	0.4826	**0.014**	0.1531
N (pairs)	12	4	5	6	4	14	33
Median (IQR) Baseline	25.75 (6.645-43.58)	39.7 (21.45-54.88)	15.6 (8.8-45)	22.75 (17.83-42.68)	34.1 (15.15-46.35)	27.95 (13.53-36.35)	30.6 (16.15-39.1)
Median (IQR) 1year after treatment	22.7 (11.19-37.48)	33.2 (15.95-43.18)	9.36 (5.335-26)	27.2 (14.88-43.3)	20.1 (7.678-29.88)	16.3 (10.87-20.65)	18.3 (10.8-32.6)
P (Baseline vs 1year after treatment)*	0.791	0.875	**0.0625**	0.8438	0.125	0.104	**0.0244**
P(Infected vs. controls)**		0.4665	0.4292	0.5427	0.4665	0.5892	0.5294
%CD4 HLA-DR+CD38-							
N (pairs)	16	5	5	5	5	13	33
Median (IQR) Baseline	9.25 (5.06-11.75)	15.2 (6.185-17.9)	7.19 (5.535-16.1)	8.44 (0.67-15.32)	5.65 (4.88-12.03)	8.72 (4.56-11)	8.43 (5.175-13.7)
Median (IQR) 3months after treatment	10.65 (4.42-13.15)	15 (6.03-17.7)	9.86 (4.055-18.45)	5.96 (4.43-16.55)	6.49 (4.655-11.49)	10.8 (5.715-15.9)	9.77 (4.955-15.5)
P (Baseline vs 3months after treatment)*	0.1706	0.8125	0.625	**0.0172**	0.8125	0.4143	0.268
P(Infected vs. controls)**		0.3423	0.9671	0.5915	0.3216	0.8436	0.639
N (pairs)	12	4	5	6	4	14	39
Median (IQR) Baseline	7.47 (3.808-11.07)	12.46 (5.95-19)	7.19 (5.535-16.1)	5.28 (1.005-12.52)	5.475 (4.67-6.633)	8.545 (6.408-12.25)	8.39 (5.17-12.2)
Median (IQR) 1year after treatment	9.6 (5.175-15.38)	12 (5.543-13.65)	13.1 (5.3-18.55)	6.26 (4.748-10.52)	7.71 (4.16-8.335)	11.45 (8.995-14.63)	10.4 (6.12-13.8)
P (Baseline vs 1year after treatment)*	0.4697	0.375	0.8125	0.4375	0.875	**0.0063**	**0.0475**
P(Infected vs. controls)**		0.1296	0.8744	0.9626	0.6712	0.2577	0.7899
%CD8 HLA-DR+CD38-							
N (pairs)	16	5	5	5	5	13	33
Median (IQR) Baseline	13.1 (6.953-19.1)	15.8 (10.77-17.8)	22.2 (15.55-43.9)	7.63 (2.485-21.65)	14.6 (8.48-20.55)	17.7 (6.43-25.75)	15.9 (7.27-23.35)
Median (IQR) 3months after treatment	12.15 (7.643-17.73)	19.2 (9.69-29.2)	23.7 (12.85-38.9)	10.2 (3.565-20.55)	14.5 (7.4-24.45)	13.3 (4.99-24.8)	14.3 (7.17-24.8)
P (Baseline vs 3months after treatment)*	0.2663	0.8125	0.4375	0.8125	0.625	0.1272	0.453
P(Infected vs. controls)**		0.1266	0.5631	0.8538	0.3859	0.5986	0.7572
N (pairs)	12	4	5	6	4	14	35
Median (IQR) Baseline	8.05 (6.493-18.13)	16.05 (13.7-18.55)	22.2 (15.55-43.9)	8.235 (2.638-17.03)	12.08 (7.94-15.58)	19.55 (9.078-25.05)	16.3 (9.09-22.2)
Median (IQR) 1year after treatment	13.3 (9.367-17.98)	24.05 (17.98-38.98)	22.1 (15.95-35)	11.05 (3.86-14.65)	15.25 (11.25-25.33)	20.85 (13-30.93)	18.8 (12.5-27.8)
P (Baseline vs 1year after treatment)*	0.6772	0.125	0.4375	0.6875	0.375	0.2412	0.2132
P(Infected vs. controls)**		0.1296	0.3166	0.6734	0.5853	0.6997	0.8357
%CD4 Total HLA-DR+							
N (pairs)	16	5	5	4	5	13	33
Median (IQR) Baseline	18.07 (11.24-27.19)	34 (14.04-36.6)	15.64 (8.4-30.75)	20.89 (10.83-29.11)	10.89 (9.685-24.88)	16.11 (9.14-32.38)	18.51 (9.14-31.58)
Median (IQR) 3months after treatment	21.36 (11.1-33.2)	32.3 (14.59-39.35)	17.54 (7.655-37.35)	29.63 (14.79-33.43)	15.28 (9.2-23.77)	26.3 (13.07-47.35)	22.12 (11.95-35.9)
P (Baseline vs 3months after treatment)*	0.2052	0.8125	0.3125	0.125	1	**0.0266**	**0.0025**
P(Infected vs. controls)**		0.8365	0.6497	0.1707	0.5087	0.3687	0.4178
N (pairs)	12	4	5	5	4	14	33
Median (IQR) Baseline	17.21 (7.84-27.19)	27.06 (12.21-34.6)	15.64 (8.4-30.75)	20.43 (6.68-26.52)	10.77 (9.208-18.51)	17.1 (11.08-31.75)	16.11 (9.14-30.73)
Median (IQR) 1year after treatment	20.46 (12.45-26.78)	22.58 (8.935-30.8)	25.5 (6.97-34.1)	19.2 (8.835-32.67)	13.01 (5.91-19.09)	22.41 (14.18-28.85)	20.12 (12.27-29.3)
P (Baseline vs 1year after treatment)*	0.9097	0.125	0.8125	0.4375	1	0.3575	0.2918
P(Infected vs. controls)**		0.4617	0.4304	0.3243	0.6919	0.3693	0.3172
%CD8 Total HLA-DR+							
N (pairs)	16	5	5	5	5	13	33
Median (IQR) Baseline	41.6 (27.87-50.93)	54.3 (42.05-61.87)	53.3 (41.77-63.73)	44.48 (28.97-51.6)	46.5 (35.67-53.75)	38.28 (32.1-62.2)	46.59 (33.85-57.87)
Median (IQR) 3months after treatment	40.4 (27.4-52.03)	52.88 (48-66.85)	51.3 (40.45-61.15)	61.19 (49.2-63.52)	47.9 (23.06-55.2)	49.4 (34.83-62.02)	52.3 (45.02-61.6)
P (Baseline vs 3months after treatment)*	**0.0465**	0.8125	0.125	0.1875	0.4375	0.7354	0.6942
P(Infected vs. controls)**		0.6497	0.7102	0.1074	0.9671	0.1811	0.1971
N (pairs)	12	4	5	6	4	14	33
Median (IQR) Baseline	39.7 (17.59-59.11)	57.5 (38.13-68.7)	53.3 (41.77-63.73)	37.49 (27.03-49.09)	46.18 (30.57-54.45)	45.49 (30.67-58.9)	46.5 (32.1-57.6)
Median (IQR) 1year after treatment	33.3 (24.2-57.8)	52.45 (39.85-81.03)	41.1 (29.49-47.98)	43.4 (23.79-51.71)	35.35 (20.25-53.88)	43.4 (25.98-57.3)	42.9 (29.49-52.33)
P (Baseline vs 1year after treatment)*	0.6772	0.875	**0.0625**	1	0.625	0.5016	0.1634
P(Infected vs. controls)**		0.9517	0.5621	0.4824	0.9517	0.8977	0.9284
%CD4 Total CD38+							
N (pairs)	16	5	5	4	5	13	33
Median (IQR) Baseline	54.09 (45.15-67.43)	58 (52.9-67.96)	48.6 (39.86-59.78)	49.1 (28.88-75.69)	49.93 (44.42-75.19)	57.6 (41.87-65.56)	55.5 (41.98-63.82)
Median (IQR) 3months after treatment	55.55 (41.38-69.25)	63.2 (53.75-66.66)	54.28 (48.7-59.3)	65.9 (42.75-71.56)	50.72 (41.26-67.5)	59.4 (50.95-67.08)	58.92 (50.36-66.96)
P (Baseline vs 3months after treatment)*	0.9382	0.8125	0.625	0.625	0.8125	0.3757	0.1503
P(Infected vs. controls)**		0.5915	0.5915	0.7409	0.7102	0.3458	0.263
N (pairs)	12	4	5	5	4	14	39
Median (IQR) Baseline	62.42 (41.44-71.95)	59.2 (52.3-71.06)	48.6 (39.86-59.78)	56.5 (33.15-76.15)	61.31 (48.96-76.45)	56.66 (42.66-63.52)	54.1 (44.19-64.05)
Median (IQR) 1year after treatment	47.39 (32.7-65.28)	57.03 (54.01-65.36)	36.2 (31.37-42.7)	50.63 (46.5-74.29)	44.18 (33.1-62.4)	45.63 (30.76-60.18)	50 (33.88-60)
P (Baseline vs 1year after treatment)*	0.3804	0.625	**0.0625**	0.625	0.25	**0.0052**	**0.0072**
P(Infected vs. controls)**		0.8557	0.4292	0.2254	0.5048	0.7381	0.9911
%CD8 Total CD38+							
N (pairs)	17	5	5	5	5	13	33
Median (IQR) Baseline	58.1 (30.3-75.35)	66.1 (51.8-77.8)	25.8 (14.74-66.5)	47 (35.11-87.95)	52 (32.39-76.3)	49.3 (34.5-70.55)	50.9 (34.5-75.75)
Median (IQR) 3months after treatment	48.7 (31.44-67.9)	62.5 (49.3-75.7)	43 (20.85-60.4)	81.5 (48.81-87.2)	39.4 (31.21-67.25)	56.4 (43.03-76.8)	56.4 (40.25-76.8)
P (Baseline vs 3months after treatment)*	0.8129	0.3125	1	0.625	0.8125	**0.0327**	0.1555
P(Infected vs. controls)**		0.3471	0.6383	0.6383	1	**0.0753**	0.3841
N (pairs)	12	4	5	6	4	14	33
Median (IQR) Baseline	49.15 (29.18-78.83)	68.4 (44.95-75.73)	25.8 (14.74-66.5)	43.2 (36.5-87.73)	61.85 (34.05-78.6)	48.22 (32.98-60.98)	49.3 (32.85-68.4)
Median (IQR) 1year after treatment	49.8 (30.12-60.08)	52.97 (35.16-57.4)	17.67 (8.365-47.1)	56.5 (31.65-71.78)	42.99 (31.74-52.45)	28.95 (20.89-53.1)	32.8 (26.45-56.7)
P (Baseline vs 1year after treatment)*	1	0.375	**0.0625**	0.4375	0.25	**0.0494**	**0.0091**
P(Infected vs. controls)**		0.5045	0.1545	0.6063	0.3023	0.2471	0.2429

*P values between baseline and 1-3 months follow up performed using the Wilcoxon-matched pairs test

**P values in median change with time between helminth infected and non-infected controls using the Mann-Whitney test

Effective treatment of *T.trichiura* co-infection was not associated with decreased frequency of immune activation markers on T cells (Table 3- 16). Instead, treatment of Hookworm co-infection seemed to either increase expression of immune activation markers (HLA-DR+CD38-, HLA-DR+CD38+ or total HLA-DR), or decrease the frequency of HLA-DR-CD38+ or total CD38 expression particularly on CD4 T cells up to either 3 months or a year after treatment (Table 3- 16). The percentage of HLA-DR expressing CD4 T cells was increased from a median of 16.11% (IQR: 9.14- 32.38%) to 26.3% (IQR: 13.07- 47.35%) up to 3 months post treatment (P= 0.0266; Figure 3- 33A). Percentage of CD38+ CD4 T cells was decreased from a median of 56.66% (IQR: 42.52- 63.55%) to 45.63% (IQR: 27.65- 60.95%; P= 0.0052; Figure 3- 33B); while that of CD8 T cells was from 48.22% (IQR: 32.85- 61.05%) to 28.95% (IQR: 20.31 - 56.2%; P= 0.0494; Figure 3- 33C) a year post treatment. These changes were insignificant compared to median changes observed in the respective control groups (Table 3- 16).

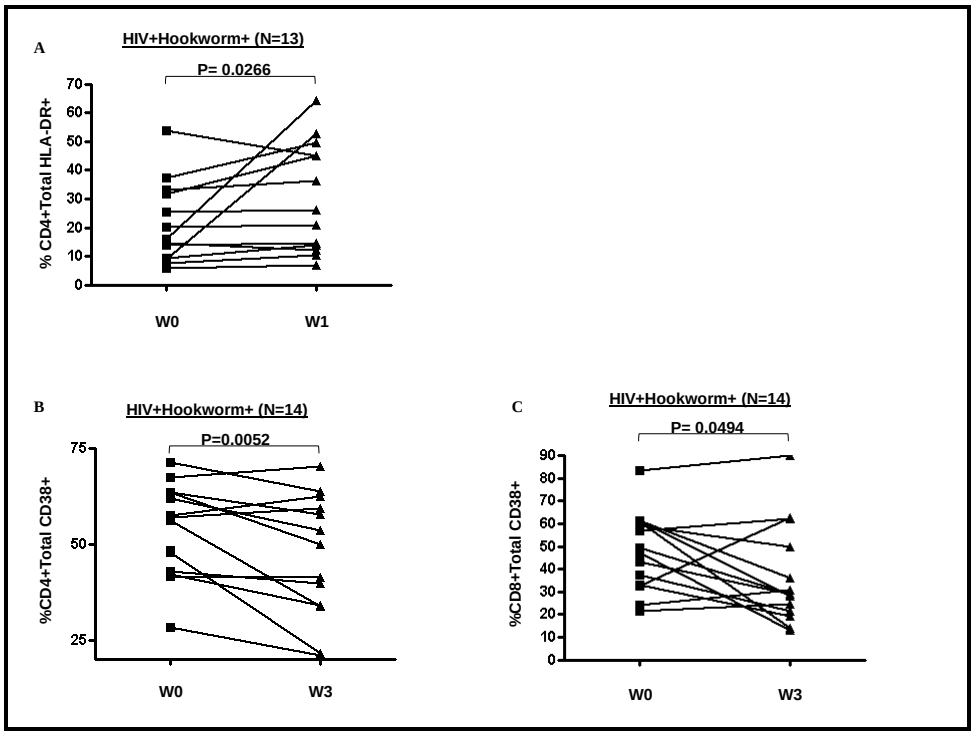


Figure 3- 33: Effect of helminth treatment on frequency of T-cell activation in the peripheral blood of HIV-1 and Hookworm co-infected volunteers. Comparison of percentage Total HLA-DR+ on CD4 T cells before (W0) and 1-3 months (W1) after helminth treatment is shown in (A). Comparison of percentage CD4+Total CD38+ (B) and CD8+Total CD38+ (C) at W0 and a year (W3) after helminth treatment are shown. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

Also, HIV+*S.haematobium*+ subjects (N=5) seem to down-regulate the frequency of immune activation parameters on T cells as illustrated in Figure 3- 34A-D with no substantial differences between the median changes in this group to the control group (Table 3- 16).

