ANALYSIS OF T CELL SUBSETS IN HIV-1 INFECTED AND UNINFECTED ETHIOPIANS ON THE BASIS OF VARIOUS DIFFERENTIATION AND ACTIVATION MARKERS IN THE CONTEXT OF INTESTINAL PARASITIC INFECTIONS

By

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List of Abbreviations

AIDS - Acquired Immunodeficiency Syndrome

CD - Cluster of Differentiation

EBSS – Earle's Balanced Salt Solution

ELISA – Enzyme Linked Immunosorbent Assay

FACS – Fluorescence Activated Cell Sorter

FCS – Fetal Calf Serum

FITC – Fluorescein Isothiocyanate

HIV - Human Immunodeficiency Virus

HLA – Human Leukocyte Antigen

IFN – Interferon

IL – Interleukin

IMDM – Iscove's Modified Dulbecco's Medium

LTR – Long Terminal Repeat

MHC – Major Histocompatibility Complex

MIP - Macrophage Inflammatory Protein

NBCS – New Born Calf Serum

PBMC – Peripheral Blood Mononuclear Cells

PE – Phycoerythrine

PerCP – Perdinin-Chlorophyl-A Protein

RANTES - Regulated on Activation Normal T Expressed and Secreted

SDF - Stromal Derived Factor

TNF – Tumour Necrosis Factor

Abstract

The present study assessed whether expansion of a specific sub-population of CD8+ T cells is responsible for variations in CD8+ T cell counts; and also investigated the effect of incident intestinal parasitic infection and treatment on the profile of various differentiation and activation markers on CD4+ and CD8+ T cells from HIV-1 infected and uninfected adult Ethiopians (n=150). The pattern of CD8+ T cell population in whole blood samples of 86 subjects (60 HIV negative and 26 HIV positive) from Akaki and Wonji were analysed. The effect of incident intestinal parasites and their treatment was studied on cryo preserved PBMCs of 64 subjects (41 HIV- and 23 HIV+). In both cases, the samples were stained with antibodies to various T cell differentiation and activation markers and naïve, memory, effector, memory/effector, activated and resting CD4+ and CD8+ T cell subsets were quantified by triple colour FACScan. Significantly higher ($P<0.05$) count of CD8+ T cells in Akaki HIV negative subjects was reflected in a proportional increase in each of the CD8+ T cell compartment studied. Incident intestinal parasitic infections resulted in a significant increase of memory CD4+ T cells both in HIV negative and HIV positive subjects ($P<0.05$). There was significant increase in percentage of CD8+HLA-DR+ ($P<0.05$) T cells in HIV positive subjects with parasites. Increase in resting CD4+ and CD8+ T cells, decline in CD4+HLA-DR+ and CD8+HLA-DR+ and increase in CD8+CD38+ T cells was observed after treatment in HIV positive subjects. In HIV negative subjects, a significant decline in activated cells and a significant increase in resting CD8+ T cells ($P<0.05$) was observed after treatment. These data suggest that the difference in the CD8+ T cells might not be attributed to a specific response of these cells to a specific endemic infectious disease. Other factors, which may lead to different adaptive changes in the immune response, could be involved

and should be a subject of further study. The study also indicated that intestinal parasitic infections could result in alteration of T cell subset counts and also in up-regulation of T cell activation markers in the peripheral blood. Treatment for the intestinal parasites showed a tendency of reducing activation of the T cell subsets studied suggesting that, together with other community based intervention strategies, it could be used to down regulate the activation process and hence protect the host T cells from being easily attacked by HIV, the major cause of morbidity and mortality in the country and the developing world at large.

1. Introduction

1.1. Scope of the global HIV/AIDS problem

Human immunodeficiency virus (HIV) has been identified as the primary cause of the acquired immunodeficiency syndrome (AIDS). Since the initial clinical reports in the summer of 1981 among patients presenting with *Pneumocystis carinni* pneumonia in previously healthy homosexual men in United States of America (Gottlieb *et al.*, 1981), a large number of human beings all over the world have been infected by the virus, and a considerable number of the patients have died as a result of the disease. Two years after the first description of AIDS, its causative agent was identified to be a virus and was variously named as human T cell lymphotropic virus type III (Gallo *et al.*, 1983), lymphadenopathy associated virus (Barre-Sinoussi *et al.*, 1983), or AIDS associated retrovirus (Levy *et al*, 1984). Then after, the International Committee on the Taxonomy of Viruses decided the virus to be called human immunodeficiency virus (Coffin *et al.*, 1986).

According to UNAIDS (2000a) estimate, as of the end of 2000, there were 36.1 million people living with HIV/AIDS worldwide. Of these, 25.3 million (more than 70%) were from Sub-Saharan Africa. The high infection rate (15, 000/day) is expected to have resulted in 5.3 million new HIV infections in that year alone. More than 95% of these new infections occurred in developing countries (UNAIDS, 2000a). Through 2000, cumulative HIV/AIDS-associated deaths numbered approximately 21.8 million, of which 17.5 million were adults and 4.3 million children younger than 15 years (UNAIDS, 2000a). In 2000 alone, HIV/AIDS-associated illnesses caused the deaths of approximately 3 million

people, including 500,000 children younger than 15 years (UNAIDS, 2000a). Worldwide, more than 80% of all adult HIV infections have resulted from heterosexual intercourse (UNAIDS 2000a and b).

1.2. The HIV/AIDS situation in Ethiopia

In Ethiopia the first HIV-1 positive sera were reported in 1984 (Tsega *et al.*, 1988) and the first AIDS cases appeared two years later (Lester *et al.*, 1988). Since then the epidemic has spread explosively to reach prevalences of 10-27% in urban pregnant women (Fontanet *et al.*, 1998), 45% and 74% among commercial sex workers respectively in 1996 (Hussein *et al.*, 2000) and 1998 (Aklilu *et al.*, 2001). According to the Ethiopian Ministry of Health (MOH) report there were 83487 reported AIDS cases and 400000 estimated AIDS cases in Ethiopia since the beginning of the AIDS epidemic through March 2000 (MOH, 2000). Further, WHO estimates indicated that by the end of 1998 about 3 million Ethiopians were expected to be infected by the virus including 100000 children and an estimated total of 750 Ethiopians seroconvert daily (UNAIDS/WHO, 1998). This figure represented 9 percent of the world's HIV infections putting the country with the third most HIV-1 infected population in the world.

The predominant HIV-1 subtype circulating since the early days of the epidemic in the country has been determined to be subtype C (Ayehunie *et al.*, 1993; Salminen *et al.*, 1996; Abebe *et al.*, 1997; Hussien *et al.*, 2000). This subtype is the major subtype that accounts for greater than 50% of HIV-1 infections worldwide (UNAIDS/WHO, 1998).

1.3. The Human Immunodeficiency Virus

HIV is an RNA virus belonging to a group called lentiviruses in the family Retroviridae. It is an enveloped virus of 100 nm size. It contains an icosahedral nucleocapsid and two copies of an RNA genome (Figure 1). It has two types identified as HIV-1 and HIV-2. They have different geographical distribution and pathogenicity. The distribution of HIV-1 is worldwide while HIV-2 is relatively restricted to western Africa and is associated with a more benign course of disease than HIV-1 (Romieu *et al.*, 1990; Marlink *et al.*, 1994). HIV-1 has three distinct groups: M (main), O (outlier), and N (not-M-not-O). The M group constitutes the major group of HIV-1 and accounts for most cases of HIV-1 infection worldwide. Members of the M group are further divided into 10 subtypes (A, B, C, D, E, F, G, H, J, K) having specific geographic distributions. Viruses of most subtypes are found in Africa (Robertson *et al.*, 2000).

HIV is transmitted through sexual contact, blood transfusion, needle sharing, and from mother to child. Entry into host cells is mediated by the binding of gp120 surface molecule of the virus to the CD4 molecule. The gp41 component mediates fusion of the viral envelop with the plasma membrane of the cell, thereby causing conformational change that exposes the V3 loop in gp120. This permits a subsequent interaction with one of the co-receptors, CXCR4 or CCR5. This allows entry of the viral genome and associated viral proteins to the cytoplasm. Following entry of the virus into the cell, reverse transcription is initiated in the cytoplasm producing a double-stranded DNA replica of the original RNA genome. The DNA replica is then transported to the nucleus and integrated into the host genome. The integrated viral genome is called the provirus. The host transcription and translation machinery is employed for production of regulatory

proteins, structural proteins and the viral RNA genome upon activation of the host cell. Structural proteins and the RNA genome are transported to and assembled at the cellular membrane, after which the newly produced virus buds off from the membrane (Greene, 1993).

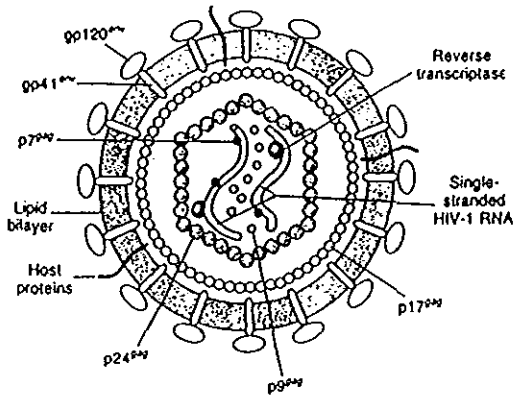


Figure 1. Structure of HIV (Greene, 1993)

1.4. Pathogenesis of HIV infection and AIDS

The course of HIV infection can be characterized by three phases: primary infection, clinical latency and AIDS (Figure 2).

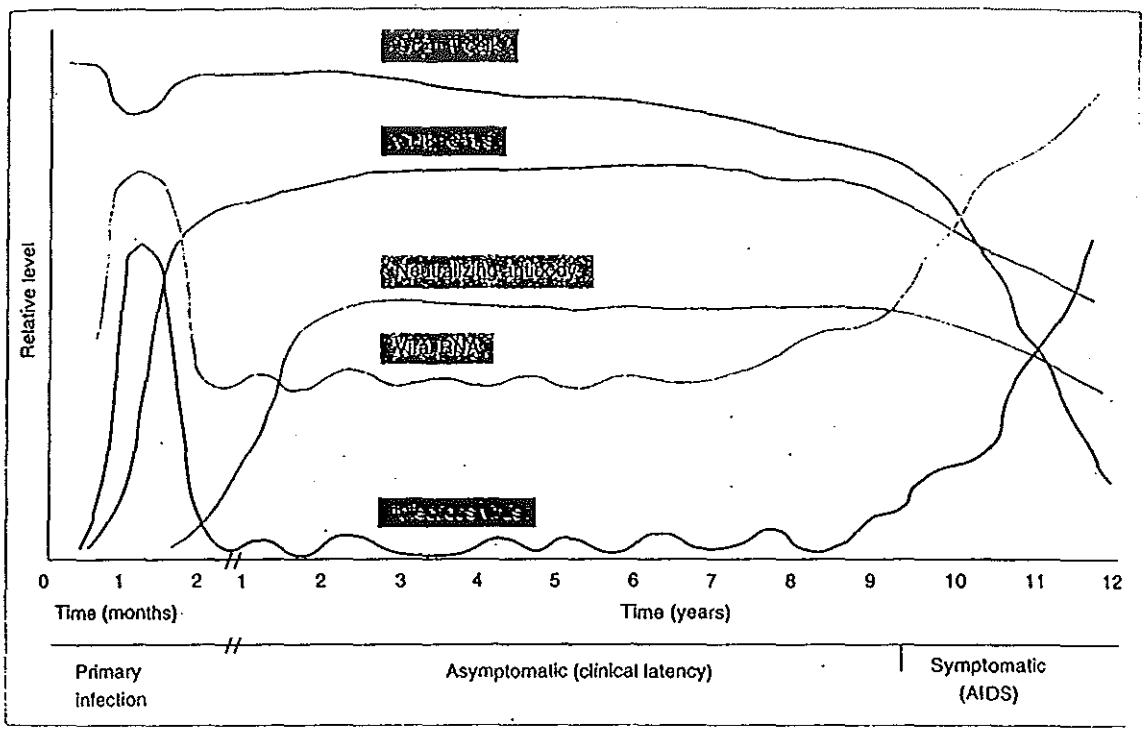


Figure 2. Typical course of HIV infection (Poignard *et al.*, 1996)

The acute infection may be either asymptomatic or associated with mononucleosis-like symptoms. This is followed by high titers of HIV RNA in plasma, high numbers of infected peripheral blood mononuclear cells (PBMC), and by early CD4+ T cell lymphocytopenia. After the acute stage, HIV RNA levels decrease and the number of CD4+ T cells increase again, although not to pre-infection levels. The level of viremia at this time, known as virologic set point, is a strong predictor for the length of the

subsequent period of clinical latency. This clinically latent period, also called asymptomatic period, varies in time from a few months to more than 15 years. The period is characterized by declining circulating CD4+ T cell numbers, increasing CD8+ T cell numbers, progressive immune dysfunction, activation of the immune system and increasing plasma viremia. Altogether, this will eventually lead to severe immunodeficiency and manifestations of clinical symptoms associated with AIDS, such as lymphadenopathy, flaring up of opportunistic infections, neoplasms and neurological symptoms (Pantaleo and Fauci, 1995; Graziosi *et al.*, 1998).

1.5. Immune Responses to HIV Infection

In the majority of infected persons, HIV infection is associated with a progressive and relentless destruction of the immune system. In response to the infection, both cellular and humoral arms of the immune responses are elicited (Burton and Montefiori, 1997; Goulder *et al.*, 1999; Gotch and Hardy, 2000). The cellular arm of the immune response comprises activation of virus-specific CD4+ helper and CD8+ cytotoxic T lymphocytes. The CD4+ T-helper cells recognize viral proteins that have been taken up in lysosomes of antigen-presenting cells, processed to smaller peptides (12-15 amino acids in length), and then presented at the cell surface within the peptide-binding groove of the class II major histocompatibility molecule. The presence of viral peptide in the class II binding groove elicits a T-helper-cell response to the virus. Then the CD4+ T cells become activated by a direct cell-cell interactions and release cytokines to orchestrate an effective immune response (Gotch and Hardy, 2000)

CD8+ cytotoxic T lymphocytes are also generated in response to HIV infection. These cells recognize viral peptides that have been processed in the cytosol into 9-10 amino acids in length, and that are presented in the context of MHC class I molecules on the cell surface. The presence of these viral proteins within the peptide-binding cleft of a class I molecule is a signal to the immune system that a foreign invader is present within that cell. This then triggers cytotoxic T lymphocytes to kill the infected cell through a direct recognition mediated by the T-cell receptor on the cytotoxic T cell. At the same time, activation of the cytotoxic T lymphocyte leads to the release of soluble antiviral factors. These two antiviral mechanisms mediate killing of infected cells through the release of granzymes and perforin (Yang *et al.*, 1996). They also mediate inhibition of progeny viruses that have already been produced by an infected cell through the release of β chemokines called regulated on activation normal T expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1α, and MIP-1β (Wagner *et al.*, 1998), that are active against macrophage-tropic viruses, as well as by stromal derived factor (SDF)-1 that are active against T cell-tropic viruses.

In addition to the cellular immune responses, the humoral immune response is also elicited against HIV infection. The majority of antibodies against HIV antigens are directed against virion debris rather than conformational epitopes on intact virions and are less likely to exert an antiviral effect (Burton, 1997). However, neutralizing antibodies that can directly neutralize free virus and are targeted against a number of different epitopes have been detected. These include antibodies directed against portions of the envelope glycoprotein that are involved in virus entry (the V3 loop antibodies), in the CD4 binding (CD4-binding-site antibodies) and other conformationally determined

epitopes that depend on the tertiary structure of the virus (Burton and Montefiori, 1997; Burton and Parren, 2000).

1.6. Infections and chronic immune activation

In areas of the world where the spread of AIDS is most dramatic, parasitic infections and HIV co-exist. Especially in the developing countries, millions of individuals are infected with chronic infectious diseases of protozoal, helminthic, bacterial, viral and fungal origin (Bentwich *et al.*, 1995; Chan, 1997; Lanzer *et al.*, 1997; Grant *et al.*, 1997; Tarantola & Schwartlander, 1997; UNAIDS, 2000a). Such individuals may harbour parasites for a very long time which would result in presence of circulating antigens and hence persistent antigenic stimulation (Miazeles *et al.*, 1993; Lanzer *et al.*, 1997).

Protozoan parasites and helminths are complex organisms and often have complex developmental cycles. Because of their large size, they carry more antigens both in number and kind in addition to the antigenic variation seen in many of them. As a result, the host mounts a complex immune response to a complex array of parasite antigens which depend largely upon the particular parasite, the stage of infection and the type of antigen (Wakelin, 1996).

