Virus and host determinants of HIV-1 infection and AIDS pathogenesis
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Chapter 11

General Discussion

HIV-1 infection (1): The two 'parties'

Multiple host and virus factors influence the rate of infection upon HIV-1 exposure as well as the rate of disease progression. HIV-1 transmissibility is determined by factors that influence the infectivity in the route of exposure and stage of disease, viral load, and factors that influence the susceptibility and the immune defense of the exposed individual. Once infection is established, the rate of disease progression is associated with viral factors that influence virus production and replication and viral pathogenesis and host factors that cause acute, subacute and chronic infection and delayed immune recovery. Immune recovery and the course of infection have been observed in the absence of treatment, but have not been established among two...

CCHS32

Upon HIV-1 transmission and during the early phase of infection, the vast majority of all HIV-1 variants have the N54 prototype.
HIV-1 infection (1): The two 'parties'

Multiple host and virus features influence the risk of infection upon HIV-1 exposure as well as the rate of disease progression. Thus, the risk of transmission is determined by factors that influence the infectivity of the potential HIV-1 donor (e.g., stage of disease, viral load), and factors that influence the susceptibility and the immune defense of the exposed individual. Once infection is established, the rate of disease progression is associated with viral factors that influence virus production, virus spread, and virus pathogenicity, and host factors that control virus production and virus spread (i.e., susceptibility and immune control), and the capacity of the host to replenish lost CD4+ T cells. In this thesis some of the virus and host factors involved in infection/transmission and the course of infection have been described. Below, these and other features that have been identified during two decades of HIV-1 research are summarized and discussed.

**Host features**

Host factors that interfere with the efficiency of infection, virus production and virus spread through the body are likely to influence transmission and the clinical course of infection. A variety of polymorphisms have been implicated in this respect and are highlighted below.

**HIV-1 susceptibility**

The idea that cases of natural resistance to HIV-1 infection and long-term survival may partly be due to differences in host genetics came amongst others from in vitro observations that PBMC of different individuals are differentially susceptible to HIV-1 infection\(^{1285,286}\). Low in vitro susceptibility was shown to be associated with a reduced risk of transmission from mother to child\(^{287}\), and total insusceptibility to NSI infection was observed in HIV-1 negative individuals with high risk of infection \(^{62,238}\). In agreement, we showed in chapter 2 that reduced in vitro susceptibility of PBMC from exposed non-infected individuals to NSI variants from their infected partners was associated with lack of transmission in (monogamous) homosexual couples.

**CCR5Δ32**

Upon HIV-1 transmission and during the early phases of infection, the fast majority of all HIV-1 variants have the NSI phenotype\(^ {191-194}\). Furthermore in half of the HIV-1-infected individuals only variants with the NSI phenotype can be detected throughout the course of infection\(^ {188,194,201}\). In contrast to SI variants, that use CXCR4 and can often additionally use CCR5 and CCR3, NSI variants almost exclusively use CCR5 as a coreceptor\(^ {97-101}\) (chapter 10), irrespective the stage of disease at which they are isolated (chapter 4).

Soon after the identification of the coreceptors, several studies reported on the existence of a common polymorphism in CCR5\(^ {291-293}\). The polymorphism comprises a 32-bp deletion in the coding region of CCR5, resulting in a truncated protein that is not transported to the cell surface. Concomitantly, individuals carrying the deletion at both alleles (CCR5 Δ32/Δ32 individuals) lack CCR5 at their cell surface\(^ {293,409}\). PBMC from CCR5 Δ32/Δ32 individuals appeared to be completely resistant to NSI infection\(^ {291,292,417}\). Approximately 20% of the Caucasian population is heterozygous for CCR5Δ32 (CCR5 Δ32/+ individuals)\(^ {292,294,296,301}\). PBMC from CCR5 Δ32/+ individuals on average contain intermediate numbers of CCR5 expressing cells and have intermediate CCR5 cell surface expression levels, compared to CCR5 Δ32/Δ32 and CCR5 +/- PBMC\(^ {409}\) (chapters 3 and 5). Concomitantly, PBMC from CCR5 Δ32/+ individuals generally have an intermediate susceptibility to infection with NSI variants in vitro\(^ {291,300,405,416}\) (chapter 2). This effect of heterozygosity for CCR5Δ32 was even stronger in vivo in PBL-SCID mice\(^ {417}\). Despite the general association between CCR5Δ32 genotype
and CCR5 cell surface expression, large variation was observed within PBMC from CCR5 Δ32/+ and CCR5 +/+ individuals, resulting in overlapping expression levels between individuals from the two genotypic groups. In chapter 3, we showed that irrespective of the genotype, the frequency of CCR5 expressing cells is correlated with in vitro susceptibility to NSI variants.

The in vitro observed effects of CCR5Δ32 on CCR5 expression and susceptibility to HIV-1 infection are translated to the in vivo situation. Compared to the total human population, the frequency of CCR5 Δ32/Δ32 individuals was higher in people that remained uninfected despite frequent exposure to HIV-1. The higher frequency of CCR5Δ32 homozygosity among ‘high-risk’ individuals implies that this mutation protects against HIV-1 infection. The basis of this would be the preferential transmission of macrophage-tropic (NSI) variants, together with the complete lack of CCR5 surface expression. This protection appeared not to be absolute, however, since some CCR5 Δ32/Δ32 individuals were shown to be infected with HIV-1. Infection of CCR5 Δ32/Δ32 individuals can be explained by transmission of (macrophage-tropic) SI variants. Indeed, one of the HIV-1-infected CCR5 Δ32/Δ32 individuals that was analyzed showed to harbor CXCR4-using viruses only.