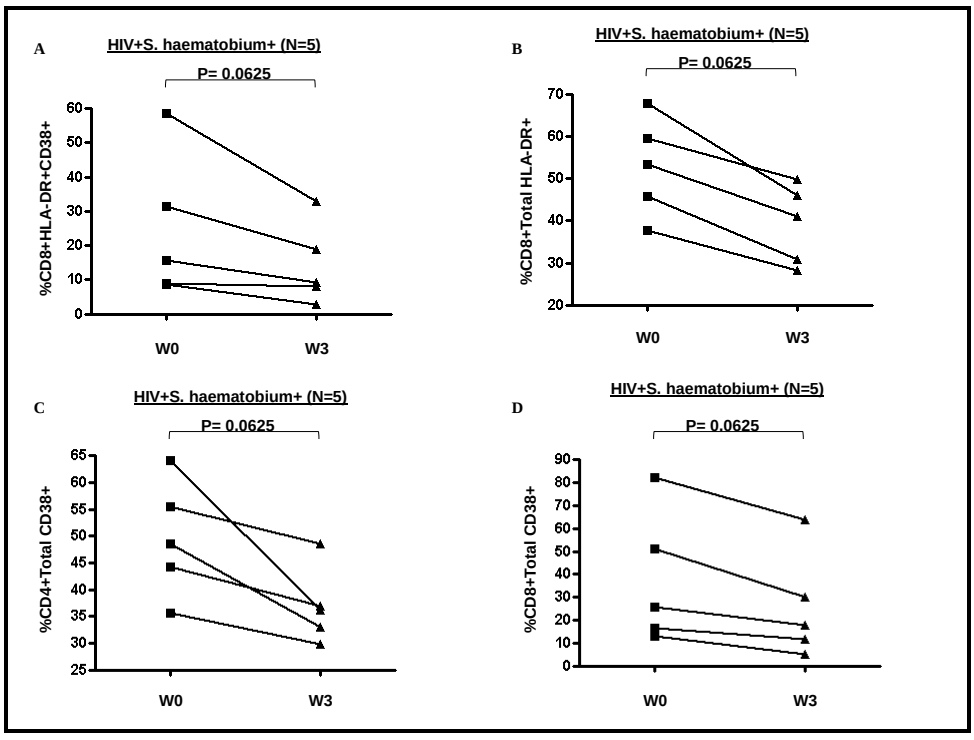


Figure 3- 34: Effect of helminth treatment on frequency of T-cell activation in the peripheral blood of HIV-1 and *S.haematobium* co-infected volunteers. Changes in the frequency of T-cell activation before (W0) and a year (W3) after helminth treatment are shown. (A) CD8+HLA-DR+CD38+; (B) CD4+Total CD38+ and (C) CD8+Total CD38+.Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

3.3.4. Expression of activation markers on CD4 and CD8 T cells in relation to chronic HIV-1 infection

Immune activation drives HIV pathogenesis. Persistent immune activation is in fact a strong predictor of decline in CD4 T cells and hence progression to AIDS (Hazenberg et al. 2003; Brenchley et al. 2004; Bentwich et al. 1998). As expected, HIV infection alone was observed to have a great impact on immune activation, measured by the frequency of expression of HLA-DR and CD38 molecules on CD4

and CD8 T cells. Higher frequencies of CD4 (Median: 8.8%; IQR: 4.285- 14.3%) and CD8 (Median: 25.2%; IQR: 14.25- 40.05%) T cells co-expressing both HLA-DR and CD38 markers were observed within HIV positive individuals compared to their respective control groups by over 3.5-fold (Median: 2.465%; IQR: 1.835- 3.555% and Median: 6.86%; IQR: 3.97- 12.4% respectively; P value for both <0.0001; data not shown). This elevation was observed also when CD4 and CD8 T cells expressing HLA-DR+CD38+ within HIV positive subjects without or with helminth infections were compared with HIV negative, non-infected subjects (P value for both <0.0001;data not shown). Similarly, frequency of CD4 T cells expressing HLA-DR marker alone was significantly higher in HIV+ subjects (Median: 8.55%; IQR: 5.005-13.25%) than in HIV- subjects (Median: 4.91%; IQR: 3.55-7.005%; P<0.0001; data not shown) with no apparent influence of helminth infection. On the other hand, the increased frequency of HLA-DR+CD38- on CD8 T cells was seen to be a function of helminth co-infection more than HIV (Figure 3- 35A-B). HIV infection did not seem to have an impact on the expression of CD38 alone on CD4 T cells (P=0.3208; data not shown). However, HIV infection independent of helminth infection showed a moderate decline in the frequency of HLA-DR-CD38+ CD8 T cells compared to HIV negative subjects (P=0.0036; Figure 3- 35C); but this observation lost its statistical power when stratified on the basis of helminth infections (Figure 3- 35D). Percentages of CD4 and CD8 T cells expressing either HLA-DR or CD38 molecules independent of each other were higher with HIV infection (Figure 3- 35E-H). Elevation of the frequency of HLA-DR expression on CD8 T cells independent of CD38 was also contributed by helminth co-infection (Table 3- 13).

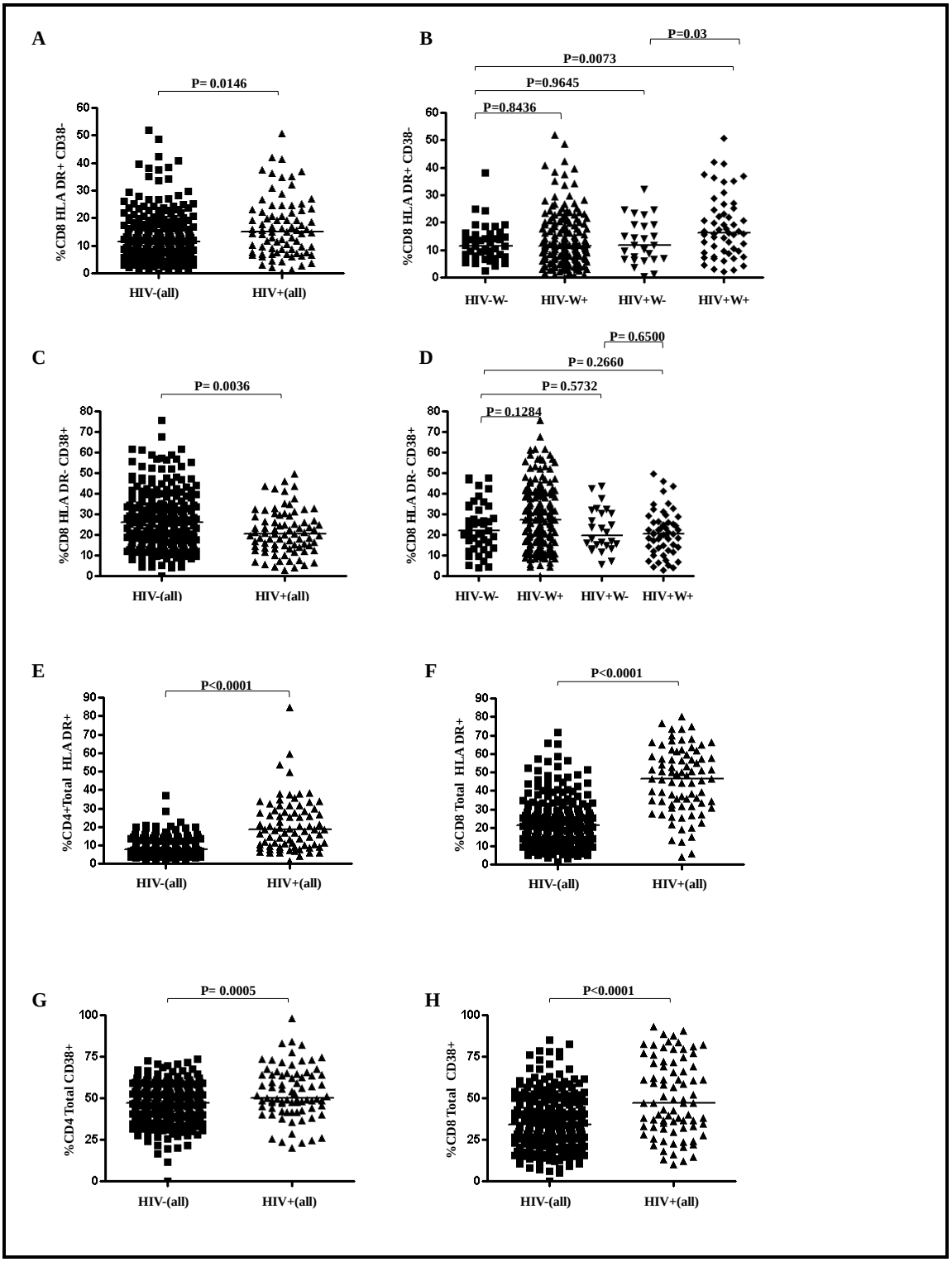


Figure 3- 35: Frequency of T-cell activation in relation to HIV-1 infection. The frequencies of CD8+HLA-DR+CD38-(A-B), CD8+HLA-DR-CD38+ (C-D), CD4+ Total HLA-DR+ (E), CD8+ Total HLA-DR+ (F), CD4+ Total CD38+ (G) and CD8+ Total CD38+ (H) T cells is shown between the HIV- and HIV+ subjects. Statistical analysis was performed using Mann-Whitney test for comparing groups.

Linear regression analysis showed a close link between the increase in frequency of immune activation markers (HLA-DR and CD38) on T cells and the loss of CD4 T cell counts within HIV positive subjects irrespective of their helminth infection status (Figure 3- 36A-D). When analysis included subjects with HIV only, only the frequency of total HLA-DR+CD4 T cells was associated with less CD4 T cell counts (P= 0.0033; r^2= 0.3066; data not shown) within HIV+Helminth- subjects; while the frequency of total CD38+CD8 T cells was closely linked to a decline in CD4 T cell counts (P= 0.224; r^2= 0.0146; data not shown).

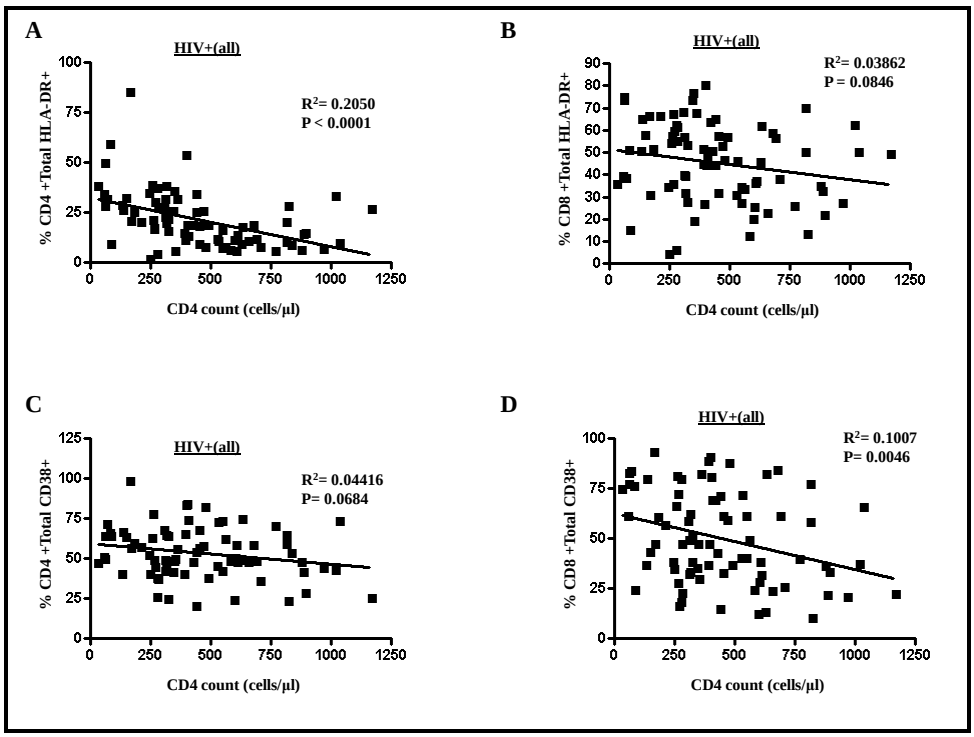


Figure 3- 36: Linear association between frequency of T-cell activation and loss of CD4 T cell count. Linear regression analyses between CD4 T cell counts and %Total HLA-DR (A-B) or % Total CD38 (C-D) on CD4 and CD8 T cells is shown in HIV infected volunteers.

3.3.5. Expression of HIV-coreceptor, CCR5 on total memory CD4 T cells in relation to chronic infection with different helminth species

3.3.5.1. Cross-sectional

Chemokine receptor CCR5 is also an important HIV-coreceptor whose lack of expression is associated with resistance to HIV acquisition (Deng et al. 1996; Liu et al. 1996). In this study, we assessed CCR5 expression intensity and frequency on total memory CD4 T cells (defined a sum of central memory, effector memory and terminally differentiating CD4 T cells) in relation to chronic helminth infections and/or HIV chronic infection. Since the expression of CCR5 is almost absent in naïve CD4 T cells (our own observation, data not shown), analysis of CCR5 expression intensity on total memory CD4 T cells is expressed as a ratio/difference between CCR5 expression intensity on total memory CD4 T cells to that of naïve CD4 T cells. In addition, the frequency of CCR5 expression on activated (defined by the expression of HLA-DR) total memory CD4 T cells was assessed between the different study groups. Figure 3- 37A-B shows a representative plot for gating total memory CD4 T cells and their CCR5 expression.

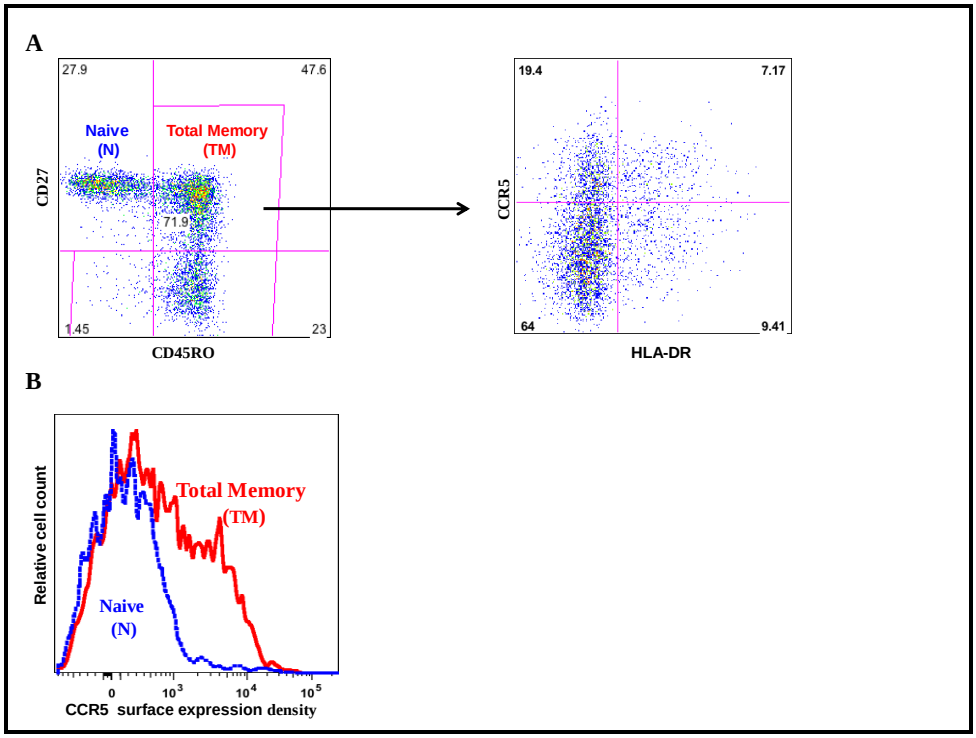


Figure 3- 37: HIV co-receptor expression on Total memory CD4 T cells. A representative dot plot for gating total memory CD4 T cells and their CCR5 expression is shown in (A). Shown in (B) is a histogram overlay for CCR5 density (MFI) expression on total memory CD4 T cells (red) and naïve CD4 T cells (blue).

Ratio of CCR5 median fluorescent intensity (MFI) between total memory and naïve CD4 T cells of HIV negative *T.trichiura* infected volunteers was higher (Median: 2.867; IQR: 2.138-3.643) than that of non-helminth infected subjects (Median: 2.234; IQR: 1.483-3.111; P=0.0198; Figure 3- 38A). Also, a higher but non-significant value was found in the median differences in CCR5 MFI between total memory and naïve CD4 T cells (P=0.0588; Figure 3- 38B). Furthermore, a tendency towards increased median frequency of CCR5 expression on activated (HLA-DR+) total memory CD4 T cells was observed in the HIV- *T.trichiura* + group (Median: 6.99%; IQR: 4.63-10.8%) compared to the control group (Median: 5.72%; IQR: 4.51-7.5%; P=0.0818; Figure 3- 38C). This trend could also be observed for *A.lumbricoides* infected individuals (P=0.0972; Figure 3- 38D). This suggests that *T.trichiura* infection might also be associated with elevation of CCR5 expression on the surface of total memory CD4 T cell in the peripheral blood.

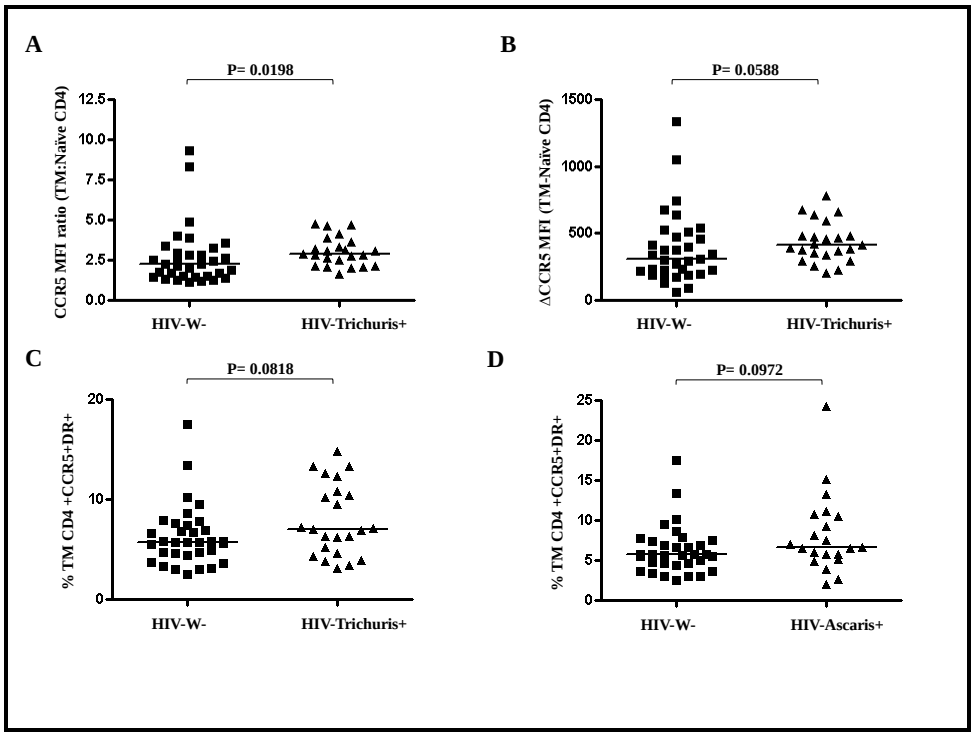


Figure 3- 38: HIV co-receptor expression on Total memory CD4 T cells in relation to Helminth infection. Median Fluorescent Intensity (MFI) ratio (A) or differences (B) in CCR5 expression between surfaces of CD4 T memory and naïve (CD4+ CD45R0- CD27+) cells amongst HIV negative subjects infected with *T.trichiura*. Comparison of CCR5+HLA-DR+ frequencies between HIV- Worm- and HIV-*T.trichiura*+ (B) or HIV-*A.lumbricoides*+ (C) subjects is shown.

