The Influence of host genetic factors on HIV-1 infection

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GENERAL INTRODUCTION:
VIRAL AND HOST DETERMINANTS OF HIV-1
ACQUISITION AND DISEASE PROGRESSION

Daniëlle van Manen, Angélique B. van ’t Wout
and Hanneke Schuitemaker

INTRODUCTION

The clinical course of HIV-1 infection can be highly variable, with extremes of disease progression within 12 months after seroconversion or continuous asymptomatic infection for more than 20 years. The loss of CD4+ T cells is one of the hallmarks of HIV-1 infection which in the absence of combination antiviral therapy ultimately leads to immunodeficiency. The mechanism by which CD4+ T cells are lost is still under debate, but the vital role of virus driven chronic immune activation and increased cell turnover is now well accepted. A high and persistent level of virus replication seems to be the driving force. The high variability in the clinical course of HIV-1 infection is therefore determined by the level of HIV-1 load, which itself is determined by the interplay between viral and host (genetic) factors. In this chapter, we will discuss these factors and their effect on HIV-1 load and disease progression in the natural course of infection.

SPECIFIC (ADAPTIVE) ANTIVIRAL IMMUNE RESPONSE

Humoral Immune Response

Upon infection with HIV-1, both cellular and humoral immune responses are mounted against the virus. The humoral immune response initially consists mainly of binding antibodies. Only after a few weeks to months, neutralizing antibodies emerge [1,2]. The importance of neutralizing antibodies in controlling infection was indicated in SIV-infected macaques by B-cell depletion [3-5], albeit not in all studies [6]. Moreover, passive transfer of three broadly neutralizing antibodies resulted in a delay in viral rebound after cessation of antiretroviral therapy in acutely HIV-1 infected individuals [7]. However, HIV-1 can escape from neutralizing antibodies and a neutralization resistant phenotype is associated with changes in the viral envelope [2,8-10]. The existence of this escape mechanism was first demonstrated in macaques [11] and in a laboratory worker who accidentally got infected with the neutralization sensitive HIV-1 variant IIIB and in whom a neutralization resistant IIIB related variant evolved [12]. A major component of the viral escape mechanism is the increase and repositioning of glycans, the so-called glycan shield [1,2]. Glycans are strategically positioned and repositioned continuously throughout infection, in a way that minimally affects the interaction of the envelope with the receptor molecules on the cell surface, but maximally hinders the binding of neutralizing antibodies [2]. Additionally, elongation of variable loops in Env has been associated with neutralization resistance [9,10,13,14].

Despite the rapid escape of HIV-1, the neutralizing antibody response was initially still considered to be important because escape from humoral immunity was thought to coincide with a loss of viral fitness [15] which would give rise to a lower viral load set-point and hence an ameliorated disease course. However, later studies found that escape from neutralizing antibodies did not coincide with a reduction in replication fitness [16]. In recent years, interest has increased for broadly neutralizing antibodies. These
antibodies can neutralize HIV-1 variants from different subtypes and are therefore considered to be directed against conserved epitopes. Based on this it was hypothesized that escape from these broadly neutralizing antibodies would be extremely difficult for the virus and would come at an extreme fitness cost. Surprisingly, escape from autologous serum with broadly neutralizing antibodies occurred rapidly and was also not associated with a loss in viral replication fitness [10]. This is most likely because escape is not associated with mutations in the epitope itself, but rather with the above mentioned changes in the viral envelope, being an increased number of glycans and an increased length of the variable loops that may occlude the antibody epitope. In agreement with the rapid escape, the presence of even broadly neutralizing activity in serum was not associated with a prolonged asymptomatic course of infection [17,18].

Cellular Immune Response: Cytotoxic T Lymphocytes
HIV-1 specific cellular immune responses, mediated by cytotoxic and helper T lymphocytes, emerge very rapidly after infection [19-21]. Several observations have suggested that HIV-1 specific CD8+ cytotoxic T cells (CTLs) contribute to the control of HIV infection. Strong CTL responses are often observed in long term asymptomatic HIV-1 infected individuals but diminish with progression to AIDS [22]. It cannot be excluded, however, that these preserved CTL responses are a consequence, rather than a cause, of prolonged asymptomatic survival. Experimental depletion of CD8+ T cells in SIV-infected macaques led to the loss of control of acute infection and to increased viral load in chronic infection [23,24]. Later studies showed, however, that CD4+ T cells under these conditions started to proliferate, providing the virus with an excellent opportunity to replicate which may explain the rise in viral load [25].

