HIV-1 escape from the HLA-B27 and HLA-B57 restricted CTL response

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List of abbreviations

AIDS	acquired immunodeficiency syndrome
CTL	CD8+ cytotoxic T lymphocyte
CsA	cyclosporin A
CTD	C-terminal domain
CypA	cyclophilin A
EC	elite controller
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
LNTP	long term non-progressor
MHC	major histocompatibility complex
NTD	N-terminal domain
RP	rapid progressor
TLR	toll like receptor
TP	typical progressor

Abstract

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), a condition in humans characterised by a progressive loss of CD4+ T-cells. The course of HIV-1 infection is highly variable between individuals and is influenced by both viral and host factors, such as the HLA class I genotype. HLA-B57 and HLA-B27 are most strongly associated with a protective effect against HIV-1 infection.

Because of the high immune pressure provided by HLA-restricted CTLs in HIV-1 progression, escape from the CTL mediated immune response is a major mechanism of adaptation of HIV-1. In CTL escape mutations can target peptide processing, the binding of the peptide to the HLA molecule, or recognition of the presented peptide by T-cells. This results in loss, or reduction of peptide presentation by the HLA molecule, thereby inhibiting proper activation of the HIV-1 CTL response.

The *in vivo* benefits of escape from the HLA-restricted CTL response may be severely limited by the loss of fitness that is often the result of the development of the escape mutations. However, the replication-impaired phenotype of the escape variants is often followed by the appearance of compensatory mutations, which restore the viral replication capacity.

In HIV-1 infected individuals expressing the protective HLA-B27 and –B57 alleles, the CTL response is mainly targeted against Gag p24, the capsid protein that encapsulates the genomic RNA-nucleocapsid complex in the HIV-1 virion. Escape mutations in the epitopes restricted by HLA-B27 and –B57 thus can affect capsid structure and stability.

Conservation of the escape and compensatory mutations upon transmission to a new host can eventually result in accumulation of these mutations in the population. The loss of epitopes able to elicit a CTL response will result in the emergence of less immunogenic HIV-1 variants, which could pose difficulties in developing new therapy options.

1
Introduction

1.1 HIV

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), a condition in humans characterized by a progressive loss of CD4+ T-cells. In 1981, AIDS was first recognized in young homosexual men who died of rare malignancies and opportunistic infections *(1)*. HIV infection as the cause of AIDS was not discovered until a few years later *(2-4)*. At the end of 2011, 34.0 million people worldwide were infected with HIV; an estimated 0.8% of adults aged 15-49 *(5)*.

HIV originated in west-central Africa in the early twentieth century. Two closely related HIV variants can be distinguished, which differ in both origin and sequence. HIV-1 originated in chimpanzees and gorillas from West-Africa, while HIV-2 is related to a virus found in the Sooty Mangabey *(6)*. HIV-1 is considered to be responsible for the AIDS epidemic and can be further categorized into three major groups: M (main), O (outlier), and N (non-M, non-O). The vast majority of HIV infections worldwide are caused by group M HIV-1 *(7)*.

1.2 Variable course of HIV-1 infection

The course of HIV-1 infection is highly variable between individuals. HIV-1 infection can be characterized by a gradual but progressive loss of CD4+ cells, which eventually results in the development of AIDS. Primary infection is characterized by a rapid rise in viral load levels, coinciding with a rapid decline in circulating CD4+ T cells *(8)*. The subsequent drop of plasma virus level to a lower steady level, known as the viral load set point, is predictive of the subsequent progression to disease *(9)*. The lower the setpoint, the longer it takes for an infected individual to develop AIDS. Primary infection is followed by a phase of clinical latency, in which no symptoms are apparent, but without adequate treatment, CD4+ cells are continuously being depleted. If CD4+ T cell counts reach $<$ 200 cells/μL, the patient is diagnosed with AIDS.

Depending on the patient's rate of progression, the period of clinical latency can span from three to twenty years. Without therapy, 50% of HIV-1 infected individuals will develop symptoms (e.g. loss of immune function and a decrease in CD4+ T cell counts below 350 cells/μL) in 10 years after HIV-1 infection *(10)*. Based on their rate of progression to HIV-1 disease, infected individuals can be classified either as rapid progressors (RP), typical progressors (TP), or long-term non-progressors (LNTP) *(11)*. Between these categories, differences in CD4+ T cell counts and viral load can be detected, as indicated in figure 1. Rapid progressors often develop AIDS within three to five years, while long-term non-progressors (5-15% of the HIV-1 infected population) often do not show symptoms for ten to twenty years and maintain CD4+ T cell counts above 500 cells/μL. Within the

subgroup of LTNPs, a small subset of individuals can be classified as elite controllers (EC), which comprise < 1% of all HIV-1 infected individuals *(12)*. These patients have undetectable loads of viral RNA (< 50 copies/mL plasma) compared to LTNPs (often < 2000 copies/mL plasma) *(13)*.