Although the actual processes of immune response elicited against parasites are much more complex, the general events are that, the invading organism, or part of it, is taken up by a macrophage as part of the normal process of phagocytosis, broken down in the phagocytic vacuole and fragments are transported to the surface accompanied by class II MHC molecules. The fragments constitute the antigen and the whole complex consisting

of the antigen and the class II molecule, is recognized by the T cell receptors, on T helper lymphocytes and binds tightly to them. The macrophage which acts as an antigen presenting cell, then secretes a lymphocyte activating factor called interleukin 1 (IL-1) which stimulates the T helper lymphocytes to secrete other cytokines which activate other sets of cells (Cox and Liew, 1992). In mice two functional groups of CD4+ lymphocytes characterized by the profile of cytokines they produce have been shown to exist and are named as T helper 1 (Th1) and T helper 2 (Th2) cells. Similar cells also exist in humans. Th1 cells secrete IL-2, T cell growth factor, which activates cytotoxic cells, or gamma interferon (IFN-γ) which activates macrophages. Th2 cells secrete IL-4 and IL-5 which activate B lymphocytes to produce antibodies (Mosman and Sad, 1996). Immunological specificity is conferred at the stage of antigen processing and presentation and is maintained by the production of memory T and B cells which continue to circulate long after the antigen has disappeared (Ahmed and Gray, 1996).

This general pattern varies in detail, since antigen presentation can be exogenous as summarized above, or endogenous in which the antigen is synthesized by the infected cell and presented in the context of Class I molecules. The overall result is the activation of B cells, cytotoxic T cells and macrophages which together constitute the three arms of the immune response in which the effector mechanisms are antibodies, cytolysis and macrophage mediated killing. These three arms, acting either independently or in combination, are instrumental in the destruction of parasites and the acquisition of immunity. Once immunity has been established, resistance to reinfection is brought about by recalling the original immune response through the activation of the circulating memory cells (Cox and Liew, 1992).

According to Bentwich and colleagues (1995) the constant and lifelong confrontation of hosts with such infectious burden results in a chronic immune activation leading to unbalanced immune state. This is associated with a markedly increased number of eosinophils and an increased serum levels of immunoglubulin E (IgE) and soluble tumor necrosis factor (TNF) receptors (Bentwich *et al.*, 1996). Increased expression of activation markers (HLA-DR) on peripheral blood T cells; increased proportion of CD4+ cells expressing CD45RO+ (memory) with decreased proportion of CD45RA+ (naïve); decreased proportion of the costimulatory molecule, CD28+, on CD8+ cells as well as increased spontaneous apoptosis (Kalinkovich *et al.*, 1998), indicating activated state of the immune system and polarization of the immune response towards a dominant Th2 profile which promotes humoral responses (Bentwich *et al.*, 1997) are the characteristic features of individuals living in parasite endemic regions. Activated immune status due to chronic helminth infections have been shown to impair signal transduction and anergy which were restored gradually following anti-helminthic treatment (Brokow *et al.*, 2000). Other studies have shown induction and secretion of cytokines such as tumor necrosis factor-alpha (TNF-α) in parasitic and bacterial infections which can stimulate HIV replication by increasing the nuclear factor-kB (NF-kB) binding in the proviral regulatory region called long terminal repeat (LTR) (Finnegan *et al.*, 1996; Sulkowski *et al.*, 1998; Gopinath *et al.*, 2000; Cooper *et al.*, 2000; Whitworth *et al.*, 2000; McDonald, 2000). This would result in induction of transcription of the viral genome in HIV infected subjects and hence replication. Furthermore, *in vitro* activation of resting immune cells in HIV uninfected subjects were shown to upregulate expression of co-receptor CCR5 for HIV, increasing the susceptibility of cells for HIV infection (Wu *et al.*, 1997). In addition, the expression of CCR5 was found to be largely restricted to memory (Helbert *et al.*, 1997)

and HLA-DR+ (Ostrowski *et al.*, 1998) T cells, which were found to be ideal candidates for preferential infection (Schnittman *et al.*, 1990) and replication (Spina *et al.*, 1997) of HIV.

All the above studies seem to indicate that parasitic infections result in activation of the immune system which further makes the host susceptible to HIV infection and hastens its disease progression, as suggested by Bentwich and colleagues (1995). Bentwich *et al.* (1995) also pointed out the need for a state of decreased activation status to prevent people of developing nations from being easily attacked by HIV and the need to consider such background when planning for medical interventions (Bentwich *et al.*, 1995). Other investigators also have shown presence of immune dysregulation and activation in African subjects (Pollak *et al.*, 1994; Worku *et al.*, 1997; Rizzardini *et al.*, 1996; Rizzardini *et al.*, 1998; Messele *et al.*, 1999; Tsegaye *et al.*, 1999; Tsegaye *et al.*, 2000; Clerici *et al.*, 2000). This background is also supported by the observations that persons infected with HIV from sub-Saharan Africa may exhibit rapid HIV disease progression as compared with their counterparts in the western world (Gilks, 1993). Several co-factors were suggested to be involved in the facilitated progression of HIV disease in Africa. One of these is an abnormal activation of the immune system resulting from parasitic and non-parasitic infections, poor hygienic conditions, and nutritional deficiencies which would thus lead to chronic activation of peripheral lymphocytes with a consequent increased susceptibility of these lymphocytes to HIV infection (Bentwich *et al.*, 1995; Rizzardini *et al.*, 1996, Bentwich *et al.*, 1997). Besides the increased susceptibility to HIV such activated status is suggested to decrease the ability of the host to cope with HIV and other subsequent infections after exposure (Bentwich *et al.*, 1995).

1.7. T cell immunophenotyping

The expression of different isoforms of the leucocyte common antigen CD45 and a T cell activation antigen CD27 has been utilized as the best characterized phenotypic criterion to discriminate distinct stages of postthymic human CD4 and CD8+ T cell differentiation (Hamann *et al.*, 1997). Postthymic T cells undergo a number of changes after encountering antigens. These alterations contribute to the enhanced effectiveness that the immune system displays upon antigenic rechallenge (Ahmed and Gray, 1996).

T cells that enter the blood stream from the thymus are naïve and can be phenotypically characterized by the expression of the high molecular weight isoform of CD45 and CD27 (CD45RA+CD27+). These cells migrate through peripheral lymphoid organs and return to the blood to re-circulate until they encounter an antigen. Upon activation with their specific antigen, the naïve T-cells start to divide and differentiate into effector cells (CD45RA+CD27-) or convert into memory cells (CD45RA-CD27+). CD45RA-CD27- cells have characteristics of both memory and effector cells (Baars *et al.*, 1995, Hamann *et al.*, 1997, Hamann *et al.*, 1999). In addition to changes in CD45RA and CD27 antigens, postthymic T lymphocytes upregulate activation markers CD38 and HLA-DR when stimulated with their specific antigen. T cells expressing both HLA-DR and CD38 are classified as activated cells while those which have downregulated their expression are termed resting cells (Giorgi *et al.*, 1994). Such distinct stages of postthymic T lymphocyte changes can be phenotyped using specific monoclonal antibodies directed against various surface antigens such as CD4, CD8, CD45RA, CD27, CD38 and HLA-DR (BD Biosciences, 2000).

Phenotypic analysis of peripheral blood T cells into functionally different T cell subsets by flow cytometry provides insight into *in vivo* T cell differentiation in man. It is an important tool in the evaluation of the immunological status of a human subject, especially of value in the management of diseases that involve alterations in lymphocyte sub-populations, such as HIV infection (Giorgi and Detels, 1989, Roederer, 1995, Roederer *et al.*, 1995; Rabin *et al.*, 1995). For example, absolute CD4+ and CD8+ T cell counts and the derived CD4/CD8 T cell ratio are important elements for monitoring HIV infection progression (Stein *et al.*, 1992, Fahey *et al.*, 1990). CD4+ T cell counts are of additional value for the initiation of prophylactic treatment for opportunistic infections and to monitor responses to antiretroviral therapy in HIV infected individuals (CDC, 1992). Although CD4 lymphocytopenia and CD8 lymphocytosis have been reported as hallmarks of HIV infection (Giorgi and Detels, 1989), preferential depletion of naïve CD4 and CD8 T lymphocytes (Roederer, 1995, Roederer *et al.*, 1995; Rabin *et al.*, 1995) and increased expression of activation markers as measured by HLA-DR and CD38 (Giorgi *et al.*, 1994; Kestens *et al.*, 1994; Messele *et al.*, 1999) has also been shown. The progressive loss of naïve cells implies a concomitant loss in the ability to mount immune responses to new antigens, including the continually mutating HIV itself. While loss of memory cells implies inability to mount immune response to previously encountered antigens. Hence immunophenotyping can give a clear picture of the immune status of an individual. Such approach has been reported useful in the study of the dynamic changes that occur within the immune system in reaction to antigenic challenge (Baars *et al.*, 1995; Hamann *et al.*, 1997; Hamann *et al.*, 1999).

Immunophenotyping studies have also indicated that African populations are not homogeneous in terms of standard immunological parameters (Urassa *et al.*, 1996; Anglaret *et al.*, 1997; Tsegaye *et al.*, 2000). For example, high CD4+ T cell counts were reported in subjects of western Africa (Anglaret *et al.*, 1997; Zekeng *et al.*, 1997) while low counts in the east including Ethiopia (Urassa *et al.*, 1996; Tsegaye *et al.*, 1999; Messele *et al.*, 1999).

However, there has been very few reports of immunological studies on HIV infected and uninfected Ethiopians. Few immunophenotyping studies compared healthy adult Ethiopians to their Swedish (Worku *et al.*, 1997), Israelis (Pollak *et al.*, 1994, Kalinkovich *et al.*, 1998) and Dutch (Tsegaye *et al.*, 1999; Messele *et al.*, 1999) counterparts. Altogether, they indicated a lower $CD4^+$ and a higher $CD8^+$ T lymphocyte counts in the former, which has been ascribed to the increased load of environmental pathogens especially intestinal parasites in the Ethiopian setting. Two of these studies (Kalinkovich *et al.*, 1998, Messele *et al.*, 1999) demonstrated significantly reduced proportions and absolute numbers of naïve $CD4^+$ and $CD8^+$ T cells and an increased proportions and absolute numbers of memory $CD4^+$ and $CD8^+$ T cells in HIV seronegative Ethiopians compared to their Israelis and Dutch counterparts. These studies also indicated increased number of HLA-DR+CD38+ CD4 and CD8 T cells, and increased count of CD27- CD4 and CD8 T cells. These have been commonly used as indications of persistent immune activation in HIV negative Ethiopians (Kalinkovich *et al.*, 1998, Messele *et al.*, 1999). Furthermore, the report by Kalinkovich and colleagues (Kalinkovich *et al.*, 1998) indicated that treating patients for intestinal parasites decreases the activation status. This was demonstrated by comparing Ethiopians with intestinal helminth infections who

arrived in Israel within a very short time to Ethiopians who lived for more than three years there and who are free from intestinal parasites. A recent study also showed an increase in T cell proliferative responses and IFN-γ production in purified protein derivative (PPD) negative Ethiopians who were Bacille Calmette-Guerin (BCG) vaccinated after deworming compared to the placebo group (Elias *et al.*, 2001). On the other hand, a comparative cross-sectional study between parasite infected and non-infected Ethiopian individuals indicated no difference in $CD4^+$ and $CD8^+$ T cells and their subset counts (Abdulkadir, 1998). Although the subjects followed for the treatment study were very few, the same study had shown that treatment of intestinal parasites did not bring significant change on the above subset values and on the activation status, despite a reduction in activated CD8+ T cells (Abdulkadir, 1998). Interestingly, reversal of impairment in cell mediated immune response 3 to 6 months following chemotherapeutic cure of intestinal parasitic infections has been reported (Zwingenberger *et al.*, 1989; Roberts *et al.*, 1993; Grogan *et al.*, 1996).

Another recent comparative study also indicated differences in T cell subset values within Ethiopians living in geographically different areas (Tsegaye *et al.*, 2000). According to this later study, which involved healthy HIV negative subjects free from intestinal parasites, the previously reported low CD4 counts in Ethiopians (Pollack *et al.*, 1994; Worku *et al.*, 1997; Kalinkovich *et al.*, 1998; Tsegaye *et al.*, 1999; Messele *et al.*, 1999) was confirmed. However, the high CD8+ T cell counts reported for Ethiopians so far did not hold true for one of the population groups studied (Tsegaye *et al.*, 2000), calling for caution in using results obtained in a particular setting applicable to other population groups within the country. Although the role of parasites require further elucidation,

immunological studies conducted so far have indicated an altered immune status in Ethiopians. Therefore, we hypothesize that an endemic infectious disease could be the cause of the increased T cell numbers in a population, since a specific immune response would probably lead to adaptive changes in the composition of CD8+ T cell subsets, which would be reflected in an expansion of a specific CD8+ population.

In addition, reports on the role of intestinal parasites on immune activation status of Ethiopians are inconclusive. Investigating the effect of incident and cured parasitic infections on the profile of $CD4^+$ and $CD8^+$ T cells based on various activation and differentiation markers in Ethiopians might allow to suggest possible ways to decrease immune activation and hence to save the people from being easily attacked by HIV. Therefore, the present work is aimed to further characterize the high CD8 phenomenon and also to determine the role of incident and cured intestinal parasitic infections on profile of T cell subsets and activation markers in HIV-1 infected and uninfected adult Ethiopians.

General objectives

To determine the profile of T cell subsets in HIV-1 infected and uninfected Ethiopians on the basis of various activation and differentiation markers.

Specific objectives

1. To determine whether the difference in the total CD8+ T cell count observed between Akaki and Wonji subjects is reflected by expansion of a specific population within the CD8+ T cell compartment.

2. To determine the profile of $CD4^+$ and $CD8^+$ T cells expressing CD45RA, CD27, CD38 and HLA-DR antigens in HIV-1 infected and uninfected Ethiopians with incident and cured intestinal parasitic infections.

2. SUBJECTS AND METHODS

2.1. Study Subjects

The subjects included in this study are participants in a long-term cohort study on the progression of HIV type 1 infection in Ethiopia, performed by the Ethiopian-Netherlands AIDS Research Project (ENARP) at the Ethiopian Health and Nutrition Research Institute (EHNRI). They are factory workers in Akaki (a town about 15 km Southeast of the, Addis Ababa) and Wonji (a town 114 km Southeast of Addis Ababa). Description of the cohort studies has been reported previously (Sahlu *et al.*, 1998, Sahlu *et al.*, 1999). Study participants were enrolled following pre-test counselling and only after signing an informed consent form. Stool examination for parasites and test for HIV antibodies was done for each participant every six months. A total of 150 (101 HIV negative and 49 HIV positive) subjects were included in this study. For the cross-sectional study, 86 apparently healthy adult subjects, 42 from Akaki and 44 from Wonji were included. For the longitudinal study, 64 (41 HIV negative and 23 HIV positive) subjects with incident and/or cured parasitic infections were included from Akaki. Samples for the longitudinal study were selected if the subjects had consecutive visits in six months interval and were positive for intestinal parasites in one of their visits. Subjects found positive for intestinal protozoa (*Entamoeba histolytica* and *Giardia lamblia*) were treated with Metronidazole and those found positive for helminths were treated with broad spectrum anthelminthics such as praziquantel, albendazole, mebendazole, levamisole by ENARP cohort site physicians. Those found negative for intestinal parasites six months after treatment were selected to determine the effect of treatment.

2.2. Sample collection and processing

Whole blood was collected with a 10ml vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant between 8:30 and 11:30am and transported to ENARP laboratory within 6 hours after collection from Akaki and Wonji. Soon after arrival, each sample was screened for presence of HIV antibodies in plasma using HIVSPOT test (Genelabs Diagnostics, Singapore). The tubes then after were mixed well and 500 µl of the whole blood was transferred into Nunc tubes for immunohaematological analyses. Plasma was then removed and the rest of the sample was reconstituted with washing medium (reconstituted from 475ml Earle's Balanced Salts Solution (EBSS), supplemented with 25ml of 5% New Born Calf Serum (NBCS), 1ml of 100U/ml penicillin (P) and 100µg/ml streptomycin (S) and 2ml of 20U/ml heparin) and used for peripheral blood mononuclear cells (PBMC) isolation. Stool examination for parasite infection was performed on fresh stool at the study sites on the same date of blood sample collection.

2.2.2. Microscopic examination of stool samples

Stool samples were collected from participants in stool collection caps and examined for presence of parasites by using direct microscopy (using normal saline and iodine preparation) and formol-ether sedimentation concentration method (Beaver *et al.*, 1984). The Baermann concentration method was also performed to detect *Strongyloides stercoralis* (Lima and Degado, 1961).