With the exception of one study, no significant differences were observed in the frequencies of heterozygous individuals among HIV-1 positive, HIV-1 negative low-risk, and HIV-1 negative high-risk individuals, suggesting that heterozygosity for CCR5Δ32 does not offer protection from infection. The lack of protection from infection in individuals with the CCR5 Δ32/+ genotype seems in contrast with the association between this genotype and reduced in vitro susceptibility. The association between reduced susceptibility and lack of transmission (chapter 2) and the association between reduced susceptibility and risk of transmission (chapter 2) can be envisaged. First, the association between reduced susceptibility and CCR5Δ32 genotype is not complete. In chapter 2 we found that all CCR5 Δ32/+ individuals had PBMC with reduced susceptibility, yet 50% of the PBMC with reduced susceptibility were CCR5 +/+. Also, a study on frequently exposed uninfected individuals showed that 3 out of 23 individuals screened were CCR5 Δ32/Δ32, while the others were CCR5 +/+ [300]. These CCR5 +/+ individuals did have cells with reduced surface expression of CCR5 and reduced in vitro infectability [302]. Because of the existence of other determinants of susceptibility besides CCR5Δ32, the absence of a higher frequency of CCR5 Δ32/+ among frequently exposed uninfected individuals does not necessarily rule out the positive effect of reduced susceptibility on transmission.

Second, additional factors may override the effect of lower susceptibility. It has been shown that the stage of disease and the viral load in the virus donor are associated with the risk of transmission [299-236]. Indeed, in chapter 2 we observed that transmission despite low in vitro HIV-1 susceptibility of recipient PBMC coincided with a relatively high infectious cellular load in the HIV-1 donor, and the other way around absence of transmission despite normal susceptibility coincided with a low infectious load in the donor. Thus, whether transmission occurs is likely to be a combination of the viral inoculum at exposure and the susceptibility of the first target cells encountered. Accordingly, frequent exposure to HIV-1 may override the effect of modestly reduced susceptibility resulting from a CCR5 Δ32/+ genotype. Frequent exposure to HIV-1 will increase the overall chance that transmission occurs based on mere statistics, as well as the chance of encountering a high viral inoculum. In promiscuous individuals the effect of CCR5Δ32 heterozygosity may therefore be too low to offer protection. In monogamous individuals however, in cases where the HIV-1-infected partner has a low viral load, the slight reduction in susceptibility may contribute to protection, as we described in chapter 2.

Alternatively (or additionally), given the association between susceptibility and CCR5 expression levels [310,405] (chapter 3), the difference in protection between promiscuous CCR5 Δ32/+ and monogamous CCR5 Δ32/+ individuals may result from differences in CCR5 surface expression levels despite the same CCR5Δ32 genotype. CCR5 is mainly expressed on cells with an activated/memory phenotype (chapters 3, 5, and 9). The presence of other STDs (associated with unsafe promiscuous sexual behavior) might increase the proportion of CCR5-expressing cells, thereby increasing the likelihood of transmission.
expressing cells through immune activation and contribute to the higher risk of getting infected upon exposure associated with the presence of STDs\cite{256,229,291}. In support of this idea, the proportion of CCR5 expressing cells appeared to be higher in pre-seroconversion PBMC from individuals that got infected later on, compared to healthy (low risk) blood donors (chapter 5).

Finally, the magnitude of the protective effect of reduced susceptibility may depend on sexual behavior and hence the route of transmission (i.e., relatively thin anal epithelial layer versus vaginal mucosa). In support of the latter, one study showed that CCR5Δ32 heterozygosity offered protection in heterosexual but not homosexual partners of HIV-1-infected individuals\cite{1426}.

Although the protective effect of CCR5Δ32 heterozygosity in HIV-1 transmission is limited, its protective effect on disease progression in HIV-1-infected individuals is evident. CCR5 Δ32/+ individuals are more frequently observed among long-term nonprogressors (LTNP) compared to progressors\cite{293,296,410,411,421}. The contribution of CCR5Δ32 heterozygosity to a more benign course of infection is likely explained by limited viral spread due to lower CCR5 expression levels and reduced susceptibility. This effect is probably of most significance during the early phase of infection, given the on average reduced viral load already early in infection of CCR5 Δ32/+ individuals\cite{294,396,410}, and the beneficial effect of a low viral load set-point on the subsequent rate of disease progression\cite{333,337,520}. Indeed, the protective effect of heterozygosity for CCR5Δ32 appeared to be confined to the pre-AIDS period, indicating that the positive influence is mainly exerted during the relatively early phase of infection\cite{521}.

Even though the mean time to AIDS is lower in HIV-1-infected CCR5 Δ32/+ individuals compared to CCR5 +/+ individuals, some CCR5 Δ32/+ individuals progress rapidly to AIDS. Inversely, about half of the individuals with a benign course of disease are CCR5 +/-. The absence of a complete correlation between CCR5 genotype and disease progression may be due to several factors. For instance, coreceptor usage other than CCR5, and/or enhanced affinity for CCR5 might accelerate disease progression despite low levels of CCR5. The protective effect of CCR5Δ32 on the rate of disease progression was also observed after the emergence of SI variants, albeit reduced compared to situations were SI variants were absent\cite{396}. This indicates that alternative coreceptor usage cannot simply explain disease progression in the presence of CCR5Δ32. Furthermore, some CCR5 Δ32/+ individuals that progress to AIDS never develop SI variants. In chapter 4, we addressed the question whether evolution towards other coreceptor usage could explain rapid disease progression in CCR5 Δ32/+ individuals with only NSI variants. A total of 173 NSI biological virus clones from CCR5 +/+ and CCR5 Δ32/+ nonprogressors and CCR5 +/+ and CCR5 Δ32/+ progressors from various stages of infection were all restricted to the use of CCR5. These observations show that rapid progression in CCR5 Δ32/+ individuals is not necessarily associated with the emergence of HIV-1 variants that are capable of using other coreceptors.

In the study described in chapter 2, we observed that some NSI variants isolated from heterozygous individuals had a similar replicative capacity on PBMC from CCR5 Δ32/+ and CCR5 +/+ healthy blood donors, suggestive of evolution towards higher affinity for CCR5. In agreement, observed differences in β-chemokine sensitivity between different CCR5-restricted viruses\cite{100,522} might point to the existence of viruses with different affinities for CCR5.

An alternative (or additional) explanation of the discrepancy between CCR5Δ32 genotype and the rate of disease progression in some individuals may result from differences in the individual levels of CCR5 surface expression. As mentioned above, both in CCR5 +/+ and CCR5 Δ32/+ individuals the number of CCR5 expressing cells and the CCR5 surface expression levels vary widely\cite{405} (chapters 3 and 5). Relatively high CCR5 expression in CCR5 Δ32/+ individuals and relatively low CCR5 expression in CCR5 +/+ individuals could therefore result in similar clinical outcome.

