Immunologic characteristics of healthy and HIV-1-infected Ethiopians
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Chapter 1

General Introduction
**HIV/AIDS**

The human immunodeficiency virus (HIV) which is the cause of acquired immuno-deficiency syndrome (AIDS) was first isolated in 1983\(^1\)\(^-\)\(^3\). AIDS is the ultimate clinical stage of infection by HIV and is characterized by opportunistic infections and specific malignant diseases in patients. HIV is an RNA virus and belongs to the family *retroviridae*, subfamily *lentiviridae*\(^4\). HIV-1 and HIV-2 are the two known types of HIV with the former being distributed worldwide and the latter found primarily in West Africa\(^5\)-\(^7\). HIV-2 was found to be closely related to the simian immunodeficiency virus (SIV)\(^8\),\(^9\). Although the degree of virulence may be less for HIV-2, both HIV-1 and HIV-2 are associated with AIDS\(^2\),\(^3\),\(^10\).

Today most of the information on HIV has been obtained from studies on HIV-1.

Structurally, HIV-1 is a spherical particle with a diameter of approximately 100 nm. The outer membrane-protein of the virus covers the inner core, which contains the viral RNA, along with several copies of the enzyme reverse transcriptase\(^11\). The envelope region of HIV-1 is highly variable. Based on the variation in the larger outer membrane protein, gp120, HIV-1 at present is divided into 10 subtypes designated as subtypes A to J\(^12\),\(^13\). There is also a heterologous group of viruses designated as subtype O, which do not match any of the subtypes described above\(^14\). The human CD4 molecule, which is a 55-kD surface glycoprotein belonging to the immunoglobulin superfamilies, serves as the primary receptor for the virus to enter susceptible cells\(^15\)-\(^18\). CD4 is expressed primarily on helper T lymphocytes and also on cells of the monocyte/macrophage lineage including dendritic cells, alveolar macrophages in the lung and Langerhans cells in the skin\(^19\)-\(^24\). It is now known that the CD4 molecule is necessary, but not sufficient for HIV entry into target cells. Recently, the chemokine receptors CCR5 and CXCR4 have been identified as the major co-receptors of HIV-1\(^25\)-\(^27\). During infection, HIV first attaches to the CD4 molecule and the chemokine receptors on the cell surface\(^28\). This step is then followed by fusion of the viral and cellular membrane, resulting in the penetration of the virus core. Uncoating is followed releasing the virus RNA which is then reverse transcribed in to DNA by the viral reverse transcriptase. The DNA provirus is then transported to the nucleus and is integrated to the host cell genome with the help of virus-encoded enzyme, integrase\(^29\). The virus depends on host transcription and translation factors for its replication. The synthesized virus genome and structural and regulatory proteins are transported and assembled at the cell membrane and progeny viruses bud from the cell membrane. A characteristic feature of HIV-1 infection is a highly variable period between infection and development of AIDS. Although at the beginning of the HIV epidemic it was thought that the viral load is low during the asymptomatic period, it is now known that a large number of viruses are produced per day throughout the infection period with continuous infection of new cells resulting in gradual CD4 depletion and ultimately development of AIDS\(^30\),\(^31\).
The global situation

World wide, over 30 million people were infected with HIV by the beginning of 1998 and 11.7 million people had already lost their lives to the disease. HIV/AIDS is among the top ten killers world wide\(^{32}\). Given the current speed of the spread of infection, it is expected that it may even move to be among the top five killer diseases. Eighty nine percent of people with HIV live in sub-saharan Africa and the developing countries of Asia. In Africa, nearly 21 million men, women and children are infected and AIDS has become one of the major causes of morbidity and mortality\(^{33}\).

The HIV problem in Ethiopia

The HIV/AIDS epidemic started relatively late in Ethiopia, the first HIV-1-positive sera being detected in 1984 and the first AIDS patients reported in 1986\(^{34}\). However, since then the HIV-1/AIDS epidemic has spread to the entire country to reach a prevalence ranging 7-20% in the 15-50 year age groups in the urban areas by 1998\(^{35}\). The circulating subtype is C\(^{36}\), which has been estimated to be the most prevalent (>48%) amongst HIV-1-infected individuals in the world \(^{37}\).