2.2.3. HIV screening and confirmatory assays

Screening for plasma antibodies against HIV infection was done by using HIV-SPOT (Genelabs Diagnostics, Singapore) and enzyme-linked immunosorbent assay (ELISA)

(Organon Teknika BV, The Netherlands). Positive and discrepant plasma samples were confirmed by Western Blot analysis (HIVBLOT 2.2, Genelabs Diagnostics, Singapore).

2.2.4. PBMC isolation and freezing

PBMC were isolated by Ficoll-Hypaque density gradient centrifugation method. In brief, the reconstituted sample with washing medium was loaded over 12.5ml ficoll in 50ml test tube, and centrifuged at 1400 rpm for 30 minutes. PBMC were then harvested into a new 50 ml test tube using sterile pastette and washed two times in the washing medium. The final volume of the pellet was adjusted to 1ml by adding 0.9 ml IF20 medium (200ml of Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 50ml of 20% Fetal Calf Serum (FCS), 0.5ml of 100U/ml penicillin and 100µg/ml streptomycin) to it. From this volume, 20µl was taken and added into a plastic beaker filled with 10ml isoton (Azide-free balanced electrolyte solution) and 3 drops of Triton-Saponin. The number of cells/ml were determined by using Coulter counter (Coulter Electronics Ltd, U.K.). Freezing medium (IF20 and dimethyl sulfoxide) was added to the PBMC and the cells were transferred into freezing vials and were frozen by using a freezing machine (KRYO 10, Biomedical Series II). The frozen PBMCs were then stored in liquid nitrogen until analysed.

2.2.5. Hematological analysis

Absolute number of leucocytes per µl of whole blood was obtained using a Coulter counter T540 (Coulter Electronics, Florida, USA). The machine automatically dilutes a whole blood sample of 29.6 µl, lyses, counts, and gives a printout result of haematological parameters.

2.2.6. Thawing of cells from liquid nitrogen and preparation for three colour FACScan analysis

Cryopreserved PBMCs were removed from liquid nitrogen and immediately put in a 37^0C water bath and left until the content is thawed. The ampules were then removed from water bath, cleaned using tissue paper soaked with 70% ethanol and immediately placed in a bucket with ice. In a laminar flow, the cell suspension was transferred from the ampule into a 50ml size polypropylene tube, on ice, using a sterile plastic pastette. While carefully shaking the 50 ml tube, thawing medium (225ml EBSS, 25ml NBCS (10% final) and 0.5ml P/S) was added drop by drop, to a final volume of 20ml. The tubes were centrifuged for 10 min at 1500 rpm at room temperature. The supernatant was aspirated and the pellet dissolved by gently tapping the 50ml tube. 20 ml IF20 medium was added at room temperature and cells kept in a dark place for 30 min. Washing medium was added to a final volume of 50ml, centrifuged for 10 min at 1500 rpm at room temperature, the supernatant aspirated and the pellet dissolved by gentle tapping. The pellet was then re-suspended with IF20 medium to a final volume of 1ml. Three drops of Triton-Saponin, 10ml of Isoton and 20µl of PBMC were added into labelled Coulter counting vessels, stirred with plastic stirrer and cells counted using Coulter counter. Viability of cells was checked by mixing 20µl of sample and 20µl of trypan blue solution in a vial and dead (blue) and alive (white) cells were counted using a microscope on a counting chamber. A total of 200 events were counted and percent viability was calculated. Viability was found to be greater than 90%. The re-suspended pellet was centrifuged for 10 min at 1500 rpm at room temperature. The supernatant was discarded and pellet dissolved in 2 ml of Isoton,

centrifuged for 10 min at 1500 rpm at room temperature. The pellet was then dissolved in 500µl of Isoton. This suspension was used for three colour flow cytometric analysis.

2.2.7. Triple colour immunophenotyping of T cell subsets

Naïve, memory and effector CD4+ and CD8+ T cells were quantified by three colour flow cytometric analysis after staining with Perdinin-Chlorophyll-A Protein (PerCP)-conjugated CD4 or CD8 monoclonal antibodies (MoAbs) in combination with Fluorescein isothiocyanate (FITC)-conjugated CD27 MoAb and Phycoerythrine (PE)-conjugated CD45RA (Becton Dickinson Immunocytometry Systems, San Jose, California) according to previous reports (Baars *et al.*, 1995; Hamann *et al.*, 1997). *In vivo* activated and non-activated CD4+ and CD8+ T cells were also quantified by three-colour flow cytometric analysis after staining with PerCP-conjugated CD4 or CD8 MoAbs in combination with FITC-conjugated CD38 MoAb and PE-conjugated HLA-DR according to Giorgi *et al.* (1994). In brief, 50 µl of whole blood (or 250000 PBMCs) were mixed and incubated at room temperature with each combination of MoAbs (5 µl from each) for 30 minutes in separate tubes in the dark. Erythrocytes were lysed by adding 1 ml of fluorescent activated cell sorter lysing solution (50% diethylene glycol and 15% formaldehyde; Becton Dickinson). After vortexing tubes were incubated in the dark at room temperature for 15 minutes and immediately centrifuged at 1640 rpm for 5 minutes. The supernatant was discarded leaving approximately 50 µl of residual fluid in the tube. Two ml of Isoton was added to the cell pellet, mixed thoroughly, and centrifuged at the same speed and time interval. The supernatant was removed and the residue re-suspended in 500 µl of Isoton. Events were acquired and analysed using a FACScan flow cytometer with Cellquest software (Becton Dickinson). For acquisition and storage, a gate was set on side scatter

and PerCP fluorescence to stop acquiring when 2000 CD4+ or CD8+ T lymphocytes were collected. To analyse the events, a live gate was set first for all live events excluding debris, and then for lymphocytes, monocytes and granulocytes using forward versus side light scattering property of the cells for the whole blood analysis, and only on lymphocytes for the PBMCs. CD4+ and CD8+ cells were gated on side scatter and PerCP fluorescence in order to collect a minimum of 1500 CD4+ or CD8+ T lymphocytes from the lymphocyte gate. To prevent monocytes or natural killer cells from being included in the analyses, only bright CD4+ or CD8+ cells respectively were considered. Gated CD4+ or CD8+ bright events were used to quantify subsets and activation markers in dot plot/contour plot by setting quadrant markers. Using the three-colour flow cytometry both CD4+ and CD8+ cell subsets were defined as follows: naïve (CD45RA+CD27+), memory (CD45RA-CD27+), effector (CD45RA+CD27-), memory/effector (CD45RA-CD27-), activated (HLA-DR+CD38+), resting (HLA-DR-CD38-). Percentage of events in each quadrant was used to calculate absolute values of the corresponding cell populations. The FACScan was calibrated with CaliBRITE fluorescent beads and FACScomp software (Becton Dickinson) weekly.

2.3. Data Analysis

Statistical analyses were performed using STATA software (Stata Corporation, Texas). The distribution of T-cell subsets and activation markers was compared between groups using the non-parametric Wilcoxon sign-rank test. Differences were considered not significant when the P-value was greater than 0.05.

2.4. Ethics

The study is part of a long-term cohort study on the progression of HIV infection in Ethiopia, which is ethically approved by both EHNRI and National Ethical Clearance Committee. Informed consent was obtained from each subject.

3. RESULTS

3.1. Comparison of T cell subsets and activation status between Akaki and Wonji subjects

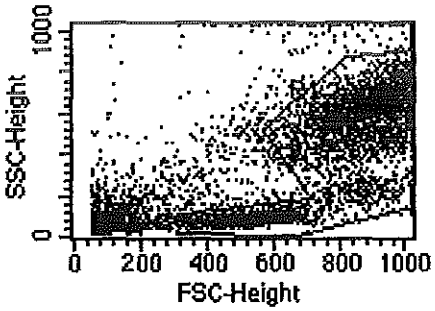
This study involved 86 subjects. Sixty were HIV negative with median age of 40 (range 27-47) and 26 were HIV positive having median age of 38 (range 28-46). All participants from Wonji were males. There were 4 HIV positive female subjects from Akaki.

3.1.1. T cell subsets and activation status between Akaki and Wonji HIV negative subjects

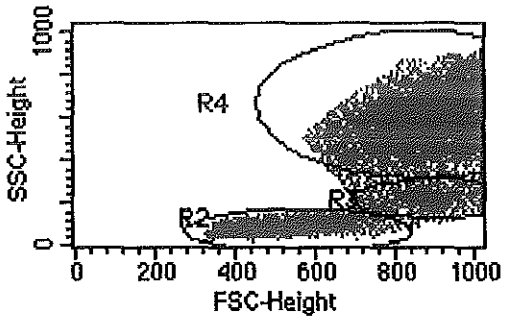
This cross-sectional study was performed on whole blood sample of 60 adult (aged 27-47) HIV negative, apparently healthy, male factory workers residing in Akaki and Wonji to study the CD4+ and CD8+ T-cell subsets and activation markers distribution between the two populations. Figure 3 shows cell gating and representative dot plots of CD4+ and CD8+ T cell subsets and activation markers.

3.1.1.1. CD4+ and CD8+ T cells and their subsets between Akaki and Wonji HIV negative subjects

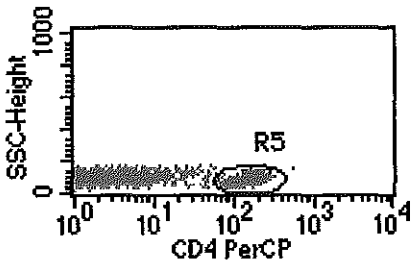
No significant difference was found in total leukocyte or absolute and percentage values of lymphocytes and granulocytes between Akaki and Wonji individuals. However



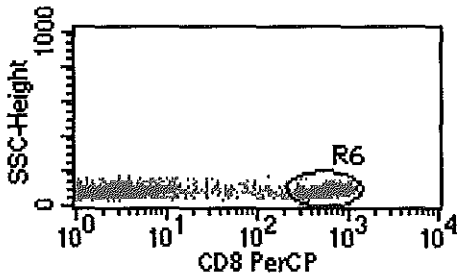
Live gate of leukocytes



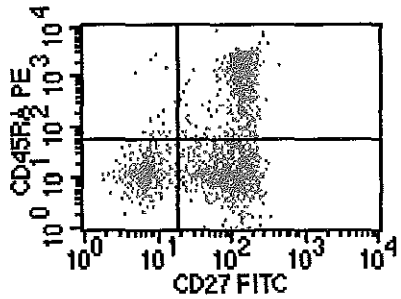
WBC subsets



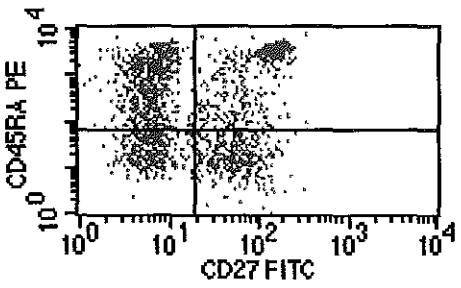
CD4+ T cells gate



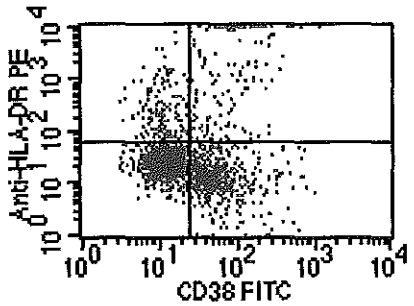
CD8+ T cells gate



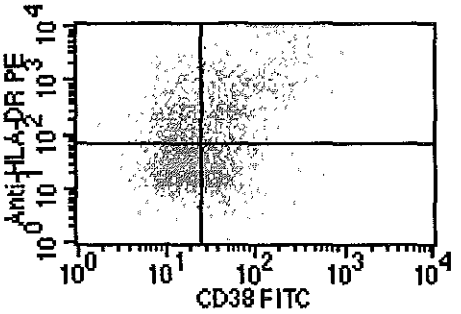
CD4+ T cell subsets



CD8+ T cell subsets



Activated & Resting CD4+ T cells



Activated & resting CD8+ T cells

Figure 3. Cell gating and subset distribution

median and 95 percentile values of lymphocytes and monocytes were relatively higher in Akaki subjects (Table 1).

There was also no significant difference in median absolute numbers and percentages of CD4+ T-cells between Akaki (661 cells/μl, 34%) and Wonji (654 cells/μl, 40%) participants. On the other hand, significantly higher absolute numbers and percentages of CD8+ T-cells were observed in Akaki (596 cells/μl, 29%) subjects than Wonji (380 cells/μl, 23%) ($P<0.05$) (Figure 4). As a result, the CD4/CD8 ratio was significantly higher for Wonji subjects ($P<0.05$).

To further study the significantly higher numbers of CD8+ T cells in males from Akaki compared to males from Wonji, CD4+ and CD8+ T cells were analysed for naïve, memory and memory/effector subsets as defined by differential expression of CD45RA and CD27 as described previously (Baars *et al.*, 1995; Hamann *et al.*, 1997). The results showed no significant difference in the absolute numbers and percentages of naïve (CD45RA+CD27+), effector (CD45RA+CD27-), memory (CD45RA-CD27+) and memory/effector (CD45RA-CD27-) CD4+ T-cells between Akaki and Wonji subjects (Table 2). With regards to the CD8+ T cell subsets compartment, the higher number of CD8+ T cells in males from Akaki appeared to be reflected by almost all CD8+ T cell subsets. As a result, there was significantly higher absolute naïve (CD45RA+CD27+), memory (CD45RA-CD27+) and cytotoxic effector (CD45RA+CD27-) CD8+ T cell populations in Akaki than Wonji subjects, $P<0.05$ (Figure 5). The memory/effector (CD45RA-CD27-) CD8+ T cell subsets were not significantly different in the two

population groups, although the values were higher for Akaki. However, there were no significant differences when percentages of CD8+ T cell subsets were compared.

3.1.1.2. Expression of CD38 and HLA-DR on CD4+ and CD8+ T cells of Akaki and Wonji HIV negative subjects

A combination of HLA-DR and CD38 monoclonal antibodies were used to study activated and resting CD4+ and CD8+ T cells in 20 subjects, 10 from each site. No significant difference was seen in absolute numbers and percentages of activated (HLA-DR+CD38+), resting (HLA-DR-CD38-), HLA-DR+CD38- and HLA-DR-CD38+ CD4+ T cell subsets between Akaki and Wonji participants (Table 3). Within the CD8+ subset, however, absolute counts of resting, HLA-DR+CD38- and HLA-DR-CD38+ T-cells were significantly higher in Akaki subjects than in Wonji, $P<0.05$ (Figure 6). No significant difference was seen in percentages of these cells and also both in absolute counts and percentages of activated cells between Akaki and Wonji subjects.

3.1.2. T cell subsets and activation markers between Akaki and Wonji HIV positive subjects

Analysis of the various T cell subsets and activation markers was also performed for 26 asymptomatic HIV positive subjects from Akaki (n=12) and Wonji (n=14). Median values and 95 percentile ranges of the parameters analysed were depicted in Tables 4 and 5. Unlike the pattern in HIV negative subjects, no significant difference was observed both

in absolute counts and percentages of all the WBC subsets and activated and resting cells studied between Akaki and Wonji HIV positive participants.

However, when values of HIV positives were compared to the HIV negative subjects, the former had significantly higher CD8+ ($P<0.05$) and significantly lower CD4+ ($P<0.05$) T cell counts (Figure 7 and 8). This resulted in a significantly lower CD4/CD8 ratio ($P<0.05$) in HIV positive subjects. In addition, the HIV positive subjects from both Akaki and Wonji had lower absoute counts of naïve, memory and memory/effector CD4+ T cells when compared to HIV negatives from both sites (Figure 9 and 10). In the CD8 compartment, there were lower naïve cells and increased memory, cytotoxic effector and memory/effector cells in subjects with HIV compared to HIV negatives (Figure 11 and 12). HIV infected subjects had also significantly higher ($P<0.05$) activated and lower resting CD4+ and CD8+ T cells as measured by HLA-DR and CD38 compared to HIV negatives (Figure 13, 14, 15, 16).

3.2. Analysis of T cell subsets and activation status in subjects with incident and/or cured intestinal parasitic infection

Previous studies on comparison of median T cell subset values between subjects with and without intestinal parasitic infections indicated no significant differences (Abdulkadir, 1998). Therefore, a more indepth study was undertaken to assess the effect of incident parasitic infection and of treatment on profile of various differentiation and activation markers on CD4+ and CD8+ T cells. For this study, cryo preserved PBMCs of 41 HIV negative and 23 HIV positive adult subjects with follow up visits were studied

longitudinally. Types of the different intestinal parasites in the study subjects is depicted in Table 6.

