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GENERAL INTRODUCTION

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In 1981, the first cases of acquired immunodeficiency syndrome (AIDS) were reported among young homosexual men in New York and Los Angeles [1,2]. Two years later, the human immunodeficiency virus type 1 (HIV-1) was identified as the causative agent of this disease [3]. Since that time, an estimated 25 million people have died of AIDS worldwide. Currently, 34 million people are living with HIV globally, and although the numbers of new infections are decreasing, approximately 2.5 million people become infected each year [www.unaids.org].

The introduction of combination antiretroviral therapy (cART) in 1996 has dramatically decreased morbidity and mortality rates. By the combinational use of at least 3 antiretroviral drugs, viral replication is suppressed and immune deterioration by depletion of CD4+ T cells is prevented. However, the viral reservoir in latently infected cells remains present. Therefore, cART alone is insufficient to stop the HIV/AIDS pandemic and the search for an effective vaccine continues.

HIV-1 TRANSMISSION
HIV-1 transmission predominantly occurs via sexual contact through the genital tract or the rectal mucosa. Other routes of transmission include transmission from mother to child during pregnancy, delivery or breast feeding and transmission via direct blood-blood contact, for instance through injection or transfusion. The transmission risk of HIV-1 is highly variable and is influenced by the viral load in the donating partner and the susceptibility of the receiving partner [4-6].

In most newly HIV-1 infected individuals, outgrowth of a single transmitted virus variant is observed, although transmission of multiple virus variants has been reported as well. The route of transmission influences the probability of transmission of multiple variants, as transmission of a single virus variant is observed in 70-90% of heterosexual transmission cases [7-12], in approximately 60% of men having sex with men (MSM) transmission couples [10,13] and in 40-70% of transmissions via injecting drug use [14,15]. In mother-to-child transmission pairs the percentage of infections by a single virus is comparable to heterosexual transmission (70-90%) [16-20], although children infected in utero seem to have a greater risk of infection with multiple variants when compared to intrapartum HIV-1 transmission [16,20]. The observation that new infections are established by a very low number of virus variants suggests a strong bottleneck for the virus during transmission.

TRANSMISSION BOTTLENECKS
The rate of HIV-1 transmission is proportionally correlated with the viral load in plasma and genital secretions in the transmitting individual [21-23]. Moreover, the number of transmitted viruses has been associated with the inoculum size in intrarectally challenged Rhesus macaques [24-26]. Indeed, lowering the plasma viral load with cART reduces the risk of sexual transmission and mother-to-child transmission of HIV-1 [27,28]. A large part of new HIV-1 infections arise from sexual transmission from individuals in the primary phase of infection [29-31] and it has been suggested that this is due to the high viral burden in early HIV-1 infection. However, Hollingsworth et al. reported a higher HIV-1 transmission rate in serodiscordant couples than would be expected when considering plasma viral loads in the transmitting partners [29]. In primary infection, the homogeneous viral quasispecies consists of recently transmitted viral
variants and may therefore harbor better biological properties for infection that might explain the high number of new infections associated with transmission from individuals with primary HIV-1 infection. A study in macaques has shown that viruses isolated during acute simian immunodeficiency virus (SIV) infection in the ramp-up stage have a much higher ratio of infectious / non-infectious virions in plasma (1:1 - 1:10) than viruses isolated during the chronic phase of infection (1:75 - 1:750) [32]. Furthermore, the virus that is transmitted from donor to recipient is not always the major virus variant present in the quasispecies of the donor [11,33]. In a recent paper by English et al. outgrowth of two different viral variants in two men who were infected by the same donor on the same night was observed [34]. These findings strongly suggest that the transmission bottleneck occurs in the receiving individual and not in the transmitting individual.

During sexual contact, HIV-1 can be transmitted directly into the blood of the recipient in the case of lesions or ruptured mucosa. However, transmission through mucosal exposure to HIV-1 can also occur, either during sexual transmission (exposure of genital or gut mucosa to HIV-1 containing genital fluids or blood) or during mother-to-child transmission (exposure of gut mucosa to HIV-1 contaminated blood or breast milk that has been swallowed by the child during or after birth, respectively [35]). Differences in morphology and availability of target cells between the different mucosal tissues may account for the variation in percentages of infections with single or multiple transmitted viruses [36]. The mucosa of the vagina and ectocervix consist of multiple layered epithelia, whereas the endocervix and rectal mucosa are lined with a single epithelial layer, and are therefore more easily breached. Also the effect of hormonal contraceptives on the acquisition risk of HIV-1 has been studied. The use of hormonal contraceptives may be associated with a higher risk of HIV-1 acquisition, although results of several studies are conflicting [38-43]. In rhesus macaques, application of hormones affects SIV infection after vaginal inoculation, possibly by influencing the thickness of the vaginal and cervical mucosa. Topical application of estrogen, leading to thickening of the mucosal layer, has a protective effect on SIV infection [44], whereas progesterone implants enhance SIV transmission [45].

