Virus and host determinants of HIV-1 infection and AIDS pathogenesis

Blaak, H.

Citation for published version (APA):
Chapter 1

General Introduction
The Disease

AIDS Epidemiology

The Acquired Immunodeficiency Syndrome (AIDS) was first recognized in 1981 and in 1983 the causative agent of this new sexually transmitted disease was identified to be a retrovirus initially named Human T-Lymphotropic Virus III (HTLV III), Lymphadenopathy Associated Virus (LAV) and AIDS associated Retrovirus (ARV) [1-4]. Based on the characteristics of the disease, the virus was renamed Human Immunodeficiency Virus (HIV) [5].

Two subtypes of HIV are identified, HIV-1 and HIV-2, which have a different geographical distribution and different pathogenicity. Thus, while the distribution of HIV-1 is worldwide, HIV-2 is most prevalent in West-African countries and is associated with a more benign disease course than HIV-1 [6].

HIV-2 is believed to have entered the human population through cross-species transmission (zoonosis) and to originate from a Simian Immunodeficiency Virus (SIV) strain that is endemic in sooty mangabey monkeys (SIVsm) [7-9]. Recently, clues pointing to a similar origin for HIV-1 have been described [10]. Phylogenetic and geographic analyses identified a particular SIVcmz strain, detected in the chimpanzee subspecies Pan troglodytes troglodytes, as a possible candidate ancestor for HIV-1. Based on the presence and characterization of HIV-1 sequences in a plasma sample dating from 1959, the introduction of HIV-1 into the human population is estimated to have occurred around the 1940s [11].

Two decades after the first recognition of AIDS, HIV-1 is responsible for the death of approximately 14 million people worldwide and the estimated number of people living with HIV/AIDS by the end of 1998 was 33.4 million (Fig. 1) [12]. In 1995 new, highly effective anti-retroviral drugs were introduced. The use of these drugs in combination regimens, termed highly active anti-retroviral therapy (HAART), has resulted in a decrease of AIDS-deaths in the western world, which can be regarded as an important victory in the human combat against HIV-1. However, the initial euphoria brought about by the prospect of HIV-1 eradication being within sight, has since been tempered considerably. Eradication of the virus is hindered by the possibly life-long persistence [13] of HIV-1 due to latent infection of long-lived resting CD4+ T cells [14-16]. Life-long viral persistence, together with drug toxicity, non-compliance, and appearance of multiple drug resistant HIV-1 variants predict that current anti-retroviral regimens, despite their effectiveness in inhibiting virus replication and their capacity to prevent and reverse disease progression, will fall short in eradicating HIV-1 infection.

Fig. 1. Adults and children estimated to be living with HIV/AIDS as of end 1998 (from reference 12)
Despite the decrease in AIDS deaths in the western world, new infections still occur in virtually every country of the world. Moreover, the epidemic is still completely out of control in many (developing) countries, for which the current anti-retroviral treatment is financially out of reach. During 1998, 11 new infections were estimated to occur every minute (90% of which occurred in Sub-Saharan Africa and South and South-East Asia), affecting men (53%), women (36%) and children (below age 15, 10%). The number of people dying of AIDS, globally 6 per minute during 1998, lags only a few years behind. The imperfection of HAART and the tremendous and continuously increasing numbers of new infections and of people living with HIV/AIDS illustrate that the battle is not yet won by far.

HIV-1 infection

HIV-1 establishes a chronic infection in humans that is characterized by a clinically asymptomatic phase unequivocally resulting in AIDS. The median time interval between seroconversion and AIDS is 9-10 years, however the length of this phase is highly variable. While some individuals develop AIDS within a few months after seroconversion, cases with lack of any signs of progression for 15 years or more have also been reported. Already during the asymptomatic phase large numbers of virus are produced every day, generally resulting in a steady increase in the viral load. Simultaneously, the numbers of CD4+ T cells decline and the functionality of the immune system deteriorates. Once beyond a threshold, the immune system is no longer able to counter opportunistic infections and control the outgrowth of malignancies. This end-stage of HIV-1 infection, thus characterized by a variety of illnesses, is called AIDS.