3.2.1. T cell subsets in HIV negative and HIV positive subjects with incident intestinal parasitic infection

Thirty four HIV negative and 21 HIV positive subjects who were diagnosed negative for intestinal parasitic infection during their base line visit but became positive in their second visit (six months latter) were included in this study.

Intestinal parasitic infection did not bring significant change in median CD4, CD8, CD4/CD8 ratio and CD4+ and CD8+ naïve and effector subset values in both HIV negative and HIV positive subjects (Tables 7 and 8). However, percentage of memory CD4+ T cells increased significantly ($P<0.05$) (Figures 17 and 18) and both CD4+ and CD8+ T cells expressing CD27 were elevated with parasitic infection regardless of HIV status (Tables 8 and 9).

3.2.2. T cell activation markers in HIV negative and HIV positive subjects with incident intestinal parasitic infection

The same subjects (n=55) were studied for the expression of activation markers, HLA-DR and CD38, on CD4+ and CD8+ T cells. As shown in Table 10, no significant changes were seen in the expression of these markers on CD4+ T cells both in HIV negative and HIV positive subjects respectively. The same phenomenon was also observed in the CD8+

compartment, except a significant increase in percentage of CD8+HLA-DR+ T cells ($P<0.05$) in HIV positive subjects with intestinal parasites (Table 11).

3.3. T cell subsets in HIV negative and HIV positive subjects after treatment for intestinal parasitic infection

To study the effect of treating intestinal parasites, 31 HIV negative and 9 HIV positive subjects, who were positive for intestinal parasitic infection in one of their visits and treated for them and became free in their next visit (six months latter) were involved in this study. Treatment did not result in a significant change in counts of CD4+, CD8+ and CD4/CD8 ratio both in HIV negative and HIV positive subjects (Table 12). However. a tendency of increased naïve CD4+, decreased memory and memory/effector CD4+ and significant increase in cytotoxic effector CD8+ T cells ($P<0.05$) was seen after treatment for intestinal parasites in HIV negative subjects. In HIV positive subjects, except an increase in absolute number of memory CD8+ T cells, no other significant change was observed in absolute counts and percentages of the CD4+ and CD8+ T cell subsets (Table 13 and 14).

3.4. T cell activation markers in HIV positive and HIV negative subjects after treatment for intestinal parasitic infection

CD4+ and CD8+ T cell activation status was compared between visits with infection and visits after treatment on 31 HIV negative and 9 HIV positive subjects. In HIV negative subjects (Table 15) a decreased expression of CD38 and HLA-DR on CD4+ T-cells was observed. In addition, in the CD8 compartment of the HIV negative subjects (Table 16),

treatment resulted in a significant decline in absolute counts and percentages of activated (HLA-DR+CD38+) cells (Figure 19) and a significant increase in percentage of resting (HLA-DR-CD38-) cells ($P<0.05$) (Figure 20). Also, there was a significant decline in counts of CD8+HLA-DR+ cells ($P<0.05$) and both absolute number and percentage of CD8+CD38+ cells ($P<0.05$). However, in HIV positive subjects (Table 15 and 16) treatment resulted in an increase in resting CD4+ and CD8+ T-cells, decline in the expression of HLA-DR on both CD4+ and CD8+ T cells and increase in CD8+CD38+ T-cells, although the difference was not statistically significant (Figure 21).

Table 1. Median values and 95 percentile ranges (in brackets) of WBC subsets in HIV negative and HIV positive subjects from Akaki and Wonji.

Cell subset	Akaki HIV negative subjects (n=30)	Wonji HIV negative subjects (n=30)	Akaki HIV positive subjects (n=12)	Wonji HIV positive subjects (n=14)
WBC	6150 (3600-21000)	6000(3300-8500)	4800(3100-6400)	5250(3300-7600)
Lymphocytes	1884 (1062-5271) [a] 31 (17-61) [a]	1662 (903-3175) 29 (14-51)	1544 (529-2400) 28 (12-54)	1223(660-2815) 29 (12-54)
Monocytes	320 (92-1428) 5 (1-11)	258 (69-442) 5 (2-9)	377 (212-916) 8 (5-18)	430 (155-1064) 7 (4-18)
Granulocytes	3399(1251-13818) 61 (31-80)	3396 (1431-6383) 65 (42-82)	3097 (1436-4192) 58 (38-83)	3686 (1045-5665) 64 (20-78)

[a]top value=absolute count (cells/µl), bottom value=proportion

Table 3. Medians and 95 percentile ranges (in brackets) of absolute numbers and proportions of activated and resting CD4+ T cell subsets in HIV negative, apparently healthy subjects from Akaki and Wonji.

T cell subset	Akaki subjects (n=10)	Wonji subjects (n=10)
CD4+HLA-DR+CD38-	92 (41-309) [a]	86 (37-124)
	13 (8-37) [a]	13 (7-18)
CD4+HLA-DR+CD38+	55 (32-123)	52 (34-94)
(activated)	7 (5-12)	8 (5-11)
CD4+HLA-DR-CD38-	326(240-831)	290 (181-583)
(resting)	48(33-62)	43(28-62)
CD4+HLA-DR-CD38+	188 (109-558)	230 (66-558)
	28 (17-38)	39 (13-46)

[a]top value=absolute count (cells/μl), bottom value=proportions.

Table 4. Median values and 95 percentile ranges (in brackets) of CD4+ and CD8+ T cells and their subsets in HIV positive subjects from Akaki and Wonji.

T cell subset	Akaki subjects (n=12)	Wonji (n=14)
CD4+	202 (47-375) [a]	284 (43-597)
	16 (10-22) [a]	21 (4-42)
CD8+	786 (231-1706)	661 (164-2058)
	54 (43-71)	52 (20-73)
CD4/CD8	0.21 (0.10-0.48)	0.36 (0.10-2.01)
CD4+CD45RA+CD27+	23 (5-136)	80 (6-205)
(Naïve)	19 (9-42)	29 (10-55)
CD4+CD45RA-CD27+	57 (19-192)	97 (27-270)
(Memory)	39 (28-63)	44 (13-63)
CD4+CD45RA+CD27-	7 (1-21)	8 (1-186)
	5 (1-12)	3 (1-36)
CD4+CD45RA-CD27-	45 (13-96)	70 (8-119)
(Memory/effector)	32 (10-51)	20 (7-41)
CD8+CD45RA+CD27+	179 (43-454)	169 (40-411)
(Naïve)	21 (11-33)	27 (8-70)
CD8+CD45RA-CD27+	161 (45-473)	120 (8-548)
(Memory)	27 (12-44)	15 (5-43)
CD8+CD45RA+CD27-	194 (93-719)	265 (40-1356)
(Cytotoxic effector)	39 (20-60)	38 (21-66)
CD8+CD45RA-CD27-	90 (19-234)	89 (1-343)
(Memory /effector)	11 (8-31)	13 (1-26)

[a]top value=absolute count (cells/μl), bottom value=proportions.

Table 5. Medians and 95 percentile ranges (in brackets) of absolute numbers and proportions of activated and resting T cell subsets in HIV positive subjects from Akaki and Wonji.

T-cell subset	Akaki subjects (n=12)	Wonji subjects (n=14)
CD4+HLA-DR+CD38-	36 (10-72) [a]	40 (11-84)
	21 (11-36) [a]	27 (8-61)
CD4+HLA-DR+CD38+	51 (15-117)	49 (16-183)
(activated)	31 (16-42)	20 (8-55)
CD4+HLA-DR-CD38-	32 (7-111)	78 (6-294)
(resting)	23 (5-32)	27 (3-56)
CD4+HLA-DR-CD38+	31 (9-143)	82 (8-230)
	24 (14-45)	27 (8-61)
CD8+HLA-DR+CD38-	192 (9-536)	142 (63-604)
	24 (2-51)	23 (15-48)
CD8+HLA-DR+CD38+	378 (140-1172)	268 (32-1076)
(activated)	48 (41-84)	41 (12-75)
CD8+HLA-DR-CD38-	46 (7-283)	71 (16-516)
(resting)	6 (2-18)	17 (1-37)
CD8+HLA-DR-CD38+	63 (7-184)	73 (10-368)
	10 (1-19)	12 (3-30)

[a]top value=absolute count (cells/μl), bottom value=proportions.

Table 6. Types of intestinal parasites in subjects included for the longitudinal study in percentage by HIV status.

Parasite	HIV status	
	HIV- (n=41)	HIV+ (n=23)
Entamoeba histolytica/dispar	31	31
Ascaris lumbricoides	29	28
Trichuris trichiura	10	3
Strongyloides stercoralis	16	17
Giardia lamblia	13	14
Taenia saginata	0	7
Enterobius vermicualris	2	0
Total helminths	56	55
Total protozoa	44	45

Table 7. Median values and 95 percentile ranges (in brackets) of lymphocyte and T cell subsets in HIV negative and HIV positive subjects with incident intestinal parasitic infection.

Cell subset	HIV negative subjects		HIV positive subjects	
	Baseline (n=34	With infection (n=34)	Baseline (n=21)	With infection (n=21)
Lymphocyte	1854 (1373-4127)[a]	1846(1340-2741)	1569(828-2526)	1414(728-3385)
	34 (17-58)[a]	33 (19-49)	30 (20-55)	31 (21-51)
CD4+	603 (345-958)	604 (346-801)	221 (59-477)	180 (64-472)
	31 (18-43)	32 (19-46)	15 (5-27)	14 (5-27)
CD8+	575 (226-2080)	552 (219-1254)	727 (285-1196)	654 (392-1840)
	32 (15-56)	31 (13-50)	43 (29-61)	45 (25-66)
CD4/CD8	0.96 (0.32-2.59)	1.11 (0.39-2.67)	0.32 (0.09-0.65)	0.32 (0.08-0.59)

[a]top value=absolute count (cells/μl), bottom value=proportions

Table 8. Median values and 95 percentile ranges (in brackets) of CD4+ T cell subsets in HIV negative and HIV positive subjects with incident intestinal parasitic infection.

CD4+ T cell subset	HIV negative subjects		HIV positive subjects	
	Baseline (n=34	With infection (n=34)	Baseline (n=21)	With infection (n=21)
CD4+CD45RA+CD27+	98 (40-290)	107 (35-241)	39 (5-146)	39 (4-143)
(Naïve)	18 (10-38)	18 (10-39)	19 (4-45)	22 (4-44)
CD4+CD45RA-CD27+	261 (132-414)	287(135-418)	83 (25-224)	71 (33-198)
(Memory)	45 (21-59)*	48 (33-61)*	33 (22-53)*	40 (28-53)*
CD4+CD45RA+CD27-	19 (4-112)	19 (2-84)	11 (2-51)	8 (1-57)
	4 (1-16)	4 (0-13)	4 (1-16)	5 (1-13)
CD4+CD45RA-CD27-	178 (65-470)	167 (51-318)	75 (23-196)	57 (21-184)
(Memory/effector)	30 (14-49)	29 (13-47)	37 (15-65)	33 (21-54)
CD4+CD27+	373 (191-649)	383(170-598)	124 (34-308)	99 (42-280)
	63 (39-84)	68 (49-85)	59 (31-82)	65 (42-76)

[a]top value=absolute count (cells/μl), bottom value=proportions.* Signrank test, P<0.05

Table 9. Median values and 95 percentile ranges (in brackets) of CD8+ T cell subsets in HIV negative and HIV positive subjects with incident intestinal parasitic infection.

CD8+ T cell subset	HIV negative subjects		HIV positive subjects	
	Baseline (n=34	With infection (n=34)	Baseline (n=21)	With infection (n=21)
CD8+CD45RA+CD27+	145 (51-287) [a]	151 (55-282)	148 (30-252)	131 (48-555)
(Naïve)	22 (8-61) [a]	26 (13-59)	18 (8-37)	19 (8-45)
CD8+CD45RA-CD27+	53 (9-290)	56 (12-170)	132 (41-262)	160 (43-253)
(Memory)	9 (2-21)	10 (5-37)	22 (5-40)	24 (5-39)
CD8+CD45RA+CD27-	312 (18-1273)	304 (15-819)	282 (73-734)	184(110-1181)
Cytotoxic effector	49 (8-74)	51 (6-70)	38 (11-62)	34 (18-64)
CD8+CD45RA-CD27-	60 (5-524)	52 (7-291)	143 (36-307)	125 (61-410)
(Memory/effector)	9 (2-36)	10 (2-35)	21 (5-40)	21 (5-41)
CD8+CD27+	200 (73-577)	215 (74-376)	328(112-436)	306 (109-690)
	31 (17-87)	37 (20-88)	42 (13-62)	46 (15-64)

[a]top value=absolute count (cells/µl), bottom value=proportions.

Table 10. Medians and 95 percentile ranges (in brackets) of absolute numbers and proportions of activated and resting CD4+ T cell subsets in HIV negative and HIV positive subjects with incident intestinal parasitic infection.

CD4+ T cell subset	HIV negative subjects		HIV positive subjects	
	Base line (n=34)	With infection (n=34)	Base line (n=21)	With infection (n=21)
CD4+HLA-DR+CD38-	80 (41-218)[a]	84 (34-150)	38(15-101)*	37 (12-83)*
	14 (8-25)[a]	14 (5-22)	21 (10-41)	19 (7-38)
CD4+HLA-DR+CD38+	34 (15-333)	38 (14-138)	53 (26-126)	45 (28-135)
(activated)	6 (3-54)	7 (3-19)	26 (9-60)	25 (11-56)
CD4+HLA-DR-CD38-	345(154-502)	334(213-552)	56 (8-218)	53 (7-205)
(resting)	57 (25-72)	57 (43-70)	30 (7-53)	28 (6-48)
CD4+HLA-DR-CD38+	114 (39-289)	130 (36-261)	39 (6-166)	32 (9-132)
	19 (7-43)	21 (10-42)	19 (6-51)	21 (6-40)
CD4+HLA-DR+	123 (62-422)	122 (66-284)	90 (41-193)	81 (40-191)
	20 (13-68)	20 (13-38)	57 (19-78)	50 (21-75)
CD4+CD38+	181 (54-376)	180 (60-325)	111 (32-242)	85 (37-251)
	30 (15-61)	29 (14-48)	47 (25-75)	52 (29-76)

[a]top value=absolute count (cells/μl), bottom value=proportions. *Signrank test, P<0.05

Table 11. Medians and 95 percentile ranges (in brackets) of absolute numbers and proportions of activated and resting CD8+T cell subsets in HIV negative and HIV positive subjects with incident intestinal parasitic infection.

CD8+ T cell subset	HIV negative subjects		HIV positive subjects	
	Base line (n=34)	With infection (n=34)	Base line (n=21)	With infection (n=21)
CD8+HLA-DR+CD38-	196(40-1003)	181 (37-640)	212 (87-577)	232 (28-817)
	33 (13-48)	33 (11-51)	32 (15-48)	33 (4-53)
CD8+HLA-DR+CD38+	81 (26-705)*	65 (25-423)*	299(111-592)	268(140-1046)
(activated)	15 (7-46)	14 (5-36)	41 (20-70)	46 (24-81)
CD8+HLA-DR-CD38-	217 (80-672)	214 (76-466)	80 (31-316)	74 (23-499)
(resting)	38 (9-55)	43 (11-58)	14 (4-40)	11 (2-28)
CD8+HLA-DR-CD38+	56 (7-134)	61 (11-139)	45 (15-153)	60 (7-113)
	9 (2-27)	9 (2-34)	9 (2-19)	9 (1-14)
CD8+HLA-DR+	289(70-1608)	270 (87-973)	547(203-858)	535(277-1251)
	47 (27-86)*	44 (28-82)*	75 (54-94)*	79 (61-89)*
CD8+CD38+	158 (39-784)	141 (41-514)	405(143-668)	322(168-1159)
	29 (9-53)	26 (7-51)	52 (25-77)	54 (28-91)

[a]top value=absolute count (cells/μl), bottom value=proportions. *Signrank test, P<0.05

Table 12. Median values and 95 percentile ranges (in brackets) of lymphocytes and T cell subsets in HIV negative and HIV positive subjects with intestinal parasitic infection and after treatment.