Within HIV positive co-infected individuals, Hookworm co-infection showed down regulation of expression of CCR5 density on total memory CD4 T cells compared to HIV positive non-helminth infected individuals (P=0.0201; Figure 3-39A). In contrast, *S.haematobium* infected people (N=6) had a slight increase in the differences in CCR5 MFI values between total memory and naïve CD4 T cells (P= 0.0455; Figure 3- 39B).

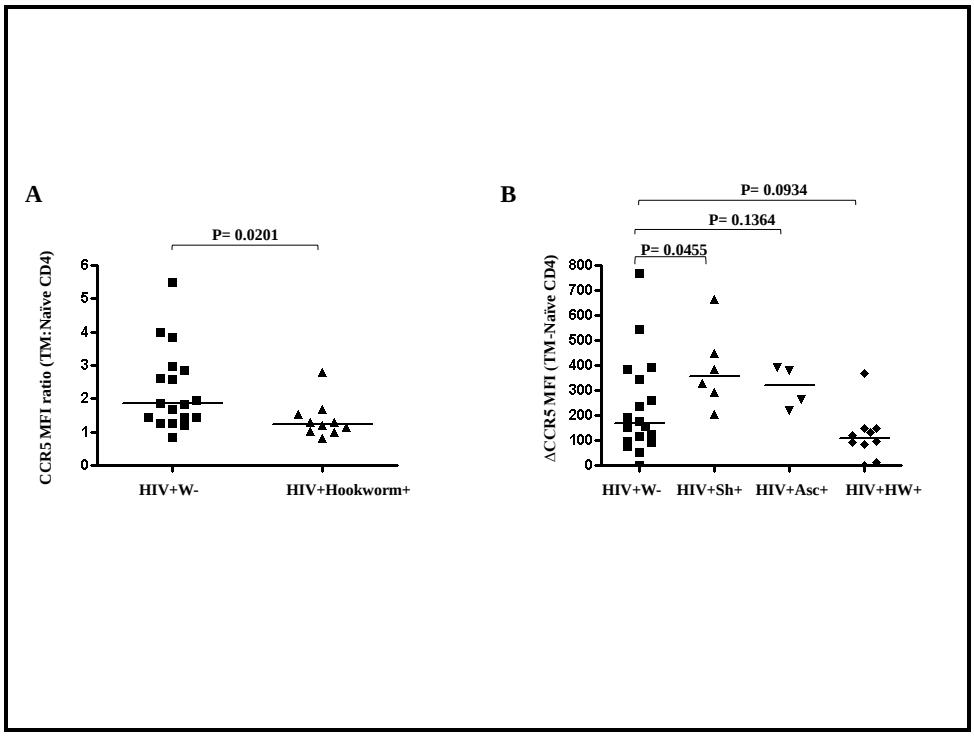


Figure 3- 39: HIV co-receptor expression on Total memory CD4 T cells in relation to HIV and Helminth co-infection. Median Fluorescent Intensity (MFI) ratio (A) or differences (B) in CCR5 expression between surfaces of CD4 T memory and naïve (CD4+ CD45R0- CD27+) cells amongst HIV+Worm- and HIV+ infected with different worm species is shown.

3.3.5.2. Effect of treatment with praziquantel and albendazole on the expression of CCR5 on total memory CD4 T cells in HIV negative and HIV positive individuals

In HIV negative individuals, relative CCR5 MFI value and frequency of CCR5 expression declined in helminth infected subjects as a result of effective helminth treatment (Table 3- 17). Most changes were insignificant when compared to the median changes between visits in CCR5 expression on non-helminth infected individuals. However, the difference in Δ CCR5 MFI between the baseline (330.5) and a year after helminth treatment (250.5, P<0.0001, Table 3- 17) in the worm-infected group was significant compared to that of the control group (P=0.0429, Table 3- 17).

Table 3- 17. Expression of a CCR5 co-receptor on CD4 total memory T cells at before and after deworming on HIV negative and HIV positive individuals

Status	WormNeg	Trichuris	S.h	S.m	Ascaris	Hookworm	All Worm
	HIV NEGATIVE VOLUNTEERS						
CCR5 ΔMFI							
N (pairs)	26	10	7	22	17	17	73
Median (IQR) Baseline	306 (193.5-486.3)	441 (351.5-522)	434 (142-486)	286.5 (184.5-427.8)	307 (228-397.5)	274 (135.5-392.7)	317.4 (213-434)
Median (IQR) 3months after treatment	348 (244-518)	290.5 (210.5-474.5)	234 (219-396)	237.5 (160.5-353.5)	208 (179-342)	165 (111.5-289.5)	232 (166.3-347)
P (Baseline vs 3months after treatment)*	0.7995	**0.0371**	0.5781	0.4954	**0.0204**	0.1514	**0.001**
P(Infected vs. controls)**		**0.0565**	0.6438	0.7721	**0.1011**	0.3983	0.1546
N (pairs)	19	10	11	18	11	10	60
Median (IQR) Baseline	375 (269-638)	464 (348.3-507.8)	434 (248-616)	271.5 (170.8-427.8)	307 (186-390)	298 (187.3-418.5)	330.5 (209.8-473.5)
Median (IQR) 1year after treatment	294 (150.3-365.8)	296.9 (248.5-400.3)	282 (198-430)	240.2 (126.1-429)	183 (102-236)	197 (102.8-324.5)	250.5 (146.3-353.5)
P (Baseline vs 1year after treatment)*	**0.001**	0.1309	0.1016	0.1701	**0.0098**	**0.019**	**< 0.0001**
P(Infected vs. controls)**		0.3239	0.1685	0.0185	0.4385	0.242	**0.0429**
CCR5 MFI Ratio							
N (pairs)	26	10	7	22	17	17	73
Median (IQR) Baseline	2.073 (1.428-3.022)	2.795 (2.121-3.354)	2.019 (1.294-3.214)	2.271 (1.77-3.392)	2.014 (1.672-2.55)	2.111 (1.501-2.869)	2.145 (1.769-3.06)
Median (IQR) 3months after treatment	2.611 (1.955-3.62)	2.843 (2.045-3.383)	1.586 (1.287-2.119)	2.237 (1.561-3.099)	1.825 (1.498-2.722)	1.601 (1.282-2.423)	1.929 (1.435-2.758)
P (Baseline vs 3months after treatment)*	0.3219	1	0.5781	0.6968	0.7049	**0.0523**	0.1334
P(Infected vs. controls)**		0.5135	0.2095	0.3057	0.4053	**0.0262**	**0.0897**
N (pairs)	19	10	11	18	11	10	60
Median (IQR) Baseline	2.54 (1.433-3.862)	3.079 (2.121-3.461)	2.848 (1.821-3.545)	2.193 (1.546-3.25)	2.198 (1.679-2.714)	2.129 (1.709-2.763)	2.229 (1.828-3.204)
Median (IQR) 1year after treatment	3.26 (2.372-3.504)	2.2 (1.697-2.742)	3.24 (1.74-4.15)	2.603 (2.043-3.568)	1.87 (1.638-2.441)	2.397 (1.336-3.673)	2.441 (1.752-3.347)
P (Baseline vs 1year after treatment)*	0.2862	0.1309	0.2061	**0.0235**	0.6377	0.2324	0.2554
P(Infected vs. controls)**		**0.0569**	0.8973	0.6161	0.2452	0.7655	0.5244
CCR5+ HLA-DR+ (% of TM)							
N (pairs)	26	11	7	22	17	17	74
Median (IQR) Baseline	5.73 (4.225-7.52)	7.12 (4.31-12.6)	6.12 (4.58-6.55)	5.46 (4.123-7.28)	6.49 (5.01-10.65)	5.52 (3.19-7.06)	5.795 (4.163-7.753)
Median (IQR) 3months after treatment	4.945 (3.683-7.043)	4.91 (3.27-7.56)	4.99 (4.36-5.93)	4.305 (3.513-5.913)	5.77 (3.295-6.75)	4.75 (2.875-5.49)	4.92 (3.543-5.935)
P (Baseline vs 3months after treatment)*	0.2977	**0.0049**	**0.0313**	**0.0221**	**0.0204**	0.2977	**< 0.0001**
P(Infected vs. controls)**		**0.0238**	0.5089	0.2866	0.1297	0.8038	0.1041
N (pairs)	19	10	11	19	11	10	61
Median (IQR) Baseline	6.87 (4.67-8.66)	7.035 (5.825-9.703)	6.12 (4.58-7.83)	5.33 (4.24-6.96)	6.51 (5.75-10.8)	5.55 (3.31-7.58)	6.11 (4.29-7.735)
Median (IQR) 1year after treatment	4.51 (3.53-5.740)	4.485 (3.15-7.215)	5.01 (2.45-7.04)	3.63 (1.82-6.11)	4.55 (2.79-8.27)	3.07 (2.18-4.48)	4 (2.41-5.84)
P (Baseline vs 1year after treatment)*	**0.0006**	**0.0645**	**0.0098**	**0.0058**	**0.0137**	**0.0039**	**< 0.0001**
P(Infected vs. controls)**		0.9087	0.1685	0.2429	0.9314	0.5977	0.2802
	HIV POSITIVE VOLUNTEERS						
CCR5 ΔMFI							
N (pairs)	13	1	5	3	3	8	23
Median (IQR) Baseline	176 (108.5-302)	240	383 (311.5-556)	520 (0-3130)	381 (220-392)	107.5 (88-149)	291 (118.9-392)
Median (IQR) 3months after treatment	139 (68.5-322)	200	248 (74-334.5)	443 (87-680)	356 (254-423)	162.5 (15-185.3)	200 (87-356)
P (Baseline vs 3months after treatment)*	0.4845		**0.0625**	0.75	0.5	0.9453	**0.0335**
P(Infected vs. controls)**			**0.03**	0.6863	0.5008	0.218	0.6329
N (pairs)	8	1	5	3	3	7	19
Median (IQR) Baseline	214.5 (87.75-352.3)	110	383 (311.5-556)	520 (0.0-3130)	381 (220-392)	96 (86-134)	220 (96-392)
Median (IQR) 1year after treatment	61.85 (18.5-203.8)	0	287.4 (111.2-330)	443 (0-473)	157.6 (104.7-172.8)	90 (58-145.3)	140.6 (80-287.4)
P (Baseline vs 1year after treatment)*	**0.0156**		0.125	0.5	0.25	0.9375	**0.0046**
P(Infected vs. controls)**			0.3543	0.9212	0.3758	**0.0059**	0.5725
CCR5 MFI Ratio							
N (pairs)	13	1	5	3	3	8	23
Median (IQR) Baseline	1.967 (1.441-2.908)	2.319	2.123 (1.785-3.331)	3.626 (0.8246-3.984)	2.256 (1.58-3.093)	1.244 (0.8973-1.653)	1.879 (1.28-2.913)
Median (IQR) 3months after treatment	1.698 (1.366-2.617)	2.961	2.183 (1.059-2.553)	1.188 (1.105-2.978)	1.902 (1.6-2.945)	1.399 (1.048-1.996)	1.6 (1.188-2.319)
P (Baseline vs 3months after treatment)*	**0.0479**		0.125	0.5	0.5	0.6406	**0.0914**
P(Infected vs. controls)**			0.5621	0.9425	0.516	**0.0409**	0.3571
N (pairs)	8	1	5	3	3	7	19
Median (IQR) Baseline	1.855 (1.318-2.438)	1.08	2.123 (1.785-3.331)	3.626 (0.8246-3.984)	2.256 (1.58-3.093)	1.209 (1.022-1.292)	1.628 (1.157-2.913)
Median (IQR) 1year after treatment	1.249 (1.076-2.726)	0.99	1.563 (0.0-2.373)	1.438 (0.8035-3.804)	2.687 (2.054-2.874)	1.629 (1.227-2.703)	1.629 (1.101-2.703)
P (Baseline vs 1year after treatment)*	0.4609		0.4375	0.75	0.5	**0.0781**	0.5596
P(Infected vs. controls)**			0.8329	0.6303	0.2788	**0.0401**	0.1982
CCR5+ HLA-DR+ (% of TM)							
N (pairs)	13	1	5	3	3	8	20
Median (IQR) Baseline	9.89 (6.465-15.65)	4.32	10.9 (7.64-19.3)	13 (6.06-19.2)	8.03 (4.26-23.7)	5.35 (2.473-10.19)	7.29 (4.275-13.3)
Median (IQR) 3months after treatment	7.9 (6.84-11.65)	1.96	7.94 (4.165-18)	18.4 (5.05-19.3)	9.26 (5.02-19.2)	7.035 (6.063-12.11)	7.715 (5.028-17.33)
P (Baseline vs 3months after treatment)*	0.1909		0.125	1	1	0.5469	0.5596
P(Infected vs. controls)**			0.375	0.893	1	0.2321	0.5756
N (pairs)	8	1	5	3	3	6	19
Median (IQR) Baseline	11.45 (7.9-15.93)	5.76	10.9 (7.64-19.3)	13 (6.06-19.2)	8.03 (4.26-23.7)	8.2 (3.625-14.15)	9.89 (5.18-16.4)
Median (IQR) 1year after treatment	4.73 (2.593-6.965)	5.1	7.62 (2.905-20.85)	9.77 (7.65-14.8)	3.85 (2.01-4.38)	3.75 (3.315-7.3)	5.1 (3.5-9.77)
P (Baseline vs 1year after treatment)*	0.0078		0.8125	0.5	0.25	0.1563	0.1031
P(Infected vs. controls)**			0.5237	0.3758	1	0.1893	0.2144

*P values between baseline and 1-3 months follow up performed using the Wilcoxon-matched pairs test

**P values in median change with time between helminth infected and non-infected controls using the Mann-Whitney test

Particularly, *T.trichiura* infected people had less differences in CCR5 MFI value between total memory and naïve CD4 T cells 3 months post treatment (Median: 290.5; IQR: 209-477) than at baseline (Median: 441; IQR: 347-568; P=0.0371; Figure 3- 40A), whereas no decline was observed in helminth negatives (Median: 306 to 348, p=0.7995). Compared to the control group, the treatment induced change in CCR5 density on memory CD4 T cells was observed in *T.trichiura* infected subjects (P=0.0565, Table 3- 17). This decline in Δ CCR5 MFI within *T.trichiura* infected subjects was less significant when compared a year post treatment (P=0.1309; Figure 3- 40B) with no substantial differences between helminth infected and the control group (Table 3- 17). Similarly, a significant decrease in frequency of CCR5 expression on HLA-DR+ total memory CD4 T cells from a median of 7.12% (IQR: 4.31- 12.6%) to 4.91% (IQR: 3.27- 7.56%) could only be observed up to 3 months after treating for helminths infections (P= 0.0049; Figure 3- 40C-D) whereas no changes were observed in the control group (P=0.2977, Table 3- 17). These changes were observed in the infected group were significant (P=0.0238, Table 3- 17) when compared to non-helminth infected controls, suggesting a positive effect of treatment on the expression of CCR5.

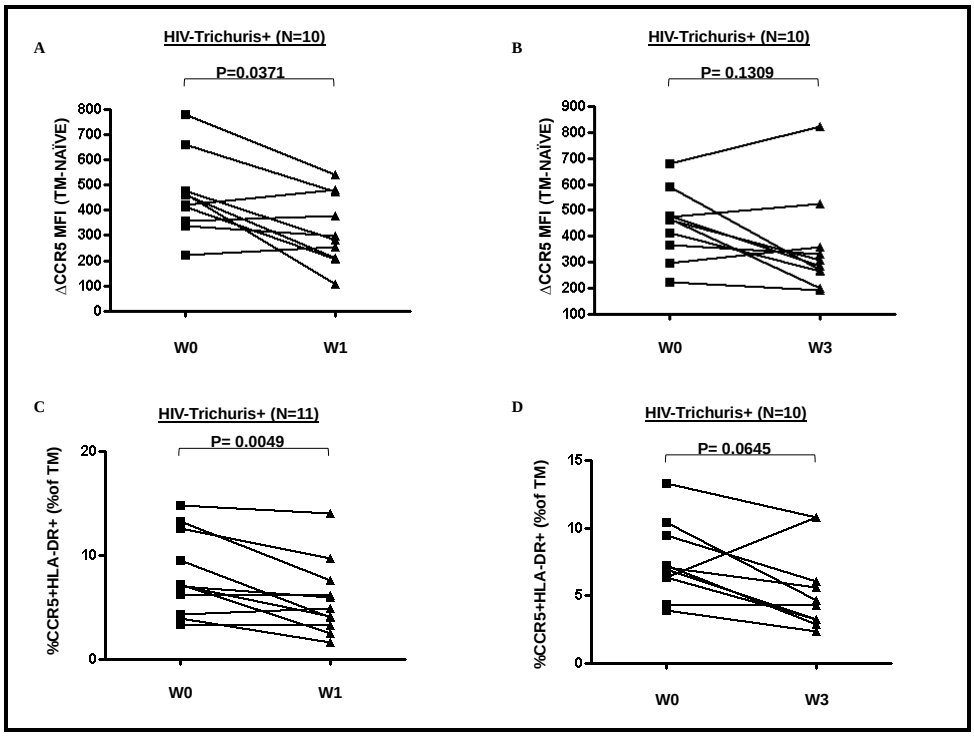


Figure 3- 40: Effect of *T.trichiura* treatment on HIV co-receptor expression on total memory CD4 T cells. Median Fluorescent Intensity (MFI) ratio (A) or differences (B) in CCR5 expression between surfaces of CD4 T memory and naïve (CD4+ CD45R0- CD27+) cells are shown before (W0) and after (W1=1-3months; W3=a year) treating for *T.trichiura*. Changes in Total memory CD4+ CCR5+HLA-DR+ frequencies between HIV- Worm-and HIV-*T.trichiura*+ at W0 and either at W1 (C) or at W3 (D) are shown. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

Figure 3- 41A-D shows the effect of treating *A.lumbricoides* on the frequency of CCR5 expression on total memory CD4 T cells 3 months and up to a year post treatment. *A.lumbricoides* co-infected volunteers also showed a significant decrease in the CCR5 density especially a year post treatment (P= 0.0098; Figure 3- 41B). Effective treatment of *A.lumbricoides* infection was associated with low frequencies of CCR5+ total memory CD4 T cells decreasing from a median of 6.51% (IQR: 5.75-10.8%) to 4.55% (IQR: 2.79- 8.27%) a year post treatment (P= 0.0137; Figure 3-41D). The observed median changes in CCR5 expression on memory CD4 T cells were very minor and insignificant when compared to the changes observed in the control group (Table 3- 17).