The Virus

HIV-1 is a retrovirus belonging to the subfamily of Lentiviruses. Retroviruses are enveloped particles of 100 nm that contain an icosahedral nucleocapsid and have a single-stranded diploid RNA genome with a molecular weight ranging from 1-3x10^6. They carry an RNA-dependent DNA polymerase, called reverse transcriptase, responsible for translating the viral genome into DNA, which is integrated into the host genome.

Like the other members of the retrovirial family, the HIV-1 virion consists of an outer membrane, formed by a lipid bilayer derived from the host cell membrane (Fig. 2). In the membrane...
and HIV-1 resides as an incomplete, labile proviral DNA species\(^{65,66}\). Furthermore, even though it has been suggested that the matrix protein, \(\text{vpr}\), and integrase mediate nuclear transport of the preintegration complex\(^{71}\) in non-dividing cells\(^{72,75}\), nuclear transport and integration of proviral DNA appear to be efficient only in activated cells\(^{68,76-78}\).

**Tropism**

The cellular receptor of HIV-1, CD4, is expressed on \(T\) lymphocytes of the helper phenotype, thymic precursors, macrophages, dendritic cells (DC) and microglial cells of the brain. Consequently, these cell types are potential target cells of HIV-1\(^{169,79-84}\). From observations that HIV-1 could not replicate in non-human cells expressing human CD4 however, it became evident that additional factors were required to establish infection\(^{85,86}\). Furthermore, in the early years of HIV-1 research it became clear that two distinct HIV-1 phenotypes could be recognized, with different tropism for \(CD4^{+}\) cell lines. These two HIV-1 variants were distinguished as slow/low and rapid/high viruses\(^{87}\), or by their differential capacity to induce syncytia, as non-syncytium-inducing (NSI) and syncytium-inducing (SI) variants\(^{88,89}\). The (rapid/high) SI variants appeared to be more \(T\)-cell-line-tropic compared to the (slow/low) NSI variants\(^{90}\). The other way around, the tropism of most NSI variants appeared to extend to macrophages, while this was only occasionally observed for SI variants\(^{91,92}\).

These observations led amongst others to the search for \(CD4^{+}\) cell-type-specific entry factors\(^{93}\), which was successfully concluded in 1996, when the chemokine receptor \(\text{CXCR4}\) (initially referred to as \(\text{FUSIN}\) or \(\text{LESTR}\)) was identified to be the coreceptor for \(T\)-cell-line-
tropic, SI, HIV-1. This discovery was just preceded by the observation that NSI HIV-1 replication is in vitro inhibited by $\beta$-chemokines. Hence, almost simultaneously to the discovery of the coreceptor for T-cell-line-tropic HIV-1 variants, the receptor of the $\beta$-chemokines, CCR5, was identified as the coreceptor for primary NSI variants. Since the identification of CCR5 as an HIV-1 coreceptor, data on coreceptor usage of primary HIV-1 variants has been accumulating and show that NSI variants are almost completely restricted to the use of CCR5. In contrast, primary SI variants are often capable to additionally use CCR5 and sometimes CCR2 and CCR3 as coreceptors. The in vivo relevance of the capability of HIV-1 to use other receptors than CCR5 and CXCR4 remains to be established.

The expression patterns of the coreceptors on cells coexpressing CD4 offered, at least partly, an explanation for differences in tropism of HIV-1 variants. Often used T cell lines like H9 and MT2 exclusively express CXCR4, explaining the incapability of NSI variants to infect these cells. Microglial cells express CCR3 and CCR5, possibly contributing to the presence of predominantly macrophage-tropic variants in the brain. The high levels of CXCR4 expressed by thymocytes during most stages of maturation agrees with the more thymocyte-tropic phenotype of SI variants compared to NSI variants, observed in a SCID-hu mouse model. Besides the different expression patterns of the HIV-1 coreceptors on different CD4+ cell types, CCR5 and CXCR4 are also differently expressed on T cell subsets. Thus, CXCR4 was shown to be mainly expressed on $T$ cells with the resting, naive phenotype, while CCR5 was mainly expressed on cells with an activated, memory phenotype.