Cell subset	HIV negative subjects		HIV positive subjects	
	With infection (n=31)	After treatment (n=31)	With infection (n=9)	After treatment (n=9)
Lymphocyte	1943 (1080-2741)[a] 34 (18-49)[a]	1828 (1138-2816) 35 (22-49)	1475 (1154-3385) 33 (22-45)	1960 (902-3846) 34 (29-50)
CD4+	563 (245-800) 28 (19-46)	513 (232-884) 29 (16-42)	221 (114-472) 14 (6-21)	217 (163-510) 14 (8-20)
CD8+	615 (236-1254) 31 (16-50)	532 (237-965) 30 (16-52)	755 (449-1840) 51 (39-66)	1005(414-2123) 49 (41-61)
CD4/CD8	0.92(0.40-2.39)	1.02 (0.40-1.89)	0.32 (0.09-0.47)	0.29 (0.14-0.46)

[a]top value=absolute count (cells/μl), bottom value=proportions

Table 13. Median values and 95 percentile ranges (in brackets) of CD4+ T cell subsets in HIV negative and HIV positive subjects with intestinal parasitic infection and after treatment

CD4+ T cell subset	HIV negative subjects		HIV positive subjects	
	With infection (n=31)	After treatment (n=31)	With infection (n=9)	After treatment (n=9)
CD4+CD45RA+CD27+	88 (35-241)	95 (27-280)	40 (19-116)	50 (25-125)
(Naïve)	19 (8-39)	19 (7-32)	25 (12-33)	25 (15-38)
CD4+CD45RA-CD27+	265 (93-418)	237(120-483)	78 (35-141)	84 (54-167)
(Memory)	47 (33-61)	48 (32-62)	33 (30-53)	33 (29-52)
CD4+CD45RA+CD27-	18 (2-94)	17 (1-113)	8 (3-31)	7 (2-46)
	4 (0-18)	4 (1-15)	4 (1-11)	2 (2-9)
CD4+CD45RA-CD27-	163 (51-273)	155 (52-244)	52 (26-184)	77 (33-218)
(Memory/effector)	32 (13-47)	28 (11-51)	30 (21-51)	35 (20-44)
CD4+CD27+	354(142-598)	337(157-754)	117 (54-257)	128(79-292)
	65 (46-85)	65 (45-87)	65 (45-78)	63 (47-78)

[a]top value=absolute count (cells/μl), bottom value=proportions.

Table 14. Median values and 95 percentile ranges (in brackets) of CD8+ T cell subsets in HIV negative and HIV positive subjects with intestinal parasitic infection and after treatment

CD8+ T cell subset	HIV negative subjects		HIV positive subjects	
	With infection (n=31)	After treatment (n=31)	With infection (n=9)	After treatment (n=9)
CD8+CD45RA+CD27+	150(55-247)[a]	126(47-317)	146 (95-555)	196 (79-453)
(Naïve)	24 (11-59)[a]	22 (13-59)	19 (10-45)	19 (14-39)
CD8+CD45RA-CD27+	56 (18-170)	59 (16-202)	126(43-230)*	149(24-427)*
(Memory)	9 (4-23)	10 (5-25)	17 (5-36)	18 (6-36)
CD8+CD45RA+CD27-	328 (81-819)	267(63-704)	257(165-1181)	268(213-1278)
	54 (23-78)	46 (26-75)	37 (31-64)	42 (19-60)
CD8+CD45RA-CD27-	52 (7-291)	57 (7-333)	125 (33-381)	113 (27-414)
(Memory/effector)	11 (2-40)	10 (2-55)	18 (5-37)	18 (5-33)
CD8+CD27+	214 (74-376)	194(65-430)	290(139-690)	382 (103-623)
	32 (16-69)	36 (18-70)	43 (15-58)	45 (20-53)

[a]top value=absolute count (cells/μl), bottom value=proportions. *Signrank test, P<0.05

Table 15. Medians and 95 percentile ranges (in brackets) of absolute numbers and proportions of activated and resting CD4+ T cell subsets in HIV negative and HIV positive subjects with intestinal parasitic infection and after treatment.

CD4+ T cell subsets	HIV negative subjects		HIV positive subjects	
	With infection (n=31)	After treatment (n=31)	With infection (n=9)	After treatment (n=9)
CD4+HLA-DR+CD38-	77(31-150) [a]	70 (36-147)	40 (25-83)	49 (25-84)
	15 (5-22) [a]	14 (7-25)	21 (11-38)	19 (15-32
CD4+HLA-DR+CD38+	35 (12-112)	32 (15-98)	45 (19-110)	56 (19-124)
(activated)	7 (3-19)	7 (4-21)	23 (8-39)	24 (12-36)
CD4+HLA-DR-CD38-	315(148-457)	295(129-532)	53 (21-158)	70 (27-173)
(resting)	58 (43-70)	60 (45-68)	28 (19-56)	32 (16-44)
CD4+HLA-DR-CD38+	105 (36-261)	98 (40-219)	58 (14-124)	70 (13-145)
	21 (7-42)	19 (9-36)	26 (6-38)	25 (8-37)
CD4+HLA-DR+	107 (48-224)	102 (56-239)	80 (48-191)	109 (44-207)
	20 (12-35)	20 (12-38)	47 (19-64)	42 (27-65)
CD4+CD38+	147 (60-325)	137 (61-270)	98 (63-234)	105 (66-253)
	27 (12-48)	28 (15-42)	52 (29-63)	50 (38-61)

[a]top value=absolute count (cells/μl), bottom value=proportions.

Table 16. Medians and 95 percentile ranges (in brackets) of absolute numbers and proportions of activated and resting CD8+ T cell subsets in HIV negative and HIV positive subjects with intestinal parasitic infection and after treatment.

CD8+ T cell subsets	HIV negative subjects		HIV positive subjects	
	With infection (n=31)	After treatment (n=31)	With infection (n=9)	After treatment (n=9)
CD8+HLA-DR+CD38-	183(37-640)[a]	156(30-454)	316 (77-817)	273(126-749)
	33 (9-51)[a]	31 (10-49)	33 (15-53)	26 (20-56)
CD8+HLA-DR+CD38+	65 (30-260)*	49(23-213)*	398(140-585)	343(124-697)
(activated)	12 (5-32)*	10 (5-27)*	47 (24-57)	46 (19-58)
CD8+HLA-DR-CD38-	247 (91-576)	239(97-610)	108 (63-499)	105 (47-728)
(resting)	46 (23-64)*	49 (23-65)*	13 (8-27)	15 (8-34)
CD8+HLA-DR-CD38+	51 (11-177)	49 (8-182)	74 (27-145)	116 (11-168)
	9 (2-26)	10 (1-21)	8 (4-19)	10 (3-23)
CD8+HLA-DR+	254(93-808)*	210(77-592)*	593(301-1251)	741(344-1227)
	44 (16-71)	42 (15-74)	77 (56-85)	74 (58-87)
CD8+CD38+	123(41-399)*	110(46-337)*	472(168-684)	492(135-795)
	24 (7-51)*	21 (8-41)*	54 (28-76)	59 (23-68)

[a]top value=absolute count (cells/μl), bottom value=proportions. *Signrank test, P<0.05

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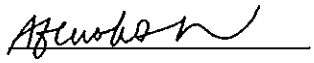
DECLARATION

I, the undersigned, declare that this thesis is my original work, has not been presented for a degree in any other university and that all sources of materials used for the thesis have been fully acknowledged.

Name: Afework Kassu Gizaw

Signature: 

Place: Addis Ababa

Date of submission: June 2001

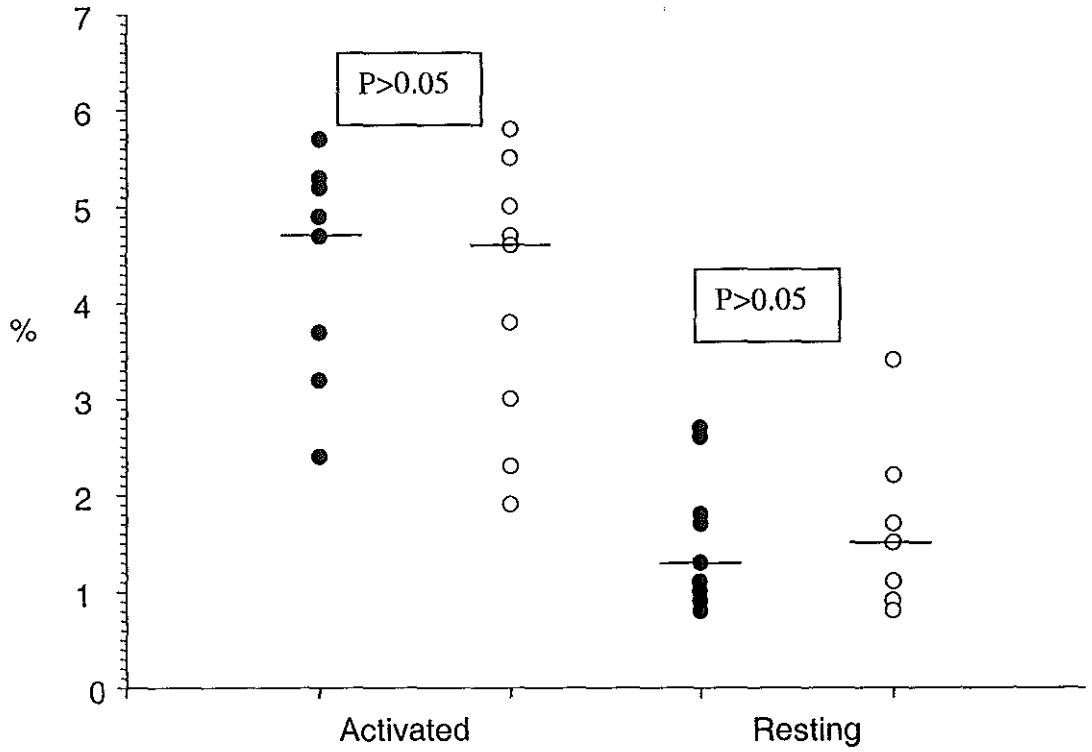


Figure 21. Activated and Resting CD8+ T cells with intestinal parasitic infections (filled circles) and after treatment (open circles) among adult HIV positive subjects (n=9)

4. DISCUSSION

The present study has generated data on total white blood cell counts, on proportions and absolute values of lymphocytes, monocytes, granulocytes, CD4+ T-cells, CD8+ T cells and CD4/CD8 ratio in the Akaki and Wonji populations. In addition, naïve, memory, effector, activated and resting CD4+ and CD8+ T cell sub-populations as measured by CD4, CD8, CD45RA, CD27, HLA-DR and CD38 antigens were quantified for subjects living in the two geographically different locations.

No significant difference was observed in absolute counts and proportions of CD4+ T-cells of HIV negative subjects from the two sites (Akaki and Wonji). In agreement with earlier studies for Ethiopians, the CD4+ T cell counts in both sites were remarkably low when compared to previously reported values for Israelis (Pollack *et al.*, 1994; Kalinkovich *et al.*, 1998), Swedish (Worku *et al.*, 1997) and Dutch (Tsegaye *et al.*, 1999; Messele *et al.*, 1999). When compared to values reported for other African countries, the Ethiopian CD4+ T cell counts, as seen from subjects of Akaki and Wonji, are lower than that of Cameroonians (Zekeng *et al.*, 1996), Ugandans (Tugume *et al.*, 1995) and Tanzanians (Levin *et al.*, 1996).

However, significantly higher percentages and absolute counts of CD8+ T-cells were observed in Akaki than Wonji HIV negative subjects, as reported earlier (Tsegaye *et al.*, 2000). The higher CD8+ T-cells in Akaki was also in agreement with previous reports for Ethiopians compared to Dutch (Tsegaye *et al.*, 1999; Messele *et al.*, 1999), Swedish (Worku *et al.*, 1997) and Israelis (Kalinkovich *et al.*, 1998). Whereas the lower CD8+ T

cells in Wonji HIV negative subjects is unlike previous reports for Ethiopian subjects, where higher CD8+ T cell values were reported (Pollack *et al.*, 1994; Worku *et al.*, 1997; Kalinkovich *et al.*, 1998; Tsegaye *et al.*, 1999; Messele *et al.*, 1999). This variation within population groups, at least in terms of CD8+ T cells, is in line with what has been observed between rural and urban Tanzanians (Urassa *et al.*, 1996; Levin *et al.*, 1996), supporting the hypothesis of Kalinkovich *et al.* (1997) on variations of basal lymphoid population according to study population and the environment. Kalinkovich *et al.* (1997) hypothesised that such variation in basal lymphoid population values in Africa may contribute to the heterogeneity of the HIV epidemic spread observed in various parts of Africa (Buve *et al.*, 1995), and stressed the potential relevance of such changes to HIV infection and its natural course. The observed variations call for the need to establish normal values for peripheral blood T cell subsets for a population in a country rather than using a general and probably non-applicable reference standards derived from different environment and populations (Anglaret *et al.*, 1997; Kam *et al.*, 1997; Kalinkovich *et al.*, 1997), since these T lymphocyte subsets are of paramount importance in the management of HIV disease progression (Stein *et al.*, 1992; Fahey *et al.*, 1990).

When Akaki and Wonji are seen as two different environments, Akaki is an urban industrial town of the country with residents exposed to high doses of environmental pollutants from the various factories and industries. While Wonji is a relatively clean environment in terms of industrial contamination. Akaki is also known for high prevalence of viral infections such as flu, hepatitis, herpes simplex infections etc. (Dr Binyam Hailu, personal communication) besides intestinal parasitic infections (Mamo *et al.*, 1989). Wonji is reported to be endemic for schistosomiasis (Tedla and Yimam, 1986)

and malaria, although the latter is also becoming common in Akaki (Adugna Woyesa, personal communication). It could, therefore, be speculated that the higher total CD8+ T cell count in Akaki might be due to the exposure of its residents to the various environmental antigens, of parasitic and non-parasitic origin, which would activate the CD8+ T cell compartment in general.

With regards to the CD4+ and CD8+ subset values, HIV negative and apparently healthy Ethiopians from both study sites had remarkably lower proportions of naïve and higher proportions and absolute values of CD4+CD27- and CD8+CD27- T-cells compared to reports for other populations such as the Dutch (Messele *et al.*, 1999). Even though the exact mechanism by which CD27 down regulation is induced is not known (Hamann *et al.*, 1999) immune cells lose CD27 antigen only after repeated antigenic stimulation (Baars *et al.*, 1995). Such an increase in CD27- cells together with reduced naïve cells and increased memory cells indicates presence of persistent immune activation in HIV uninfected Ethiopians confirming previous reports by Kalinkovich *et al*, (1998) and Messele *et al*, (1999).

However, in the HIV positive subjects, all the median values of the various WBC subsets, including CD8+ T cells, were not significantly different between subjects residing in the two geographically different locales, probably pointing to similar effect of the virus on the immune response of individuals although their basal subset values are different. But clear effect of the virus is reflected when T cell subset values of HIV positive and HIV negative residents of Akaki or Wonji were compared to each other. HIV infection resulted in a paramount decline in CD4+ T cells and increase in CD8+ T

cells. These have been considered as hallmarks of HIV infection (Fauci, 1993). In addition, as demonstrated by Roederer *et al.* (1995) counts of naïve CD4+ and CD8+ T cells, are cells responsible for mounting immune response to all newly encountered antigens (Janeway *et al.*, 1999), and were decreased in HIV positives. Besides the virus resulted in a significant activation of both the CD4+ and CD8+ T cells as reported before (Weiss, 1993; Fauci, 1993; Giorgi *et al.*, 1994; Kestens *et al.*, 1994; Graziosi *et al.*, 1998; Messele *et al.*, 1999).

The expression of HLA-DR and CD38 has been used as marker for T-cell activation (Giorgi, *et al.*, 1994; Kestens *et al.*, 1994). Expression of these markers on CD4+ and CD8+ T-cells revealed no significant difference in percentages of activated, resting, HLA-DR+CD38- and the HLA-DR-CD38+ cells between Akaki and Wonji HIV negative subjects. However, the higher total CD8+ T cell count in Akaki is reflected by the observed significantly higher absolute values of resting, HLA-DR+CD38- and HLA-DR-CD38+ CD8+ T-cells in Akaki HIV negative subjects than in Wonji. As reported before for HIV uninfected Ethiopians (Messele *et al.*, 1999) and Kalinkovich *et al.* (1998), remarkably higher expression of HLA-DR and CD38 was seen on CD4+ and CD8+ T cells in Ethiopian subjects of the present study, independent of geographical area of sample collection. This is in agreement with a study on HIV negative adult healthy Ugandans compared to Italians, where increased expression of HLA-DR and CD45RO were seen in the former (Rizzardini *et al.*, 1998).