HIV infection and the immunological response

HIV infection is characterized by dynamic and long-lasting interactions between the virus and the immune system. Both humoral and cellular immune responses are mounted to HIV infection.

Humoral immune response

Early after infection, before seroconversion, there is high level viremia and as this decreases a substantial antibody response, including an early and transient IgM response and later a sustained IgG response specific for several proteins of the infecting virus is seen\(^{38}\). The antibodies are directed to all the major antigens of the virus. As time goes on more broadly neutralizing antibodies are produced. However, these antibodies do not prevent HIV disease progression and the generally accepted explanation for this phenomenon has been the occurrence of antibody-escape mutant viruses\(^{39}\) partly because of the error-prone polymerase of the virus, which is thought to generate an average of one point mutation in each genome copy\(^{40}\) and partly due to mutations as a result of immune pressure.
Cell-mediated immune response

Cell-mediated immunity does play an important role in the immune defenses against intracellular pathogens such as viruses. Cytotoxic T lymphocytes (CTL) destroy infected cells after recognizing antigen presented by major histocompatibility (MHC) class- molecule on the surface of such cells. High frequencies of HIV-specific CTL have been detected in peripheral blood of HIV-infected patients. The activity of CTL has been found to decline as HIV disease progresses, and this has been also associated with impairment of IL-2 production and a reduced clonogenic potential of CD8+ T lymphocytes. A correlation has been observed between generation of strong CTL response and persistence of an asymptomatic state in adults. Another study in infants born to HIV-positive mothers showed that seroconversion of the infants is associated with disappearance of HIV-specific CTL in peripheral blood. Specific CTL responses, but not humoral immune responses are detected in exposed but uninfected individuals. Taken together, these observations suggest that CTL responses play a significant role in protection against infection and in the regulation of HIV disease. On the other hand, there are also a few studies which suggested a negative role of CTL on the immune system during HIV infection mediated by killing of infected cells and disruption of normal tissue architecture. Furthermore a non-lytic antiviral response which is active against HIV-1, HIV-2 and SIV and which is not MHC-restricted was indicated in CD8+ T cells. These cells are found at highest levels in asymptomatic individuals and which decreases with disease progression. The non-cytolytic CD8+ T cells anti-HIV response, termed CD8+ T-cell anti-viral factor (CAF), was initially identified to be composed of β-chemokines. However, subsequent studies have shown that the anti-viral response mediated by CAF can block both SI and NSI viruses and this effect cannot be suppressed by antibodies directed to β-chemokines suggesting that CAF's activity is not due exclusively to β-chemokines.

Differentiated CD4 T-helper subsets, which are associated with distinct types of immune response, have been identified. These T-helper subsets designated as T-helper 1 (TH1) and T-helper 2 (TH2) were first described in mouse. A third subset designated as T-helper 0 (TH0) with both Th1 and Th2 properties was also described in humans. Th1 cells produce cytokines such as IFN-γ and IL-2 that increase cellular immune response, whereas Th2 cells produce cytokines such as IL-4, IL-5, IL-6 and IL-10 that enhance antibody production. A change in the balance of production of especially IL-4 and IFN-γ has been observed in several disease conditions. There are also reports suggesting that a shift from Th1 to Th2 type of response contributes to the immune dysregulation observed in HIV infection and progression to AIDS is dependent on Th2 cytokine phenotype dominance. In contrast, there is also a study arguing for the absence of an in-vivo Th1 to Th2 phenotype shift with HIV disease progression.
Viro-immunological markers of HIV disease progression