Finally, the quality of T cell responses early in infection was not associated with AIDS free survival [26]. As for neutralizing humoral immunity, this lack of effect of CTLs may be due to viral escape. Escape mutations in even a single epitope may lead to loss of immune control by CTLs [27]. Viruses with CTL escape mutations can be rapidly selected and overtake the viral quasispecies in as little as 6 weeks after first emergence [28]. The rapid selection of these escape variants is in fact the strongest argument that CTLs are effective in vivo. Certain CTL escape mutations have an impact on viral fitness [29,30], also demonstrated by complete reversal of the escape mutation to the wild type sequence upon transmission to a new individual [31]. The selective pressure of CTLs is such that at a population level, the virus strains circulating in a population have adapted to the most common Human Leukocyte Antigen (HLA) types in that population [32,33]. The complex influence of HLA on HIV-1 disease course will be discussed later in the section on host polymorphisms. Mutations outside CTL epitopes have also been described to influence CTL function, by affecting antigen processing and presentation [34].
Cellular Immune Response: Helper T Lymphocytes

The functioning of HIV-1 specific CTL depends on the presence of functional CD4+ helper T cells. The presence of CD4+ helper T cells that proliferate and produce both IL-2 and IFN-γ in response to HIV-specific peptide pools early in infection are not predictive for prolonged AIDS free survival [35]. However, during the course of infection, the loss of these helper T cells, together with the loss of all functional CD4+ T cells, causes a less efficient immune response, thus allowing opportunistic infections to bypass the immune system, finally resulting in disease progression and AIDS. In mouse models it was demonstrated that the presence of CD4+ T cells during priming of CTLs is essential for the maintenance of immunological memory after the acute infection phase whereas their continuous presence was not required for CTL expansion [36,37]. It can thus be envisioned that with the loss of CD4+ T cells during HIV-1 infection no efficient new CD8+ T cell memory is generated in later phases of the infection.

Chronic immune activation and CD4+ T cell loss

Depletion of CD4+ T cells, which is preceded by a loss of proliferative responses to polyclonal stimuli [38] and recall antigens [39], is one of the major characteristics of HIV-1 infection [40]. The fact that HIV-1 uses CD4 as a cellular entry receptor together with the observation that CD4+ T cells are specifically lost in HIV-1 infection led to the logical conclusion that virus-mediated killing of target cells was the main cause for this cell loss. However, the frequency of infected cells in peripheral blood in the chronic phase of infection is too low to account for the ongoing depletion of CD4+ T cells by viral infection [41,42]. Several converging lines of evidence now suggest that hyper-activation of the immune system in response to chronic HIV-1 infection may be the culprit [43]. Hyperactivation is responsible for an increased naive T cell turnover, leading ultimately to the exhaustion of the naive T cell compartment, which then cannot compensate for the enhanced death of memory CD4+ T cells into which the activated CD4+ T cells mature. Indeed, the level of immune activation was found to be at least as good of a predictor of disease progression as the level of viral replication [44,45]. Additional evidence came from the comparison of SIV infection in Sooty Mangabeys and African Green Monkeys on the one hand and in Asian Macaque species on the other [46]. SIV infection in Asian Macaques generally leads to high virus loads, declining CD4+ T cell numbers and disease within 2 years [47]. Sooty Mangabeys and African Green Monkeys can both be infected with SIV and despite overt viral replication these animals have stable CD4 counts and do not progress to disease [48,49]. The stable CD4 counts in the presence of high viral load is in line with the assumption that virus mediated killing has no large effect on total CD4+ T cell numbers. Despite the high viral load, these animals show no evidence of hyperactivation of their immune system as observed in SIV infected macaques and HIV-1 infected humans [49]. This suggests that although the increased turnover of naive T cells in HIV-1 infected individuals is virus driven, the responsiveness is determined by host factors. More recently it was demonstrated that the initial immune response against the virus was similar in both
the non-pathogenic and pathogenic macaque model. Interestingly, this initial response was then down-regulated only in the non-pathogenic macaque model but not in the pathogenic SIV macaque model [50-52]. The underlying mechanism for the differential regulation of the immune response needs to be elucidated. It has been suggested that at least two populations of CD4+ T cells are important in mediating this immune response: Th17 cells, defined by the secretion of cytokine IL-17, which are thought to be critical in the defense against bacterial and fungal pathogens, and regulatory T cells (Tregs), expressing FoxP3, which are able to induce tolerance against self antigens and prevent autoimmunity. The pro-inflammatory Th17 cells and immunoregulatory Tregs have antagonistic effector functions; a shift in this balance might be critical in the outcome of HIV disease. Indeed, the loss of Th17/Treg balance was associated with chronic immune activation and pathogenic SIV infection in nonhuman primates [53]. The mechanisms responsible for selective Th17/Treg imbalance during pathogenic infection are as yet undefined.

