HIV-1 evolution and adaptation to the host during the course of infection
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8

GENERAL DISCUSSION
HIV-1 exhibits a high degree of genetic variability. This genetic diversity is caused by frequent mutations that occur due to the high replication rate of the virus and the error-prone nature of the reverse transcriptase of HIV-1 that lacks proof-reading [1-4]. In the majority of newly infected individuals, transmission and outgrowth of a single HIV-1 variant is observed [5-10]. Viral diversity expands during the course of infection and multiple viral variants co-exist as quasispecies within an individual. The composition of the quasispecies is shaped by competition and selection for the fittest viral variant at that time in that specific environment, and is specific for the individual patient. Genetic diversity of HIV-1 varies substantially among individuals and has been associated with progressive disease (chapter 6, [11,12]).

One of the major selective forces driving HIV-1 evolution is the host immune response and viral variants that have escaped immune pressure are rapidly selected. Despite the extreme mutational capacity of the virus, there are constraints on viral evolution when it comes to target cell infectivity and viral replication capacity.

**VIRAL ESCAPE FROM CTL RESPONSES**

Cytotoxic T Lymphocyte (CTL) responses play an important role in viral control. The drop in plasma viremia during acute HIV-1 infection coincides with HIV-1-specific CTL activity [13-19]. The selection pressure mediated by CTLs is one of the major forces driving viral evolution and diversity during the course of infection. Mutations that confer escape from CTL-mediated killing are rapidly selected and may result in loss of control of viremia and disease progression [20-23]. Escape from CTL responses can be mediated by mutations that disrupt viral epitope processing and presentation via HLA molecules or by mutations that diminish CTL recognition of the epitope. In the case of escape from CTL recognition, new CTLs recognizing the mutated epitope can be generated [24,25] and this mechanism of CTL escape therefore seems to have only a temporary effect.

CTL responses targeting the viral Gag protein are associated with lower viremia as compared to CTLs targeting the viral Env protein [26-28]. The Gag protein is very conserved and structural constraints limit CTL escape mutations due to the attenuation of viral replication, whereas the Env protein is more variable. Furthermore, HIV-1 particles contain large quantities of Gag protein which can immediately be processed and presented via HLA molecules upon infection, before HLA class I is downregulated by the viral Nef protein. The same holds true for Pol, although the amount of Pol protein in the incoming virion is much lower than for Gag. The Env protein remains on the cell surface upon infection and therefore de novo synthesis is required for efficient presentation of this protein. Nef, Tat, Rev, Vpu, Vif and Vpr are not present in the viral particle and synthesis of these viral proteins by the infected host cell machinery is required before epitopes derived from these proteins can be presented [29-31].

Viral control is strongly associated with HLA-B alleles and these alleles preferentially present epitopes from the conserved Gag protein [27,32-34]. Furthermore, HLA-B alleles are more resistant to Nef-mediated downregulation of HLA alleles when compared to HLA-A alleles, which may also contribute to the better control of HIV-1 infection associated with HLA-B-restricted CTLs [35].

Sharing of HLA alleles between donor and recipient is reported to be disadvantageous for the recipient, as the transmitted viral variant has already adapted to shared HLA alleles, hampering control of viral replication exerted by de novo CTL responses. HLA concordance between donor
and recipient increases the risk of HIV-1 transmission, both for sexual transmission couples [36,37], as well as for mother-to-child transmission [38-40]. This may suggest that HLA-restricted cellular immunity plays an important role in the establishment of infection and outgrowth of HIV-1. Apparently, viral variants that have escaped through mutations at anchor residues that abrogate presentation by HLA alleles have a survival advantage in a new host that is concordant for at least part of the HLA alleles, likely explaining the higher probability of HIV-1 transmission in partial HLA concordant donor-recipient pairs. These findings may also imply that cellular HIV-specific immunity, for instance elicited by a vaccine, could indeed contribute to protection against infection.

Sharing of HLA class I alleles between donor and recipient has only a modest impact on viral load levels in the recipient after sexual transmission [41]. In mother-to-child transmission pairs, genetic similarity between mother and child compromises HLA-mediated viral control [42-45] and viral evolution in children is dominated by mutations within CTL epitopes restricted by HLA alleles that are inherited from the father (chapter 5, [46]). Furthermore, children carrying a protective HLA allele experienced a slower disease progression only when the protective allele was paternally inherited [44,45]. These results suggest a prominent role for de novo CTL responses in viral control and the clinical course of infection.

However, in mother-to-child transmission pairs, the probability exists that the mother was infected by the father of her child, and that she obtained HIV-1 variants with adapting mutations to the paternal HLA. Although these mutations may revert in the mother, they potentially are still present in the virus that is transmitted to the child, resulting in an even more hampered de novo CTL response in the child who has maternal and paternal HLA alleles. This obviously could have implications for the disease course in the child.