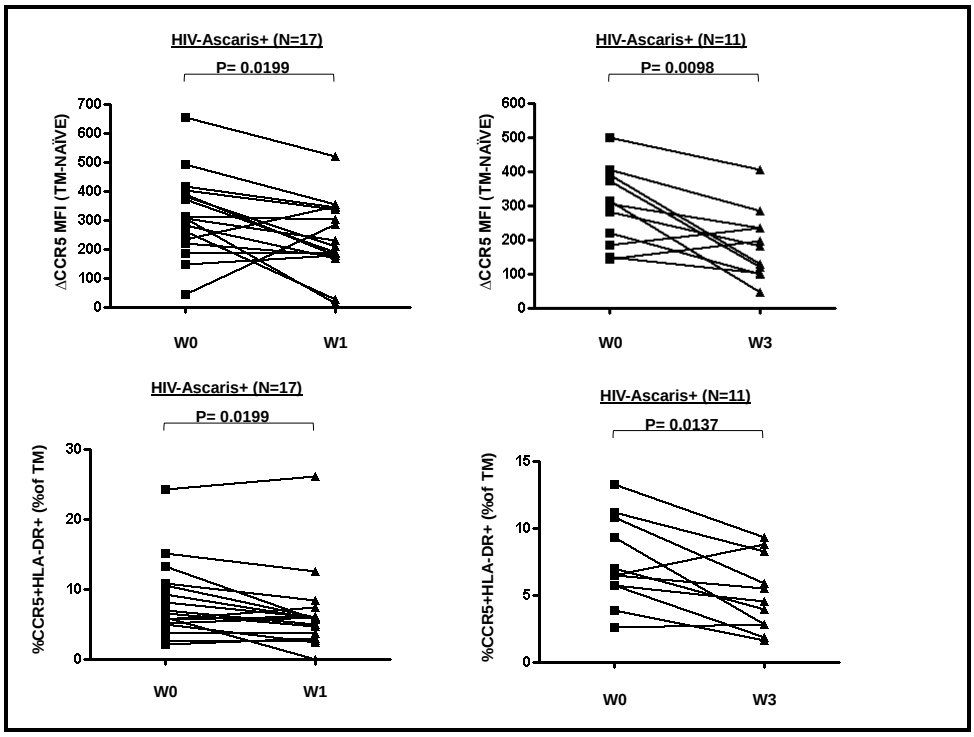


Figure 3- 41: Effect of Treating for *A.lumbricoides* on HIV co-receptor expression on Total memory CD4 T cells. Median Fluorescent Intensity (MFI) differences in CCR5 expression between surfaces of CD4 T memory and naïve (CD4+ CD45R0- CD27+) cells are shown before (W0) and either 1-3 months (A) or a year (B) after treating for *A.lumbricoides*. Changes in Total memory CD4+ CCR5+HLA-DR+ frequencies between HIV- Worm-and HIV- *A.lumbricoides* + at W0 and either at W1 (C) or at W3 (D) are shown. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

Surprisingly, effective treatment of Schistosome and hookworm infections were also seen to be associated with changes in the relative CCR5 MFI value and frequency of CCR5 expression (Table 3- 17) even though such infections did not influence the expression of CCR5 when compared with non-infected subjects at baseline. Most of these changes were insignificant when compared to the median changes between visits in CCR5 expression on non-helminth infected individuals. However, the difference in CCR5 MFI ratio between the baseline (2.111) and up to 3 months after treating for Hookworm (1.601, P=0.0523, Table 3- 17) in the Hookworm infected group was significant compared to that of the control group (P=0.0262) which showed no such changes in response to treatment (Table 3- 17).

In HIV positive, worm co-infected individuals, effective worm treatment had an impact particularly on the relative CCR5 MFI expression on memory CD4 T cells (Table 3- 17) with no substantial differences between helminth infected and the control group. Effective treatment of Hookworm infection in HIV positive subjects led to a non-significant, slight increase in the expression of CCR5 MFI ratio on total memory CD4 T cells a year post treatment (Median: 1.629; IQR: 1.227- 2.703) compared to that at baseline (Median: 1.209; IQR: 1.022- 1.292; P= 0.0781; Table 3- 17) whereas no changes were observed in the control group (P=0.4609, Table 3- 17). The observed median changes between visits in the Hookworm infected subjects was significant when compared to that observed in the non-helminth infected group (P=0.0401, Table 3- 17). In contrast, HIV+*S.haematobium* co-infected individuals seemed to have less relative CCR5 MFI expression on memory CD4 T cells up to 3 months post treatment (P= 0.0625; Figure 3- 42). This difference was also significant compared to that observed in the control group (P=0.03), which showed no such changes in response to treatment (P=0.4845, Table 3- 17).

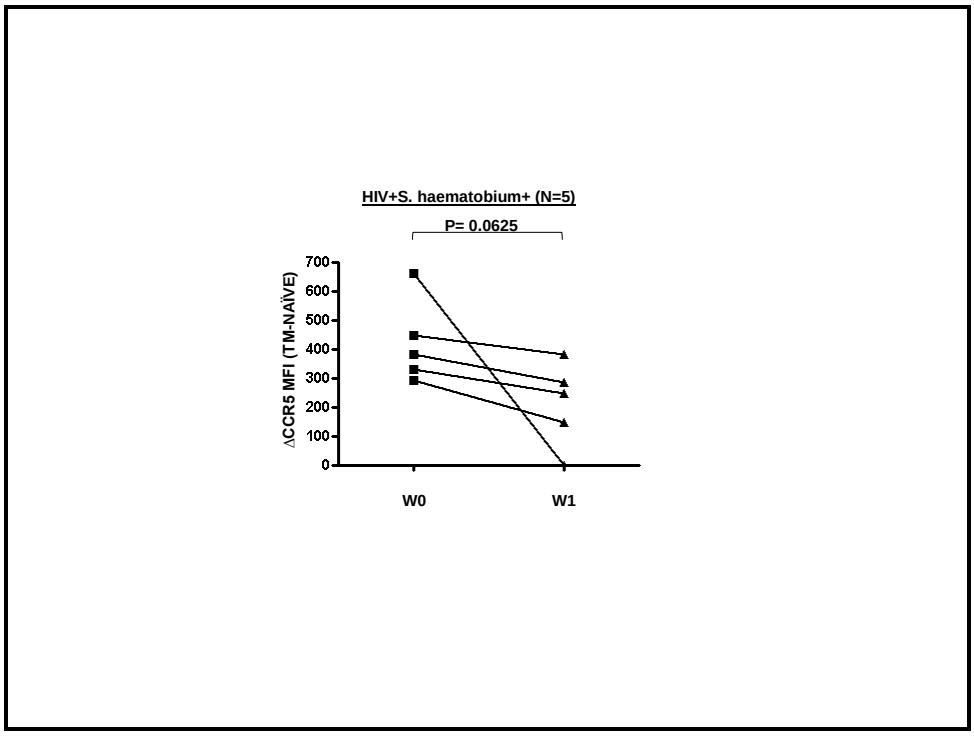


Figure 3- 42: Effect of Helminth treatment on HIV co-receptor expression on Total memory CD4 T cells of HIV+*S.haematobium* co-infected volunteers. Median Fluorescent Intensity (MFI) differences in CCR5 expression between surfaces of CD4 T memory and naïve (CD4+ CD45R0- CD27+) cells are shown before (W0) and 1-3 months (W1) after treating for *S.haematobium*. Statistical analysis was performed using Wilcoxon matched pairs test for comparison of pairs.

3.3.6. Expression of HIV-coreceptor, CCR5 on total memory CD4 T cells in relation to chronic HIV-1 infection

When CCR5 expression intensity on total memory CD4 T cells was assessed in relation to HIV chronic infection, CCR5 expression density was reduced in HIV infected individuals (P<0.002; Figure 3- 43A-B). However, the frequency of CCR5 expression in immune activated memory CD4 T cells was significantly higher in HIV positive individuals (Median: 9.08%; IQR: 5.47- 13.5%) compared to HIV negative ones (Median: 5.92%; IQR: 4.495-7.88%; P= 0.0002; Figure 3- 43C). Interestingly, frequency of CCR5 expression on total memory CD4 T cells was 31.96% and 26.04% in HIV negative and HIV positive individuals respectively. This level of CCR5 expression by memory T cells is much less than that of Treg cells reported in the previous chapter (50% for both HIV negative and positive subjects).

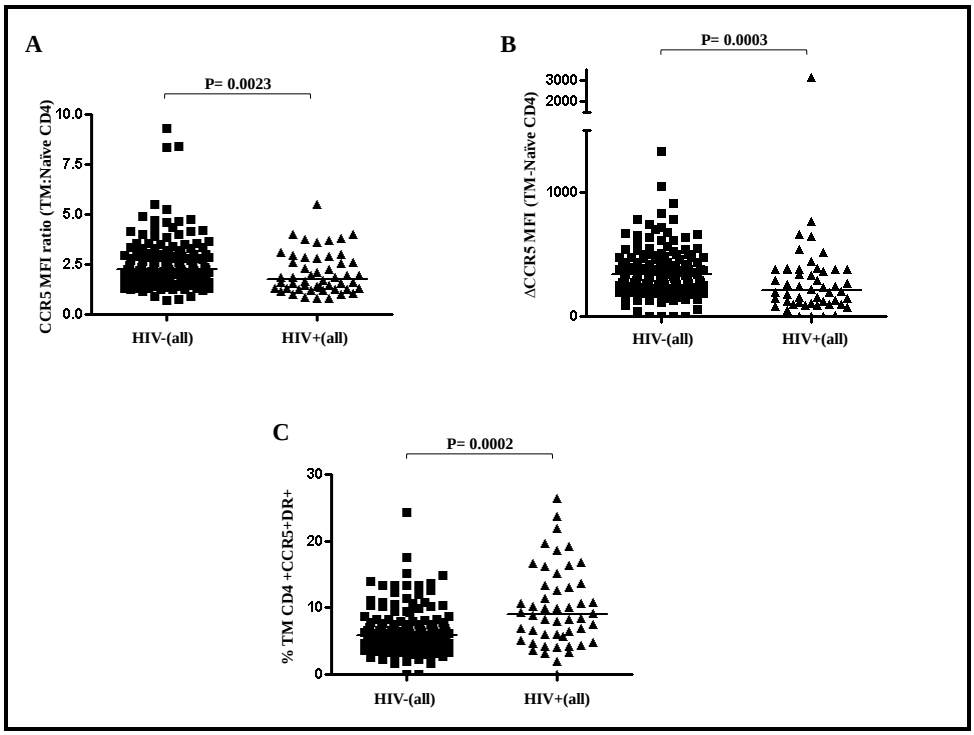


Figure 3- 43: HIV co-receptor expression on Total memory CD4 T cells in relation to HIV-1 infection. Median Fluorescent Intensity (MFI) differences (A) or ratio (B) in CCR5 expression between surfaces of CD4 T memory and naïve (CD4+CD27+CD45R0-) cells between HIV- and HIV+ volunteers are shown. Comparison of CCR5+HLA-DR+ frequencies on Total memory CD4 T cells between HIV- and HIV+ volunteers is shown in (C). Statistical analysis was performed using Mann-Whitney test for comparing groups.

4. Discussion

4.1. Pathogen-specific T cell responses during infection with HIV-1, Helminth or HIV-Helminth co-infection

Loss of pathogen-specific CD4 T cells and impaired functions of pathogen specific CD8 T cells caused by HIV contributes to the failure of the immune system to control infections with herpes virus such as CMV (Komanduri et al. 1998). Chronic helminth infections may as well induce T cell hyporesponsiveness and impair immune responses to other pathogens such as HIV and MTB (Borkow et al. 2001; Wammes et al. 2010). However, modulation of specific T cell responses to pathogens by helminth infections has not been fully explored. This study therefore examined the immune modulation of IFN-γ secreting specific T cell responses to HIV, MTB, Influenza, *T.gondii* and herpes viruses in relation to HIV and helminths.

Although McElroy *et al.* observed modulation of frequency of HIV-specific cytolytic (CD107) CD8 T cell responses by *S. mansoni*, they found no such association when analysing IFN-γ release as a consequence of CD8 T cell responses to HIV (McElroy et al. 2005). Similar to their observation, no changes in the frequency and magnitude of IFN-γ+HIV-specific CD8 T cell responses in relation to any helminth specie were observed in this study. As expected, there was a linear relation between HIV-specific T cells and total number of CD4 T cells, confirming the importance of functional HIV-specific CD8 T cell responses in controlling the disease's clinical outcomes (Jin et al. 1999; Schmitz et al. 1999; Ogg et al. 1998). A decrease in HIV-TL9- and FRP-specific T cells was observed in the study group a year after de-worming. This observation is most likely attributed to HIV infection, as a decline of HIV-specific CD8 T cells in chronic HIV individuals with time has been previously reported (Geldmacher et al. 2007).

MTB co-infections with *A.lumbricoides* or *T.trichiura* have recently been reported to be associated with reduction of IFN-γ cytokine in the supernatant after *in vitro* re-stimulation of whole blood of helminth infected individuals with MTB antigen (Resende Co et al. 2007). Also, helminth infected children have lower T cell proliferative responses to the BCG vaccine compared to uninfected children (Wammes et al. 2010). This study found no association of helminth infections on the

magnitude of IFN-γ+PPD-specific CD4 T cell responses in HIV negative and HIV positive study subjects with no signs of active TB infection. This suggests that worms have no apparent influence on IFN-γ MTB-specific T cell responses in latently infected people although it might be different in people with active TB (Resende Co et al. 2007). However, effective treatment of Hookworm infection in HIV negative volunteers demonstrated a moderate reduction on the quantity of IFN-γ+PPD-specific CD4 T cells, especially 3 months post treatment. In line with a previous report (Geldmacher et al. 2010), HIV alone had a great impact on the decline of frequency and magnitude of IFN-γ production in PPD-specific CD4 T cells, emphasizing the role of HIV in compromising pathogen specific responses.

Also, HIV infection alone was related to a decrease in frequency of detectable responses to herpes viruses analysed in this study, with the exception of CMV. The quantity of HSV-1-specific T cells declined markedly with HIV infection while HIV infection was associated with a moderate decline of CMV- and EBV-specific T cell responses with no apparent influence of worms. The observed persistence of CMV-specific T cells in chronic HIV infection has been demonstrated before (Waldrop et al. 1997; Casazza et al. 2009; Geldmacher et al. 2010). De-worming however seemed to have an impact on the reduction of CMV-specific T cells in HIV negative and HIV positive subjects.

Similarly, HIV infection was associated with a decrease in Influenza-specific T cell responses, with an inverse association to plasma viremia. Treatment of *T.trichiura* infection led to a moderate decrease in IFN-γ expressing Influenza a-specific T cells up to 3 months post treatment. A moderate decline in *T.gondii* specific T cells was also observed in relation to HIV with no apparent influence of worms.

Taken together, these results suggest that helminth infections have no impact on the frequency and the quantities of IFN-γ secreting pathogen-specific T cells. However, HIV alone has a great impact on the quality and quantity of most IFN-γ releasing pathogen-specific T cell responses and co-infection with helminths does not appear to influence these changes. Also, worm treatment does not seem to have any profound effect on the quantities of pathogen-specific T cells of volunteers as measured by IFN-γ release ELISpot assay up to a year after helminth treatment.