Taken together, analysis of the CD8+ T cell subsets using different combinations of monoclonal antibodies well known to separate T cells in to functional subsets, like naïve,

memory, effector, activated and resting cells, however, did not detect qualitative differences, comparing subjects from Akaki to subjects from Wonji. The quantitative differences detected for CD8+ T cells as a whole were reflected only in absolute numbers of most CD8+ T cell subsets, which made these subsets of limited value for explaining the observed differences in the two population groups. In contrast a dramatic change in the CD8 subset composition, mainly expansion of the memory cell types, has been observed in acute viral infections (Roos *et al.*, 2000). In addition, chronic antigenic stimulation has been shown to result in the loss of CD27 antigen expression (Hintzen *et al.*, 1993; Baars *et al.*, 1995; Hamann *et al.*, 1999). Since we observed no specific expansion of certain T cell subsets and activation marker status in one of the study group, our data suggest that the difference in CD8+ T cell counts may not be attributed to a specific response of these cells to an endemic infectious disease. Such imbalances in the CD8 subset composition would have been expected in Akaki subjects if acute or chronic viral infections were the underlying causes for the observed differences between the two populations. Other environmental factors or genetic differences between the study populations could be involved and should be the subject of further studies. In summary, it can be concluded that caution should be taken presenting immunological reference data on particular groups of Ethiopians as valid for the entire population.

The low CD4 count together with the low CD4/CD8 ratio and the changes in CD4+ and CD8+ subset composition in Ethiopians observed at both sites supports earlier reports (Messele *et al.*, 1999), and indicates presence of factors that lead to reduced naïve and increased memory and memory/effector and activated cells in both Ethiopian populations. These changes have been interpreted as immune responses to an increased

load of environmental pathogens (Bentwich *et al.*, 1995; Worku *et al.*, 1997; Kalinkovich *et al.*, 1998; Messele *et al.*, 1999). Nutritional factors (Semba *et al.*, 1993; Fawzi *et al.*, 1998) or ethnic composition (Choong *et al.*, 1995) could also be involved. Although genetic factors were reported (Choong *et al.*, 1995) to play a role, Clerici *et al.* (2000), had demonstrated that immune activation in Africans is environmentally driven and not genetically predetermined. This was demonstrated by studying HIV infected and uninfected Ugandans and Italians living in Uganda or Italy and detected presence of immune activation both in Italian and African (Ugandan) HIV uninfected individuals living in Africa but not in African subjects living in Italy as measured by augmented CCR5 specific mRNA and surface expression of CCR5. In general, the potential influence of such an activated immune status should be taken into account when addressing issues of immunological competence among Ethiopians.

Intestinal parasitic infections have been suggested to cause persistent immune activation leading to unbalanced immune state. Such state has been proposed as a major factor in the pathogenesis of AIDS in African context, by making the host more susceptible to HIV infection and less able to cope with it once infected (Bentwich *et al.*, 1995). Activation of the immune system is an essential component of an appropriate immune response to a foreign antigen. Once the immune response adequately deals with and clears the foreign antigen, the system returns to a state of relative quiescence until the next stimulus is introduced (Abbas *et al.*, 1994). This is essential for the optimal functioning of the immune response. However, in parasite endemic regions, this state of relative quiescence is disturbed. Besides, parasites are known to shed antigens in the host which would persistently stimulate the immune system (Cox, 1997). Such exposure of

the host to a multitude of parasite antigens besides other environmental antigens would make the host more susceptible and less able to cope with subsequent infections (Bentwich *et al.*, 1995). And, in HIV infection as multiple components of the immune system are persistently activated including spontaneous lymphocyte proliferation; expression of T cell activation antigens on CD4+ and CD8+ T cells; activation of monocytes and increased cytokine expression, the activation status is maintained throughout the course of HIV disease (Fauci, 1993). In parasite endemic regions this is further worsened due to parasite induced immune activation which can modulate HIV infection (Bentwich *et al.*, 1995).

Intestinal parasitism is widely distributed in Ethiopia, the most frequent parasites being *Ascaris lumbricoides* and *Trichuris trichiura* (Mamo *et al.*, 1989; Erko and Tedla, 1993; Roma and Worku 1997; Jemaneh, 1997; Jemaneh, 1998; Jemaneh, 1999). Furthermore, the prevalence of HIV/AIDS is also rapidly increasing all over the country (MOH, 2000), putting the country as the third with the highest number of total HIV infected subjects (UNAIDS, 2000b).

Kalinkovich and colleagues (1998) showed that HIV negative Ethiopian immigrant Jews to Israel who were infected with intestinal parasites had substantial markers of immune activation which was associated with remarkable changes in the distribution and phenotype of peripheral blood T cell populations. These phenotypic and functional cellular changes observed were reported to resemble those described in HIV infected people. These investigators ascribed the observed immune dysregulation as caused by the exposure of the subjects to intestinal parasites, specifically helminths.

In this study too, intestinal parasitic infections resulted in a statistically significant increase in memory CD4+ T lymphocytes both in HIV infected and uninfected individuals indicating that immune response to parasite antigens, where naïve cells change to memory cells upon antigenic stimulation (Janeway *et al.*, 1999), has taken place. Similar effect of HIV on T cell activation is also indicated since the observed increase in memory cells is lower in HIV negative subjects compared to HIV positives.

Expression of CD27 antigen increased significantly on CD4+ T cells with intestinal parasite infection in HIV seronegative subjects, but not significantly in HIV positives, indicating the sole effect of parasites in bringing immune activation. Elevated expression of CD27 has been reported to occur whenever there is activation of the immune cells by antigens and its down regulation after repeated antigenic stimulation (Hintzen *et al.*, 1993; Baars *et al.*, 1995).

Decline in resting cells and increase in activated cells was noted both on CD4+ and CD8+ T cells in subjects with both intestinal parasitic infection and HIV. Furthermore, in agreement with the observation of Kalinkovich *et al.*, (1998), increase in HLA-DR or CD38 expression on CD8 cells was observed for HIV positive subjects with intestinal parasites in the present study. Other investigators had also noted an increase in activated CD8+ T cells in HIV positive subjects (Miedema 1992; Ho *et al.*, 1993), indicating the expansion of these cells against the virus. Such increased activity is to be expected since the CD8+ T cells were reported to have higher HIV specific cytotoxic activity (Giorgi *et al.*, 1994). Whereas in HIV negatives no change in CD4 compartment was seen. Unlike

the CD8 compartment where a significant decline in activated cells and a significant increase in resting cells was observed, there was no change in the CD4 compartment in the HIV negatives. This is contrary to what is expected for parasite infected individuals and might be due to other environmental antigens, other than intestinal parasites, prevailing in the community during their base line visit.

In a follow up study of helminth infected immigrants to Israel, Kalinkovich *et al.* (1998) demonstrated that eradication of the parasites have been associated with decrease in immune activation status which gradually becomes normal like the parasite negative Israelis. In this study, however, treating intestinal parasites showed different outcomes on expression of the subset markers. For example, a decrease in cytotoxic effector cells was seen in HIV negatives after treatment for intestinal parasites. On the contrary, increase in these cells was seen in HIV positives after treatment, further confirming the role of the virus in continuous activation of the CD8 compartment, although the difference was not statistically significant. These findings point to importance of treating intestinal parasites to bring a decreased activation status as seen by a reduction in effector cells, since the ratio of effector cells to memory cells reflects how successfully virus replication is contained.

A decline in CD4+ T cells expressing HLA-DR and CD38 and an increase in resting CD4+ T cells were seen after treatment both in HIV positive and HIV negative subjects, although the difference was not statistically significant. This is in agreement with the report by Kalinkovich *et al.*, (1998) where a decline in expression of HLA-DR was seen in Ethiopian immigrants treated for intestinal parasites. This report is re-enforced by the

present finding that treatment of intestinal parasites results in a statistically significant increase in resting CD8+ T cells and in a significant decrease in activated cells in HIV seronegative subjects. Furthermore, our determination that, in HIV positive subjects, although the difference was not significant, a tendency of increase in resting cells and decrease in activated cells after treating intestinal parasites substantiates the hypothesis of immune interaction between intestinal parasites and HIV (Bentwich *et al.*, 1999). This, however, does not discount the fact that HIV by itself is potent enough to maintain the persistent activation of the immune system (Weiss, 1993; Fauci, 1993; Bentwich *et al.* 1998). Every information taken together, the present finding is in line with the proposal of Bentwich and colleagues (1995) where intestinal parasites, besides other endemic infections, were suggested to cause chronic immune activation and that their eradication would be necessary to reverse the activation. This has a clear implication for HIV disease progression in Ethiopia where the burden of intestinal parasite infection is very high.

5. CONCLUSIONS AND RECOMMENDATIONS

In the present study, the higher total CD8+ T cell count observed in subjects from Akaki resulted in a proportional increase of every compartment of the CD8+ cells studied. Since no single expanding population was observed, the elevated CD8 count in Akaki may not be specifically associated to any one endemic infectious disease. Various environmental factors could be involved and hence further study on the phenomenon is needed.

The study also points out that the Ethiopian population, as seen between Akaki and Wonji residents, might not be homogeneous in terms of the peripheral CD8 population while the CD4 population is comparable. This has a bearing in HIV disease progression since the CD4 and CD8 counts and the CD4/CD8 ratios are key parameters in management of HIV infection and AIDS. It also calls for a cautious use of immunological reference values determined for a single region or population of an area as applicable for all others in Ethiopia.

Although the results of this study may be used by researchers to compare laboratory data, in depth studies involving various Ethiopian population groups from different regions in the country could be recommended to get a clearer and more comprehensive picture of T cell subsets and immune activation profile of Ethiopians, with an emphasis on CD4 and CD8 T cell counts in terms of HIV infection progression monitoring.

Our data also indicates that intestinal parasitic infections seemed to result in alteration of CD4+ and CD8+ T cell subsets, and also in the up-regulation of T cell activation markers in the peripheral blood. Treating intestinal parasites was seen to have a tendency of reducing activation of the T cell subsets. Hence, treating intestinal parasites and other endemic infections, besides other community based intervention strategies such as health education, improved water supply and environmental sanitation programmes, could be considered as a possible strategy in bringing a state of decreased activation and hence protecting the T cells from being easily attacked by HIV, the major cause of morbidity and mortality in the country and the developing world at large.

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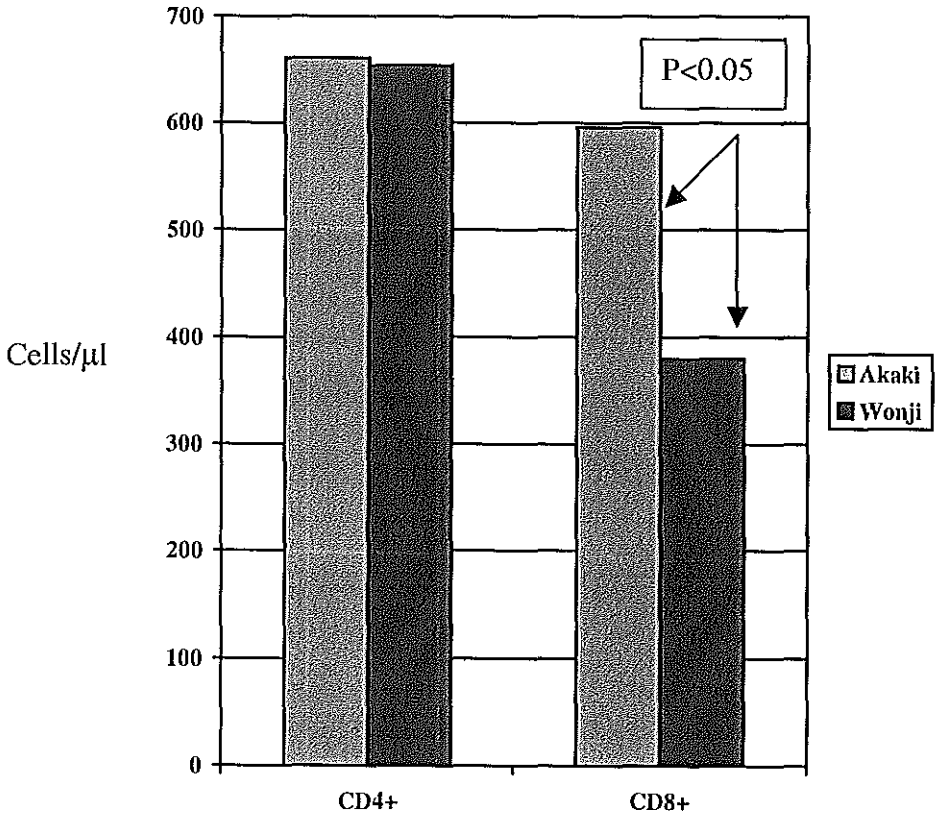

Figure 4. Median absolute CD4+ and CD8+ T cells of HIV negative adult subjects from Akaki and Wonji

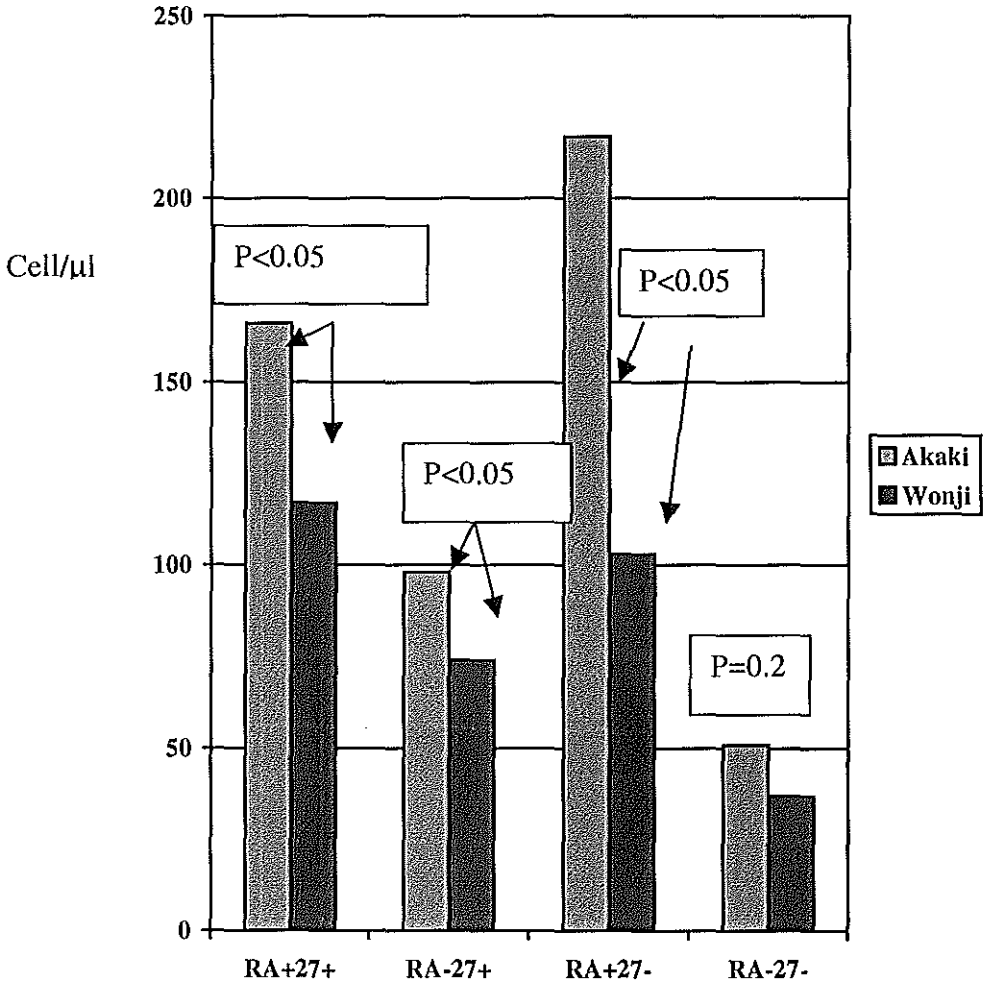

Figure 5. Median Absolute CD8+ naïve, memory and effector T Cell subset counts of HIV negative adult subjects from Akaki and Wonji