The course of HIV infection and development of disease is characterized by a wide variation among infected individuals. Studies in the West indicated that approximately 5% of HIV-infected persons develop AIDS within three years, whereas approximately 12% of the HIV-infected persons are expected to remain AIDS-free for more than twenty years\textsuperscript{68-71}. In contrast, HIV disease progression in general is reported to be faster and the time of survival after the onset of AIDS is shorter in African patients\textsuperscript{72,73}. Identification of factors which are changing with HIV infection and which predict and possibly contribute to the outcome of the infection and which could be useful in designing therapeutic strategies for appropriate patient care has been a major part of HIV research. Several cellular and serologic markers have been seen to have a strong predictive value for HIV disease progression. The level of HIV replication as expressed by viral loads is believed to be important in the immunologic decline that characterize HIV disease and that have a major impact on the course of the disease\textsuperscript{74,75}. Some studies have suggested that plasma HIV-1 RNA levels are better predictors of progression to AIDS and patients with elevated plasma HIV-RNA levels are at high risk of poor outcome\textsuperscript{76,77}. Consistent with those findings, it was reported that HIV disease progressors had significantly higher HIV- RNA copy numbers than did either slow progressors or long-term asymptomatic HIV-infected patients\textsuperscript{78}. In contrast, a recent study by Rizzardi et al on HIV-infected Africans shows that viral load is lower in the Africans compared to the Europeans, suggesting that HIV pathogenesis in this population is mainly immunopahtologically driven\textsuperscript{79}. The emergence of more virulent virus strains is also implicated to determine HIV disease progression\textsuperscript{80,81}. These strains have syncytium-inducing (SI) capacity are thought to evolve from the non-syncytium-inducing (NSI) strains detected early at infection. As the disease progresses SI viruses appear in approximately 50% of patients. The appearance of SI viruses seems, however, to be rare in HIV-1 subtype C infections and the majority of AIDS patients infected by these genotype harbor only NSI viruses\textsuperscript{82}.

CD4 T cells are the main targets of HIV infection and their depletion is the hallmark of the deteriorating immune system\textsuperscript{83-85}. The quantitative decline of CD4\textsuperscript{+} T cells expressed as an absolute number or percentage of total lymphocytes is frequently used for staging of patients. Apart from progressive decline in the number of CD4\textsuperscript{+} T cells, HIV infection is also associated with changes in the representation of many different subset of T cells. Following seroconversion, the number of CD4\textsuperscript{+} lymphocytes declines rapidly and less rapidly thereafter, while the number of CD8\textsuperscript{+} lymphocytes increases with similar kinetics\textsuperscript{86}. However, within the CD8 population there are also subsets which decline in parallel with CD4\textsuperscript{+} subsets. Furthermore, expansion of both CD4\textsuperscript{+} and CD8\textsuperscript{+} T-cell memory subsets, loss of naïve CD4\textsuperscript{+} and CD8\textsuperscript{+} T cells\textsuperscript{87,88}, increase of CD4 and CD8 subsets expressing certain cell surface antigens, especially those reflecting immunologic activation have been implicated in HIV-infected persons from studies in Europe and North America\textsuperscript{89-92}. The expression of activation associated cell surface antigens HLA-DR and CD38
especially on CD8+ T cells increases dramatically with disease progression and have been shown to have a prognostic value for AIDS. In addition to the described quantitative changes, qualitative alterations of cells of the immune system are observed in HIV infection. A functional impairment of T cells from HIV-infected subjects can be detected early at infection. Loss or reduction of T-cell proliferative capacity to in-vitro stimulation is one of these qualitative changes. The loss of in-vitro recall antigen response is detected early at infection. Also, it has been reported that T-cell proliferation in response to stimulation with CD3 and CD3+CD28 MoAbs decreases shortly after seroconversion, before the decline in CD4+ T-cell number is observed. The response to mitogens, such as phytohaemagglutinin (PHA), remains unaffected in the early phases, but is significantly reduced later in infection. It has also been demonstrated that loss of T-cell reactivity to CD3 and CD3+CD28 MoAbs in vitro is a strong predictive marker for progression to AIDS, independent of declining CD4 counts. Thus, T-cell proliferative capacity not only has been shown to be an important independent predictor of progression to HIV disease, but also has been used to monitor immunological improvement after therapy.