4.2. Regulatory T cells during HIV-1, Helminth or HIV-Helminth co-infection

Tregs are essential for controlling auto immune responses and inflammation induced by chronic infections like HIV (Sakaguchi et al. 2008; Card et al. 2009). However, there has been compelling evidence implicating Tregs interference with protective immunity against HIV (Aandahl et al. 2004; Kinter et al. 2007). FoxP3 T cells tend to accumulate in lymphoid tissues of HIV infected individuals and their increased accumulation is associated with disease progression (Nilsson et al. 2006). In this study, a moderate increase in the frequency of circulating CD4+CD25+FoxP3+ Tregs in HIV infected individuals by 1.17 fold was observed when compared to the HIV negative ones. This finding is in agreement with previous studies that have reported varying increase level in the proportion of Tregs in peripheral blood (Presicce et al. 2011; Angin et al. 2012). Increase in Treg frequencies negatively correlated with CD4 count showing an association between increase in Treg frequencies with disease progression. A reason for an increase in the frequency for Tregs in HIV infected people could be due to HIV-1 induced proliferation of Tregs.

Absolute numbers of Tregs however decreased markedly in HIV infected individuals and this loss strongly correlated positively with a loss of total CD4 T cells. This suggests that the Treg numbers are lost at the same rate as the total CD4 T cells. In addition, about 50% of Tregs of our HIV negative and HIV positive study subjects expressed the HIV-co receptor CCR5, which is higher than in total memory CD4 T cells as reported in the next chapter. Treg depletion could therefore be due to direct infection of Tregs.

Memory Tregs of healthy individuals have a rapid *in vivo* turnover (Vukmanovic-stejic et al. 2006) and are actively dividing (Booth et al., 2010). Since *in vitro* productive HIV infection of CD4 T cells is most efficient in cycling CD4 T cells and CD4 T cells that express CD25 (Douek et al. 2002; Geldmacher et al. 2010), it is likely that a higher frequency of memory Treg cells in HIV positive subjects are dividing *in vivo*, which might potentially provide substrates for HIV infection and replication. Indeed, HIV infection is associated with a significant increase in the frequency of Ki67+ memory Tregs which correlates with increase in HIV progression

demonstrated by the decline of CD4 numbers (unpublished data from Osei Kuffour *et al.*). Taken together, these data strongly suggest that Tregs could potentially serve as HIV target.

Oswald-Ritcher *et al.* developed an *in vitro* model for Treg infection using TCR mediated stimulation well known to induce HIV transcription and showed a high susceptibility of Tregs to HIV with a ~3 fold higher HIV infection rate in Tregs than other memory CD4 T cells. However, *in vitro* TCR stimulation may not reflect true HIV infection and thus has to be validated *in vivo.* A few other studies have recently demonstrated discrepant results regarding the level of HIV infection in Tregs *in vivo*. While some studies showed a similar infection level between Tregs and other CD4 T cell subsets *in vivo* (Dunham et al. 2008; Chase et al. 2008; Moreno-Fernandez et al. 2009), Tran *et al.* observed a higher infection rate in Tregs than non Tregs (Tran et al. 2008). Since memory CD4 T cells are predominantly infected by HIV (Brenchley et al. 2004; Dai et al. 2009), comparison of the level of infection between the Tregs and memory CD4 T cells was made in this study. Thus, CD4 T cell populations were sorted on the basis of their memory (CD45RO) and Treg marker expression (CD25+FoxP3).

Strikingly, HIV-gag DNA loads could be detected in >80% of sorted memory Treg populations of the HIV positive study subjects confirming that Tregs are indeed frequent targets of HIV. Interestingly, a 15-fold higher median gag DNA level was detected in memory Tregs as compared to CD25-FoxP3- memory CD4 T cells demonstrating for the first time to our knowledge, an *in vivo* preferential infection of memory Tregs by HIV.

Furthermore, in this study helios, a member of the Ikaros transcriptional factor family which has been proposed as an additional Treg marker due to its preferential expression on Tregs (Sugimoto et al. 2006; Thornton et al. 2010; Getnet et al. 2010) was included. Here too, co-expression of helios in ~75% of circulating memory Tregs was observed. However, helios expression was not restricted to CD25+FoxP3+CD4 T cells as we also observed its expression in CD4 T cells. Nonetheless, the majority (~98%) of memory CD25-FoxP3-CD4 T cells did not express helios.

The role of helios on Tregs is unclear. T. Akimova *et al.* has recently demonstrated a high helios expression on activated cells and importantly, dividing CD25+CD4 T cells that were induced to proliferate *in vitro* co-expressed of both Helios and FoxP3 while non dividing Tregs lost the expression of both molecules-suggesting helios as a marker of recently divided cells and is expressed *in vitro* after a few cycles of proliferation (Akimova et al. 2011). More recent data suggest that helios is a key negative regulator of IL-2 expression and proliferation in Tregs (Baine et al. 2012). This might explain the presence of helios on cells that have already divided enough to stop further antigen driven division by silencing IL-2 expression on Tregs. In any case, helios expression has no profound effect on Treg infection by HIV. Higher gag DNA levels in memory FoxP3+CD25+ were detected in both helios positive (26 fold increased) and negative (119 fold increased) as well as FoxP3-CD25- Helios+ memory CD4 T cells (104 fold increased) compared to FoxP3-CD25-Helios- memory CD4 T cells, which constitute the largest of these memory CD4 T cell populations. FoxP3-CD25- Helios+ memory CD4 T cells are probably cells that have recently divided which might explain their higher observed gag DNA level in comparison to FoxP3-CD25- Helios- memory CD4 T cells.

Correlation of HIV replication to pVL has been reported before (Verhofstede et al. 1994; Wei et al. 1995). In this study, pVL correlated strongly with cell associated gag HIV levels in memory CD25-FoxP3- CD4 T cells but not with Tregs. Notably, amongst the 4 HIV positive subjects with no detectable pVL, proviral DNA could be detected in memory Tregs of 3 of the individuals (and 1 in CD25-FoxP3-CD4 T cells). This poses a question of whether proviral DNA in Tregs is only passed on by cell division. It should be noted however that in this study, total proviral DNA was measured in the T cell subsets studied. Since different forms of proviral HIV DNA have different impact on HIV pathogenesis (Koelsch et al. 2008), future studies should therefore differentiate different forms of HIV infecting Tregs.

Although high frequency of circulating CD4+CD25+ (FoxP3) Tregs has been associated with *S.mansoni* (Watanabe et al. 2007) and Hookworm (Ricci et al. 2011) infections in humans, this study only observed a significant elevation of numbers and frequency of Tregs in relation to *T.trichiura* infection in HIV negative subjects. Effective treatment of *T.trichiura* infection led to a 2-fold and a 1.7-fold decline in

frequency and numbers of Treg cells respectively a year post treatment. The median changes observed a year after anti helminthic treatment in *T.trichiura* infected subjects were insignificant when compared to the median changes observed in the non-helminth infected controls. This shows that de-worming has relatively little effect on reducing potential HIV substrates in the blood of helminth infected individuals. The relatively minor effect of helminth-treatment in *T. trichiura* infected volunteers on Treg levels, might be explained by the fact that Albendazole/Praziquantel treatment might not have completely eradicated *T. trichiura* infection as it is known that albendazole is ineffective for treating Trichuris infection (Keiser & Utzinger 2008). Effect of treatment on Treg counts and/or frequency was also observed in *S.mansoni* and Hookworm HIV negative treated groups. The frequency of CCR5 expressing Treg cells was not related to helminth infections, but effective treatment of *T.trichiura* and Hookworm infections was associated with an increase in proportion of CCR5+ Tregs. Helminth co-infection with HIV and de-worming was not associated with changes in the Treg population. However, the observed median changes between visits in Treg counts, frequency or their CCR5 expression were generally very minor and in most cases insignificant in helminth infected when compared to control groups.

For logistic reasons, all study groups including controls were de-wormed. Since the effect of treatment on absolute numbers and frequency of Tregs was also observed with the control subjects, there are three possible explanations for the observed longitudinal findings. First, it is possible that not all individuals in the control group were worm free since kato-katz is not a very sensitive method of worm detection (Basuni et al. 2011). Also, experimental artifacts such as variation of staining quality with time or gating of population cannot be excluded. But it is also possible that albendazole and praziquantel act on factors other than worms.

In conclusion, this study reports a substantial depletion of Tregs associated with HIV infection and a high *in vivo* expression of proportion of HIV coreceptor-CCR5 on Tregs also during HIV infection. Al together, this data demonstrates Tregs as good target for HIV infection *ex vivo* and indeed this was confirmed by the detection of high cell associated viral loads in Tregs. Also an elevation of numbers and proportion of Tregs was observed in relation to particularly *T.trichiura* infection,

which was reduced after helminth treatment. Infection of Tregs with HIV could have a strong impact on immune regulation and may contribute to HIV disease progression.

4.3. T cell activation profile during HIV, Helminth or HIV-Helminth co-infection

It has been hypothesized that systemic immune activation caused by chronic helminth infection contributes to increased HIV transmission risk in Sub-Saharan Africa (Bentwich et al. 1995) and therefore to the high HIV prevalence rates in this region. This hypothesis is supported by observations that low systemic T cell activation is linked to HIV resistance in highly exposed HIV uninfected individuals (Card et al. 2009; Koning et al. 2005; Bégaud et al. 2006) . Furthermore, it is well established that T cell activation and proliferation facilitate efficient AIDS virus replication *in vivo* and *in vitro* (Geldmacher & Koup 2012; Zack et al. 1990; Zhang et al. 1999). Previous studies support the concept that helminth infections are associated with systemic T cell activation (Kalinkovich et al. 1998; Kalinkovich et al. 2001; Secor et al. 2003). However, whether helminths are a primary cause of systemic T cell activation in populations from endemic areas of Africa is not entirely clear, because these studies did not specifically investigate immune activation before and after helminth eradication/treatment, nor did they differentiate between different helminth species. To fill this gap, we studied systemic T cell activation and HIV co-receptor expression in relation to helminth infection within the large WHIS cohort from Mbeya region, Tanzania, before and after albendazole/praziquantel treatment.

This study shows that *T.trichiura*, but also *A.lumbricoides* and *S.mansoni* infections are linked to increased frequencies of activated CD4 and/or CD8 T cells defined by expression of HLA-DR alone or in combination with CD38. Of note, increased T cell activation was quite dramatic for CD8 T cells during *T.trichiura* infection, whereas infection with *A.lumbricoides* was rather associated with more activated CD4 T cells. In contrast, infection with Hookworms was associated with substantial decrease in the frequency of HLA-DR^+ CD8 T cells. Thus, while these results partially agree with previously published data that helminth infections are associated with T cell activation (Kalinkovich et al. 2001; Kalinkovich et al. 1998),

our results demonstrate that different helminth species can have opposing associations in regard to systemic T cell activation.

The etiology of helminth-associated T cell activation is not known. *T.trichiura* and *S.mansoni* egg counts correlated positively with the frequency of HLA-$DR^+/CD38^+$ CD8 and CD4 T cells (data not shown), respectively, suggesting that high parasite burdens contribute to systemic T cell activation. Moreover, an elevation of Treg cells within *T.trichiura* infected study volunteers with >50% CCR5 expressing Tregs was also observed in this study. Interestingly, a linear positive association was seen between the frequency of Tregs and the frequency of activated (HLA-DR+) CD4 T cells (R^2=0.2763; P=0.0012; data not shown), suggesting that the increase in Treg cells is a function of CD4 T cell activation. Tregs are control immune responses induced by chronic pathogens by producing suppressive regulatory cytokines such as IL-10 (reviewed in (Belkaid & Tarbell 2009). In line with previous findings (Faulkner et al. 2002), this study similarly observed that *T.trichiura* infection was associated with increased plasma levels of pro-inflammatory (IL-1β and IL-17α), anti-helminthic (IL-13) and regulatory (IL-10) cytokines (data not shown), which closely correlated with each other; showing a mixed cytokine response to infection with *T.trichiura*. Of interest, IL-1β and IL-10 concentrations in our *T.trichiura* infected volunteers positively correlated with the frequency of HLA-DR+ CD4 and/or CD8 T cells, linking systemic T cell activation to the pro-inflammatory IL-1β and simultaneously to the regulatory IL-10 (data not shown). It is therefore possible that the immune response to *T.trichiura* infection causes immune activation through the induction of pro-inflammatory cytokines, but also evoke a systemic regulatory and anti-helminthic cytokine response. Our data thus confirm previous reports that *T.trichiura* infections are associated with increased regulatory T cells and cytokines (Faulkner et al. 2002; Turner et al. 2008) and provide a possible link between helminth associated systemic immune activation, hyporesponsiveness and anergy (Borkow et al. 2000; King et al. 1996).

To determine whether helminth-associated systemic immune activation was primarily caused by helminth infections, we studied the effect of a single dose of Albendazole/Praziquantel treatment on reducing systemic immune activation 3 months and up to a year after treatment. HLA-DR+ T cell frequencies most profoundly dropped in subjects infected with *T.trichiura* and *A.lumbricoides* but

increased in those infected with Hookworm, which is consistent with this study's cross-sectional observations. Nonetheless, the changes were insignificant when directly compared to the helminth negative control subjects, who were also treated. The relatively minor effect of helminth-treatment in *T.trichiura* infected volunteers on T cell activation might be explained by the fact that Albendazole/Praziquantel treatment might not have completely eradicated *T.trichiura* infection. Indeed, it is well known that albendazole is ineffective for treating *T.trichiura* infection (Keiser & Utzinger 2008). Supporting this argument, 30% (8 of 27) of *T.trichiura* infected subjects had detectable *T.trichiura* eggs post-treatment as per Kato-Katz test and a more sensitive diagnosis probably would have detected more infections. A recent study has demonstrated only 10% cure rate using an identical albendazole treatment as used during the WHIS study. More effective treatment options (Knopp et al. 2010) could help to clarify the effect of *T.trichiura* eradication on systemic immune activation. Based on our data, we cannot completely exclude the possibility that other environmental factors associated with *A.lumbricoides* or *T.trichiura* also contributed to increased systemic T cell activation in WHIS study volunteers.

To our knowledge, only one other longitudinal study has studied the effect of worm treatment on reduction of T-cell activation in HIV negative individuals (Kassu et al. 2003). Kassu et al. observed no significant changes in the expression of HLA-DR and CD38 on CD4 T cells in HIV negative subjects six months after helminth treatment but a significant decline in frequencies and numbers of HLA-DR+/CD38+ CD8 T cells. This study however did not distinguish between helminth and other intestinal parasites and was limited by a small sample size. Our study therefore provides for the first time extensive evidence on helminth associated systemic T cell activation and the impact of Albendazole/Praziquantel treatment. Is it possible that these activated T cells are helminth-specific? After Yellow fever (YF) vaccination co-expression of HLA-DR and CD38 is characteristic for recently activated, proliferating ($Ki67^{+}$) YF-specific CD8 T cells during the peak response (Querec et al. 2009) and thus this is one possible explanation. However, it is counterintuitive that during infection with *T.trichiura* such large fractions of CD8 T cells participate in the anti-helminthic immune responses.

A moderate increase in CCR5 surface expression on memory CD4 T cells and a tendency towards increased frequency of CCR5+HLA-DR+ memory CD4 T cells was observed with *T.trichiura* infection, which was in line with previous reports (Kalinkovich et al. 2001; Mkhize-Kwitshana et al. 2011). This observation further supports an indirect link between *T.trichiura* infection and HIV acquisition, because viral transmission is linked to viral CCR5 tropism (Moore et al. 2004; Sattentau et al. 1988; Deng et al. 1996; Liu et al. 1996). Upon entry, HIV replication is efficiently facilitated by proliferating and activated CD4 T cells *in vitro* and *in vivo* (Geldmacher & Koup 2012; Zack et al. 1990; Zhang et al. 1999) and activated HLA-DR+ CD4 T cells have been shown to support productive HIV infection in lymphoid tissue explants (Biancotto et al. 2008). Collectively, these study results support the hypothesis that helminth infections linked to increased levels of activated CD4 T cells might facilitate early systemic dissemination of HIV upon viral entry.

Surprisingly, within HIV negative, helminth positive individuals, there was a linear, negative association between frequency of HLA-DR expressing CD4 T cells and CD4 T cell counts (P= 0.0116; r^2= 0.035), suggesting an influence of CD4 T cell activation on the loss of CD4 T cells. This phenomenon has been observed before (Kalinkovich et al. 1998) in healthy people with chronic helminth infections, but the mechanism for such T cell depletion by helminths is still unclear. One possible explanation might be that helminth induced CD4 T cell activation not only cause CD4 T cell proliferation but also increases CD4 T cell death rate.

Evidence regarding the influence of HIV co-infection with different helminth species on systemic immune activation is scarce and with some discrepancy. Kassu *et al.* observed a significant increase in the frequency of HLA-DR expression, but not CD38 on CD8 T cells of HIV positive subjects co-infected with different intestinal helminth species (Kassu et al. 2003). In contrast, a more recent study conducted in South Africa demonstrated a high level of CD4 and CD8 T cell activation in HIV subjects co-infected with *A.lubricoides* and/or *T.trichiura* compared to HIV positive, helminth negative subjects as indicated by the expression of CD38, HLA-DR and CCR5 (Mkhize-Kwitshana et al. 2011). The reason for such discrepant findings in the two studies could be in the study design. While both studies were limited by a small sample size, Kassu et al. study did not distinguish between helminth and other

intestinal parasites whereas, the latter study was also limited in a multi-faceted definition of helminth-positivity through stool-results and specific IgE-levels (Mkhize-Kwitshana et al. 2011).