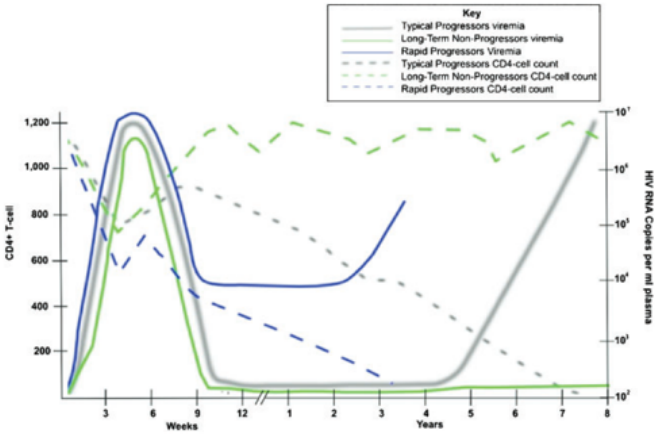

Figure 1. Disease progression in HIV-1 infected individual differs between individuals *(11)*. Based on their rate of clinical progression, patients can be characterized as either rapid progressors, typical progressors or long-term non-progressors. Between these groups, there are major differences in CD4+ T-cell counts and viral load.

The highly variable clinical course of HIV-1 infection is influenced by both viral and host factors. An important viral factor is the ability to switch co-receptor usage *(14)*. Host factors include polymorphisms of chemokine receptors and chemokines (11), and expression of certain HLA alleles *(15, 16)*.

1.3 HLA alleles

The major histocompatibility complex (MHC), known as the human leukocyte antigen (HLA) system in humans, is the most polymorphic region of the entire human genome. HLA molecules are glycoproteins expressed on the cell surface, and play an indispensable role in the immune response by presenting both intracellular and extracellular antigens *(17)*.

The HLA class II loci, HLA-DR, -DQ, and –DP, code for molecules that bind extracellular peptides and present to CD4+ T cells. The HLA class I loci, known as HLA-A, -B, and –C, encode molecules involved mainly in presentation of fragments of intracellular host or pathogen derived proteins to CD8+ cytotoxic T lymphocytes (CTLs). Each HLA molecule can bind a specific set of peptides, thus enabling efficient recognition and elimination of the pathogen.

Interestingly, the HLA class I genotype has been shown to influence in HIV-1 disease progression. A homozygous HLA class I genotype has been associated with a faster progression of HIV-1 disease compared to a heterozygous HLA class I genotype *(18, 19)*. In addition, specific HLA I alleles are known to influence progression rate to disease after HIV-1 infection. Out of all HLA-A, HLA-B, and HLA-C molecules described so far, HLA-B alleles are associated the most with differences in control of HIV-1 disease *(20)*. One of the most consistent associations has been with HLA-B57 and the closely related HLA-B5801 *(21, 22)*. HLA-B27 has also been associated with a protective effect after

HIV-1 infection *(23)*. In addition to HLA-B57 and –B27, at least one of the HLA class I alleles -B13, -B15, -B44, B51, or B58 are carried by as many as 90% of all LNTPs *(24)*. Although HLA-B57 and –B27 can individually influence progression of HIV-1 disease, HLA-B alleles can also exert an additive effect, indicating that control of HIV-1 infection can be the result of the additive effect of some or all of the HLA alleles present in individuals that carry one or more of these protective alleles *(25)*.

1.4 Cytotoxic T cells

CTLs recognize both a HLA molecule and a presented viral antigen, a phenomenon known as HLA restriction. CTLs are important in controlling HIV disease progression. HIV-1 specific CTLs can kill infected cells via HLA class I restricted recognition in in vitro experiments *(26, 27)*. Corresponding with this observation, resolution of acute viremia coincides with a major expansion of HIV-1 specific CTLs *(28, 29)*. In addition, qualitative differences in the CTL mediated response to HIV-1 can be observed when elite controllers are compared to patients with progressive HIV-1 disease *(30-33)*.

Because of the high immune pressure provided by HLA-restricted CTLs in HIV-1 progression, escape from the CTL mediated immune response is a major mechanism of adaptation of HIV-1. Escape happens during the acute, as well as the more chronic phase of HIV-1 infection and is associated with a more rapid progression to AIDS *(34-36)*. CTL escape mutations can target peptide processing, the binding of the peptide to the HLA molecule, or recognition of the presented peptide by T-cells *(37-40)*. This results in loss, or reduction of peptide presentation by the HLA molecule, thereby inhibiting proper activation of the HIV-1 CTL response.

In HIV-1 infected individuals expressing protective HLA alleles, the CTL response is mainly targeted against p24 Gag, which contains a highly conserved region *(41, 42)*. In addition, Gag epitopes among the earliest presented targets on HIV-1 infected cells *(43)*.

1.5 HIV-1 Gag

After cellular entry, reverse transcription and integration into the host genome, retroviral messenger RNA is generated by the host cell mRNA synthetic machinery. Among the produced viral transcripts is the Gag polyprotein p55. During viral maturation, the Gag polyprotein p55 is cleaved into three structural proteins: the matrix (MA) protein p17, the capsid (CA) protein p24, and the nucleocapsid (NC) protein p15, as well as two spacer peptides (SP1 and SP2) and the C-terminal peptide p6 (figure 2B) *(44)*. The Gag capsid protein p24 forms the core that encapsulates the genomic RNA-nucleocapsid complex in the virion, as indicated in figure 2A.