Mutations in viral proteins that result in escape from CTL-mediated killing are generally associated with loss of viral control [20-23]. However, this is not true for all CTL escape mutations, as some of these mutations come at a fitness cost, particularly when located in conserved viral regions [47-49]. In this way, the host can benefit from a lower viral replication rate despite escape from immune control [50]. When CTL escape mutations that come at a fitness cost are transmitted to a new host in whom antiviral immune responses have not yet developed, rapid reversion of these mutations is observed as a consequence of selection for the fittest viral variant [16,51-53]. It has been reported, however, that the presence of viral variants with lower fitness in the period before reversion of mutations occurs, can still be beneficial for the recipient, resulting in a lower viral load set point and higher CD4+ T cell counts in the early phase of infection [44,54-57].

It has been described that viral evolution during the initial phase of infection after sexual transmission is dominated by reversion of transmitted mutations [58-60], although others did not corroborate with these findings [52]. In contrast, after HIV-1 transmission from mother to child, viral evolution is not driven by reversion of transmitted mutations (chapter 5, [46]). However, it cannot be excluded that escape mutations in the transmitted virus did revert in the child, but were not detected, because de novo CTL responses in the child rapidly reselected the escape mutations. Reversion of mutations located in epitopes restricted by HLA alleles not shared between mother and child, were observed to some extent, but not for all transmitted mutations, which may indicate that the fitness cost associated with certain CTL escape mutations is limited, or that the fitness cost of the CTL escape mutation was already compensated in the mother before the transmission event.
Instead of reversion of a transmitted mutation that comes at a fitness cost, selection of forward mutations that restore viral replication capacity can also occur (chapter 3). As mutations are incorporated at random, a forward mutation that restores viral fitness can occur prior to reversion of the transmitted mutation, and compensation for the fitness cost associated with the transmitted mutation can circumvent the need for reversion of the escape mutation. It remains unclear why these compensatory mutations have not developed in the donor before the transmission event occurred. A large part of new HIV-1 infections arise from sexual transmission from individuals in the primary phase of infection [61-63], and therefore compensatory mutations may not have developed yet.

Not all CTL escape mutations come at a fitness cost and these mutations can remain present in viral variants upon transmission. In addition, compensation of CTL escape mutations associated with a fitness cost in the donor can also lead to transmission of a stable CTL escape mutation. In this way, CTL escape mutations can persist in circulating viral strains, resulting in the accumulation of CTL escape mutations and possible compensatory mutations in the virus at a population level over time [64-66]. Adaptation to CTL responses can have important implications for viral control in newly infected individuals and must be considered in the development of a T cell-based HIV vaccine.

### CTL RESPONSES AND VIRAL ESCAPE IN LONG-TERM NONPROGRESSORS

A small percentage of HIV-1 infected individuals show spontaneous control of HIV-1 infection and maintain high CD4+ T cell counts for more than 10 years without the use of antiretroviral therapy (reviewed in [67]). Numerous studies in these so-called long-term nonprogressors (LTNPs) have demonstrated the relationship between certain HLA class I alleles and a better clinical course of infection, with the highest impact for HLA-B57 and HLA-B27 [68-73]. Control of viremia in LTNPs is associated with the presence of HIV-1-specific CTLs [74-81] which better defines disease progression than HLA genotype alone [74]. In LTNPs, CTLs recognize and kill infected cells at lower antigen concentrations and have a better proliferative capacity [81-87]. There is, however, no correlation between the number of HIV-specific CTLs and viral control [88,89]. The CTL responses in patients that can control HIV-1 infection do have superior functional capacity and polyfunctionality compared to CTL responses in non-controllers, including the capacity to secrete perforine, degranulate and produce multiple cytokines [78,81,90,91]. Furthermore, HIV-1-specific CTLs maintain their proliferative capacity and functionality in LTNPs, whereas these CTL functions are lost in progressors during the course of infection [75-78,81,86]. Recently, it was demonstrated that CTL responses restricted by protective HLA alleles are not suppressed by CD4+ regulatory T cells (Tregs), whereas CTL responses restricted by other HLA alleles in the same individual are [92]. The low expression of the inhibitory Tim-3 receptor on activated CTLs restricted by protective HLA alleles prevents an interaction between CTLs and Tregs. Moreover, the protective CTLs kill Tregs, thereby escaping suppression and maintaining viral control [92]. Another recent report showed that LTNPs possess a larger population of HIV-specific CD8+ T cells that are resistant to apoptosis, through upregulation of anti-apoptotic molecules [93]. These findings indicate that patients capable of controlling HIV-1 infection maintain strong
CTL responses of high quality throughout infection, whereas CD8+ T cells from progressors lose functionality and become exhausted during prolonged infection. The question whether persistence of polyfunctional CTLs in LTNPs is a cause or a consequence of viral control remains unanswered. The fact that the proliferative capacity of HIV-specific CD8+ T cells and their ability to suppress viral replication are not restored in patients on suppressive cART [94] may seem to suggest that the prolonged strong CTL responses in LTNPs are not only the consequence of a low viral load or preserved CD4+ T cell help. However, patients that start cART may have less functional CTLs prior to start of therapy, and the fact that these functions are not restored during cART still does not give a definitive answer whether prolonged polyfunctionality of CTL responses in LTNPs are cause or consequence of viral control.