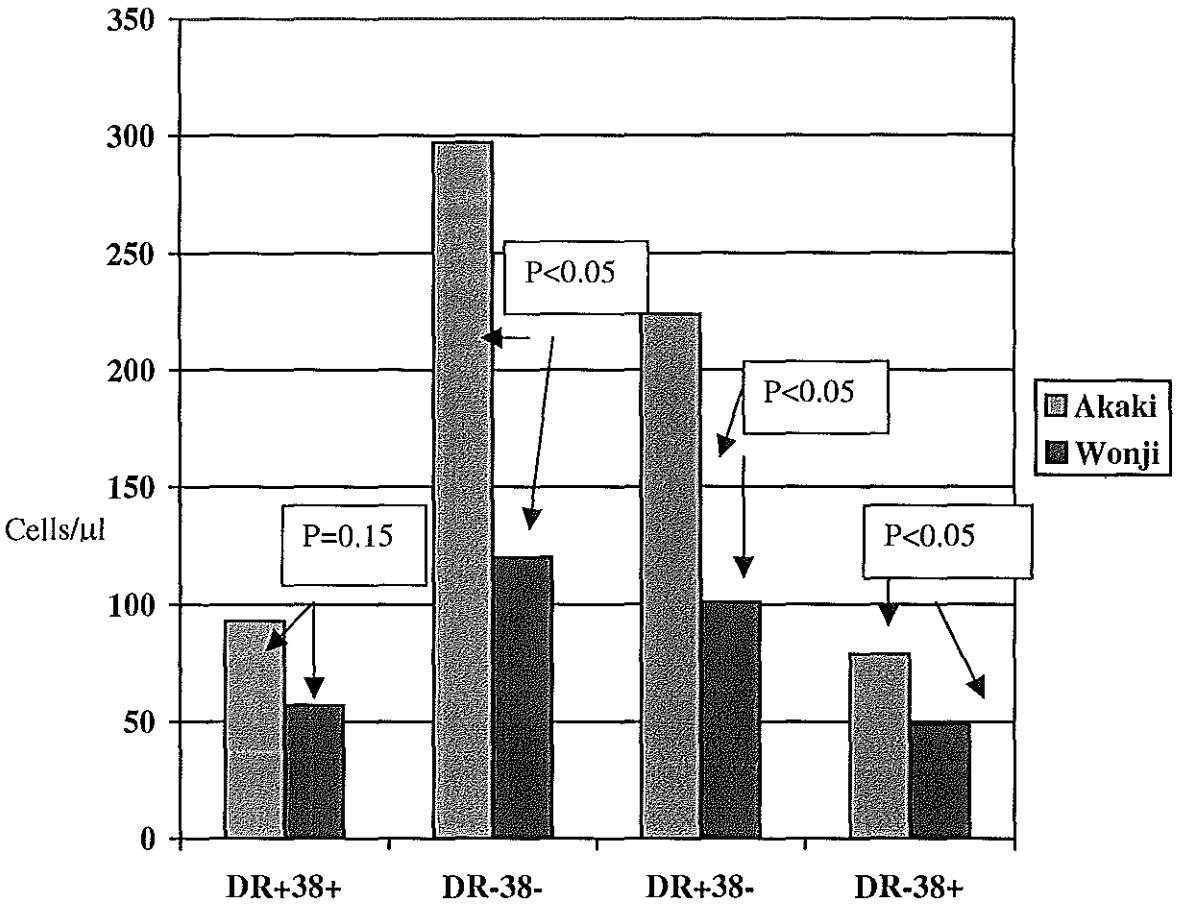

Figure 6. Activated and resting CD8+ T cell subsets of HIV negative adult subjects from Akaki and Wonji

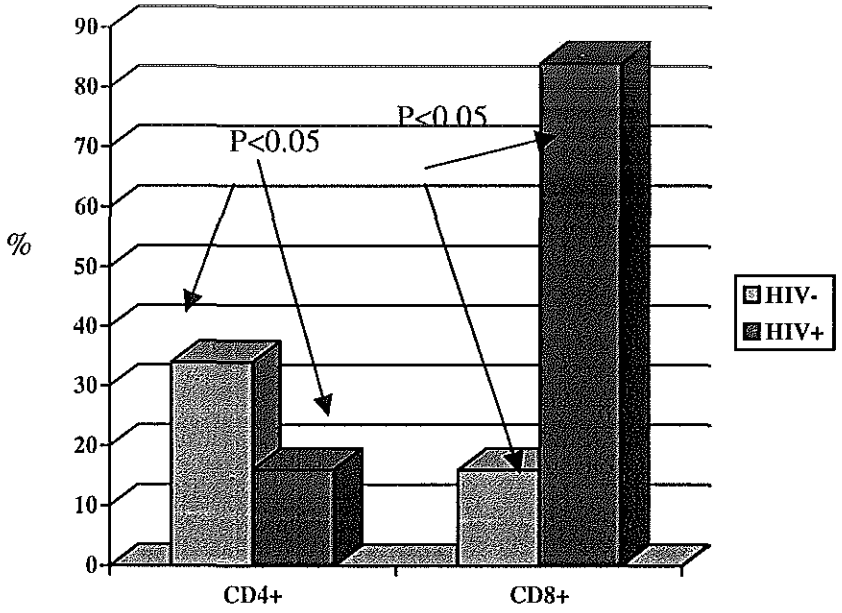

Figure 7: CD4+ and CD8+ T cell subsets of HIV negative and HIV positive adult subjects from Akaki

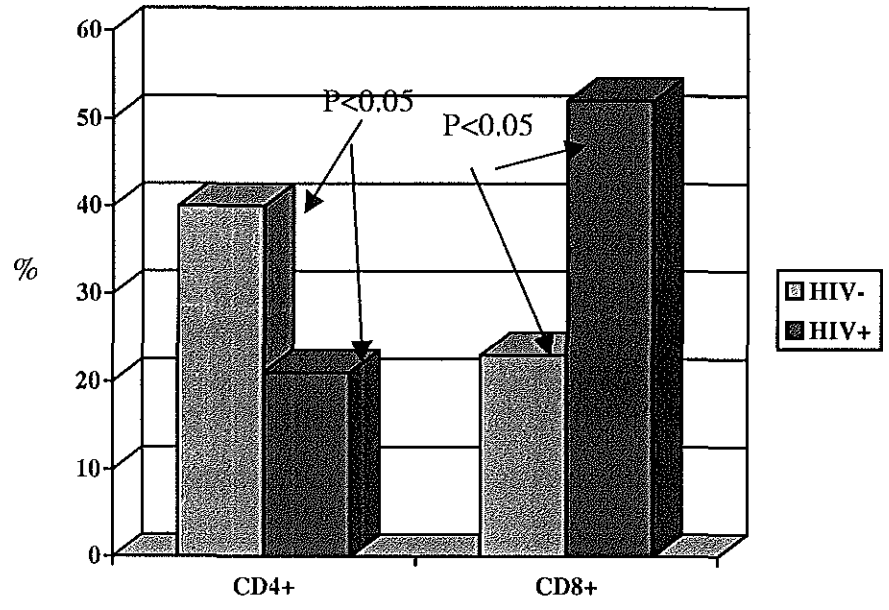

Figure 8: CD4+ and CD8+ T cell subsets of HIV negative and HIV positive adult subjects from Wonji.

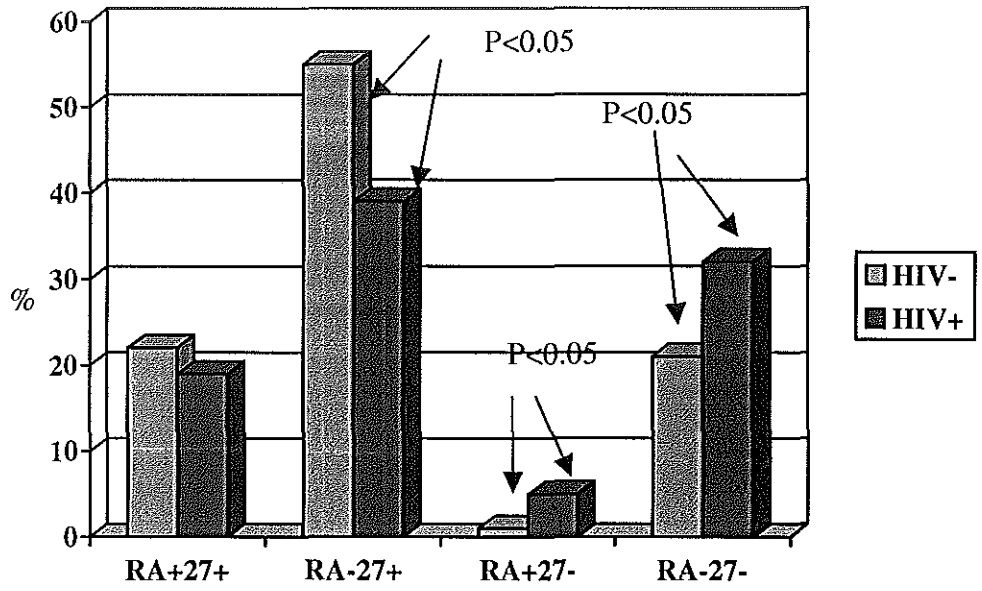

Figure 9: CD4+ naïve, memory and effector like T cell subsets of HIV negative and HIV positive adult subjects from Akaki

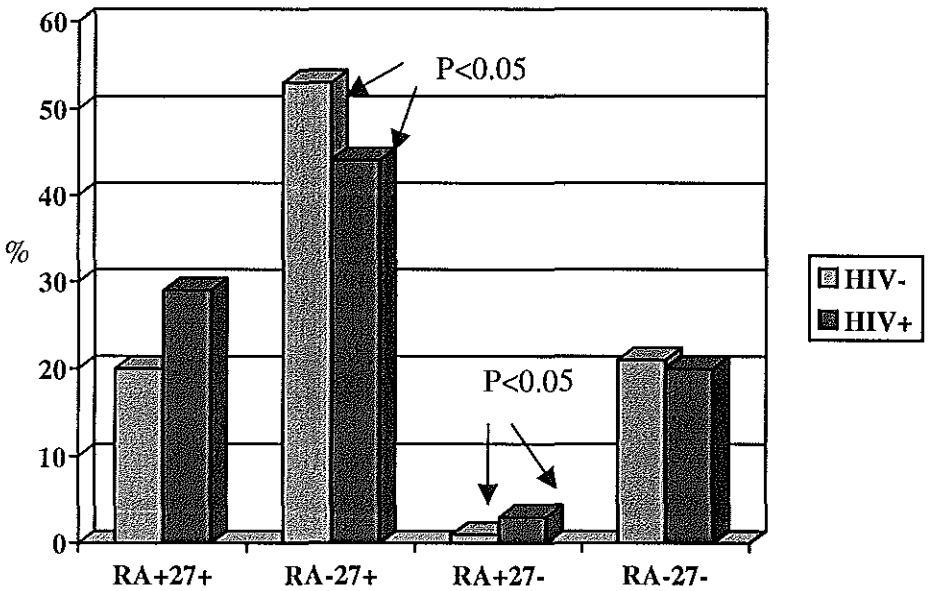

Figure 10: CD4+ naïve, memory and effector like T cell subsets of HIV negative and HIV positive adult subjects from Wonji

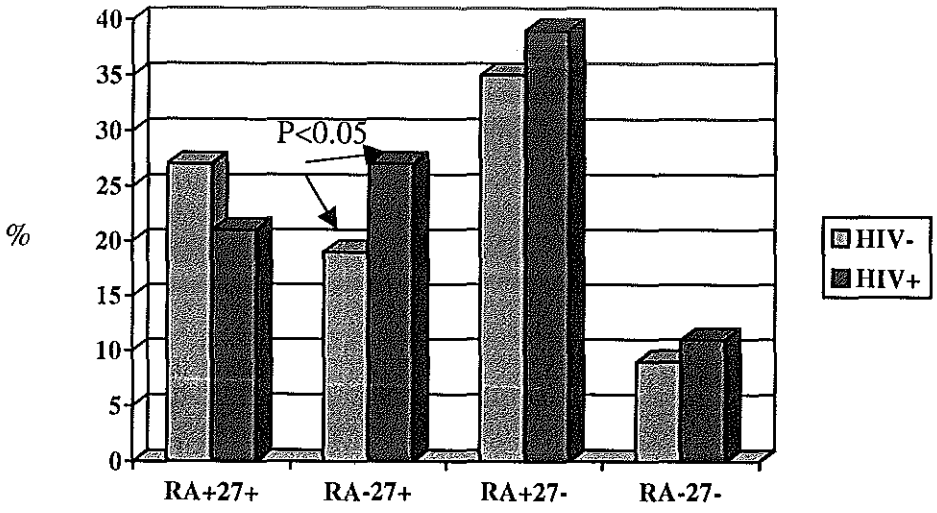

Figure 11: CD8+ naïve, memory and effector T cell subsets of HIV negative and HIV positive adult subjects from Akaki

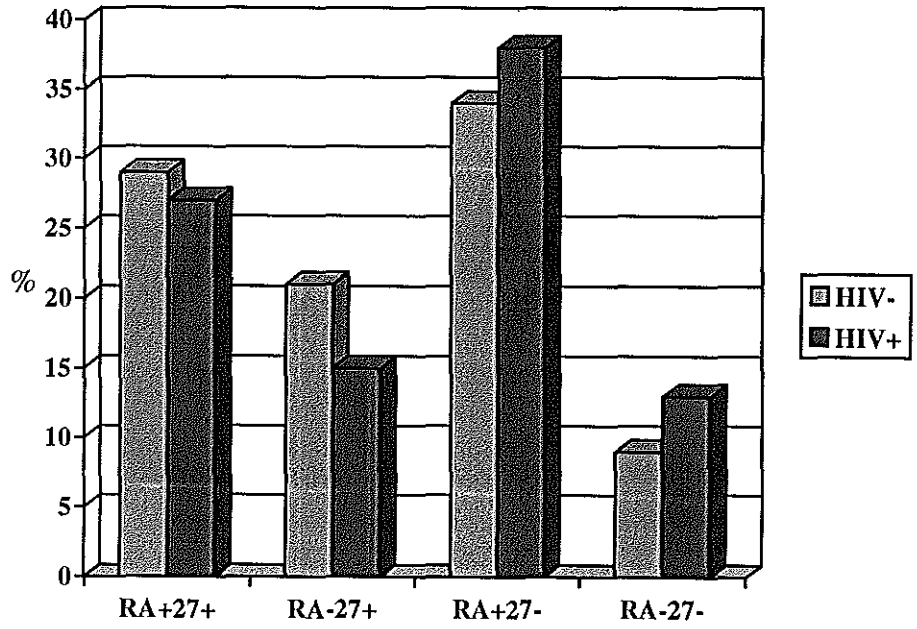

Figure 12: CD8+ naïve, memory and effector T cell subsets of HIV negative and HIV positive adult subjects from Wonji

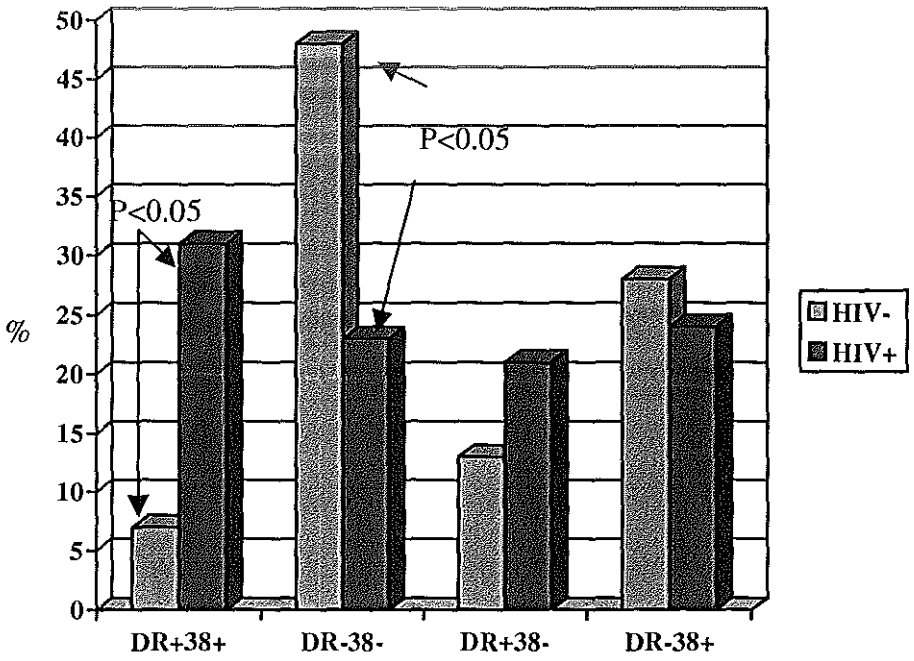

Figure 13: Activated and resting CD4+ T cell subsets of HIV negative and HIV positive adult subjects from Akaki