The role of CCR5 expression levels in differential clinical course is described in chapter 5. In HIV-1-infected individuals the frequency of CCR5 expressing cells was inversely correlated with CD4* T cell numbers and hence with ongoing infection. The increase in the proportion of CCR5 expressing cells during HIV-1 infection likely
reflects a general increase in immune activation. This idea is based on the observation that CCR5 is mainly expressed on T cells with an activated/memory phenotype (chapters 3, 5, and 9), together with the increase in the proportion of memory cells which is observed during HIV-1 infection (chapter 5). The increasing frequencies of CCR5 expressing cells late in infection both in CCR5 A32/+ and CCR5 +/+ individuals may contribute to a decreasing protective effect of CCR5A32 heterozygosity with ongoing infection.

At similar stages of disease, as reflected by similar numbers of CD4+ T cells, the proportion of CCR5 expressing cells was higher in individuals who progressed to AIDS than individuals who did not progress to AIDS in a similar time span (chapter 5). Furthermore, the difference in CCR5 expression between CCR5 +/+ progressors and slow progressors was already apparent prior to seroconversion. Thus, the proportion of CCR5 positive cells appears to influence the course of infection, irrespective of CCR5A32 genotype. The higher proportion of CCR5 expressing cells already prior to seroconversion in individuals that progress to AIDS more rapidly, are also in agreement with the early effect of CCR5 expression.

Other coreceptor polymorphisms

The impact of CCR5A32 on HIV-1 transmission and disease progression and the wide variation in CCR5 surface expression in CCR5 A32/+ and CCR5 +/+ individuals led to a search for other polymorphisms in CCR5. Also polymorphisms in other chemokine receptors and in the natural ligands became subjects of comprehensive study. Indeed, additional polymorphisms in CCR5 were identified, both in the coding region (chapter 12), and in the promotor region (chapter 12), that are associated with disease progression. Also, a commonly occurring mutation in the CCR2b gene, a valine to isoleucine substitution at position 64 (CCR2-64I), influences the rate of HIV-1 disease progression. The presence of CCR2-64I at one or both alleles was associated with delayed disease progression and concomitantly occurred more often in nonprogressors than progressors (chapter 12). This finding was not confirmed by all studies and contradicting results on the magnitude of the effect of CCR2-64I were reported. A recently performed analysis on the combined HIV-1 cohorts however, showed that the protective effect on the progression to AIDS conferred by CCR5 A32 and CCR2b-64I is the same.

Similar to CCR5A32, the presence of CCR2-64I was associated with a lower plasma viremia early in infection. However, the effect of the CCR2-64I was not detected in cohorts of seroprevalent individuals in contrast to cohorts of seroconverters. These combined observations led to the hypothesis that the effect of CCR2-64I, even more than that of CCR5A32, is restricted to the early phase of infection.

In agreement with the positive effect of CCR2b-64I on disease progression, this mutation was retrospectively identified in 3 out of 3 individuals described in chapter 2, who were CCR5 +/+ but who were among the individuals with the least susceptible PBMC (data not published).

The mechanism through which the mutation in CCR2b exerts its effect is not clear. CCR2b does not appear to be a receptor commonly used by HIV-1, and the 64I mutation does not alter CCR2b surface expression or its function as coreceptor for a CCR2b using variant. It has been suggested that protection is accomplished through genetic linkage between the mutation in CCR2b and mutations in the promotor region of CCR5. More recent studies however, showed that the CCR5 cell surface expression is only slightly decreased on CCR2-64I PBMC and no association between CCR2-64I and the functionality of CCR5 as an HIV-1 coreceptor was observed. Furthermore, the mutations in the CCR5 promotor region associated with CCR2-64I had no effect on the promotor activity and CCR5 transcription levels.

No dysfunctions in the SI HIV-1 coreceptor, CXCR4, have been identified so far making its role in HIV-1 pathogenesis less clear. The absence of major mutations in CXCR4 is probably related to its essential role in haematopoiesis, cardiogenesis, and vascular and cerebellar development, because of which CXCR4 lacking individuals would die early during embryonic development.

CAF and chemokines

Already a decade prior to the identification of
CCR5 as an HIV-1 coreceptor and the \( \beta \)-chemokines as HIV-1 suppressive factors produced by CD8* T cells, the existence of non-cytolytic anti-HIV-1 activity of CD8* T cells had been recognized. This HLA non-restricted non-cytolytic soluble component was called CD8* T lymphocyte antiviral factor (CAF)[532-537]. In HIV-1-infected individuals the antiviral activity was observed predominantly in asymptomatic individuals, and was undetectable or low in AIDS patients[535,538-542], suggestive of a protective effect in HIV-1 infection. Initially, the antiviral activity was thought to be HIV-1 induced, since it was not detected in CD8* T cells from healthy individuals[536,538] and increased during primary HIV-1 infection[541]. However, other studies did report CAF activity in CD8* T cells from healthy, low-risk individuals[535,543-545]. The discrepancies may result from the fact that CD8* T cells produce multiple HIV-1 suppressive factors.

Increased insight into the identity of suppressive factors secreted by CD8* T cells came in 1995, when almost simultaneously IL-16[546] and the \( \beta \)-chemokines[94] were identified as HIV-1 suppressive factors secreted by CD8* T cells. Exogenously added[61,63,54,297,405] and endogenously produced \( \beta \)-chemokines[530,419] were shown to inhibit NSI HIV-1 infection in vitro. In agreement, we observed an association between the susceptibility of healthy donor PBMC to NSI HIV-1 infection and their production levels of MIP-1\( \alpha \), MIP-1\( \beta \) and RANTES (chapter 3). Since CAF can inhibit replication of both NSI and SI variants[14,15,53,539,543-545] and CAF activity cannot be abrogated by antibodies directed against \( \beta \)-chemokines, the \( \beta \)-chemokines are not likely to be the only constituents of CAF[547-548]. The inhibitory effect of IL-16 did appear to affect both NSI and SI variants[546,550]. However, the suppressive effect of IL-16 on SI HIV-1 was lower compared to CAF, and like it was observed for \( \beta \)-chemokines, a monoclonal antibody directed against IL-16 could not abrogate the effect of CAF[551], suggesting that CAF contains additional SI inhibitory components.