Although the HLA-B57 and HLA-B27 alleles are overrepresented in LTNPs, most patients carrying these protective HLA alleles show a progressive course of infection in the absence of cART. The differential clinical course in HLA-B57 positive patients cannot be explained by differences in CTL activity against Gag epitopes or development of CTL escape mutations [50]. Viral escape from CTL responses against the immunodominant TW10 Gag epitope occurs via the T242N mutation [50,95,96]. Selection for this mutation is usually observed early in infection and is associated with reduced viral fitness [16,50,72,73]. This indicates a role for strong polyfunctional CTL response early in infection, resulting in the selection of an escape mutation that comes at a high fitness cost. During the course of infection additional mutations within or flanking the TW10 epitope in Gag occur, which compensate for the lower viral replication capacity of the T242N viral variant (chapter 2, [96,97]). The accumulation of these compensatory mutations restores viral fitness and may lead to disease progression (chapter 2, [98]).

In contrast, in HLA-B27 positive patients the R264K CTL escape mutation in the immunodominant KK10 epitope occurs late in infection and is associated with higher viral load and disease progression [99-108]. This suggests that viral replication in HLA-B27 individuals is controlled by a strong polyfunctional CTL response and that escape from presentation by HLA-B27 is unfavorable for the virus. Indeed, the R264K mutation has a very high fitness cost, even higher than that seen for the HLA-B57 associated T242N mutation [47]. It has been suggested that this escape mutation is not viable on its own, but requires the presence of the S173A compensatory mutation, which may explain occurrence of the R264K mutation late in infection [108]. The R264G CTL escape mutation, however, has a lower fitness cost as compared to the R264K mutation, and this mutation can develop without the presence of the associated E260D compensatory mutation (chapter 4). However, the R264K/G CTL escape mutation is not seen in all HLA-B27-positive patients that progress to disease (chapter 4, [105-110]). This suggests that other factors, for instance lower TCR diversity and CTL exhaustion, may influence disease progression despite the presence of a protective HLA allele [111].

FUTURE PERSPECTIVES

The introduction of cART has dramatically prolonged survival of HIV-1 infected individuals and viral replication is controlled in the majority of treated individuals. Treatment with cART efficiently inhibits viral replication, however, HIV-1 infected cells are not eliminated. Although activated CD4+ T cells in which virus replication is blocked by cART will eventually undergo apoptosis, quiescent latently
infected cells remain present despite long-term cART. Strategies to purge the HIV reservoir are under study and the first compounds that activate latently infected cells are being tested in clinical trials. However, recent clinical studies using histone deacetylases (HDAC) inhibitors failed to detect a reduction in the viral reservoir despite reactivation of latent virus [112,113]. This suggests that circulating CTLs are not able to directly kill reactivated HIV-1 infected cells, which may indicate that these cells reside in immune privileged sites where CTLs are unable to kill these infected cells, or that circulating CTLs are functionally impaired and exhausted. It has been demonstrated that after antigen-specific prestimulation, CTLs were able to eliminate infected cells after reactivation of the viral reservoir [114]. Boosting of CTL responses in patients treated with cART by vaccination strategies and subsequent reactivation of latent HIV-1 may be a promising strategy for eradication. However, circulating CTLs might be unable to recognize reactivated HIV-1 infected cells due to the presence of CTL escape mutations, or the location of these cells.

Furthermore, therapeutic vaccines may help to reduce viral burden and delay start of therapy [115,116]. A therapeutic vaccine could also be used to redirect CTL responses towards proteins that have been associated with viral control and viral attenuation once CTL escape mutations emerge. Patients may benefit from the reduction in viral replication capacity with lower viral load and less severe CD4+ T cell depletion, even though compensatory mutations that restore viral fitness may be selected over time.

Although therapeutic vaccine strategies may help to reduce disease burden and HIV-1 transmission risk, a vaccine focused on the prevention of new infections is needed to halt the HIV-1 pandemic. Preventive strategies mainly focus on sterilizing immunity via neutralizing antibodies. However, CTL responses may be capable of eliminating virally infected cells before establishment or outgrowth of infection, as demonstrated by the fact that CTL responses against Gag or Pol epitopes can kill virally infected cells approximately 6 hours after infection of a cell, before the replication cycle has been completed and new virions are produced [29-31]. Indeed, it was recently shown that a vaccine inducing specific effector memory T cells prevented the establishment and outgrowth of SIV infection in rhesus macaques after mucosal challenge [117]. However, a vaccine eliciting both strong CTL responses and broadly neutralizing antibodies will be needed to prevent transmission and outgrowth of new infections. In depth studies providing new mechanistic insights in the development of strong polyfunctional CTL responses and the development of broadly neutralizing antibodies may ultimately provide key information leading to the development of a protective vaccine against HIV-1.
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