The mere presence of CCR5 or CXCR4 (or other coreceptors) is not the sole factor influencing tropism however. The multi-factorial basis of cell tropism is very well illustrated by HIV-1 infection of macrophages and dendritic cells. In general, macrophages do not support SI replication. Yet, some primary SI variants can use CCR5, and macrophages express CXCR4. Infection of macrophages by some exclusively CXCR4-using HIV-1 variants is restricted at the level of entry. The constraints on (X4) virus entry into macrophages may be explained by a higher CD4 dependency of CXCR4 using isolates compared to CCR5 using isolates, given the relatively low CD4 expression on macrophages. In addition, due to cell-type dependent differences in post-translational processing CXCR4 exists in different biochemical forms or the surface of different cells. In macrophages, CXCR4 is present in a high molecular weight form, which fuses poorly with X4 HIV-1 envelopes. HIV-1 infection of macrophages is not always restricted at the level of entry. Primary SI variants have been reported to enter macrophages via CXCR4 and in the case of dual-tropic viruses also via CCR5. In most of these cases, however, replication was shown to be restricted at post-entry events.

Similarly, contradictory observations have been made with respect to the infectability of DC and Langerhans cells. Some studies showed that these cells can be productively infected in vitro, albeit at low levels, while others reported viral entry, but no virus production. In addition, while some reported that DC and LC can be infected by both SI and NSI variants, others detected infection by NSI variants only. Clues directing towards an explanation for the different observations come from studies showing that infectability of DC and the expression patterns of CCR5 and CXCR4 are associated with different stages of differentiation. The association between productive HIV-1 infection of DC and the stage of differentiation is further confirmed by the observation that productive infection of DC is achieved only upon interaction with CD4+ T cells. In parallel to what has been observed for macrophages and T cells, host factors associated with proliferation and the stage of maturation probably determine HIV-1 replication in DC and LC.

**HIV-1 phenotype**

The process of reverse transcription is error-prone, due to the absence of proofreading activity, resulting in approximately one misincorporation per replication cycle. In combination with the enormous production rate of $10^{10}$ virions each day, this accounts for the fact...
that HIV-1 changes its appearance continuously.\textsuperscript{[149]} Thus, large genotypic variation can be observed between HIV-1 isolated from different infected individuals, and even within each HIV-1-infected individual a population of distinct, yet closely related variants exists. Therefore, rather than to think of HIV-1 as one virus, it is more appropriate to regard HIV-1 as a quasispecies with a multitude of different genotypic and phenotypic characteristics. As mentioned above, HIV-1 phenotypic variation is manifested in differences in tropism, syncytium-inducing capacity, and coreceptor usage. In addition, differences in replication capacity\textsuperscript{[126,150,151]} and cytopathicity\textsuperscript{[160]} have been observed.

**Molecular aspects of HIV-1 variation**

The HIV-1 envelope protein (gp120) consists of 5 constant (C1-C5) and 5 variable (V1-V5) domains. Syncytium-inducing capacity, cell tropism and coreceptor usage which are, as indicated above, highly inter-dependent viral properties, are largely determined by the first, second and third variable domain.

V3 was shown to be the minimal determinant for tropism\textsuperscript{[152-160]}, syncytium-inducing capacity\textsuperscript{[160-163]} and co-receptor usage\textsuperscript{[164-167]}. The presence of 1 or 2 positively charged amino acids at 2 specific positions of the V3 region appeared sufficient to confer a SI phenotype and CXCR4 usage\textsuperscript{[162,163,167,168]}. The sequences conferring the SI phenotype and determining tropism are partly independent\textsuperscript{[160,169]}.

Next to V3, specific sequences in V1, V2, V4 and V5 have been implicated in differential tropism, coreceptor usage and syncytium-inducing capacity\textsuperscript{[169,166,169-175]}. These regions of the envelope protein were shown to be involved in increasing the efficiency of infection of specific cell types, and enhancing syncytium-inducing capacity. Furthermore, the length of V2 was shown to transiently increase during transition from NSI to SI phenotype in some HIV-1 variants\textsuperscript{[176,177]}.

The accessory genes, nef, rev, vif, vpu, vpr, and tat regulate HIV-1 production at different levels of the viral life cycle. Hence mutations in these regions influence virus production, albeit to different extents and dependent on cell type\textsuperscript{[178-190]}.