This study observed that helminth (mainly *T.trichiura* and Hookworm) infections only associated with an apparent up-regulation of percentages of CD8 T cells expressing HLA-DR+CD38- and HLA-DR independent of CD38. HIV co-infection with *T.trichiura* was also associated with higher frequencies of CD4 and CD8 T cells expressing HLA-DR+CD38+. Linear regression analysis showed a negative association between frequency of total HLA-DR+CD4 expressing T cells and CD4 T cell counts (P= 0.0017; r^2= 0.1845); and that frequency of total CD38+CD8 T cells is linked to a decline of CD4 T cell counts (P= 0.0706; r^2= 0.064), suggesting a role of immune activation contributed by HIV-Helminth co-infection on HIV disease progression. This is in agreement with previous findings which reported increased immune activation in HIV co-infection with other pathogens such as (but not limited to) helminths correlates with progression to AIDS as indicated by CD4 T cell decline (Eggena et al. 2005). Of note, Hookworm co-infection showed down regulation of expression of CCR5 density on total memory CD4 T cells compared to HIV positive non-helminth infected individuals suggesting an influence of Hookworm co-infection on modulation of CCR5 expression which may have an impact on prolonging HIV disease progression.

In some cases, treating for helminth infections reduced frequencies of immune activated T cells of helminth infected HIV negative and positive subjects 3 months to a year post treatment. It should be noted however, that there was a very moderate impact of de-worming observed in such a way that the state of immune activation was still raised. This suggests that they may be factors other than worms that contribute to systemic T cell activation in the study volunteers. In addition, in co-infected individual, the impact of HIV infection in driving HIV disease progression through modulation of the immune system might be greater than helminth induced T-cell activation as reports showing on only minor changes if at all in HIV progression markers following anti helminthic treatment exist (Webb et al. 2012; Sangaré et al. 2011). Therefore, our and previous findings show that deworming might not delay ARV treatment substantially in subjects with helminth co-infections (Walson et al.

2012). Thus, helminth co-infection should be treated in HIV+ patients, but only ARV treatment is a viable and reliable strategy to stop or slow down HIV disease progression.

It is well established that persistent immune activation strongly predicts the decline of CD4 T cells and hence progression to AIDS (Brenchley et al., 2004; Hazenberg et al., 2003). As expected, HIV infection alone was observed to have a great impact on immune activation as measured by the frequency of expression of HLA-DR and CD38 molecules on CD4 and CD8 T cells. Median frequencies of CCR5+ HLA-DR+ memory CD4 T cells were significantly higher in HIV positive than in HIV negative individuals although CCR5 density on the surface of memory CD4 T cells was reduced; possibly indicating their HIV co-receptor mediated specific depletion. In addition, linear regression analysis showed a relation between increase in frequency of immune activation markers on CD4 (HLA-DR) and CD8 (CD38) T cells and the loss of CD4 T cell counts within HIV positive subjects irrespective of their helminth infection status, emphasizing the role of immune activation on progression to AIDS. The etiology of systemic immune activation during HIV infection is obscure and most likely multi-factorial. Other factors potentially contributing to activation include: persistent antigen-specific stimulation of T cells specific for HIV and other persistent pathogens, such as HHVs (Giorgi et al. 1999; Ascher & Sheppard 1988), translocation of microbes and microbial compounds, such as Lipopolysaccharide (LPS), that activate PAMP-receptors (Brenchley et al. 2006) and chronically elevated levels of Type 1 Interferons (Bosinger et al. 2009; Jacquelin et al. 2009; Manches & Bhardwaj 2009).

A gradual decline in CD4 T cells is a major characteristic of chronic HIV infection. Depletion of a pool of memory CD4 T cells defined by the expression of CD27 and CD45RO is affected the most during the course of HIV infection (Hazenberg et al. 2003). Similarly, the depletion of CD4 T cells in HIV (independent of helminth) infection was mainly attributed to the decline in the proportion of central memory CD4 T cells. The frequency of CD8 T cells is elevated during the course of HIV and its elevation is linked to the control of HIV replication (Ogg et al. 1998). In line with previous studies (Hazenberg et al. 2003; Roederer et al. 1995; Sousa et al.

2002), an increase in frequencies of CD8 memory T cell subsets and a decline in the proportion of naïve CD8 T cells was seen in relation to HIV infection.

It has previously been reported that chronic infections with helminth species are associated with high frequency of memory (CD45RO+) and low frequency of naïve (CD45RA+) CD4 T cells in the blood of HIV negative people (Kalinkovich et al. 1998). In this study, no apparent influence of helminths was associated with changes in the proportion of T cell subsets of HIV negative subjects except for a significant increase in the frequency of terminally differentiated CD4 T cells, particularly, in *T.trichiura* and *S. haematobium* infected subjects. There was however a non-significant and significant decrease in frequency of central memory CD4 T cells for *T.trichiura* and *S. haematobium* infection respectively, increased frequency of effector memory CD4 T cells in association with *T.trichiura* infection and increased frequency of effector memory CD8 T cells with *S. haematobium* infection, suggesting that infections with such species modify the systemic distribution of T cell subsets. Helminth infections are characterized by a polarized CD4 T helper 2 (TH2) responses (reviewed in (Maizels & Yazdanbakhsh 2003)). Moreover, the level of memory CD4 T cells (defined by their lack of CD27 expression) is increased in filarial infection and contains TH2 cells (Yazdanbakhsh et al. 1993; Elson et al. 1994). These reports may therefore imply that the increased frequency of different memory T cell subsets in relation to different helminths that was observed in previous (Kalinkovich et al. 1998) and this study are most likely TH2 cells necessary for anti-helminth immune responses.

Within HIV positive subjects, again, helminths infections were generally not associated with changes in the proportion of T cell subsets. However, a significant increase in the frequency of terminally differentiating CD8 T cells, associated with *T.trichiura* and *S.haematobium* infections could be observed. In addition, *T.trichiura* co-infection was also related to a moderate increase in frequency of effector memory CD4 T cells. Our findings are to some extent similar to what Kassu *et al.*(Kassu et al. 2003) observed previously, although contrary to their observation of an increased proportion of memory (CD27+CD45RA-) T cells in association with particularly *A. lumbricoides*, this study found no such association. The difference observed between this and the previous study might be in the study design.

Only one study to our knowledge has documented on the effect of helminth treatment on the frequency of CD4 and CD8 T cell subsets in relation to HIV infection (Kassu et al. 2003). They reported a significant increase in the absolute numbers of effector (CD45RA+CD27-) CD8 T cells of HIV negative, helminth positive volunteers while HIV-helminth co-infected subjects showed increased frequency of naïve (CD45RA+CD27+) and memory (CD45RA-CD27+) CD8 T cells (Kassu et al. 2003). But as mentioned above, this study also had several limitations. In this study, an increase in percentage effector memory CD4 T cells was observed in effectively treated HIV negative subjects a year after treatment, accounted mainly by the treatment of *S.mansoni* and Hookworm. In addition, effective treatment of especially Hookworm and *A.lumbricoides* infections was also associated with increased frequency of terminally differentiated CD4 T cells. This was unexpected as helminth infection was observed to be associated with increased frequency of terminally differentiated CD4 T cells when compared HIV negative chronically infected with worms with non-infected subjects. A decline in the frequency of central memory CD4 and CD8 T cells and that of terminally differentiated CD8 T cells was also seen in relation to mainly treatment of *T.trichiura* infection. Contrary to a previous report (Kassu et al. 2003), the influence of treatment on the decline in proportion of naïve and central memory CD8 T cells was apparent in the blood of HIV-helminth co-infected subjects. In addition, increased frequency of effector memory and terminally differentiated CD4 T cells was observed a year after treatment in subjects with HIV-helminth co-infection. Surprisingly, a further increase in the frequency of terminally differentiated CD8 T cells was seen in relation to helminth treatment. However, effect of helminth treatment on the profile of T cell subsets was relatively little compared to helminth-negative controls. It should also be noted that for logistic reasons, all study groups including controls were de-wormed. Since the effect of treatment on T-cell subsets (and T-cell activation) was also observed with the control subjects, it is impossible to exclude experimental artifacts and other reasons mentioned previously as confounding effects for the longitudinal findings observed.

In conclusion, not all studied helminth species modulated systemic immune system in the same manner. Particularly, *T.trichiura*, *A.lumbricoides* and *S.mansoni*

infections correlate with increased expression of T cell activation markers with relatively little effect of helminth treatment compared to helminth-negative controls. Contrary, Hookworm infection was associated with a decreased frequency of HLA-DR expressing CD8 T cells. Because systemic T cell activation potentially contributes to increased HIV transmission risk (Card et al. 2009; Koning et al. 2005; Bégaud et al. 2006), this data support the concept that helminth infections, which are linked to systemic Immune activation (and increased CCR5 density on memory CD4 T cells), such as *T.trichiura* infection, could indeed also contribute to increased HIV transmission risk. Since the effect of helminth treatment on T cell activation was very minor in such a way that the state of immune activation was still raised it is likely that there may be factors other than worms that also contribute to the observed systemic T cell activation. Nonetheless, the link between parasite burden and activated T cells during *S.mansoni* and *T.trichiura* infections still suggest a causal link between helminths and systemic immune activation.

5. Summary

HIV/ AIDS is the most serious infectious disease to have infected humankind with most cases being reported in the Sub Saharan Africa. It has been hypothesized that helminth infections modify HIV susceptibility and disease progression by altering the human immune system and thus might contribute to the high prevalence of HIV-1 in Africa. However, Immunological evidence regarding whether and to what extent different helminth infections modify HIV susceptibility and disease progression remains controversial and it is only poorly understood, which immune system parameters might contribute to such alterations. Helminths might alter the systemic immune activation associated with HIV disease progression during the chronic phase of HIV infection but they might also change parameters associated with cellular susceptibility to HIV infection and replication. It has also been shown that chronic helminth infections can induce T cell hyporesponsiveness and impair other pathogen-specific T cell responses but it is still unclear how such changes influence cellular susceptibility to HIV infection. Simultaneously the fraction of $CD25^{+}$ (IL2 receptor alpha-chain) CD4+ T cells that contains a large proportion of regulatory T cells can be expanded in helminth infected individuals. CD25+ CD4+ regulatory T cells (Treg cells) have been shown to express the HIV co-receptor CCR5, thus may also serve as a preferential target of HIV infection. However, it is unclear whether such mechanisms contribute to susceptibility to HIV acquisition or disease progression in helminth and HIV co-infected subjects.

In order to study immune system modulation of different helminth infections in relation to HIV-1 susceptibility and disease progression, 381 adult volunteers from Mbeya region in Tanzania were enrolled and grouped according to their HIV-1 and helminth infection status. Participants were followed up at 3 months and 1 year after helminth treatment. Expression of regulatory (CD25, FoxP3, Tregs), memory (CD45RO, CD27) and activation markers (CCR5, HLA-DR/CD38) on T cells were studied *in vivo* using polychromatic flow cytometry in fresh anti-coagulated whole blood. HIV- and other pathogen-specific T cell responses were quantified in freshly isolated peripheral blood mononuclear cells using an Interferon gamma ELISPOT assay after stimulation with a peptide pool of 15 HIV frequently recognized Gag and

Nef peptides or respective studied pathogen antigens. Results were analysed in relation to helminth and HIV infection status. HIV+ subjects on ART were excluded from analysis.

Neither concurrent helminth infections nor their treatment had a significant effect on HIV- or other pathogen-specific T cell responses. However, HIV infection alone correlated with depletion of specific T cell responses to studied pathogens. HIV alone had a great impact on the decline of frequency and magnitude of IFN-γ production in PPD-specific CD4 T cells ($P \leq 0.0001$). Also, the quantity of HSV-1-specific T cells declined markedly with HIV infection ($P<0.0001$) while HIV infection was associated with a moderate decline of CMV- and EBV-specific T cell responses ($P= 0.0419$ and 0.0979 respectively) with no apparent influence of worms. Similarly, decrease in Influenza- and *T.gondii*-specific T cell responses in relation to HIV was also observed ($P<0.0001$ and 0.0576 respectively) with no apparent influence of worms.

Treg frequencies were increased especially in subjects infected with *T.trichiura* ($p=0.008$) but were also moderately high in HIV+ subjects, independent of their helminth infection status ($p=0.1612$ for HIV+Worm- and $p=0.0478$ for HIV+Worm+). In contrast, Treg count declined markedly with HIV infection ($P<0.0001$). Interestingly, a substantial fraction of Tregs (Median: 50%) in the blood of HIV negative and HIV positive volunteers expressed the HIV co-receptor CCR5, which potentially could support HIV entry into Tregs. Quantification of HIV-DNA copies in sorted CD4 T cells then demonstrated a 15 fold higher HIV infection rate in memory Tregs as compared to CD25-FoxP3- memory CD4 T cells ($p=0.0032$).

It is generally accepted that increased systemic immune T cell activation is associated with increased susceptibility to HIV acquisition. All studied helminth species were associated with systemic immune modulation but only *T.trichiura* infection correlated with substantially increased expression of HLA-DR on CD4 and CD8 T cells (both $p<0.005$); and increased density of CCR5 expression on the surface of memory CD4 T cells ($P=0.02$). A moderate increase in frequency of activation marker, CD38 on T cells was associated with *S.mansoni* infection ($P=0.04$). *S.haematobium* and *A.lumbricoides* infections also correlated with slight increased in T cell activation. This data shows that the studied helminth species modify HIV

susceptibility by increasing the systemic immune activation. On the Contrary, Hookworm infection was associated with decline in the frequency of HLA-DR expressing CD8 T cells (P=0.0283). Increase T cell activation was observed in relation to HIV co-infection with particularly *T.trichiura* showing the influence of *T.trichiura* infection on accelerating HIV disease progression by elevating T cell activation. HIV infection also correlated with immune activation and high proportion of CCR5+HLA-DR+ CD4 cells independent of helminth co-infection. Treating for helminth had a moderate impact on decreasing T-cell activation status.

6. Acknowledgement

I would like to declare and acknowledge that all the immunological procedures and analysis performed leading to these findings were conducted/coordinated by myself under the supervision of the study principle investigators (Dr. Christof Geldmacher and Dr. Elmar Saathoff) whereas, the establishment of the WHIS cohort, sample collection and diagnosis of worms were conducted by other colleagues under the leadership of the principle investigators. I would therefore like to thank members of the molecular diagnostic laboratory especially Wilbroad Nyembe and the WHIS field team for their excellent work throughout the study. I would also like to extend my gratitude to the NIMR-Mbeya Medical Research Centre team, particularly the main laboratory team members for their support and hard work, which led to these exciting findings. Last but not least, many thanks to the study principle investigators and my PhD supervisors especially Dr. Geldmacher for their close supervision, support and mentorship throughout the study.

The research leading to these results received funding from the German Research Foundation (DFG) programme for German-African cooperation projects in Infectiology (DFG, grant SA 1878/1-1) with additional support from the European Community's Seventh Framework Programme ([FP7/2007-2013] [FP7/2007-2011]) under EC-GA n° [241642].

7. References

Aandahl, E.M. et al., 2004. Human CD4+ CD25+ regulatory T cells control T-cell responses to human immunodeficiency virus and cytomegalovirus antigens. *Journal of virology*, 78(5), pp.2454–9.

Akimova, T. et al., 2011. Helios expression is a marker of T cell activation and proliferation. *PloS one*, 6(8), p.e24226.

Andersson, J. et al., 2005. The prevalence of regulatory T cells in lymphoid tissue is correlated with viral load in HIV-infected patients. *Journal of immunology (Baltimore, Md. : 1950)*, 174(6), pp.3143–7.

Angin, M. et al., 2012. Preserved function of regulatory T cells in chronic HIV-1 infection despite decreased numbers in blood and tissue. *The Journal of infectious diseases*, 205(10), pp.1495–500.

Antons, A.K. et al., 2008. Naive precursors of human regulatory T cells require FoxP3 for suppression and are susceptible to HIV infection. *J. Immunol.*, 180(2), pp.764–773.

Ascher, M.S. & Sheppard, H.W., 1988. AIDS as immune system activation: a model for pathogenesis. *Clinical and experimental immunology*, 73(2), pp.165–7.

Baecher-Allan, C. et al., 2003. CD4+CD25+ regulatory cells from human peripheral blood express very high levels of CD25 ex vivo. *Novartis Foundation symposium*, 252, pp.67–88; discussion 88–91, 106–14.

Baecher-Allan, C. et al., 2001. CD4+CD25high Regulatory Cells in Human Peripheral Blood. *J. Immunol.*, 167(3), pp.1245–1253.

Baine, I. et al., 2012. Helios Induces Epigenetic Silencing of Il2 Gene Expression in Regulatory T Cells. *The Journal of Immunology*.

Bancroft, A.J., McKenzie, A.N. & Grencis, R.K., 1998. A critical role for IL-13 in resistance to intestinal nematode infection. *Journal of immunology*, 160(7), pp.3453–61.

Barré-Sinoussi, F. et al., 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). 1983. *Revista de investigación clínica; organo del Hospital de Enfermedades de la Nutrición*, 56(2), pp.126–9.

Basuni, M. et al., 2011. A pentaplex real-time polymerase chain reaction assay for detection of four species of soil-transmitted helminths. *The American journal of tropical medicine and hygiene*, 84(2), pp.338–43.

Bégaud, E. et al., 2006. Reduced CD4 T cell activation and in vitro susceptibility to HIV-1 infection in exposed uninfected Central Africans. *Retrovirology*, 3, p.35.

Belkaid, Y. & Tarbell, K., 2009. Regulatory T cells in the control of host-microorganism interactions (*). *Annual review of immunology*, 27, pp.551–89.

Bentwich, Z. et al., 1998. Immune activation in the context of HIV infection. *Clinical and experimental immunology*, 111(1), pp.1–2.