Several other potential factors, so called surrogate markers, including inflammatory cytokines have also been suggested for use in monitoring disease progression in HIV-infected patients. Neopterin, which is a metabolite of guanosine triphosphate is produced by macrophages when they are stimulated by interferon gamma from activated T cells. The level of soluble interleukin-2 receptor reflects the activation of T cells and that of β2-microglobulin reflects lymphoid activation more generally. The levels of β2-microglobulin and neopterin are elevated in HIV infection and strongly correlated with the risk of progression to AIDS. Cytokines are integral components of the immune response and their role in HIV disease progression has been extensively investigated. Cytokines, such as IL-2 and IL-12, are crucial for cell-mediated immunity, whereas it is well documented that cytokines, like tumor necrosis factor-α (TNF-α), upregulates HIV replication in both T lymphocytes and monocytes/macro-phages via activation of cellular transcription factor NF-κB. Tumor necrosis factor α (TNF-α) has two specific cell surface receptors and these two receptors, sTNFαRI and sTNFαRII are released from cells as a result of high level of TNF-α and are detectable in soluble forms in body fluids. Although sTNFαRII is not specific for HIV infection, serum levels of sTNFαRII are a strong predictor for disease progression in asymptomatic HIV-positive persons.

**HIV-1 co-receptors and chemokines**

Chemokine receptors, which belong to a family of seven transmembrane spanning G-protein-coupled receptors also serve as co-receptors for HIV-1 entry. It is now known that HIV-1 uses a number of chemokine receptors for its entry. The CC-chemokine receptor, CCR5, is used by macrophage-tropic primary isolates, the viruses that predominate early in infection and are thought to be important for transmission of HIV-1. The CXC-chemokine receptor,
CXCR4, is used by T-cell-tropic or SI viruses that occur late during disease progression to AIDS. HIV can also use other chemokine receptors, CCR2b and CCR3, albeit to a lesser extent. Both CCR3 and CCR5 are expressed on microglia and it is suggested that both receptors are involved in HIV infection of the central nervous system. Bleul et al have shown that HIV co-receptors are differentially expressed on human T lymphocytes. CXCR4 is predominantly expressed on naïve T cells and CCR5 is mainly expressed on previously activated memory cells. The expression pattern of the HIV-1 co-receptors of the surface of CD4+ T cells is believed to have an influence on susceptibility of CD4+ T cells to HIV-1 infection, viral tropism and rate of disease progression. A study by Zhang et al indicated that CCR5 and CXCR4 serve as the major co-receptors for different HIV-1 subtypes and co-receptor usage is determined by viral phenotype irrespective of viral genotype. However, less frequent use of CXCR4 is reported for subtype C virus.

The natural ligands of the HIV-1 co-receptors are chemokines, which are soluble factors thought to direct the migration of different leukocyte subsets to sites of inflammation. They can be subdivided into two groups. The α-chemokines also known as CXC chemokines include SDF-1 and the β-chemokines or C-C-chemokines include RANTES, macrophage inhibitory protein-1α (MIP-1α) and MIP-1β. The CC-chemokines were shown to block infection of susceptible cells into vitro by macrophage-tropic primary HIV isolates and SDF-1 was shown to inhibit T-cell-tropic viruses.