**HIV-1 evolution**

In recently infected individuals, a homogenous population of predominantly macrophage-tropic viruses is detected\textsuperscript{[191-194]}. In the course of infection the virus population gets more heterogeneous\textsuperscript{[195-200]} forming the quasispecies.

Evolution of the virus is driven by Darwinian selection, favoring the fittest phenotype given a specific environment. The selective forces can be envisaged to come from the genetic background of the host, the immune response and anti-retroviral drugs. The high mutation rate of HIV-1 results in accumulation of random mutations throughout the genome. These mutations either change (non-silent mutations) or do not change (silent mutations) the amino acid sequence. Most mutations will have little or no effect on the fitness of the virus. Such mutations particularly cause the heterogeneous appearance of the virus\textsuperscript{[149]}. Some of the mutations however, will be deleterious to the virus and will therefore not persist (i.e. mutations in conserved sequences). On the other hand, some of the mutations will be highly beneficial, dependent on environmental factors, and viruses bearing this phenotype will increase proportionally. The role of environmental pressure is illustrated by the observation that in individuals with rapid disease progression, and thus probably with failing immune control, less virus evolution is observed\textsuperscript{[159,201]}.

**A few examples of HIV-1 evolution**

SI variants, CTL escape mutants and drug resistant mutants are examples of HIV-1 evolution and are highlighted below.

In general, SI variants are not among the viruses that establish a new infection\textsuperscript{[191-194]}, during disease progression however, SI variants emerge in about 50% of the infected individuals\textsuperscript{[68,194,202]}. In general SI variants have higher replication capacity than NSI variants\textsuperscript{[26,87,151]} and because of the capacity to use CXCR4 in addition to CCR5\textsuperscript{[97-100,102,103]}. SI variants have a broader range of target cells. The absence of SI variants early in infection is thought to result from selective transmission of macrophage-tropic variants in combination with adequate immune pressure before the onset of immunodeficiency. It has been suggested that since macrophage-tropic
variants can spread through cell-cell contact, they may better equipped to escape immune surveillance compared to SI variants\(^{(203-205)}\). In this view, SI variants can prosper only when the immune surveillance weakens with progressive infection. In agreement with this, SI variants appear at a higher rate when CD4\(^+\) T cell counts are below a certain threshold\(^{(206)}\). Still, not in all individuals with low CD4\(^+\) T cell counts SI variants emerge\(^{(206)}\), suggestive of the existence of additional constraints on SI evolution. A possible explanation might be that NSI to SI transition is accomplished via an intermediate genotype with low fitness. In support of this theory is the scarce observation of variants with intermediate genotypes\(^{(207)}\). Another remarkable feature of SI evolution is that, while after their emergence the proportion of SI variants increases over time, NSI variants persist and even still increase in number\(^{(208)}\). This might be explained by their partially different coreceptor usage, and indicate that both variants have their own niche within the human body.

More important adaptations HIV-1 has to make are the ones necessary to prevail, and are those dealing with immune response and anti-retroviral drugs. When CTL responses are directed against one single variable epitope, the virus can easily adapt, while in contrast, CTL directed against conserved epitopes would prevent viral escape\(^{(209-211)}\). The emergence of CTL escape mutants has been documented, and was associated in vivo with rapid expansion of virus 'despite' the presence of a strong CTL response\(^{(212-219)}\).

During the early days of anti-retroviral treatment, when patients generally received only 1 or 2 drugs, the emergence of resistant variants was the major obstacle interfering with success of treatment\(^{(220-224)}\). Still, in the era of protease inhibitors, resistance is easily acquired in vitro\(^{(225-229)}\) and HIV-1 variants with reduced sensitivity to protease inhibitors have been detected in treated patients\(^{(230-233)}\). The current treatment strategy encompasses a combination of 3 or more drugs, consisting of both reverse transcriptase and protease inhibitors, and is termed highly active retroviral therapy (HAART). HAART has been very successful in reducing virus production to undetectable levels\(^{(234-236)}\) and might thus prevent the emergence of resistance. Recently however, it was shown that even during HAART virus replication and viral evolution go on in some patients\(^{(237)}\), raising the pessimistic thought that even HAART may ultimately appear to merely slow down but not entirely stop the emergence of multiple-drug resistant variants.