Bentwich, Z. et al., 1995. Immune activation is a dominant factor in the pathogenesis of African AIDS. *Immunology today*, 16(4), pp.187–91.

Bethony, J. et al., 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet*, 367(9521), pp.1521–32.

Betts, M.R. et al., 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. *Blood*, 107(12), pp.4781–9.

Biancotto, A. et al., 2008. HIV-1 induced activation of CD4+ T cells creates new targets for HIV-1 infection in human lymphoid tissue ex vivo. *Blood*, 111(2), pp.699–704.

Booth, N.J. et al., 2010. Different proliferative potential and migratory characteristics of human CD4+ regulatory T cells that express either CD45RA or CD45RO. *Journal of immunology*, 184(8), pp.4317–26.

Borkow, G. et al., 2000. Chronic immune activation associated with intestinal helminth infections results in impaired signal transduction and anergy. *The Journal of clinical investigation*, 106(8), pp.1053–60.

Borkow, G. et al., 2001. Helminths, human immunodeficiency virus and tuberculosis. *Scandinavian journal of infectious diseases*, 33(8), pp.568–71.

Borrow, P. et al., 1994. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *Journal of virology*, 68(9), pp.6103–10.

Bosinger, S.E. et al., 2009. Global genomic analysis reveals rapid control of a robust innate response in SIV-infected sooty mangabeys. *The Journal of clinical investigation*, 119(12), pp.3556–72.

Brenchley, J.M., Schacker, T.W., et al., 2004. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *The Journal of experimental medicine*, 200(6), pp.749–59.

Brenchley, J.M. et al., 2006. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nature medicine*, 12(12), pp.1365–71.

Brenchley, J.M., Hill, B.J., et al., 2004. T-cell subsets that harbor human immunodeficiency virus (HIV) in vivo: implications for HIV pathogenesis. *Journal of virology*, 78(3), pp.1160–8.

Brown, M. et al., 2004. Helminth infection is not associated with faster progression of HIV disease in coinfected adults in Uganda. *The Journal of infectious diseases*, 190(10), pp.1869–79.

Brown, M. et al., 2005. Treatment of Schistosoma mansoni infection increases helminth-specific type 2 cytokine responses and HIV-1 loads in coinfected Ugandan adults. *The Journal of infectious diseases*, 191(10), pp.1648–57.

Burchill, M.A. et al., 2007. Interleukin-2 receptor signaling in regulatory T cell development and homeostasis. *Immunology letters*, 114(1), pp.1–8.

Cao, W. et al., 2009. Regulatory T cell expansion and immune activation during untreated HIV type 1 infection are associated with disease progression. *AIDS research and human retroviruses*, 25(2), pp.183–91.

Card, C.M. et al., 2009. Decreased immune activation in resistance to HIV-1 infection is associated with an elevated frequency of CD4(+)CD25(+)FOXP3(+) regulatory T cells. *The Journal of infectious diseases*, 199(9), pp.1318–22.

Casazza, J.P. et al., 2009. Autocrine production of beta-chemokines protects CMV-Specific CD4 T cells from HIV infection. *PLoS pathogens*, 5(10), p.e1000646.

CDC, Parasites - Soil-transmitted Helminths (STHs). Available at: http://www.cdc.gov/parasites/sth/.

Chapman, A.L.N. et al., 2002. Rapid detection of active and latent tuberculosis infection in HIV-positive individuals by enumeration of Mycobacterium tuberculosis-specific T cells. *AIDS (London, England)*, 16(17), pp.2285–93.

Chase, A.J. et al., 2008. Preservation of FoxP3+ regulatory T cells in the peripheral blood of human immunodeficiency virus type 1-infected elite suppressors correlates with low CD4+ T-cell activation. *Journal of virology*, 82(17), pp.8307–15.

Chou, C.S. et al., 1997. Highly purified CD25- resting T cells cannot be infected de novo with HIV-1. *Proceedings of the National Academy of Sciences of the United States of America*, 94(4), pp.1361–5.

Cooper, A.M. et al., 1993. Disseminated Tuberculosis in Interferon gamma Gene-disrupted Mice. *Journal of Experimental Medicine*, 178, pp.2243–2247.

Cooper, P.J. et al., 2000. Human infection with Ascaris lumbricoides is associated with a polarized cytokine response. *The Journal of infectious diseases*, 182(4), pp.1207–13.

Cox, F.E.G., 2002. History of human parasitology. *Clinical microbiology reviews*, 15(4), pp.595–612.

Currier, J.R. et al., 2002. A panel of MHC class I restricted viral peptides for use as a quality control for vaccine trial ELISPOT assays. *Journal of immunological methods*, 260(1-2), pp.157–72.

Dai, J. et al., 2009. Human immunodeficiency virus integrates directly into naive resting CD4+ T cells but enters naive cells less efficiently than memory cells. *Journal of virology*, 83(9), pp.4528–37.

Demoustier, A. et al., 2002. In patients on prolonged HAART, a significant pool of HIV infected CD4 T cells are HIV-specific. *AIDS (London, England)*, 16(13), pp.1749–54.

Deng, H. et al., 1996. Identification of a major co-receptor for primary isolates of HIV-1. *Nature*, 381(6584), pp.661–666.

Dieckmann, D. et al., 2001. Ex vivo isolation and characterization of CD4(+)CD25(+) T cells with regulatory properties from human blood. *The Journal of experimental medicine*, 193(11), pp.1303–10.

Douek, D.C. et al., 2002. HIV preferentially infects HIV-specific CD4+ T cells. *Nature*, 417(6884), pp.95–8.

Dunham, R.M. et al., 2008. CD127 and CD25 Expression Defines CD4+ T Cell Subsets That Are Differentially Depleted during HIV Infection. *Journal of immunology*, 180(8), pp.5582–5592.

Eggena, M.P., Barugahare, B., Jones, N., et al., 2005. Depletion of regulatory T cells in HIV infection is associated with immune activation. *Journal of immunology (Baltimore, Md. : 1950)*, 174(7), pp.4407–14.

Eggena, M.P., Barugahare, B., Okello, M., et al., 2005. T Cell Activation in HIV-Seropositive Ugandans : Differential Associations with Viral Load , CD4 + T Cell Depletion , and Coinfection. *Journal of Infectious Diseases*, 191, pp.694–701.

Elias, D. et al., 2001. Effect of deworming on human T cell responses to mycobacterial antigens in helminth-exposed individuals before and after bacille Calmette-Guérin (BCG) vaccination. *Clinical and experimental immunology*, 123(2), pp.219–25.

Elias, D. et al., 2005. Schistosoma mansoni infection reduces the protective efficacy of BCG vaccination against virulent Mycobacterium tuberculosis. *Vaccine*, 23(11), pp.1326–34.

Eller, M. a et al., 2012. Single-cell level response of HIV-specific and cytomegalovirus-specific CD4 T cells correlate with viral control in chronic HIV-1 subtype A infection. *Journal of acquired immune deficiency syndromes (1999)*, 61(1), pp.9–18.

Elson, L.H. et al., 1994. T cell subpopulation phenotypes in filarial infections: CD27 negativity defines a population greatly enriched for Th2 cells. *International immunology*, 6(7), pp.1003–9.

Faulkner, H. et al., 2002. Age- and infection intensity-dependent cytokine and antibody production in human trichuriasis: the importance of IgE. *The Journal of infectious diseases*, 185(5), pp.665–72.

Finberg, R.W. et al., 1991. Selective elimination of HIV-1-infected cells with an interleukin-2 receptor-specific cytotoxin. *Science (New York, N.Y.)*, 252(5013), pp.1703–5.

Fontenot, J.D. et al., 2005. A function for interleukin 2 in Foxp3-expressing regulatory T cells. *Nature immunology*, 6(11), pp.1142–51.

Fontenot, J.D., Gavin, M. a & Rudensky, A.Y., 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nature immunology*, 4(4), pp.330–6.

Fontenot, J.D. & Rudensky, A.Y., 2005. A well adapted regulatory contrivance: regulatory T cell development and the forkhead family transcription factor Foxp3. *Nature immunology*, 6(4), pp.331–7.

Gallegos, A.M. et al., 2008. Delayed protection by ESAT-6-specific effector CD4+ T cells after airborne M. tuberculosis infection. *The Journal of experimental medicine*, 205(10), pp.2359–68.

Geldmacher, C., Gray, C., Nason, M., et al., 2007. A high viral burden predicts the loss of CD8 T-cell responses specific for subdominant gag epitopes during chronic human immunodeficiency virus infection. *Journal of virology*, 81(24), pp.13809–15.

Geldmacher, C., Currier, J.R., Herrmann, E., et al., 2007. CD8 T-cell recognition of multiple epitopes within specific Gag regions is associated with maintenance of a low steady-state viremia in human immunodeficiency virus type 1-seropositive patients. *Journal of virology*, 81(5), pp.2440–8.

Geldmacher, C. et al., 2008. Early depletion of Mycobacterium tuberculosis-specific T helper 1 cell responses after HIV-1 infection. *Journal of Infectious Diseases*, 198(11), pp.1590–1598.

Geldmacher, C., Currier, J.R., Gerhardt, M., et al., 2007. In a mixed subtype epidemic, the HIV-1 Gag-specific T-cell response is biased towards the infecting subtype. *AIDS (London, England)*, 21(2), pp.135–43.

Geldmacher, C. et al., 2009. Minor viral and host genetic polymorphisms can dramatically impact the biologic outcome of an epitope-specific CD8 T-cell response. *Blood*, 114(8), pp.1553–62.

Geldmacher, C. et al., 2010. Preferential infection and depletion of Mycobacterium tuberculosis-specific CD4 T cells after HIV-1 infection. *The Journal of experimental medicine*, 207(13), pp.2869–81.

Geldmacher, C. & Koup, R. a, 2012. Pathogen-specific T cell depletion and reactivation of opportunistic pathogens in HIV infection. *Trends in immunology*, 33(5), pp.207–14.

Getnet, D. et al., 2010. A role for the transcription factor Helios in human CD4(+)CD25(+) regulatory T cells. *Molecular immunology*, 47(7-8), pp.1595–600.

Giorgi, J. V et al., 2002. Predictive value of immunologic and virologic markers after long or short duration of HIV-1 infection. *Journal of acquired immune deficiency syndromes (1999)*, 29(4), pp.346–55.

Giorgi, J. V et al., 1999. Shorter survival in advanced human immunodeficiency virus type 1 infection is more closely associated with T lymphocyte activation than with plasma virus burden or virus chemokine coreceptor usage. *The Journal of infectious diseases*, 179(4), pp.859–70.

Goletti, D. et al., 1996. Effect of Mycobacterium tuberculosis on HIV replication. Role of immune activation. *Journal of immunology (Baltimore, Md. : 1950)*, 157(3), pp.1271–8.

Hazenberg, M.D. et al., 2003. Persistent immune activation in HIV-1 infection is associated with progression to AIDS. *AIDS (London, England)*, 17(13), pp.1881–8.

Hazenberg, M.D. et al., 2000. T-cell division in human immunodeficiency virus (HIV) -1 infection is mainly due to immune activation : a longitudinal analysis in patients before and during highly active antiretroviral therapy (HAART) T-cell division in human immunodeficiency virus. *Blood*, 95, pp.249–255.

Hoffmann, D. et al., 2010. Elevated Epstein-Barr virus loads and lower antibody titers in competitive athletes. *Journal of medical virology*, 82(3), pp.446–51.

Holland, M.J. et al., 2000. Proteins secreted by the parasitic nematode Nippostrongylus brasiliensis act as adjuvants for Th2 responses. *European journal of immunology*, 30(7), pp.1977–87.

Hori, S., Nomura, T. & Sakaguchi, S., 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science (New York, N.Y.)*, 299(5609), pp.1057–61.

Hori, S., Takahashi, T. & Sakaguchi, S., 2003. Control of autoimmunity by naturally arising regulatory CD4+ T cells. *Advances in immunology*, 81, pp.331–71.

Hotez, P.J. et al., 2007. Control of neglected tropical diseases. *The New England journal of medicine*, 357(10), pp.1018–27.

Hunt, P.W. et al., 2003. T cell activation is associated with lower CD4+ T cell gains in human immunodeficiency virus-infected patients with sustained viral suppression during antiretroviral therapy. *The Journal of infectious diseases*, 187(10), pp.1534–43.

Jackson, J.A. et al., 2004. T Helper Cell Type 2 Responsiveness Predicts Future Susceptibility to Gastrointestinal Nematodes in Humans. *Journal of Infectious Diseases*, 190.

Jacquelin, B. et al., 2009. Nonpathogenic SIV infection of African green monkeys induces a strong but rapidly controlled type I IFN response. *The Journal of clinical investigation*, 119(12), pp.3544–55.

Jassoy, C. et al., 1993. Human immunodeficiency virus type 1-specific cytotoxic T lymphocytes release gamma interferon, tumor necrosis factor alpha (TNF-alpha), and TNF-beta when they encounter their target antigens. *Journal of virology*, 67(5), pp.2844–52.

Jin, X. et al., 1999. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. *The Journal of experimental medicine*, 189(6), pp.991–8.

Kalinkovich, A. et al., 1998. Decreased CD4 and increased CD8 counts with T cell activation is associated with chronic helminth infection. *Clinical and experimental immunology*, 114(3), pp.414–21.

Kalinkovich, A. et al., 2001. Increased CCR5 and CXCR4 expression in Ethiopians living in Israel: environmental and constitutive factors. *Clinical immunology (Orlando, Fla.)*, 100(1), pp.107–17.

Kallestrup, P. et al., 2005. Schistosomiasis and HIV-1 infection in rural Zimbabwe: effect of treatment of schistosomiasis on CD4 cell count and plasma HIV-1 RNA load. *The Journal of infectious diseases*, 192(11), pp.1956–61.

Kamal, S.M. et al., 2001. Specific cellular immune response and cytokine patterns in patients coinfected with hepatitis C virus and Schistosoma mansoni. *The Journal of infectious diseases*, 184(8), pp.972–82.

Kassu, A. et al., 2003. Role of incidental and/or cured intestinal parasitic infections on profile of CD4+ and CD8+ T cell subsets and activation status in HIV-1 infected and uninfected adult Ethiopians. *Clinical and experimental immunology*, 132(1), pp.113–9.

Keiser, J. & Utzinger, J., 2008. Efficacy of current drugs against soil-transmitted helminth infections: systematic review and meta-analysis. *JAMA : the journal of the American Medical Association*, 299(16), pp.1937–48.

Kiepiela, P. et al., 2007. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. *Nature medicine*, 13(1), pp.46–53.

King, C.L. et al., 1996. Cytokine control of parasite-specific anergy in human urinary schistosomiasis. IL-10 modulates lymphocyte reactivity. *Journal of immunology (Baltimore, Md. : 1950)*, 156(12), pp.4715–21.

Kinter, A.L. et al., 2007. CD25+ regulatory T cells isolated from HIV-infected individuals suppress the cytolytic and nonlytic antiviral activity of HIV-specific CD8+ T cells in vitro. *AIDS research and human retroviruses*, 23(3), pp.438–50.

Knopp, S. et al., 2010. Albendazole and mebendazole administered alone or in combination with ivermectin against Trichuris trichiura: a randomized controlled trial. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 51(12), pp.1420–8.

Koelsch, K.K. et al., 2008. Dynamics of Total, Linear Nonintegrated, and Integrated HIV-1 DNA In Vivo and In Vitro. *The Journal of Infectious Diseases*, 197(3), pp.411–419.

Komanduri, K. V et al., 1998. Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. *Nature medicine*, 4(8), pp.953–6.

Koning, F.A. et al., 2005. Low-Level CD4+ T Cell Activation Is Associated with Low Susceptibility to HIV-1 Infection. *The Journal of Immunology*, 175, pp.6117–6122.

Korber, B. et al., 2000. Timing the ancestor of the HIV-1 pandemic strains. *Science*, 288(5472), pp.1789–96.

Koup, R. A et al., 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *Journal of virology*, 68(7), pp.4650–5.

Lalvani, a et al., 2001. Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. *American journal of respiratory and critical care medicine*, 163(4), pp.824–8.

Lazenby, G.B., 2012. Opportunistic infections in women with HIV AIDS. *Clinical obstetrics and gynecology*, 55(4), pp.927–37.

Levacher, M. et al., 1992. The significance of activation markers on CD8 lymphocytes in human immunodeficiency syndrome : staging and prognostic value mrkersai ptory Thereists aneed for biologica monitoring oe disethusasneedprogre ionoand th reresp to therapye Emoitologic adcinical. *Clinical and experimental immunology*, 90, pp.376–382.

Lichterfeld, M. et al., 2004. Loss of HIV-1-specific CD8+ T cell proliferation after acute HIV-1 infection and restoration by vaccine-induced HIV-1-specific CD4+ T cells. *The Journal of experimental medicine*, 200(6), pp.701–12.

Liu, R. et al., 1996. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell*, 86(3), pp.367–77.

Liu, Z. et al., 1997. Elevated CD38 antigen expression on CD8+ T cells is a stronger marker for the risk of chronic HIV disease progression to AIDS and death in the Multicenter AIDS Cohort Study than CD4+ cell count, soluble immune activation markers, or combinations of HLA-DR. *Journal of acquired immune deficiency syndromes and human retrovirology : official publication of the International Retrovirology Association*, 16(2), pp.83–92.