Inflammatory genital infections caused by the human papilloma virus (HPV), herpes simplex virus type 2 (HSV-2) and other sexual transmitted infections, have been reported to be associated with a higher susceptibility to HIV-1 infection [46,47] and outgrowth of multiple HIV-1 variants after transmission [11,37]. Infections in the genital region can cause genital ulcerative disease which can lead to breaching of the mucosa, or can cause an increased cellular influx to the infection site.

HIV-1 transmission in the absence of a mucosal barrier via direct blood-blood contact is more efficient and is related to a higher risk of infection with multiple viral variants [14,15,48]. However, a genetic bottleneck is still observed during HIV-1 transmission via blood-blood contact, which is most likely related to the inoculum size.

**VIRAL ADAPTATION TO HOST IMMUNE RESPONSES**

After HIV-1 infection has been established in the new host, the viral population expands rapidly. The viral reverse transcriptase enzyme is error-prone and lacks proofreading and the frequent misincorporation of nucleotides in the viral genome during reverse transcription results in diversification of the viral quasispecies [49-52]. In the early phase of HIV-1 infection the viral
quasispecies remains rather homogenous, but more and more mutations accumulate in the viral genome over time. Selection pressure exerted by the host immune responses drive viral adaptation and evolution of the viral quasispecies (Figure 1 [53]).

Natural killer (NK) cells play an important role in the first line of defence in viral infections. They recognize infected cells through a variety of activating and inhibitory receptors, like KIR receptors, and secrete pro-inflammatory cytokines to shape the adaptive immune response. In HIV-1 infection, NK cells recognize infected cells through interaction of KIR receptors and HLA molecules presenting HIV-1 derived short peptides, and NK cell activity is directly correlated with the level of viral replication during acute HIV-1 infection [54]. The NK receptors KIR3DS1 and KIR3DL1 and their ligand HLA-Bw4-80I are correlated with higher production of interferon-γ and strong inhibition of HIV-1 replication [55-58]. Recently, it was demonstrated that HIV-1 can adapt to the NK mediated immune response and evade recognition through selection of HIV-1 sequence polymorphisms. NK cell recognition via the KIR2DL2 inhibitory receptor is avoided through selection of mutations that enhance binding of the infected cell to this receptor and

**Figure 1.** Bottlenecks of HIV-1 transmission and evolution of the viral population. From the viral quasispecies in the donor, a small number of viruses will be transferred to the recipient. In most cases, only a single viral variant will establish HIV-1 infection in the recipient due to a severe transmission bottleneck. In the recipient, the virus population will expand and due to the error-prone reverse transcriptase minor changes are incorporated. The homogeneous viral population will diversify and evolve under immune pressure by cytotoxic T lymphocytes (CTLs), Natural Killer (NK) cells and neutralizing antibodies, resulting in a diverse quasispecies.
thereby inhibiting NK directed killing of the infected cell [59]. This may indicate that NK cells play a more prominent role in viral evolution and adaptation than previously anticipated.

Neutralizing antibodies against HIV-1 typically develop within a few months after primary infection [60-63]. These antibodies bind to the viral envelope and block attachment to the receptors on the target cells or prevent the conformational changes necessary for fusion of the viral and cellular membrane. The first HIV-1 neutralizing antibodies have a narrow repertoire and are usually only specific for the transmitted viral variant. Escape from these neutralizing antibodies is very quick, resulting in longer variable regions, differences in charge and a more extended glycosylation of the envelope, shielding it from antibodies [64-70].