These examples demonstrate the various constraints presented by a hostile environment, with which HIV-1 deals quite easily, through fast and error-prone replication.

### The Course of Infection

#### HIV-1 transmission

HIV-1 can be transmitted via heterosexual\(^{(238)}\) and homosexual contact\(^{(239)}\), through blood transfusions\(^{(240,241)}\) and needle sharing (in particular) among intravenous drug users\(^{(242)}\), and from mother to child\(^{(243)}\), either in utero, intrapartum or post-partum via breast milk\(^{(244-248)}\). The diverse routes of transmission have resulted in infection of men, women and children, thereby affecting the entire human population and not, as believed during the early days of the epidemic, 'only' the homosexual community.

Whether upon exposure to HIV-1 transmission ensues and infection is established, depends on numerous factors influencing the infectiousness of the virus donor and susceptibility of the exposed individual. The stage of disease of the HIV-1 donor is an important risk factor of transmission\(^{(249,252)}\). The association between transmission frequency and the stage of disease is explained by the positive effect of a high viral load on the transmission rate\(^{(253-259)}\). An extremely high viral load is mainly observed in individuals experiencing acute infection and individuals with AIDS\(^{(26-28,259-264)}\), rendering these individuals very infectious. In cases of sexual transmission, especially the viral load in semen and vaginal fluid\(^{(265-271)}\) is likely to be an important risk factor, which does not necessarily correlate with the viral load in the periphery\(^{(272-279)}\). The extent of HIV-1 shedding in genital secretions has been associated with reduced immune status and the presence of other sexually transmitted diseases (STD)\(^{(273,274,276-279)}\). In line with the above, other
factors associated with the stage of disease of the potential HIV-1 donor, such as high CD4+ T cell counts and the presence of neutralizing antibodies have been found to correlate with reduced risk of transmission. 

The degree of susceptibility of individuals exposed to HIV-1 influences the risk of transmission. Susceptibility to infection is based on genetic polymorphisms and is enhanced by the presence of other infections (STD) that cause lesions at the sites of transmission and attract potential HIV-1 target cells to the site of transmission. Some individuals remain uninfected despite frequent exposure to HIV-1. In some individuals the basis of natural resistance appeared to be a 32-bp deletion in both alleles of the CCR5 gene (CCR5Δ32), resulting in total lack of CCR5 cell surface expression. Given the major role of CCR5 in HIV-1 infection, its natural ligands MIP-1α, MIP-1β and RANTES, which inhibit viral replication in vitro, may also be of influence during transmission. Mutations influencing the level of RANTES production have been identified, and high concentrations of β-chemokines at the site of transmission might offer protection from infection.

The 32-bp deletion in CCR5 accounts for only a minority of all recorded cases of HIV-1 resistance. Furthermore, the CCR5Δ32 polymorphism is common only in the Caucasian population, and could not explain absence of infection for instance in frequently exposed African prostitutes. Therefore, it is evident that other host genetic factors contribute to the natural resistance to HIV-1 infection. Various studies showed that a strong cellular immune response can be detected in exposed uninfected individuals. Also, a non-HIV-1-specific, allogeneic humoral immune response directed against HLA molecules incorporated in the virion membrane has been suggested to explain cases of protection. In a monkey model, immunization with human HLA molecules conferred resistance to infection with SIV grown in human cells. In human HIV-1 infection however, conflicting data have been obtained regarding this mechanism of protection.

The selective transmission of macrophage-tropic variants and the importance of CCR5 in transmission suggest that macrophages are among the first cells encountered by HIV-1. Also LC have been suggested to act as initial targets of HIV-1 infection. LC from skin of infected individuals were shown to contain proviral DNA and mRNA, but the level of productive infection is low. An important role for LC in transmission does not necessarily involve high levels of productive infection however. DC carry high amounts of virions on their membrane, either as immune complexes captured by Fc receptors or, as it was recently shown, via ICAM-1 and LFA-1 interactions. Migration of such virus laden DC from the site of transmission to lymph nodes would facilitate dissemination of the virus. Both CD4+ T lymphocytes and macrophages were shown to be readily infected and produce high levels of virus upon exposure to HIV-1 pulsed DC and LC. The role of DC and LC in transmission and routing of the virus to lymphoid tissue is confirmed in monkey and mouse models.