**CD4 Depletion in the Gut**

In both the SIV macaque model and in HIV-1 infected humans it was demonstrated that already during the phase of primary infection a massive depletion of CD4+ T cells occurs in all tissues, including the gut associated lymphoid tissues (GALT) where more than 80% of T cells reside [54-57]. This depletion of lymphocytes from the GALT occurs within days and seems to be irreversible [58]. It was shown that HIV-1 infection and depletion in the acute phase was not restricted to activated memory cells as also a high frequency of infected resting CD4+ T cells could be demonstrated [59,60]. The unique capacity of HIV-1 to replicate in non-dividing cells is in agreement with this observation [61]. Recently it has been reported that Th17 cells are preferentially lost from the GI tract. Importantly, this loss is observed in HIV-1 infection and pathogenic SIV infection of rhesus macaques, but not in nonpathogenic SIV infection of sooty mangabeys [62,63].

**Viral Factors that Influence Viral Load: Biological Phenotype**

HIV-1 can vary with respect to biological properties such as replication rate, cell tropism, coreceptor use, and neutralization sensitivity. The use of different coreceptors by HIV-1 is a general phenomenon for viruses from all different subtypes and circulating recombinant forms (CRFs), although quantitative differences in the prevalence of HIV-1 variants that can use the CXC chemokine receptor 4 (CXCR4-using variants) among subtypes exist. Virus variants with different biological properties dominate in different phases of infection. New infections are generally established by HIV-1 variants that use CD4 and additionally the CC chemokine receptor 5 (CCR5) as a coreceptor (R5 variants) [64-68]. Even if both R5 and CXCR4-using variants are present in the donor, most often only the R5 variants are detected in the recipient [65,66,69]. With progression of disease, a shift in the viral quasispecies towards more rapidly replicating T cell-tropic R5 variants is observed,[64] In the natural course of approximately half of
HIV-1 subtype B infected individuals this is associated with the appearance of CXCR4-using HIV-1 variants prior to AIDS diagnosis [70]. CXCR4-using variants are associated with an accelerated CD4+ T cell decline and a more rapid disease progression [71,72]. The accelerated loss of CD4+ T cells after appearance of CXCR4-using variants can be explained from the fact that naive T cells express CXCR4 but not CCR5, while memory cells express both CXCR4 and CCR5 [73]. This coreceptor expression pattern makes naive T cells a unique target cell population for CXCR4-using variants. Indeed, clonal virus isolation from naive T cells resulted in predominantly CXCR4-using variants, whereas both R5 and CXCR4-using biological virus clones could be obtained from the memory T cells from the same individuals [74]. It can be envisaged that infection and subsequent virus-mediated killing of naive T cells by CXCR4-using virus variants directly interferes with T cell ontogeny as the infected and killed naive T cell will no longer give rise to a daughter cell population. Considering the potential beneficial effect of an expanded target cell population and the limited number of amino acid substitutions in V3 that is sufficient to confer CXCR4-using capability to R5 variants, it is puzzling that CXCR4-using variants emerge prior to AIDS diagnosis in only a proportion of infected individuals [75,76]. It has been hypothesized that the absence of CXCR4-using variants early in HIV-1 infection may be due to their higher vulnerability to the host adaptive immune responses, in particular neutralizing antibodies. The fact that these variants appear more frequently after the initial decline of CD4+ T cells indeed suggests that they may represent a peculiar, congenic form of opportunistic infection. The first appearing CXCR4-using variants are more sensitive to neutralizing antibodies directed against the CD4 binding site than their co-existing R5 variants [77]. Moreover, a conserved neutralization epitope, designated D19, is invariably cryptic in R5 variants of different genetic subtypes, but it is consistently exposed in CXCR4-using variants, rendering such variants sensitive to neutralization by a specific antibody [78].