**Immune activation and HIV pathogenesis**

Activation of all components of the immune system is the major feature of HIV infection. The activated state of the immune system is reflected by increased expression of antigens on cells which are otherwise expressed at a reduced level on resting cells and by increased level of soluble proteins which are released from activated cells. Several in-vitro studies have established the role of cellular activation in the propagation of HIV infection in CD4 cells. However, it seems paradoxical that on one hand immune activation is most probably involved in the immune control of HIV-1 infection and, on the other hand, several lines of evidence support that activation of immune cells may lead to enhanced HIV-1 replication. Activated CD4+ lymphocytes are found to be more susceptible to HIV infection compared to their resting state and in-vitro activation of latently infected lymphocytes triggers active viral replication. In vivo, CCR5 is mainly expressed on memory or primed (CD45RO*) T cells and these cells are indicated to be selectively infected by HIV-1. There is evidence that CCR5 expression on this subset of cells is associated with HLA-DR expression and increases with disease progression. The strong association observed between the decline of CD4+ T cells and increased levels of activation markers on CD4+ T cells further support the view that cellular activation promotes HIV disease progression. Another study by Weissman et al demonstrated that 100 times less virus is required to initiate HIV infection in cell culture from an individual after immunization than before immunization.
indicating the potential contribution of cellular activation associated with an ongoing antigen-specific immune response to the pathogenesis of HIV disease.\textsuperscript{144} It is suggested that activation of CD4\textsuperscript{+} T cells facilitates HIV infection in a number of ways either by enhancing HIV entry into the cell, triggering the completion of reverse transcription and viral integration or by stimulating viral transcription from proviral DNA.\textsuperscript{145} In light of these observations, chronic immune activation due to, among others, highly prevalent parasitic infections has been suggested to explain, at least in part, the reported accelerated HIV disease progression in African patients.\textsuperscript{146,147}

Finally, there is evidence to support that there is persistent CD4\textsuperscript{+} and CD8\textsuperscript{+} T-cell activation in HIV-infected individuals, which provides an optimal environment for continuous HIV replication. CD4 and CD8 lymphocytes are crucial for the maintenance of appropriate immunological response against a wide range of pathogens. In addition, CD4 cells secrete factors that affect the growth and differentiation of lymphoid cells and haematopoietic cells and the function of non-lymphoid cells as well. Therefore, it is clear that quantitative or qualitative abnormalities of the CD4 and CD8 populations, as a result of HIV disease and abnormal immune activation, can have profound effects on the immune system function.

**HIV infection susceptibility/resistance**

From the observation that some individuals remain uninfected despite high-risk exposure, it has been concluded that host factors exist that determine susceptibility to HIV infection. Studies conducted in various population groups, including commercial sex workers, discordant couples, infants born to HIV-infected mothers and exposed health care workers, have described several host factors which possibly contribute to HIV infection resistance.\textsuperscript{148-151} CCR5 and CXCR4 are the main co-receptors used by HIV.\textsuperscript{25,120} Polymorphism of the gene encoding CCR5 is one of the factors found to be associated with resistance.\textsuperscript{150} The Δ32 base pair deletion mutation in the CCR5 gene is shown to result in a premature stop codon and loss of HIV-1 co-receptor activity. However, some individuals with this mutation were found to be HIV-infected, indicating that the protection conferred by this mutation is not absolute.\textsuperscript{152,153} In addition, HIV-1-infected individuals, heterozygous for the Δ32bp deletion mutation, have been shown to have slower rates of CD4 decline, have lower viral loads and survive longer compared to individuals with the wild genotype.\textsuperscript{154,155} Moreover, reduced cell surface expression of CCR5 has been reported in individuals with heterozygous deletion compared to the wild genotype group.\textsuperscript{156} The Δ32bp deletion is common in Caucasians but rare in Africans. Other mutations on the CCR5 than the Δ32bp deletion have also been reported. A mutation in the promoter region of the CCR5, CCR5P1, has been found to have a negative effect, in that infected individuals homozygous for this mutation progress rapidly to AIDS.\textsuperscript{157,158} In contrast, polymorphism of the coding region of CCR2b has been suggested to be associated with a delay in disease progression but not with reduced transmission risk.\textsuperscript{159,160} This mutation, CCR2-64I, changes valine to isoleucine and is linked to a CCR5
promoter polymorphisms (CCR5-59653T). It is shown that CCR2-64I effect on AIDS progression is not mediated by a negative effect on the CCR5 co-receptor function, although a slightly reduced expression of CCR5 is reported in individuals with CCR2-64I mutation\textsuperscript{161}. Furthermore, a mutation on the 3' untranslated region of the SDF-1 chemokine gene (SDF1-3A) is indicated to be associated with rapid disease progression\textsuperscript{162,163}. No polymorphism is reported for CXCR4, which is mainly expressed on naïve cells and is used by SI viruses.