The identity of additional SI inhibitory component(s) of the CAF is still not clear. The natural ligand of CXCR4, the \( \alpha \)-chemokine stromal cell derived factor-1 (SDF-1), was shown to inhibit infection by SI variants[560,552,553]. However, the low level of SDF-1 expression by CD8* T cells and the absence of an association between SDF-1 expression levels and CD8* suppressive activity, ruled out this \( \alpha \)-chemokine as the SI suppressing factor[554,555]. A natural ligand of CCR4, the \( \beta \)-chemokine, MDC[556], produced by a CD8* T cell clone, was shown to inhibit replication of both SI and NSI variants[557]. At which level this inhibition occurs is not clear. Since CCR4 is not a main coreceptor of HIV-1 (and especially not of NSI variants), the mode of inhibition is not likely to be at the level of entry, unless MCP and HIV-1 appear to share another, yet unidentified receptor.

The multi-factorial nature of CAF is also illustrated by the level of action of the different CAF components. The activity associated with inhibition of SI variants (including IL-16) appeared to be at the level of transcription[550,558,559] in contrast to the \( \beta \)-chemokines, which act at the level of entry.

Two mechanisms have been proposed by which the chemokines might exert their effects: through induction of signal transduction upon coreceptor binding and through blocking of HIV-1-coreceptor binding. The coreceptors are G protein-coupled proteins and binding of the ligands results in activation associated with a (G protein dependent) calcium flux response. Coreceptor activity (fusion) [560,561] as well as the inhibitory effect of \( \alpha \)- and \( \beta \)-chemokines on HIVenv-coreceptor fusion appeared to be independent of the capacity of CXCR4[562,563] and CCR5[564] to induce a calcium flux upon ligand binding. In contrast, when the process of coreceptor internalization was prevented, the inhibitory effect of chemokines was reduced[562,562,554]. Furthermore, the extent of internalization was associated with the inhibitory capacity of chemokines and chemokine analogues[562,565]. These data show that for both CCR5 and CXCR4, the inhibitory effect of the ligands is associated with the induction of down-regulation of surface expression. In addition, since the inhibitory effect of chemokines is not completely abrogated when coreceptor down-regulation is prevented, direct blocking of coreceptors likely provides an additional mechanism of chemokine mediated inhibition of HTV-1 infection[562,566]. In agreement with the negative influence of \( \beta \)-chemokines on CCR5 surface expression we showed in chapter 3 that
production levels of RANTES were inversely correlated with percentages of CCR5 expressing cells.

Despite the clear inhibitory effect of MIP-1α, MIP-1β and RANTES on NSI HIV-1 replication in vitro, the role of these β-chemokines in vivo is not irrefutable. Analysis of serum β-chemokine levels in progressors and nonprogressors resulted in quite unexpected findings. In stead of a protective effect, either no differences or even elevated levels of chemokines were found to be associated with AIDS. Similarly, studies on in vitro induced β-chemokine production by PBMC from progressors and nonprogressors resulted in conflicting observations. The observed discrepancies are likely explained by the influence of the source of the chemokine production, rather than the absolute levels, as it was suggested by Saha et al. Thus it was shown that CD8+ T cells from progressors produced high levels of β-chemokines, while in contrast the CD4+ T cells are the main β-chemokine producers in nonprogressors. The negative effect of production of β-chemokines by CD4+ T cells, but not by CD8+ T cells, on disease progression, might be explained by direct CCR5-β-chemokine binding upon secretion, thereby directly influencing the CCR5 expression on CD4+ T cells. These data suggest that even though CD8+ T cells produce β-chemokines, the protective effect of CAF in disease progression and transmission, is not mediated by β-chemokine production. In line with this, CD4+ T cells of frequently exposed uninfected individuals produced elevated levels of RANTES in vitro. However, another study showed that the production of all 3 β-chemokines was elevated in total PBMC of exposed uninfected individuals. Nonetheless, these studies show that the capacity to produce β-chemokines, which correlates with NSI HIV-1 susceptibility in vitro (chapter 3), is associated with protection from infection and disease progression in vivo. The in vivo relevance of β-chemokines was supported by the identification of a polymorphism in the promotor region of the RANTES gene that associates with reduced RANTES secretion and reduced CD4+ T cell decline (but not HIV-1 transmission).

The influence of CXCR4 and its natural ligand, SDF-1, on disease progression is unclear at the moment. A polymorphism in the 3' untranslated region of the gene encoding SDF-1 has been identified. However, its reported influence varied from a beneficial and no effect on the rate of disease progression to (slightly) accelerated progression to AIDS and death.

**HIV-1 specific immune response**

The major histocompatibility complex (MHC) contains many genes that regulate immune function. The human leukocyte antigens (HLA) are encoded by MHC genes and comprise a highly polymorphic complex of molecules. HLA diversity is clustered geographical and ethnic, based on the different pathogens encountered through human history. Different HLA alleles or haplotypes are associated with increased or reduced susceptibility to different pathogens. Thus, particular HLA haplotypes have been linked to a more benign acute HIV-1 infection and delayed disease progression, while on the opposite other HLA haplotypes are associated with accelerated disease progression.

The association of certain HLA types with HIV-1 pathogenesis point to an important role of the immune response in controlling HIV-1 infection. The importance of an HIV-1 specific cellular immune response as the first line of defense is suggested from the observation of strong cellular immune responses in HIV-1 exposed uninfected individuals. The early onset of an HIV-1 specific CTL response during acute HIV-1 infection and its association with low viremia and slower disease progression in the subsequent course of infection further support this notion.

In HIV-1-infected individuals experiencing a benign course of HIV-1 infection, both humoral and cellular immune responses are detected. Even though both types of responses were also detected in progressors, high titers of neutralizing antibodies and frequencies of CTL were mainly persistent throughout infection in nonprogressors only. As it was observed for CTL, the presence of HIV-1 specific CD4+ help response was inversely associated with viral load, further supporting...
the role of the cellular immune response in containment of viral replication

Other host factors
Polymorphisms in other genes have been studied with respect to their influence on the rate of HIV-1 disease progression. In these cases, both the association with disease progression and the mechanism of action are not always clear. Genetic linkage to genes that are associated with progression (like it was suggested for CCR2b-64I) has been offered as an explanation. Two such examples are tumor necrosis factor (TNF) and mannose binding lectin (MBL).