Cytotoxic T lymphocytes (CTL) recognize infected cells through viral epitopes presented by HLA molecules and subsequently induce lysis of the infected cell. CTL responses play an important role in viral control during acute HIV-1 infection, indicated by the drop in plasma viremia coincident with HIV-1-specific CTL activity [71-75]. However, HIV-1 is able to escape from CTL responses through selection of escape mutations in CTL epitopes abrogating CTL mediated killing [76]. These CTL escape mutations may result in loss of CTL recognition or may interfere with presentation of the viral epitope by the HLA molecule through disruption of the binding sites or altered intracellular processing of viral proteins by the proteasome or the transporter associated with antigen processing (TAP) proteins. CTL responses targeting variable regions of HIV-1 will rapidly result in viral escape, whereas targeting of conserved proteins is more effective in viral control. Indeed, early in infection, CTL targeting of Gag epitopes is associated with lower viremia as compared to targeting of the envelope [77,78].

Viral control has also been associated with HLA-B-restricted CTL responses. HLA-B alleles are able to present viral epitopes of a more conserved nature, suggesting that these alleles confer a greater impact on viral evolution [79-81].

ROLE OF CTLS IN THE CONTROL OF HIV-1 REPLICATION IN VIVO

Certain HLA alleles, most notably HLA-B57 and HLA-B27, are associated with prolonged survival after HIV-1 infection [82-85]. These HLA molecules present viral peptides from the Gag protein that are very conserved in the circulating virus population. CTL escape mutations in these regions have an impact on viral replication due to functional constraints on those proteins. In patients carrying the HLA-B27 allele, the R264K CTL escape mutation in the KK10 Gag epitope usually occurs late in the infection and is associated with loss of control of viremia [86-89]. It has been suggested that this CTL escape mutation does not occur in earlier stages of infection due to the high fitness cost. Individuals carrying HLA-B57 on the other hand can benefit from CTL escape [76,90,91]. The described T242N CTL escape mutation in the TW10 Gag epitope occurs quickly after infection and is known to reduce the replication capacity of the virus, leading to a lower viral load [76,92]. Transmission of these mutations to a new host lacking similar immune pressure can lead to reversion of the mutations, as optimal replication fitness drives selection for the consensus sequence [76,92-94]. The presence of certain mutations in the same viral region, however, can (partially) restore viral replication ability and compensate for the fitness loss associated with these CTL escape mutations [92,95-97].
VIRAL ADAPTATION AT THE POPULATION LEVEL

HIV-1 is continuously adapting to its host. These adaptations can revert to wild type sequences after transmission to a new host, driven by an increase in replication ability. However, not all mutations revert after transmission and may accumulate in the population over time.

An increase in viral load at set point at a population level has been described [98-100], which may indicate that HIV-1 has adapted to its host and gained in fitness over time, although others have not confirmed this observation [101-103]. Recently, the protective effect of genetic host factors, such as HLA-B57, was reported to have faded over time, coinciding with an increased viral load at set point [104]. Also, an enhanced resistance against neutralizing antibodies over time was reported comparing early viruses from individuals who seroconverted in the 1980s and viruses isolated from individuals who seroconverted between 2003 and 2006. This observation was accompanied by an increase in both length and number of potential N-linked glycosylation sites, making the envelope less accessible to antibody binding [105]. Furthermore, accumulation of CTL escape mutations and loss of CTL epitopes restricted by protective HLA alleles have been described over time [106-108]. These findings are suggestive of the adaption of HIV-1 to its host at a population level.

SCOPE OF THIS THESIS

In this thesis, we studied HIV-1 evolution after transmission and the effect of viral adaptation to the host on disease progression. First, we studied the viral adaptation to HLA alleles and CTL pressure. In chapter 2 we analyzed differences in amino acid composition of the Gag protein in HLA-B57 long-term nonprogressors and progressors that were associated with CTL escape and affect the replication kinetics of the virus. Chapter 3 describes the sequence variation in the HIV-1 capsid protein in three homosexual men infected with the same HIV-1 variant who show large variation in the course of infection. Viral evolution and escape from HLA-B27 restricted CTLs was studied in four HLA-B27 positive individuals during disease progression in chapter 4. HIV-1 sequence evolution in CTL epitopes after transmission was studied in mother-to-child transmission couples in chapter 5.

In the second part of this thesis, we studied the effect of viral diversity and evolution on disease progression and treatment response. In chapter 6, the effect of viral envelope sequence diversity in the first year after infection on the subsequent disease progression was studied. The effect of HIV-1 coreceptor usage prior to start of cART therapy on the treatment response was analyzed in chapter 7.

The results and implications of these studies are discussed in chapter 8.
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GENERAL INTRODUCTION