The role of the cell surface expression levels of the co-receptors on cellular susceptibility and tropism of the virus is also implicated. A correlation between low expression of CCR5 and reduced infectability of T cells \textit{in vitro} is also reported\textsuperscript{164,165}.

\textit{In-vitro} studies have shown that the CC-chemokines RANTES, MIP-1\textalpha{} and MIP-1\beta{} can block the entry of macrophage-tropic viruses into susceptible cells\textsuperscript{127,128}. This inhibition is thought to be mediated by blocking env-driven HIV fusion through competition for the chemokine receptors or receptor downregulation. In relation to this, high level of chemokines was detected in exposed but uninfected individuals\textsuperscript{149,164}. Other host genetic factors have also been implicated to play a role and increased frequencies of certain HLA alleles were detected in exposed but uninfected individuals\textsuperscript{166}. Furthermore, acquired protective immunity after exposure to HIV was also suggested to play a role in HIV-1 infection resistance. HIV-1-specific cytotoxic T lymphocytes are detected in exposed health workers, commercial sex workers and was indicated as one of the mechanisms of natural protective immunity\textsuperscript{167,168}. From studies on commercial sex workers in Kenya and Thailand mucosal immunity was also shown to be highly associated with HIV-infection resistance\textsuperscript{168,169}. HIV-specific mucosal IgA, in the absence of systemic IgG response, was detected in mucosal sites of high proportions of HIV-1 exposed but uninfected women. This response was found rarely in HIV-infected women it's involvement in mediating protection against HIV infection. Recently, another study by Mazzoli \textit{et al} reported HIV-specific IgA in the serum of exposed seronegative partners of HIV-seropositive persons, implying the involvement of not only mucosal but also systemic IgA-mediated immunity to HIV\textsuperscript{170}.

\section*{ENARP and scope of this thesis}

The Ethio-Netherlands AIDS Research Project (ENARP) is a collaborative project between the governments of Ethiopia and the Netherlands. It started in 1994 with three main objectives:

i. training of Ethiopian scientists

ii. capacity development and

iii. conducting research on HIV.

The project is based at the Ethiopian Health and Nutrition Research Institute (EHNRI) in Addis Ababa and is supported by three research groups in Amsterdam, the Netherlands: 1) The Division of Public Health and Environment, Municipal Health Service (Prof Roel Coutinho, Epidemiology); 2) Department of Viro-Immunology at CLB (Prof Frank Miedema, Immunology) and 3) The
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Department of Human Retrovirology, Academic Medical Center (Prof Jaap Goudsmit, Virology). The project has established a well-equipped laboratory. ENARP has also started a cohort study on HIV infection progression in two factories, Akaki and Wonji, 20 km and 100 km away from Addis Ababa, respectively. Research for this thesis was conducted as part of the immunology research program of ENARP.

Scope of this thesis

HIV infection is associated with several changes of the immune system. In chapter 2 CD4 and CD8 values as well as haematological parameters are compared between Ethiopian and other populations. Since the base line values of some of these parameters in Ethiopians were different in proportions and numbers compared to Dutch and other published values for Africans, immunohaematological reference ranges established for adult Ethiopians is shown also in chapter 2. The representation of several T-cell subsets are studied in HIV-negative Ethiopians and compared to HIV-negative Dutch subjects in Chapter 3. Furthermore, HIV-associated changes in these subsets are analyzed in Ethiopians who are HIV-negative, HIV-positive with and without AIDS in chapter 3. Chapter 4 levels of soluble viro-immunological markers in HIV-infected and -non-infected Ethiopians are presented. In addition, the prognostic value of these markers for HIV disease progression in Ethiopians is discussed.

A study on the existence of possible host factors associated to HIV infection resistance in high-risk HIV-1-negative Ethiopian commercial sex workers is presented in chapter 5. Chapter 6 shows the expression levels of co-receptors in different CD4 and CD8 T-cell subsets of HIV-infected and -non-infected Ethiopian commercial sex workers. The co-receptor expression is correlated to a polymorphism in the CCR2b gene. In chapter 7, the work included in this thesis is discussed in relation to current literature.

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