TNF-α enhances HIV-1 replication in vitro through activation of nuclear factor κB (NFκB). Particular polymorphisms in TNF-α are genetic linked with specific HLA haplotypes that are associated with faster disease progression (DR3, DR4) and increased levels of TNF-α. Nevertheless, no association was found between the TNF-α polymorphisms and HIV-1 disease progression. A variation of the TNF-β gene however, appeared to be associated with slow disease progression.

Mannose-binding lectin (MBL) is a protein that can activate the complement system and acts as an opsonic factor. MBL can bind many pathogens including HIV. Variant alleles result in low serum MBL levels and are therefore associated with defects in opsonization and increased susceptibility to infections. In line with this, individuals homozygous for the MBL variant alleles were at higher risk of HIV-1 infection, and the presence of one or two variant alleles was associated with shorter survival after AIDS diagnosis. The mechanism by which the variant MBL acts is not clear. Low levels of MBL could increase the susceptibility to HIV-1 itself, or to other (AIDS-related) pathogens. However, serum MBL levels, although more often undetectable in HIV-infected compared to uninfected individuals, were not associated with stage of HIV-1 disease. Furthermore, another study showed contrasting results, with a weak protective effect prior to AIDS, and no enhancement after AIDS. Individuals homozygous for the variant allele lack any functional protein, in contrast to heterozygous individuals in whom the levels are reduced, but where the protein is still functional. Concomitantly, the effect of variant MBL on increased susceptibility to (non-HIV-1) infections was more pronounced in homozygous individuals. The low frequency of homozygous mutant individuals may contribute to the observed discrepancies with respect to the effect of the variant MBL on HIV-1 pathogenesis.

Virus features

The multiple variable features of HIV-1 already intuitively point to an association between the clinical course of HIV-1 infection and the HIV-1 phenotype. Thus, viral features that increase HIV-1 virulence will be reflected by a higher viral load and a steeper CD4+ T cell decline.

SI versus NSI HIV-1
The replacement of primarily macrophage-tropic variants by more T-cell-tropic variants is associated with disease progression. When this shift is accompanied by acquisition of the SI phenotype at some point during infection, the rate of progression accelerates, associated with an accelerated decline in CD4+ T cells and increase in viral load. The higher pathogenicity of SI variants compared to NSI variants has been the subject of many studies. Besides the difference in SI capacity, these variants generally differ with respect to replication kinetics, coreceptor usage and tropism. These factors likely contribute to the enhanced virulence of SI variants.

NSI versus NSI HIV-1
Despite the lower pathogenicity of NSI compared to SI HIV-1, approximately 50% of all HIV-1-infected individuals develop AIDS in the absence of SI variants. While the rate of progression is generally slower in individuals with solely NSI variants, some individuals progress to AIDS with similar kinetics as observed after SI switch. The differences in the rate of disease progression also among individuals solely harboring NSI HIV-1 might suggest that also among NSI variants, differences with respect to at least some of the mentioned virulence factors can occur.
Replication kinetics

The level of virus in the periphery is associated with the stage of disease\textsuperscript{[26,28,201-204,350,351]}. Longitudinal studies showed that the viral load increases during the course of infection in individuals with decreasing CD4\textsuperscript{+} T cells\textsuperscript{[26,27]}. In chapter 6, we analyzed the dynamics of viral load during the course of infection of 23 individuals. We observed 3 different patterns in the dynamics of viral load as measured by HIV-1 RNA copies in serum and the frequency of productively infected CD4\textsuperscript{+} T cells. In 80% of the individuals both measures of viral load showed the same kinetics: either both measures remained stable at low, high or intermediate levels, or both increased gradually during infection.

Increasing numbers of circulating free virus and frequencies of productively infected cells could theoretically have a number of causes. If a cell, once infected, would produce virus for extended periods of time, the overall increase in virus load could merely be a result of accumulation of infected cells, while the virus production per cell (burst size) and the numbers of newly infected cell per time unit remain constant. HIV-1-infected cells however, have a very short life span of approximately 2 days\textsuperscript{[28]}. Hence, the increase in viral load implies that either the production of virus per cell increases and/or that more cells are newly infected per time unit, either due to enhanced infectivity, host susceptibility, or weakening immune surveillance. An increase in production rate and/or enhanced infectivity is reflected by in vitro replication kinetics. Indeed, longitudinal analysis of in HIV-1 mRNA levels in PBMC from HIV-1-infected individuals and in vitro analysis of the replication kinetics of virus variants isolated from different stages of infection provided evidence for evolution of HIV-1 replicative capacity\textsuperscript{[26,87,150,151,336,350]}. In most studies high replicative capacity was associated with the SI phenotype\textsuperscript{[26,87,151]}, or the phenotype was not determined, making it impossible to dissociate the replication capacity from the SI phenotype. Yet, increases in viral load can be observed prior to the appearance, and even in total absence, of SI variants\textsuperscript{[26]} (chapter 6). Therefore we determined whether replication kinetics of NSI variants evolve during the course of infection, and whether absence of evolution is associated with a stable course of infection. This study is described in chapter 7 of this thesis. As shown previously for SI variants, high replication kinetics and high viral load were observed around the moment of AIDS diagnosis. In most individuals we observed evolution from low towards high viral replication kinetics during the course of infection, yet viruses from 4/7 long-term nonprogressors (LNTP), showed no or only a slight increase in replicative capacity. The 3 LTNP in which virus evolution was detected all had increasing viral load, despite stable CD4\textsuperscript{+} T cell counts. In all instances the replication kinetics were associated with viral load.