**Evolution of Coreceptor Use**

Phylogenetic analyses of HIV-1 envelope sequences have shown that CXCR4-using variants directly evolve from R5 variants, after which the CXCR4-using and R5 viruses co-exist within the same individual [79,80]. After the first appearance of CXCR4-using variants, both R5 and CXCR4-using variants continuously evolve away from the common ancestor and each other. However, this is only evident for the Env gene as CXCR4-using and R5 virus populations cannot be distinguished on the basis of Gag sequences due to frequent recombination events outside the Env gene.[81] In general, the first CXCR4-using variants can still use CCR5 on primary cells albeit it far less efficient [82]. Recent studies have shown that failure of Maraviroc, a small molecule CCR5 antagonist, is caused by the presence of R5 variants that can use maraviroc-bound CCR5 for entry [83] or by the presence of CXCR4-using variants, which upon closer inspection were existent pre-therapy [84,85]. The continued evolution of CXCR4-using and R5 variants is also evident from changes in their biological properties over time. Early CXCR4-using variants are more sensitive to the inhibitory effect of coreceptor
antagonists AMD3100 and T22 than late-stage obtained CXCR4-using variants [82]. In analogy, late stage R5 variants from individuals who never developed CXCR4-using variants are less sensitive to inhibition by the natural ligand of CCR5, RANTES [86]. The coreceptor inhibitor resistance of R5 and CXCR4-using variants is correlated with the immune status of the host. Although the exact mechanism of resistance remains to be established, the observation is suggestive for selection of HIV-1 variants that use their coreceptor with increasing efficiency as the infection progresses. In a recent study we have observed that viruses with this late stage phenotype can be transmitted to a new host after which evolution continued [87]. So, while R5 variants are preferentially transmitted over CXCR4-using variants, there does not seem to be a preferential transmission phenotype within R5 variants. The fact that HIV-1 envelope is changing at a population level over calendar time is in line with this observation [88].

Viral Accessory Genes

The HIV-1 genome encodes for three structural proteins (Gag, Pol and Env), two regulatory proteins (Tat and Rev) and four accessory proteins: Vpr, Vpu, Nef and Vif. Initially the function of the accessory genes was not clear and they are dispensable for viral replication in some cell types. It is believed that their main function is dedicated to mechanisms to escape or manipulate adaptive and innate immunity. Here we describe how Vpr, Vpu, Nef and Vif each suppress the antiviral activity of specific host cell factors.

**Nef**

In the SIV macaque model it was demonstrated that inoculation with SIV that lacked the Nef gene resulted in infection but not disease progression [89]. A cohort of individuals in Sydney infected with HIV-1 from the same source and classified as long-term non-progressors, all carried a ΔNef HIV-1 variant [90], although some have ultimately progressed to AIDS-defining events [91].

Multiple Nef activities are known, which are mostly genetically separable and make use of distinct elements located throughout the Nef molecule. Nef alters the intracellular trafficking of important immune molecules, such as class I and II major histocompatibility complex proteins (MHC-I, MHC-II), CD4 and DC-SIGN [92-95]. Down regulation of MHC-I proteins on the surface of the cell by Nef protects HIV-infected cells from recognition and killing by CTLs, while Nef-dependent CD4 removal enables optimal release of infectious virions. More directly, Nef also triggers apoptotic pathways, affecting survival of bystander CD4+ T cells [96]. Furthermore, Nef promotes the induction of cellular transcription factors that can elevate viral replication [97] and Nef intersects with the macrophage CD40L signaling pathway to promote infectivity [98]. Recently it has been reported that Nef can transfer from infected cells into B cells, leading to impaired class switching [99]. Thus, Nef supports viral replication via both direct and indirect mechanisms.
**Vif**

The HIV-1 protein Vif is essential for viral replication by counteracting the effects of Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) 3G and APOBEC3F, mediators of one aspect of the innate immunity, a potent cellular defense system against retroviral infection [100,101]. APOBEC3F and APOBEC3G are members of the APOBEC superfamily of cytosine deaminases which, in the absence of Vif, are incorporated into the virion. During reverse transcription of the viral genome in a new cell, they deaminate cytidine to uracil, inducing lethal G-to-A hypermutation in the viral DNA [102,103]. Vif can bind both APOBEC3F and APOBEC3G and redirect it by ubiquitination to degradation in the proteasome, thereby preventing the viral DNA from mutation [104,105]. More recently it was reported that the expression of truncated or misfolded viral proteins due to APOBEC3G editing enhances the recognition of HIV-1-infected cells by CTLs, linking the innate and adaptive immune responses [106].