Primary HIV-1 infection

Within a few weeks after infection, 50-70% of individuals experience an acute retroviral syndrome (ARS), which is characterized by flu and mononucleosis-like symptoms. During this acute HIV-1 infection an enormous amount of virus is produced, with peak titers of 10^7 RNA copies per ml of serum. The 'viral burst' is accompanied by a decline in the number of CD4+ T cells. After 2-3 months the viral load declines, and the CD4+ T cell numbers regain subnormal levels. The reduction in viral load varies between individuals and results in a viral 'set-point' ranging from <500 to 10^6 copies per milliliter of serum. The viral load at this moment in infection greatly influences the subsequent rate of progression to AIDS.
found to be lagging behind the decrease in viremia[338,341,343,344].

Specific HLA types[345], high frequencies of HIV-1 specific CTL[340,346], and a broad immune response[347] during the first 1-2 years of infection are associated with a low baseline RNA load and a better prognosis. This underscores the importance of an adequate immune response during the acute phase of infection.

**Chronic phase of HIV-1 infection**

After viral load has declined and CD4* T cell numbers rebounded to subnormal levels, the chronic phase of infection starts. A gradual decrease in CD4* T cell numbers and a gradual increase in viral load, ultimately resulting in AIDS, characterize this clinically asymptomatic phase of variable length. In most individuals, the period between seroconversion and AIDS diagnosis is 8 to 10 years, which is called typical disease progression. At the one extreme, there are individuals who show a very rapid CD4* T cell decline from seroconversion onwards. In most of these cases the viral load is already high from the start, and does not necessarily further increase. On the other extreme, there are individuals who maintain stable and subnormal levels of CD4* T cells and viral load under current detection levels for periods ranging from 10 to over 15 years. In some of these cases, after long stable periods a rise in viral load and drop in CD4* T cells (either rapid or slow) are suddenly initiated, resulting in progression to AIDS after all. The individuals categorized in this group are called long term non-progressors or long-term survivors of HIV-1 infection. The different rates of CD4* T cell decline and increase in viral load, and the virtually absence of these characteristics in some individuals point to the existence of variables that influence the course of HIV-1 infection.

**I. Viral load**

In the early days of the HIV-1 epidemic, researchers were faced with the paradox of progressive immunodeficiency in the presence of only a low viral burden[348,349]. With the development of more sensitive methods to detect viral RNA in serum and virus in cell cultures, it became evident however, that a substantial amount of virus was produced not only in AIDS patients but also during the asymptomatic phase[28,261-264]. It was not until the introduction of potent inhibitors of HIV-1 replication in 1995, however, that the real magnitude of virus production was recognized. Based on the quasi steady state of plasma virus levels prior to anti-retroviral therapy and the rapid decrease in plasma virus levels after HAART, it was estimated that $10^7-10^{10}$ virones are produced every day[23-26]. The high level of virus replication results in an accumulation of virus in the body. The levels of virus in the periphery commonly measured as cell-free RNA in plasma or serum, proviral DNA, or infectious virus or mRNA levels in PBMC reflect the stage of disease and predict future disease progression[26-28,251-254,335,350,351].

Longitudinal studies have shown that the viral load in the periphery gradually increases during the asymptomatic phase of infection[26,27].

The lymphoid tissues are thought to be the primary sites of viral replication[319,357,358]. The presence of viruses in the lymph nodes harboring mutations that cannot (yet) be detected in the PBMC quasispecies supports this[354,355]. Besides lymphoid tissues and peripheral blood, HIV-1 is also detected in other body tissues[356-359]. The presence of HIV-1 in non-lymphoid tissues likely represents migration of infected lymphocytes/macrophages to places with ongoing opportunistic infections coinciding with AIDS[356,357].

**II. CD4* T cell decline**

The decline of CD4* cells is the main immunological feature of HIV-1 infection[26-31], yet the mechanisms responsible for this decline are still not fully understood. The loss of CD4* T cells may be explained by increased cell death. Increased death is partly due to direct virus mediated killing of infected cells[360-363], and partly to death of uninfected cells through activation induced apoptosis[364-367]. The accelerated death of CD4* T cells can be envisaged to result in CD4* T cell depletion, if the body cannot make up for the loss. However, until recently, not much information was available on the regenerative capacity of human T cells.