Thus, absence of an increase in replicative capacity of virus variants appears to be associated with maintenance of low viral load and high CD4\textsuperscript{+} T cell counts and hence with slow disease progression. This is also nicely illustrated by cases where individuals are infected with attenuated HIV-1 variants. These viruses have inactivating mutations in one or more of the regulatory genes. The most striking example consists of a group of 7 HIV-1-infected individuals who received blood products contaminated by the same HIV-1-infected blood donor. The recipients remained asymptomatic for at least 11-14 years. The donor and three of the recipients that were analyzed had in common that they harbored viruses with multiple deletions in the nef gene\textsuperscript{[470]}. Functional nef was shown to be associated with viral infectivity in vitro\textsuperscript{[179-181]} and viral replication and pathogenesis in HIV-1-infected SCID-hu mice\textsuperscript{[482,622,623]} and SIVmac-infected rhesus monkeys\textsuperscript{[632,624]}. The basis of the positive effect of nef on infectivity is not quite clear. Nef appeared to be (indirectly) involved in the process of reverse transcription\textsuperscript{[625,626]}. Furthermore, mutations that abolish the capability of nef to interact with cellular protein kinases reduced virus infectivity\textsuperscript{[627]}, suggestive of a role of cellular signaling. The effect of nef on CD4 down-modulation\textsuperscript{[628,632]} did not appear to be associated with virus infectivity\textsuperscript{[625,627,633]}. More recently it was shown that nef is also responsible for MHC class I down modulation\textsuperscript{[634,635]}, a property that results in the escape of infected cells from CTL surveillance\textsuperscript{[636]}.
viruses is likely a combination of enhanced infectivity and CD4 and MHC class I down-modulation. Even though the effect of nef deletion mutants on a benign course of infection is likely not totally due to reduced virus replication\textsuperscript{622,623}, reduced replication capacity likely contributes to the low viral load in the mentioned LTNP. Similarly, other studies, among which the one described in chapter 7, reported on LTNP carrying HIV-1 variants with deletions in nef, or other regulatory genes.\textsuperscript{463,471-473,480,481}

The combined observations that SI variants have high replication capacity, and that during the course of infection NSI variants can gain higher replication capacity, raised the question whether evolution towards the SI phenotype is preceded by evolution of higher replication capacity. In any case, as indicated above, the presence of NSI variants with high replication kinetics does not necessarily result in evolution of a SI phenotype. The sequential events in NSI to SI evolution with respect to replication kinetics were studied in chapter 8. The emergence of slow-replicating SI variants in the presence of slow-replicating NSI variants in one of the analyzed individuals showed that evolution of the SI phenotype can occur prior to and independent of the appearance of variants with high replication kinetics. Furthermore, it appeared that NSI variants do not necessarily gain higher replication capacity in the presence of SI variants. In agreement with chapter 7, evolution of replication capacity was observed during the course of infection. However, the more detailed study at several time points in chapter 8 showed that viruses with high replication kinetics were present already a few years prior to AIDS diagnosis. This observation and the existence of LTNP with fast replicating viruses (chapter 7) may indicate that the presence of viruses with rapid replication rate by itself is no prerequisite for development of AIDS. This does not exclude however, that carrying a virus population with only slow replicating viruses can contribute to a benign course of infection.

In vivo, NSI variants remain present after the emergence of SI variants\textsuperscript{12081}, despite the fact that SI variants outgrow NSI variants in vitro (unpublished). More remarkably, after the emergence of SI variants, NSI and SI variants contribute equally to the viral load, even when the replication kinetics of the NSI variants are lower than that of the SI variants (chapter 8). The other way around, emerging SI variants can obviously grow out, despite the presence of very replication competent NSI variants. The latter suggests that SI variants have an additional feature, which results in higher fitness compared to NSI variants regardless of their equal replication competence. This apparently higher in vivo fitness of SI variants seems in sharp contrast with their inability to replace the NSI population. The obvious explanation would be that SI and NSI variants have their own niche within the body, omitting the necessity to compete with one another. This theory is in accordance with the increase in evolutionary distance between NSI and SI variants over time (chapter 8). In contrast, high homology of variants within the NSI and SI population at any given time point might point to fierce competition within each variant subpopulation.

**Tropism and coreceptor usage**

The different tropism of SI and NSI variants, partly resulting from the dependency of NSI variants on the presence of CCR5, while SI variants can additionally use other coreceptors\textsuperscript{97-103} (chapters 4 and 10), offers an explanation for their apparent occupancy of different niches. The high viral load of both variants in peripheral blood and CD4\(^{+}\) T lymphocytes being the main target of both NSI and SI variants, suggest that the differences in tropism are not restricted to specific compartments as macrophages\textsuperscript{91-92} and thymocytes\textsuperscript{1116-1118}. Since CCR5 and CXCR4 are differentially expressed on CD4\(^{+}\) T cell subsets\textsuperscript{119,120}, we studied whether NSI and SI variants have different target cells within the CD4\(^{+}\) T cell population (chapter 9). Indeed, while NSI variants appeared to reside mainly in the memory subset (identified by surface expression of CD45RO), SI variants were detected in both memory as well as naive (identified by surface expression of CD45RA) CD4\(^{+}\) T cells. The distribution of the variants over the different CD4\(^{+}\) T subsets agrees with the almost exclusive expression of CXCR4 on naive cells, while memory cells can express CCR5 and/or CXCR4. Within the memory cells of HIV-1-infected individuals the...
The surface expression of CCR5 and CXCR4 appeared to be largely mutually exclusive, providing two major and distinct HIV-1 target cell populations (chapter 10). The existence of almost completely separated NSI and SI HIV-1 niches within the CD4+ T cell compartment is consistent with the coexistence of NSI and SI HIV-1 and their independent evolutionary pathways.

Individuals with SI variants have a higher viral load compared to individuals with only NSI variants (chapter 6). This is not merely a result from the association between the presence of SI variants and late stage infection, since the difference was also observed between individuals with similar numbers of CD4+ T cells. With the emergence of SI variants, that have an additional (X4R5 viruses) or alternative (X4 viruses) array of target cells compared to NSI variants, the range of total HIV-1 target cells expands considerably. The higher viral load in individuals with SI variants might thus result from increased infectivity of SI variants, due to a high replication rate and the capability to infect a wider range of cells, together with the continuous expansion of NSI infected cells within their own niche (chapter 8).