Mahalingam, M. et al., 1995. Cell cycling in HIV infection: analysis of in vivo activated lymphocytes. *Clinical and experimental immunology*, 102(3), pp.481–6.

Maizels, R.M. & Yazdanbakhsh, M., 2003. Immune regulation by helminth parasites: cellular and molecular mechanisms. *Nature reviews. Immunology*, 3(9), pp.733–44.

Manches, O. & Bhardwaj, N., 2009. Resolution of immune activation defines nonpathogenic SIV infection. *The Journal of clinical investigation*, 119(12), pp.3512–5.

Mashishi, T. & Gray, C.M., 2002. The ELISPOT assay: an easily transferable method for measuring cellular responses and identifying T cell epitopes. *Clinical chemistry and laboratory medicine : CCLM / FESCC*, 40(9), pp.903–10.

McElroy, M.D. et al., 2005. Coinfection with Schistosoma mansoni is associated with decreased HIV-specific cytolysis and increased IL-10 production. *Journal of immunology (Baltimore, Md. : 1950)*, 174(8), pp.5119–23.

McKenzie, G.J. et al., 1998. A distinct role for interleukin-13 in Th2-cell-mediated immune responses. *Current biology*, 8(6), pp.339–42.

Mkhize-Kwitshana, Z.L. et al., 2011. The influence of different helminth infection phenotypes on immune responses against HIV in co-infected adults in South Africa. *BMC infectious diseases*, 11(1), p.273.

Moore, J.P. et al., 2004. The CCR5 and CXCR4 coreceptors--central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS research and human retroviruses*, 20(1), pp.111–26.

Moreno-Fernandez, M.E. et al., 2009. Human regulatory T cells are targets for human immunodeficiency Virus (HIV) infection, and their susceptibility differs depending on the HIV type 1 strain. *Journal of virology*, 83(24), pp.12925–33.

Moreno-Fernandez, M.E. et al., 2011. Regulatory T cells control HIV replication in activated T cells through a cAMP-dependent mechanism. *Blood*, 117(20), pp.5372–80.

Murphy, K., Travers, P. & Walport, M., 2007. *Janeway's Immunobiology* 7th ed. E. Lawrence, ed., Garland Publishing Inc.

Ng, W.F. et al., 2001. Human CD4+CD25+ cells: a naturally occurring population of regulatory T cells. *Blood*, 98(9), pp.2736–2744.

Nilsson, J. et al., 2006. HIV-1-driven regulatory T-cell accumulation in lymphoid tissues is associated with disease progression in HIV/AIDS. *Blood*, 108(12), pp.3808–17.

Ogg, G.S. et al., 1998. Quantitation of HIV-1-Specific Cytotoxic T Lymphocytes and Plasma Load of Viral RNA. *Science*, 279(5359), pp.2103–2106.

Oswald-Richter, K. et al., 2004. HIV infection of naturally occurring and genetically reprogrammed human regulatory T-cells. *PLoS biology*, 2(7), p.E198.

Picker, L.J. et al., 2004. Insufficient production and tissue delivery of CD4+ memory T cells in rapidly progressive simian immunodeficiency virus infection. *The Journal of experimental medicine*, 200(10), pp.1299–314.

Portales, P. et al., 2012. The intensity of immune activation is linked to the level of CCR5 expression in human immunodeficiency virus type 1-infected persons. *Immunology*, 137(1), pp.89–97.

Presicce, P. et al., 2011. Frequency of circulating regulatory T cells increases during chronic HIV infection and is largely controlled by highly active antiretroviral therapy. *PloS one*, 6(12), p.e28118.

Qin, S. et al., 1998. The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. *The Journal of clinical investigation*, 101(4), pp.746–54.

Querec, T.D. et al., 2009. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. *Nature immunology*, 10(1), pp.116–25.

Ramilo, O. et al., 1993. Role of CD25+ and CD25-T cells in acute HIV infection in vitro. *Journal of immunology*, 150(11), pp.5202–8.

Resende Co, T. et al., 2007. Intestinal helminth co-infection has a negative impact on both anti-Mycobacterium tuberculosis immunity and clinical response to tuberculosis therapy. *Clinical and experimental immunology*, 147(1), pp.45–52.

Ricci, N.D. et al., 2011. Induction of CD4(+)CD25(+)FOXP3(+) regulatory T cells during human hookworm infection modulates antigen-mediated lymphocyte proliferation. *PLoS neglected tropical diseases*, 5(11), p.e1383.

Rivière, Y. et al., 1995. Gag-specific cytotoxic responses to HIV type 1 are associated with a decreased risk of progression to AIDS-related complex or AIDS. *AIDS research and human retroviruses*, 11(8), pp.903–7.

Roederer, M. et al., 1995. CD8 Naive T Cell Counts Decrease Progressively in HIV-infected Adults CD4 T cells CD8 T cells CD62L CD62L. *Journal of Clinical Investigation*, 95, pp.2061–2066.

Roncador, G. et al., 2005. Analysis of FOXP3 protein expression in human CD4+CD25+ regulatory T cells at the single-cell level. *European journal of immunology*, 35(6), pp.1681–91.

Rosenberg, E.S. et al., 1997. Vigorous HIV-1-Specific CD4+ T Cell Responses Associated with Control of Viremia. *Science*, 278(5342), pp.1447–1450.

Sachsenberg, N. et al., 1998. Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen. *The Journal of experimental medicine*, 187(8), pp.1295–303.

Sadlack, B. et al., 1993. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell*, 75(2), pp.253–61.

Sakaguchi, S. et al., 1995. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *Journal of immunology (Baltimore, Md. : 1950)*, 155(3), pp.1151–64.

Sakaguchi, S. et al., 2008. Regulatory T cells and immune tolerance. *Cell*, 133(5), pp.775–87.

San-Andres, F. et al., 2003. Incidence of Acquired Immunodeficiency Syndrome – Associated Opportunistic Diseases and the Effect of Treatment on a Cohort of 1115 Patients Infected with Human Immunodeficiency Virus , 1989 – 1997. *Clinical infectious diseases*, 36, pp.1177–1185.

Sandler, N.G. et al., 2011. Plasma levels of soluble CD14 independently predict mortality in HIV infection. *The Journal of infectious diseases*, 203(6), pp.780–90.

Sangaré, L.R. et al., 2011. Species-specific treatment effects of helminth/HIV-1 co-infection: a systematic review and meta-analysis. *Parasitology*, 138(12), pp.1546–1558.

Sattentau, Q.J. et al., 1988. The human and simian immunodeficiency viruses HIV-1, HIV-2 and SIV interact with similar epitopes on their cellular receptor, the CD4 molecule. *AIDS (London, England)*, 2(2), pp.101–5.

Schmitz, J.E. et al., 1999. Control of Viremia in Simian Immunodeficiency Virus Infection by CD8+ Lymphocytes. *Science*, 283(5403), pp.857–860.

Schnittman, S.M. et al., 1990. Preferential infection of CD4+ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individuals. *Proceedings of the National Academy of Sciences of the United States of America*, 87(16), pp.6058–62.

Schorle, H. et al., 1991. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. *Nature*, 352(6336), pp.621–4.

Schuetz, A. et al., 2012. Pathogen prevalence may determine maintenance of antigen-specific T-cell responses in HIV-infected individuals. *AIDS (London, England)*, 26(6), pp.695–700.

Secor, W.E. et al., 2003. Increased Density of Human Immunodeficiency Virus Type 1 Coreceptors CCR5 and CXCR4 on the Surfaces of CD4+ T Cells and Monocytes of Patients with Schistosoma mansoni Infection. *Infection and Immunity*, 71(11), pp.6668–6671.

Seddiki, N. et al., 2006. Expression of interleukin (IL)-2 and IL-7 receptors discriminates between human regulatory and activated T cells. *The Journal of experimental medicine*, 203(7), pp.1693–700.

Sester, U. et al., 2006. Improved efficiency in detecting cellular immunity towards M. tuberculosis in patients receiving immunosuppressive drug therapy. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association*, 21(11), pp.3258–68.

Shapira-Nahor, O. et al., 1998. Increased susceptibility to HIV-1 infection of peripheral blood mononuclear cells from chronically immune-activated individuals. *AIDS (London, England)*, 12(13), pp.1731–3.

Sousa, A.E. et al., 2002. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. *Journal of immunology (Baltimore, Md. : 1950)*, 169(6), pp.3400–6.

Stevenson, M. et al., 1990. HIV-1 replication is controlled at the level of T cell activation and proviral integration. *The EMBO journal*, 9(5), pp.1551–60.

Suchard, M.S. et al., 2010. FOXP3 expression is upregulated in CD4T cells in progressive HIV-1 infection and is a marker of disease severity. *PloS one*, 5(7), p.e11762.

Sugimoto, N. et al., 2006. Foxp3-dependent and -independent molecules specific for CD25+CD4+ natural regulatory T cells revealed by DNA microarray analysis. *International immunology*, 18(8), pp.1197–209.

THMIS, 2011. *HIV/AIDS and Malaria Indicator Survey 2011-12*,

Thornton, A.M. et al., 2010. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. *Journal of immunology (Baltimore, Md. : 1950)*, 184(7), pp.3433–41.

Tran, T.-A. et al., 2008. Resting regulatory CD4 T cells: a site of HIV persistence in patients on long-term effective antiretroviral therapy. *PloS one*, 3(10), p.e3305.

Turner, J.D. et al., 2011. CD4+CD25+ regulatory cells contribute to the regulation of colonic Th2 granulomatous pathology caused by schistosome infection. *PLoS neglected tropical diseases*, 5(8), p.e1269.

Turner, J.D. et al., 2008. Intensity of intestinal infection with multiple worm species is related to regulatory cytokine output and immune hyporesponsiveness. *The Journal of infectious diseases*, 197(8), pp.1204–12.

Turner, J.D. et al., 2003. Th2 cytokines are associated with reduced worm burdens in a human intestinal helminth infection. *The Journal of infectious diseases*, 188(11), pp.1768–75.

Ulrichs, T. et al., 1998. Differential T cell responses to Mycobacterium tuberculosis ESAT6 in tuberculosis patients and healthy donors. *European journal of immunology*, 28(12), pp.3949–58.

UNAIDS, 2012. *Global Report: UNAIDS Report on the global AIDS epidemic*,

Verhofstede, C. et al., 1994. Evaluation of proviral copy number and plasma RNA level as early indicators of progression in HIV-1 infection: correlation with virological and immunological markers of disease. *AIDS (London, England)*, 8(10), pp.1421–7.

Vukmanovic-stejic, M. et al., 2006. Human CD4+CD25hiFoxp3+ regulatory T cells are derived by rapid turnover of memory populations in vivo. *The Journal of Clinical Investigation*, 116(9), pp.2423–2433.

Vukmanovic-Stejic, M. et al., 2008. The kinetics of CD4+Foxp3+ T cell accumulation during a human cutaneous antigen-specific memory response in vivo. *The Journal of clinical investigation*, 118(11), pp.3639–50.

Waldrop, S.L. et al., 1997. Determination of antigen-specific memory/effector CD4+ T cell frequencies by flow cytometry: evidence for a novel, antigen-specific homeostatic mechanism in HIV-associated immunodeficiency. *The Journal of clinical investigation*, 99(7), pp.1739–50.

Walson, J. et al., 2012. Empiric deworming to delay HIV disease progression in adults with HIV who are ineligible for initiation of antiretroviral treatment (the HEAT study): a multi-site, randomised trial. *The Lancet infectious diseases*, 12(12), pp.925–32.

Walson, J.L. et al., 2008. Albendazole treatment of HIV-1 and helminth co-infection: A randomized, double blind, placebo-controlled trial. *AIDS*, 22(13), pp.1601–1609.

Wammes, L.J. et al., 2010. Regulatory T cells in human geohelminth infection suppress immune responses to BCG and Plasmodium falciparum. *European journal of immunology*, 40(2), pp.437–42.

Watanabe, K. et al., 2007. T regulatory cell levels decrease in people infected with Schistosoma mansoni on effective treatment. *The American journal of tropical medicine and hygiene*, 77(4), pp.676–82.

Webb, E.L. et al., 2012. The effect of anthelmintic treatment during pregnancy on HIV plasma viral load: results from a randomized, double-blind, placebo-controlled trial in Uganda. *Journal of acquired immune deficiency syndromes (1999)*, 60(3), pp.307–13.

Wei, X. et al., 1995. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature*, 373(6510), pp.117–22.

WHO, Intestinal worms. Available at: http://www.who.int/intestinal_worms/more/en/index.html.

WHO, 2013. TB/ HIV FACTS 2012-2013. Available at: http://www.who.int/tb/publications/factsheet_tbhiv.pdf.

Yazdanbakhsh, M. et al., 1993. Elevated levels of T cell activation antigen CD27 and increased interleukin-4 production in human lymphatic filariasis. *European journal of immunology*, 23(12), pp.3312–7.

Zack, J.A. et al., 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell*, 61(2), pp.213–22.

Zhang, Z. et al., 1999. Sexual transmission and propagation of SIV and HIV in resting and activated CD4+ T cells. *Science (New York, N.Y.)*, 286(5443), pp.1353–7.

Curriculum Vitae

I. PERSONAL DETAILS

Surname**:** **Chachage**
First names: Mkunde Seithy
Date of Birth: June 2, 1984
Sex: Female
Nationality: Tanzanian
Languages: English (fluent) & Kiswahili (Mother Tongue)
Address: NIMR-Mbeya Medical Research Programme
P. O. Box 2410
Mbeya
Tanzania
E-mail: mchachage@nimr-mmrc.org; mkundec@yahoo.com
Phone: (+255) (0)754 099520

II. SUMMARY OF COMPETENCES

Academic

2010-To date PhD Candidate in International Health (Ludwig-Maximilians-Universität München)

2008 BSc (Hon) Degree in Medicine-Infectious Diseases and Immunology (University of Cape Town, Republic of South Africa)

2005 – 2007 BSc degree in Molecular and Cellular Biology (University of Cape Town, Republic of South Africa)

2002 - 2004 Advanced Level Certificate of Secondary Education (Aga Khan Mzizima Secondary School, United Republic of Tanzania)

1998 - 2001 Ordinary Level Certificate of Secondary Education (Aga Khan Mzizima Secondary School, United Republic of Tanzania)

Research

2009-To date NIMR-MBEYA MEDICAL RESEARCH CENTRE- TANZANIA
Immunology of Helminth Infections in relation to HIV-1 susceptibility and disease progression in Mbeya Region, Tanzania (WHIS)

2008 UNIVERSITY OF CAPE TOWN (UCT) - SOUTH AFRICA
Genetic characterization of methicilin resistant *Staphylococcus aureus* from Western Cape hospitals and their susceptibility to natural products

Conference and Poster Presentations

Immunology of Helminth Infections in relation to HIV-1 susceptibility and disease progression in Mbeya Region, Tanzania (WHIS). *NIMR 26th Annual Joint Scientific Conference (AJSC).* Arusha, Tanzania. 16-19 April 2012.

Immunology of Helminth Infections in relation to HIV-1 susceptibility and disease progression in Mbeya Region, Tanzania (WHIS). *German Society for Tropical Medicine and International Health (DTG) Conference.* Heidelberg, Germany. 14-16 March 2012

The Worms and HIV interaction study (WHIS). *Follow up German-African Cooperation Projects in Infectology (DFG) Conference.* Accra, Ghana 24-26 March 2011 & Bonn, Germany 27-30 June 2012

Independent Projects

2012 *Planning a symposium on "Fighting the Scourge of TB/HIV Co-Infection. Are Vaccine and Novel Diagnostics the Solution?"*
Details of this event can be found on the following link: **http://www.international-health.uni-muenchen.de/conferences_workshops/symposium_infect_immu/index.html**

2011 *Facilitating an Immunology Workshop in flow cytometry technique*

PUBLICATIONS

Melissa J. Jansen van Rensburg; V. Eliya Madikane; Andrew Whitelaw; **Mkunde Chachage**; Sumayya Haffejee; B. Gay Elisha. **The Dominant Methicillin-Resistant *Staphylococcus aureus* Clone from Hospitals in Cape Town has an Unusual Genotype: ST612. *Clin Microbiol Infect* 2011; 17: 785–792**

IV. WORK EXPERIENCE

2009- MBEYA MEDICAL RESEARCH PROGRAMME- TANZANIA
Research Scientist investigating the impact of HIV co-infections on the immune system.

V. AWARDS & CERTIFICATES

2012 Dr. Maria Kamm Best Young Woman Scientist Award
2008 Golden Key International Honour Society member for Academic Excellency
2007, 2005 UCT Dean's merit list certificate for Academic Excellency
2006-2008 Forest Hill Residence Academic Award for three consecutive years (2006, 2007&2008)
2007 Orientation Leadership Training Certificate

VI. REFERENCES

1. Doctor Christof Geldmacher
   Address: LMU, Georgenstr. 5, 80799 Munich, Germany
   Email: **geldmacher@lrz.uni-muenchen.de**
   Phone: (+49) (0)89 2180-17601

2. Doctor Lucas Maganga
   Address: NIMR-MMRP, P. O. Box 2410, Mbeya-Tanzania
   Email: **lmaganga@nimr-mmrc.org**
   Phone: (+255) (0) 784-514311

List of Manuscripts for Publication

1. CD25+FoxP3+ memory CD4 T cells are frequent targets of HIV infection *in vivo.*
2. Increased systemic T cell activation by helminth infections in relation HIV-1 susceptibility.