**Vpr**

Monkeys infected with SIV without Vpr function had severely attenuated infections with much lower viral burden and no evidence of disease progression [107], confirming the role of Vpr in viral pathogenesis. Multiple functions of the viral protein Vpr have been reported. First, Vpr has been shown to interact with cellular factors leading to the inhibition of host cell proliferation by a G2 cell cycle arrest of infected cells,[108] although the virological role remains unclear. Vpr also has a nuclear localization signal and facilitates nuclear localization of the viral pre-integration complex [109,110]. By interaction of Vpr with various transcriptional factors on the LTR promoter, the viral protein induces HIV-1 viral gene transcription [111]. Additionally, Vpr causes cell death by inducing apoptosis [112].

**Vpu**

Two distinct functions have been associated with the viral protein U (Vpu). Vpu downregulates CD4 cell surface expression by targeting CD4 for degradation in the endoplasmic reticulum of infected cells [113]. Vpu is also known to enhance efficient viral particle release, by antagonizing the action of tetherin [114,115]. Tetherin is a transmembrane protein that blocks the release of budding HIV-1 virions by directly anchoring the viral particle to the surface of the cell. The retained virions are internalized by endocytosis and subsequently degraded.

**HOST FACTORS THAT INFLUENCE HIV-1 ACQUISITION AND DISEASE PROGRESSION**

Several host factors have been identified that influence the clinical course of HIV-1 infection. Initially, these host genetic factors were discovered in candidate gene studies (see Table 1), in which gene variants that were already known or suspected to play a role in HIV-1 pathogenesis and immune regulation were tested for association with
### Table 1. Host factors that influence HIV-1 acquisition and disease progression discovered by candidate gene studies

<table>
<thead>
<tr>
<th>Host factor</th>
<th>Function</th>
<th>Polymorphism</th>
<th>Effect on gene</th>
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<td><strong>Chemokine receptors</strong></td>
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<tr>
<td>CCR5</td>
<td>Viral coreceptor</td>
<td>32bp deletion</td>
<td>Truncation, no membrane expression</td>
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<tr>
<td></td>
<td></td>
<td>m303</td>
<td>Truncation, no membrane expression</td>
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<td></td>
<td></td>
<td>CCR5P1</td>
<td>Increased expression</td>
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<td>CCR2</td>
<td>Viral coreceptor</td>
<td>V64I</td>
<td>Interaction with CXCR4</td>
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<td>HHE haplotype</td>
<td>Increased CCR5 expression</td>
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<tr>
<td>DARC</td>
<td>Trans-receptor</td>
<td>-46C</td>
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<td>Secondary coreceptor</td>
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<td>IL-8 receptor</td>
<td>Haplotype Ha</td>
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### Effect on HIV

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HIV-1 infection and/or disease progression. Examples of these are genes that encode proteins necessary for HIV-1 entry in a cell or for efficient replication and propagation of the virus. In addition, variations in innate and adaptive immune-regulatory genes and in specific viral-restriction genes have been studied for association with HIV-1 disease. Of these, the HLA genes are discussed in more detail below. The variants that were identified in most of these candidate gene studies turned out to have large effects on disease risk, even in small cohorts (see Table 1) and most have been reviewed extensively before [116-118]. In the case of CCR5, this has even resulted in the development of new antiviral strategies to block CCR5 in HIV-1 infected individuals, as uninfected individuals without CCR5 function (i.e. those homozygous for the 32 base pair deletion in CCR5) show no overt clinical symptoms.

The more recent genome-wide association studies (GWAS) offer a hypothesis-free analysis of the genome to find novel factors influencing HIV-1 infection and disease course. GWAS are a tool to examine the complete genome of different individuals to determine variation between individuals, mostly at the level of single DNA mutations, the so called single-nucleotide polymorphisms (SNPs). Associations between these SNPs and HIV-1 susceptibility or HIV-1 disease course and thus identification of genes essential to HIV-1 and of gene variants present in healthy individuals that affect HIV-1 may ultimately lead to new prevention strategies and therapies, similar to the development of CCR5 antagonists.

### Table 1. Continued

<table>
<thead>
<tr>
<th>Host factor</th>
<th>Function</th>
<th>Polymorphism</th>
<th>Effect on gene</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human Leukocyte Antigens</strong></td>
<td></td>
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</tr>
<tr>
<td>All HLA types</td>
<td>Ag recognition</td>
<td>homozygosity</td>
<td>Decreased recognition</td>
</tr>
<tr>
<td>HLA-B*27</td>
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<td></td>
<td>Protective epitope recognition</td>
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<tr>
<td>HLA-B*57</td>
<td>Ag recognition</td>
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<tr>
<td>HLA-B*35Px</td>
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<tr>
<td>HLA-C</td>
<td>Ag recognition</td>
<td>rs9264942</td>
<td>Increased expression</td>
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<tr>
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<tr>
<td>KIR3DS1</td>
<td>NK activity</td>
<td></td>
<td>Increased activity</td>
</tr>
<tr>
<td>KIR3DS1 + HLA-B Bw4-80I</td>
<td>NK activity</td>
<td></td>
<td>Increased activity</td>
</tr>
</tbody>
</table>