The increased pathogenicity associated with the presence of SI (chapter 9) is probably not solely due to a broader host cell range. In chapter 9 we showed that the frequency of infected naive cells was related to the rate of CD4+ T cell decline. In contrast, the viral load in memory cells or total PBMC was not associated with CD4+ T cell decline during the particular period analyzed, suggesting a greater impact of infected naive cells. The capacity of SI variants to infect and kill CD4+ T cell precursors, like thymocytes and naive cells, may interfere with the renewal capacity of the host. A decreasing capacity to generate CD4+ T cells (due to SI infection of progenitors) in the face of increasing numbers of infected and killed cells (due to expansion of the target cell range) can easily be envisaged to result in accelerated CD4+ T cell decline.

The broader and alternative host cell range of SI variants likely contributes to the worse prognosis after their appearance. While increased virulence resulting in accelerated disease progression is not beneficial to the virus, the high fitness associated with the SI phenotype is. It is not totally clear therefore, why SI variants appear only in half of the infected individuals, and apparently only once during the course of infection (chapter 8). Given the high production and mutation rate of HIV-1, it is hard to imagine that the required mutations do not appear sooner and more often than SI variants are actually detected. Hence, the emergence of SI variants is likely rather a matter of getting the opportunity to grow out than the chance of accumulating the right mutations. The fact that variants with an intermediate genotype are only rarely detected, suggests that the evolutionary path leading to the SI phenotype moves via a stage associated with a less fit phenotype. The already relatively high genetic distance between the first SI variants and the NSI variants isolated from the same time point further confirms the scarcity of intermediate genotypes (chapter 8). Assuming that SI variants can only grow out when immune surveillance weakens, the presence of NSI variants with high replication kinetics at later stages of infection (chapters 7 and 8) may explain the relatively low frequency with which SI variants appear. The only way for newly emerging SI variants to efficiently compete with the well-established NSI viruses may be to use CXCR4 and to infect target cells out of reach for the NSI viruses. The capacity to use CXCR4 at a high affinity might be a gradual process however, and in this line of reasoning the presumed intermediate variants with low fitness might be variants with the capacity to use CCR5 but with low affinity for CXCR4 (or another coreceptor). Chapter 10 provides clues supporting this theory. Around the moment of SI switch, variants could be detected that were discordant with respect to their capacity to infect MT2 and U87CD4/CXCR4 cell lines, and that were in addition incapable to infect Δ32/Δ32 PBMC, indeed suggesting inefficient CXCR4 and preferential CCR5 usage. These variants had an NSI V3 genotype, which may indicate that regions outside V3 encode the first step towards the SI, CXCR4-using phenotype.

In general, the first efficiently CXCR4-using SI variants could also efficiently use CCR5 (and sometimes CCR3), however, at later stages of infection the majority of SI variants lost the capacity to use CCR5. The shift from R5X4(R3) to
X4(R3) viruses appeared to be temporarily associated with a relative increase in the frequency of SI-infected naive cells compared to the frequency of SI-infected memory cells. The increasing phenotypic difference thus creates an increasing physical barrier between NSI and SI variants over time, and is reflected in the separate evolutionary pathways of NSI and SI variants (chapters 8 and 10).

It is not known whether the (early stage) dual-tropic SI variants infect memory cells via CCR5 and/or via CXCR4. The existence of mainly CXCR4*CCR5* (naive and memory) and CXCR4* CCR5* (memory) CD4+ T cell populations (chapter 10), however, favor the option that also dual-tropic SI variants preferentially use CXCR4 in vivo. Infection of CXCR4 positive cells provides the means to avoid competition with the NSI variants and hence infection of CXCR4* cells may offer a growth advantage compared to infection of CCR5* cells. If infection of CXCR4* cells is attended by an increased affinity for CXCR4, a gradual increase in the preference for CXCR4* T cells is likely to evolve. Loss of CCR5 usage is compatible with this model and may be a side effect of gaining increased affinity for CXCR4.

**Cytopathicity**

Accelerated disease progression associated with the emergence of SI variants may partly result from enhanced cytopathicity. At this moment, only 2 studies really addressed cytopathicity of NSI and SI variants within the particular cellular subsets they can infect. The first study showed that some T cell clones supported NSI replication, while others did not. In contrast, SI variants could infect all T cell clones. Virus replication in the T cell clones infected by SI variants and the T cell clones infected by NSI variants was similar. However, while in the SI-infected clones the number of viable cells decreased during culture to approximately 10% of control cells, the viability of clones infected with NSI variants was similar to the uninfected control[50]. This study was performed well before the identification of both coreceptors, and hence does not provide any information on the expression of the coreceptors or coreceptor usage of the variants used. Nevertheless, the incapability of the NSI variants to infect certain T cell clones altogether might indicate that the clones are a fairly homogeneous cell population with respect to coreceptor expression.

The other study appeared recently, and showed that in vitro infection of tonsil tissue with NSI variants results in high mortality among the cells that express CCR5 but not CXCR4, and to a lesser extent among double positive cells[498]. In contrast, in SI-infected tonsil tissue all CD4+ T cell subsets defined by CCR5 and CXCR4 expression are killed. Thus, the cytopathicity of the analyzed NSI and SI variants appeared very similar within the subsets they are capable to infect, suggesting that increased host cell range might be the sole explanation to increased pathogenicity of SI variants. However, also among NSI variants differences in pathogenicity are observed. It is therefore conceivable that some NSI variants (e.g. viruses of LNTP or variants present during early stages of infection) are less cytopathic than others, which would contribute to different rates of disease progression among individuals with only NSI variants (chapter 7).
HIV-1 infection (2): The 'battlefield'

The summarized virus and host features all contribute to the rate of disease progression. Virus and host strategies are indissolubly connected, since HIV-1 infection is a dynamic process in which HIV-1 responds to the pressures the host imposes. In this final section interactions between virus and host during the course of infection are discussed.