The introduction of the potent anti-retroviral protease inhibitors provided a tool to estimate the dynamics of T cell turnover. Based on the initial
increase of CD4+ T cells in the blood after initiation of therapy, it was calculated that $2 \times 10^9$ CD4+ T cells were being destroyed and replaced each day. Based on the idea that this turnover rate was severely increased compared to healthy individuals, it was suggested that physical exhaustion could explain the CD4+ T cell loss in HIV-1-infected individuals. This highly elevated turnover could not be confirmed however, by analysis of biological measures for cell turnover, like telomeric length and Ki-67 expression. Telomeres are the ends of chromosomes that shorten with each cell division, and consequently the telomeric length is supposed to be a measure of turnover, Ki-67 is a nuclear antigen that is expressed only in cycling cells. These latter studies suggested a maximal 2- to 3-fold increase in CD4+ T cell turnover in HIV-1-infected individuals. The high expansion rate of CD4+ T cells after potent anti-retroviral therapy while proliferation is only slightly elevated, might partly be explained by a release of CD4+ T cells formerly trapped in lymph nodes when, as a result of therapy, viral antigen disappears from these sites and immune activation decreases.

Even though the 2- to 3-fold increase in T cell turnover appears to be only marginal, it is unknown whether the human body can handle such production rates for extended periods of time. It has been suggested that in healthy adult individuals the level of CD4+ T cell production at normal turnover rates is already close to its limits. The natural limitations to renewal capacity may be enhanced by viral interference with CD4+ T cell production. In line with this, HIV-1 has been shown to infect thymocytes in vitro as well as in vivo. In SCID-hu mice implanted with fetal human liver and thymic tissue, infection of thymocytes was associated with depletion of (particularly CD4CD8 double positive) thymocytes and disruption of the thymic microenvironment, thereby interfering with thymopoiesis. Besides direct viral effects, interference with thymopoiesis could be attributed to apoptosis of uninfected cells. The direct and indirect effects of HIV-1 infection likely account for the severe involution of the thymus observed in adults and children who died of AIDS.

Furthermore, in adult HIV-1-infected individuals, the abundance of thymic tissue was inversely associated with the stage of disease, suggestive of reduced CD4+ T cell generative capacity with ongoing disease.

The renewal capacity of T cells may also be affected at earlier stages of T cell development. It was shown that the generation of CD4+ T cells from peripheral hematopoietic progenitors was impaired in HIV-1-infected individuals, and in particular in individuals progressing to AIDS. Also, in HIV-1-infected SCID-hu mice a depletion of hematopoietic progenitors was observed, which was independent of direct infection.

It is easily envisaged that increased cell death and the incapability to make up for this loss, either because of natural and/or virus-induced constraints, together would result in the gradual CD4+ T cell decline. Finally, since the decrease in CD4+ T cells is measured in the peripheral blood, a part of the decline may be explained by trapping of cells in the lymph nodes, rather than by actual depletion.

**Scope of this thesis**

Both the odds of getting HIV-1-infected upon exposure and the course of HIV-1 infection are highly variable among individuals. This thesis focuses on a number of virus and host factors that influence HIV-1 infection and pathogenesis.

Chapter 2 describes determinants associated with homosexual transmission. Studied was the role of viral load and susceptibility of PBMC to NSI HIV-1. The bases of differential susceptibility to NSI variants were studied in chapter 3. Associations between the NSI phenotype, CCR5 cell surface expression, and HIV-1 pathogenesis were further analyzed in chapters 4 and 5. One of the main parameters identifying differences in the course of HIV-1 infection is the rate of increase in viral load. Chapter 6 compares the kinetics of two commonly used measures of viral load in individuals with varying clinical courses. Viral features contributing to differences in virus production are described in chapters 7-10. Chapters 7 and 8
describe replication kinetics of HIV-1 in relation to disease progression and viral load (chapter 7) and during the process of NSI to SI evolution (chapter 8). Finally, the role of differential coreceptor usage in determining tropism and pathogenesis (chapter 9), and coreceptor evolution during NSI to SI transition (chapter 10) are described.