**Table 1.**
Human Leukocyte Antigens

The HLA genes map to chromosome 6 and form one of the most polymorphic regions in the human genome. HLA class I loci, HLA-A, B and C all encode for a large number of different alleles. Each individual expresses two HLA-A, two HLA-B and two HLA-C alleles on the surface of all their cells. These HLA molecules present viral antigens to CD8+ T cells, thereby initiating a cytotoxic T cell response. Due to the large variation that is created by 6 different HLA molecules, a large diversity of viral peptides can be presented which supports potent immunity. Homozygosity for HLA alleles reduces the repertoire that can be presented to the immune system, thereby limiting the number of epitopes recognized by CTLs. Indeed, homozygosity has been associated with faster disease progression.[119,120] Moreover, certain class I alleles have been implicated in the variable clinical course of HIV-1 infection. HLA-B*27 [27] and B*57 are consistently associated with effective control of HIV-1 and delayed disease progression albeit that this association is not absolute [29,30]. HLA-B*5701 has been associated with long-term non-progression in Caucasian populations [121,122], while the closely related HLA-B*5703 is associated with delayed disease progression after HIV-1 infection in individuals from African descent [123,124]. This was confirmed in a GWAS in African Americans [125]. Additionally, HLA-B*57 seems to be associated with control of HIV-1 already early in infection as the prevalence of this allele was significantly lower in individuals with symptomatic acute infection, when compared to a chronically infected population [126]. HLA-B*27 and HLA-B*57 restricted CTL select for HIV-1 variants that have escape mutations in certain epitopes in more conserved
regions that come at a relatively high fitness cost to the virus. This is supported by the observation that escape mutations in some epitopes that are restricted by HLA-B*57 immediately revert to wild-type sequences after transmission to a non-HLA-B*57 individual [31]. Interestingly, HIV-1 variants of HLA-B*57 typed individuals with a typical disease course had additional mutations that compensated for the fitness cost associated with the mutations in CTL epitopes. These compensatory mutations were not observed in HLA-B*57 typed long term non-progressors [29]. Another mechanism for HLA-B*57-related control involves induction of strong CTL responses against the escaped epitopes [127].

HLA-B*35Px, a subgroup of HLA-B*35 based on peptide-binding properties, is associated with a more benign disease course after HIV-1 infection [120], for which the underlying mechanism is not fully clear.

While it seems that HLA-B is most frequently implicated in the course of HIV-1 disease, a GWAS revealed genetic variation in the HLA-C gene region to be associated with viral control and slower progression to AIDS [128,129] and confirming earlier data [130]. The protective allele of this polymorphism is associated with high HLA-C cell surface expression, possibly through affecting a 3’UTR miRNA binding site that can degrade or repress translation of the HLA-C gene [129,131].

**Genome-wide association studies**

To date, the disease-associated phenotypes that have been used in the search for novel host genetic factors involved in HIV pathogenesis are largely overlapping and correlated with each other. The first reported GWAS that was performed in the EURO-CHAVI cohort used two phenotypes: HIV RNA viral load at set-point, with viral load set-point defined as the steady-state viral load after the acute phase of the primary HIV-1 infection, or extrapolated time to a CD4+ T cell count below 350 cells/ml [128]. In the EURO-CHAVI cohort two loci were genome-wide significantly (P<5x10^-8) associated with viral load set-point. One of these loci is tagged by SNP rs2395029 near the HLA complex 5 gene (HCP5), a gene that is localized within the MHC class I region. SNP rs2395029 is in nearly absolute linkage disequilibrium (LD) with HLA-B*57, whose protective effect was known already, as discussed above [121,122]. The other locus is tagged by SNP rs9264942, located 35kb upstream of HLA-C.