In the 'battle' between man and HIV-1, the host is obviously at a disadvantage since HIV-1 undermines the most important line of defense, the immune system, and because of the genetic flexibility of the virus. The basis of the flexibility is the high and error-prone replication rate. As a result the intra-host virus population diversifies and it has been theorized that the mere diversification of HIV-1 can explain the ultimate failing immune defense, since an indefinitely broad immune response would be required to ultimately keep virus replication in control. Next to the random accumulation of mutations, the virus acquires mutations that increase the fitness and allow for adaptation to the hostile environment. Hence, while the basis for evolution is formed by intrinsic characteristics of the virus, virus evolution is driven by the host and will result in increased viral fitness (virus production) and immune escape contributing to disease progression. Thus, if the host constraints are not strong enough to keep virus replication in control from the start, but are strong enough to promote virus evolution, HIV-1 infection appears to proceed as a downward spiral, ultimately resulting in the defeat of the host.

The main strategies of man to keep HIV-1 replication in control are a strong immune response and a low susceptibility to infection. Low susceptibility is achieved via factors inhibiting virus entry, such as low levels of CCR5 surface expression and a high production of β-chemokines, and factors inhibiting virus replication. If virus production is not sufficiently suppressed, immune escape mutants may emerge, as well as viruses that are adapted to the inhibitory factors. Thus, variants may emerge with increased affinity for CCR5 and/or a reduced sensitivity for β-chemokines, that can use other coreceptors, or that have adapted to the intracellular environment of the host, resulting in higher replication kinetics. Although low CCR5 expression in CCR5 Δ32/+ individuals does not appear to promote the emergence of SI variants, a strong inhibitory RANTES analogue was able to drive NSI to SI evolution in a human PBL-SCID mouse model.

All the mentioned virus adaptations will result in an increase in virus production. Furthermore, as a result of immune activation the proportion of CCR5 expressing cells increases, and hence the susceptibility to NSI infection, additionally contributing to enhanced virus production. Besides rapid evolution, HIV-1 may have additional strategies to increase virus production. One example is the down-regulation of MHC class I molecules on the infected cell surface, thus directly limiting the capability of the immune system to adequately respond to the infection.

The increasing virus burden affects the immune system both quantitatively and qualitatively, through direct (virus-mediated killing) and indirect (induction of apoptosis, disturbance of immune regulatory processes) mechanisms. The weakening immune surveillance will result in even more virus production, and so on. If SI variants emerge, either due to high levels of β-chemokines or to weakening immune surveillance, the target cell range of HIV-1 broadens, mainly due to the fact that both SI and NSI variants persist, both in their own niche. As a result of the broadening target cell range, either in combination with an increased cytopathicity or not, the frequency of infected and killed cells will increase even further. More importantly, increasing numbers of CD4+ T cell progenitors may get infected and killed, and the immune system is faced with an attack at two sites simultaneously. On the one hand more cells are killed, on the other, the capacity to regenerate the CD4+ T cell population decreases.

The gradual process of the immune system loosing and the virus gaining ground is absent in
some individuals that progress to AIDS very rapidly as well as in long-term nonprogressors (LNTP). The absence of an adequate immune response resulting in an out-of-control virus growth (with no need for evolution) explains rapid disease progression. In LNTP, the numbers of CD4+ T cells as well as the functioning of CD4-helper and CTL largely stay intact and simultaneously the virus load remains low, suggesting that virus replication is kept in control. In some LNTP, the success of keeping virus replication in control be may be attributed to the presence of attenuated, slow-replicating viruses. It is not clear however, why in some instances virus evolution appears to be absent. Restoration of gene function and repair of deletions has been reported in vitro[641], and in vivo for SIV[642]. It might be that a low viral replication rate, resulting from specific mutations, slows down the process of virus evolution, and that it is merely a matter of time of accumulating the right mutations. Alternatively, an immune response directed at conserved epitopes might control viral replication, prevent the emergence of escape mutants and lower the rate of viral evolution in general. Whether the immune response is adequate in controlling virus replication, or results in emergence of escape mutations, likely depends on host genetics.

The capacity of the virus to evade host attacks and circumvent host defenses appears to be a major obstacle in controlling infection, and hence, prevention of rapid increase in virus production and evolution seems to be the best host 'strategy'. This would explain the impact of the outcome of the acute phase of infection on the subsequent course of infection. Vigorous anti-HIV-1 immune responses and low susceptibility to infection confine the initial damage afflicted by the virus, and prevent the virus from getting a head start. The importance of a favorable balance between host and virus at the end of the acute phase of infection is substantiated by a couple of observations: 1) The early effect of CCR5Δ32 and CCR2b-64I; 2) The beneficial effect of a strong and broad HIV-1 specific CTL response during the acute phase; 3) The prognostic value of viral load after the acute phase; 4) The positive effect of early and intermittent anti-HIV-1 treatment on subsequent immune control (possibly due to a head start of the immune system)[643].

The interaction between HIV-1 and man during infection might be compared with a battlefield, where both parties react to one another's actions in an effort to survive. The struggle can go on for variable periods of time, depending on the 'aggression and flexibility' of the virus and the 'strength and endurance' of the host. In most cases, the virus appears to be better equipped and the host is defeated sooner or later. Man has an obvious advantage over HIV-1 however, which is its capability to learn from battles lost and won. Thus the knowledge on factors involved in protection from infection and disease progression, as well as successes and failures in anti-HIV-1 treatment can be used against the virus. So far, the major step forwards in the battle against HIV-1 has been the success of HAART in reducing virus replication for extended periods of time, which shows that there may be limits to the flexibility of HIV-1. Furthermore, current vaccination strategies may prove that an effective vaccine against HIV-1 is within reach. The battle has not been won yet, but the odds may be changing...
In this context, we can also consider the role of a more efficient immune system in protecting against viral infections. The immune system's effectiveness is enhanced by increased expression of MHC (Major Histocompatibility Complex) class I and II molecules, which present viral peptides on the cell surface to activate cytotoxic T lymphocytes. Additionally, the production of interferons and other cytokines is crucial for the immune response. These factors work together to limit viral replication and prevent the spread of infection.

Furthermore, studies have shown that the presence of certain cytokines, such as interleukin-10 (IL-10), can inhibit the production of pro-inflammatory cytokines, thereby reducing the immune system's responsiveness to viral infections. This balance is critical in maintaining an effective immune response without causing excessive inflammation.

In summary, the immune system's susceptibility to infection can be influenced by various factors, including genetic predispositions, environmental exposures, and the specific pathogen encountered. Understanding these interactions can help in developing more effective strategies for infection prevention and control.