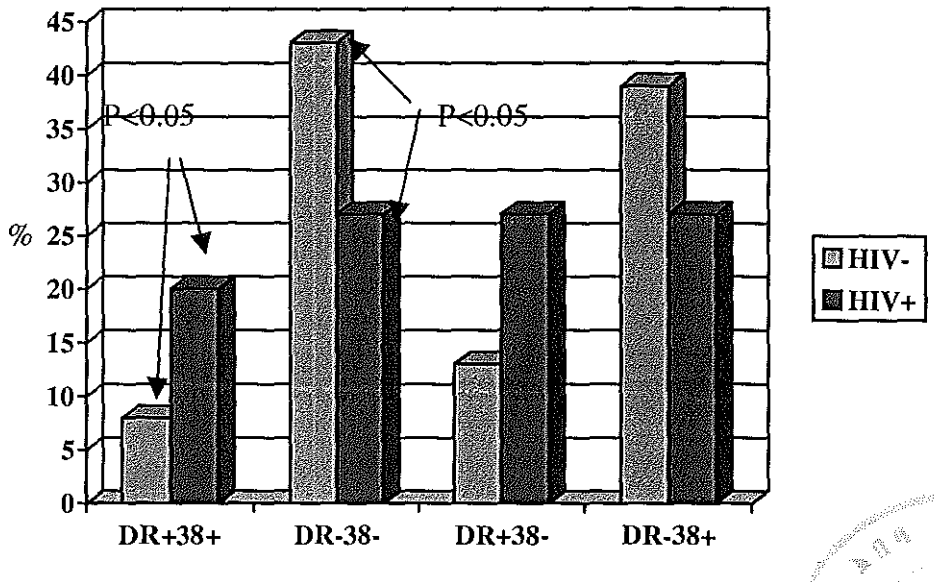

Figure 14: Activated and resting CD4+ T cell subsets of HIV negative and HIV positive adult subjects from Wonji

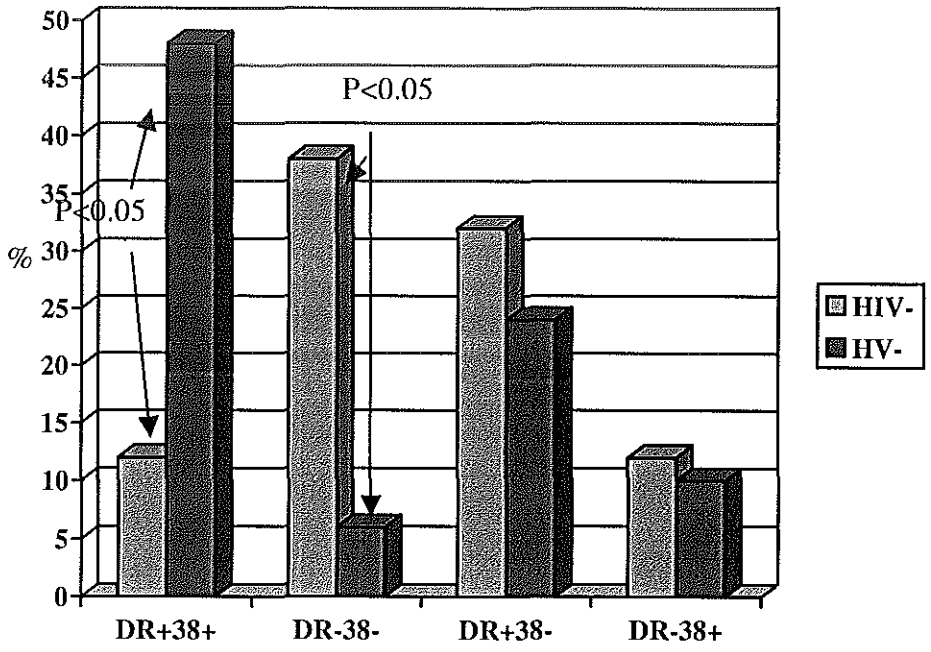

Figure 15: Activated and resting CD8+ T cell subsets of HIV negative and HIV positive adult subjects from Akaki

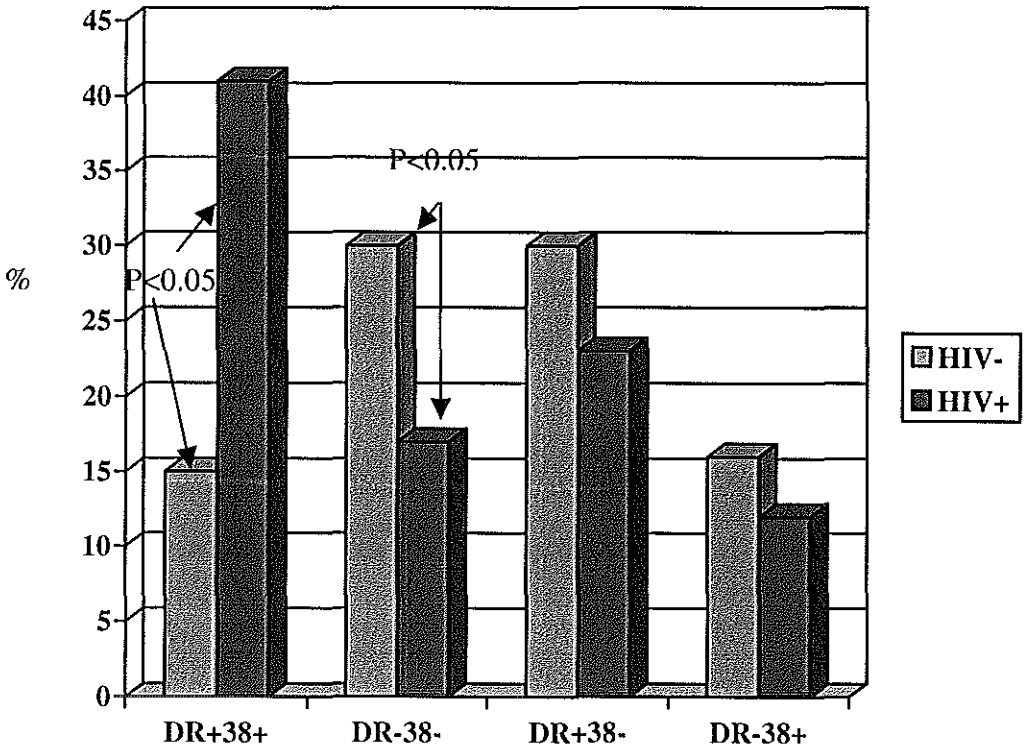

Figure 16: Activated and resting CD8+ T cell subsets of HIV negative and HIV positive adult subjects from Wonji

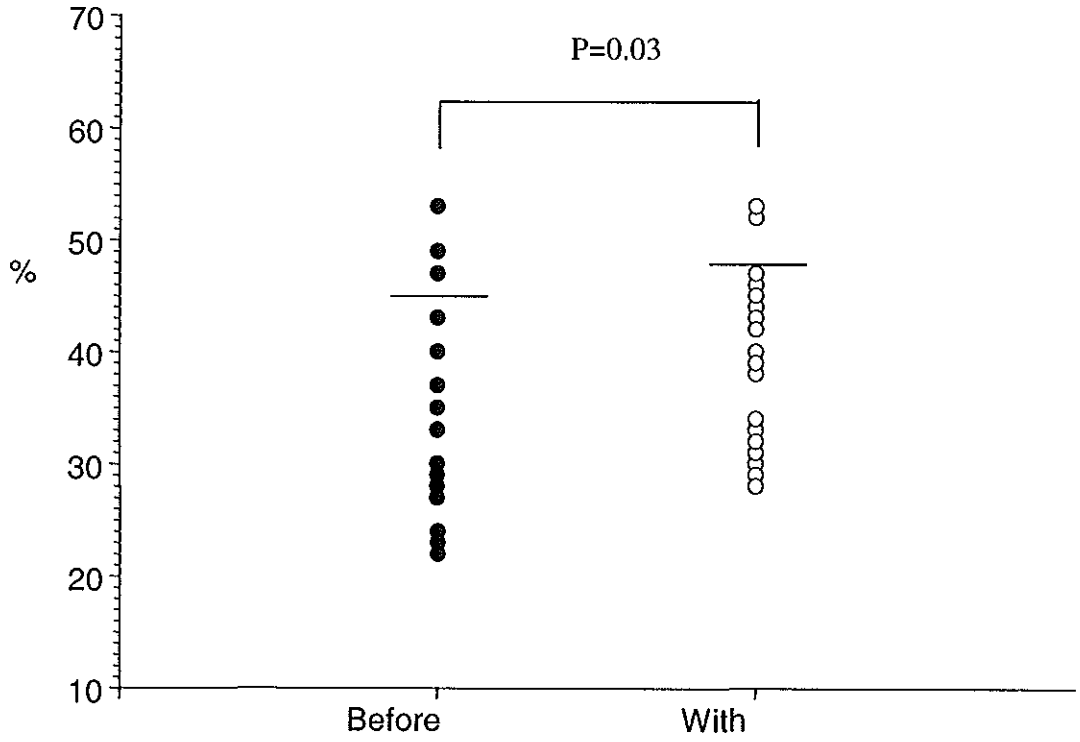

Figure 17. Memory CD4+ T cells before and with intestinal parasitic infections among adult HIV negative subjects (n=34)

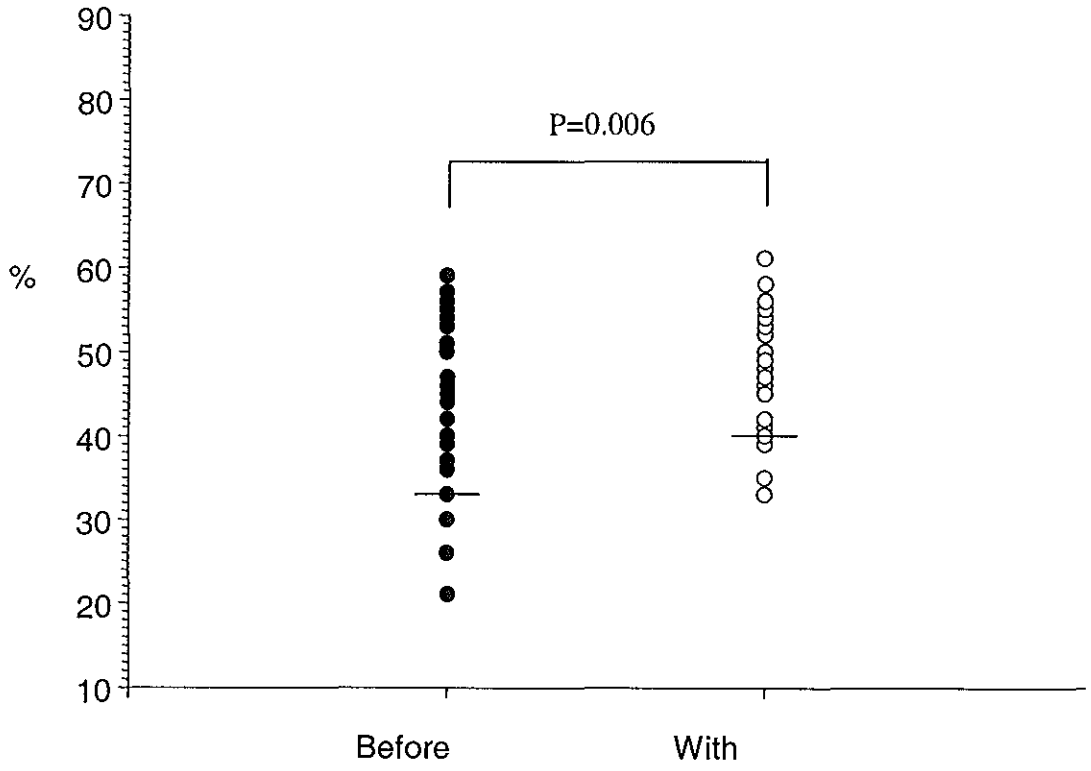

Figure 18. Memory CD4+ T cells before and with intestinal parasitic infections among adult HIV positive subjects (n=21)

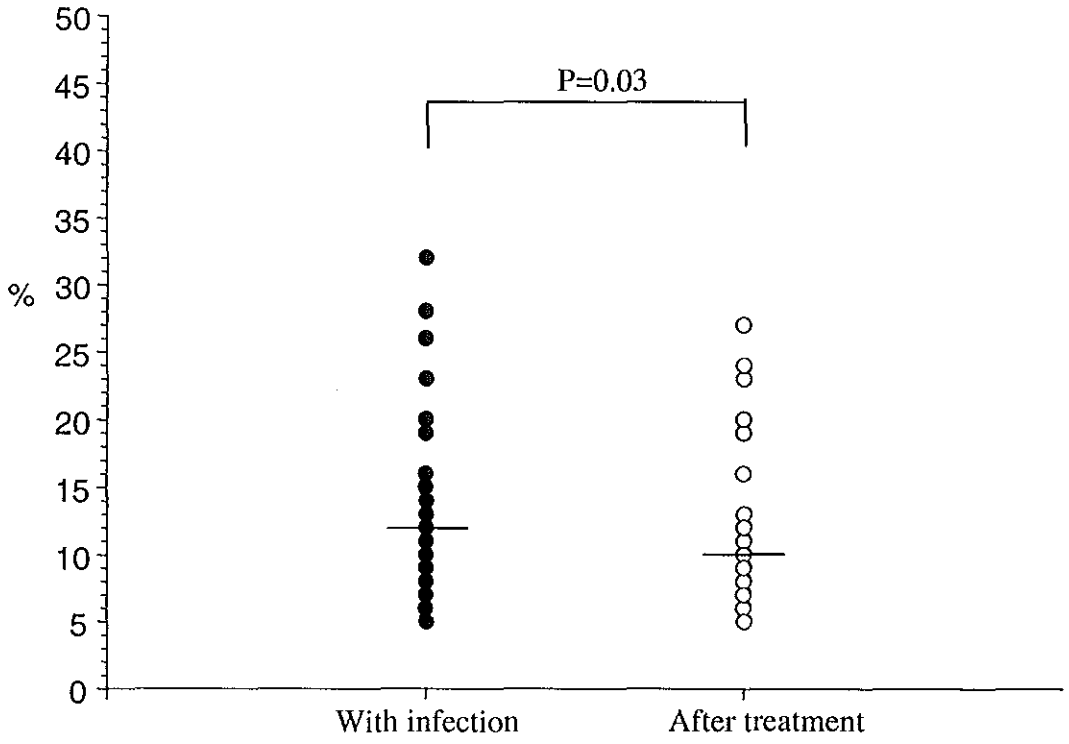

Figure 19. Activated CD8+ T cells with intestinal parasitic infections and after treatment among adult HIV negative subjects (n=31)

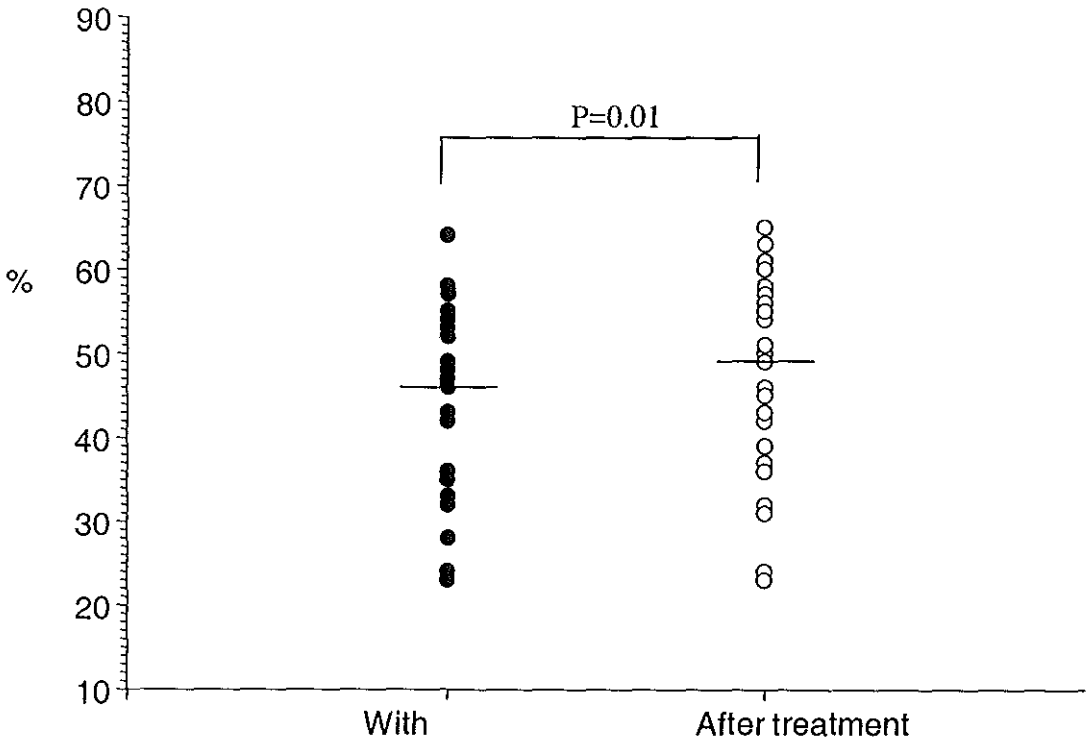

Figure 20. Resting CD8+ T cells with intestinal parasitic infections and after treatment among adult HIV negative subjects (n=31)