This first GWAS additionally identified variants in the zinc ribbon domain-containing protein 1 (ZNRD1) to be associated with progression to CD4+ T cell count below 350 cells/ml. Dalmasso et al. [132] also used viral load as a disease phenotype in their GWAS, but evaluated plasma HIV-RNA and cellular HIV-DNA levels during primary infection rather than at set-point. Most of the variants that were strongly associated with HIV-RNA and HIV-DNA levels were localized in the MHC region, including rs2395029 in the HCP5 region. Limou et al. [133] and Le Clerc et al. [134] looked for genetic associations with extreme phenotypes in HIV-1 infection in long-term non-progressors (LTNPs) and rapid progressors (RP), in the Genomics of Resistance to Immunodeficiency Virus (GRIV) cohort. The non-progression GRIV GWAS identified
mainly associations between the clinical course of infection and genetic variation in chromosome 6 and could confirm both the HCP5 and the ZNRD1 locus identified by the EURO-CHAVI cohort. The analysis of RP revealed several interesting loci, but these need to be replicated in other cohorts. Viral load was also used as a phenotype in the multinational HIV Controllers study. A large cohort was divided into HIV-1 controllers, who are able to control viral load after infection to levels below 50 copies of viral RNA/ml plasma, and HIV-1 progressors, those who failed to ever control viremia. Over 300 SNPs that were genome-wide significantly associated with viral load were identified and all were located within the MHC gene region. Specific amino acids in the HLA-B peptide binding groove (associated with rs2395029 HCP5 SNP), as well as an independent HLA-C effect (associated with rs9264942 HLA-C SNP), were found to be associated with the capacity to control HIV-1 [135].

A multi-stage GWAS in US seroconverters compared RP, moderate progressors and LTNPs, followed by replication of interesting signals in another cohort [136]. Variation upstream of PROX1, a negative regulator of IFN-γ expression in T cells, was associated with slower progression to AIDS. Another GWAS amongst US seroconverters identified a cluster of SNPs in the gene PARD3B to be associated with a delayed survival time to AIDS [137]. One of the variants in this cluster could be confirmed in two European cohorts of rapid progressors.

The majority of GWAS performed have focused on populations from European descent. The first published GWAS on a non-European population searched for associations with viral load at set-point in African Americans [125]. Although no loci were genome-wide significantly associated with viral load at set-point, one of the strongest associations was a SNP tagging the HLA-B*5703 allele. This confirms the important association between HLA-B*57 and viral load variation, both in African Americans and in individuals of European ancestry.

In a mother-to-child transmission cohort in Malawi, in which HIV-negative children and HIV-positive infants from HIV-infected mothers are compared, several regions were identified to be potentially associated with vertical transmission of HIV-1. However, these findings still need further examination and replication [138].

A linkage analysis in a cohort of SIV-infected macaques [139] revealed MHC class I markers and an unknown X chromosomal locus to be associated with progression to AIDS. The association between the signal on the X chromosome and AIDS progression could be replicated in a cohort of HIV-1 infected patients.

Two studies performed a genetic association analysis of in vitro susceptibility to HIV-1 infection in lymphoblastoid B cell lines from a family cohort (CEPH) [140] and in primary monocyte-derived macrophages [141]. The first study identified the LY6 gene family and the SNP in LY6 subsequently turned out to be associated with accelerated disease progression in one of two cohorts of HIV-1-infected patients. In the second study we observed a strong association between a SNP intronic of DYRK1A and in vitro HIV-1 replication in monocyte-derived macrophages. This SNP appeared to be associated with HIV-1 disease progression in vivo in two independent cohort studies.
Genome-wide scanning of RNA for more than 20,000 human proteins has been performed to identify genes required for HIV-1 replication. Three studies used siRNA transfection to knock down gene expression [142-144] and one study used short hairpin RNA transduction for gene silencing [145]. These four studies identified over 1,000 proteins that may be required for optimal viral replication. However, only three genes were identified in all four studies [146]. These were mediator complex subunit 6 (MED6), which is involved in transcription of RNA polymerase II-dependent genes, mediator complex subunit 7 (MED7), which is required for efficient transcription of Sp1, and v-rel reticuloendotheliosis viral oncogene homolog A (RELA), which is part of the NF-κB complex. The minimal overlap in outcome of these studies may be caused by differences in cell types used for analysis and the steps in the replication cycle of HIV-1 that were studied. Interestingly, several genes that were identified to be involved in HIV-1 replication could be grouped in pathways or categories of genes, such as nuclear import and export, transcription factors, components of the NF-κB complex, and kinases.

In another approach, a cDNA library representing 15,000 unique genes was used to find novel factors that when overexpressed could enhance HIV-1 infection [147]. The mixed lineage kinase 3 (MLK3) was identified as one of the strongest enhancers of HIV-1 replication, confirmed by RNAi gene expression silencing.

Conclusion
As is clear from the above, the clinical course of HIV-1 infection is influenced by many host genetic factors as well as viral factors. This is logical from the point of view that the genome of HIV-1 only encodes a limited number of proteins rendering it dependent on cellular proteins for replication. On the other hand, HIV-1 needs to protect itself from the host’s innate and adaptive antiviral defense mechanisms in order to persist.

Multiple interactions between viral proteins and host cellular factors have been observed, and may be pursued for the design of new antiviral therapies. As described, polymorphisms in several host factors have been identified and some variants have now been convincingly associated with disease progression in several cohorts. However, it will require meta-analyses to complete and validate the identification of host genetic factors that are associated with disease course.
SCOPE OF THESIS

As described above, the clinical course of HIV-1 infection is highly variable between individuals, and host genetic variations may at least account for part of this variation. This thesis contains studies in which a closer look was taken on the involvement of host genetic factors in HIV-1 infection and disease progression. In Chapter 2, the effect of polymorphisms in the tripartite interaction motif 5 α (Trim5α) gene on the clinical course of HIV-1 infection in participants of the Amsterdam Cohort Studies (ACS) on HIV-1 infection and AIDS was analyzed. The first genome-wide association study (GWAS) on HIV-1 infection identified three SNPs to be genome wide associated with viral load at set-point. These three SNPs were located in the gene regions of HLA-C, HCP5 and RNF39/ZNRD1. In Chapter 3 it was studied whether these associations could be replicated in HIV-1 infected individuals from the ACS and whether these SNPs were also associated with other clinical outcomes of HIV-1 infection. To reveal additional host genetic factors that are associated with disease progression, a GWAS on survival to AIDS-diagnosis and AIDS-related death in HIV-1-infected participants of the ACS was designed. This GWAS is described in Chapter 4. In Chapter 5, a multi-cohort study on genetic association with the extreme phenotype long-term nonprogression after HIV-1 infection is described. To specifically look for rare variants that could influence the clinical course of HIV-1 infection, GWAS data from two European and an American cohort were reanalyzed in Chapter 6, targeting specifically SNPs with a minor allele frequency below 5%. In the AIDS pandemic, rare individuals have remained persistently HIV-uninfected despite multiple high-risk exposures to HIV-1. In Chapter 7, an attempt to identify mechanisms of resistance to infection was made. To this end a GWAS was performed in men having sex with men (MSM) from the ACS who remained uninfected despite high-risk sexual behavior and compared them with HIV-1 positive individuals and healthy seronegative controls. In order to reveal whether host genetic variation may play a role in the HIV-1-specific cross-neutralizing activity in after HIV-1 infection, a GWAS was performed in participants of the ACS with available information on neutralizing activity in serum as is described in Chapter 8. Recent studies have shown that over calendar time, viral load at set-point has significantly increased at a population level. In Chapter 9 the impact of host genetic markers over time in the epidemic was studied. Therefore, associations between viral load at set-point and the host genetic markers CCR5Δ32, HCP5 rs2395029 and -35HLA-C rs9264942 were studied in HIV-1 infected individuals in the Netherlands who seroconverted before 2003 or individuals who seroconverted post-2003. In Chapter 10, all findings are put in perspective, also in relation to novel developments in the field.
REFERENCES


37. Sun JC, Williams MA, and Bevan MJ: CD4+ T cells are required for the maintenance, not programming, of memory CD8+ T cells after acute infection. *Nat Immunol* 2004, 5:927-933.


77. Bunnik EM, Quakelaar ED, van Nuenen AC, Boeser-Nunnink B, and Schuitemaker H: Increased neutralization sensitivity of recently emerged CXCR4-using human immunodeficiency virus type 1 strains compared to coexisting CCR5-using


84. Westby M, Lewis M, Whitcomb J, Youle M, Pozniak AL, James IT et al.: Emergence of CXCR4-using human immunodeficiency virus type 1 (HIV-1) variants in a minority of HIV-1-infected patients following treatment with the CCR5 antagonist maraviroc is from a pretreatment CXCR4-using virus reservoir. *J.Virol.* 2006, 80:4909-4920.


5. Multi-stage genomewide association study identifies a locus at 1q41 associated with slower HIV-1 disease progression to AIDS in Malawian women. AIDS 1999, 13:1900-1901.


160. He W, Neil S, Kulkarni H, Wright E, Agan BK, Marconi VC et al.: Duffy antigen receptor for chemokines mediates trans-infection of HIV-1 from red blood cells to...