The Gag capsid protein monomer comprises two predominantly alpha-helical domains, a N-terminal (NTD; 133-283), and a C-terminal (CTD; 284-363) domain. The NTD is composed of seven alpha-helices and a beta-hairpin. Via a flexible linker, it is connected to the CTD, as shown in figure 2D *(45)*.

The core surrounding the RNA-nucleocapsid complex is built up from capsid protein hexamer subunits. Three interfaces can be recognized. The NTD-NTD interface creates hexameric rings via interaction of the first three helices of the NTD. This hexamer is reinforced by interaction of the NTD and CTD domains. The created hexameres are then connected via interaction of two CTD domains, which interact by parallel packing of helix 9 of each capsid monomer *(45, 46)*.

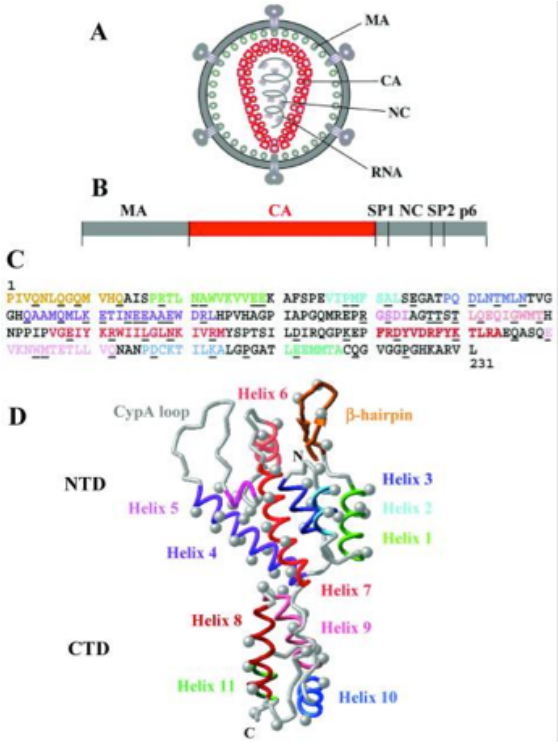

Figure 2. Structural properties of HIV-1 Gag *(47)*. (A) Model of HIV-1 virion. The capsid surrounding the viral core is indicated in red. (B) Domains of HIV-1 Gag polyprotein. The domain encoding the capsid protein (CA) is shown in red. (C) Consensus sequence of HIV-1 CA. The beta-hairpin and alpha helices are coded with colors corresponding to the secondary structures shown in figure D. (D) Model of HIV-1 Gag capsid protein. The NTD consists of the B-hairpin and helices 1-7, while the CTD is formed helices 7-11.

1.6 Role of CypA

Replication of WT HIV-1 depends on the interaction of the host protein cyclophilin A (CypA) with the viral capsid. Via the interaction of the proline-rich CypA binding loop in Gag p24 and CypA, CypA is incorporated in virions *(48, 49)*. Disruption of this interaction, either by mutating the binding loop or the addition of CsA, a CypA binding agent, results in the inhibition of HIV-1 replication *(49, 50)*. CypA is thought to be essential during early replication *(51, 52)*. While the exact role of CypA is not yet completely elucidated, it has been shown that the CypA-CA interaction takes place following entry of the viral core into the cell, and incorporation of CypA into virions is biologically irrelevant *(53, 54)*. CypA can catalyze the cis/trans isomerization of prolyl-peptide bonds in the HIV-1 capsid protein *(55)*, which suggests that CypA has a possible role in uncoating of the viral core following entry into the cytoplasm.

The proline-rich loop is also a target for the restriction factor Trim5α. This restriction factor interferes with the early stages of viral uncoating during replication by targeting incoming virions to the

proteasome. HIV-1 overcomes binding of TRIM5a by binding a molecule of CypA to the same target site *(56, 57)*. Mutations in the CypA binding site or treatment of target cells with CsA allows binding of Trim5α to HIV-1 capsids *(58, 59)*. Thus, acquisition of CypA by incoming HIV-1 capsids may be a defense against restriction factors *(60)*.

1.7 Adaptation of HIV-1 to its host

Transmission between individuals usually occurs through a single viral variant *(61, 62)*. To adapt to the immune system of the new host, or to compensate for the fitness lost in the previous host, the newly transmitted viruses often must mutate. During HIV infection, viral replication and turnover are in general high *(63, 64)*. In addition, the reverse transcriptase involved in the creation of DNA from the RNA template, a process necessary during viral replication, is error-prone *(65, 66)*. Combined with the lack of proof-reading, this will result in the development of the so-called viral quasispecies: a group of highly related, but slightly different, viral variants that are present in one host. This allows the virus to adapt and select for viruses with the most optimal biological properties, e.g. that are capable of evading the CTL response in the host.

1.8 Aim of thesis

Escape from the CTL mediated immune response is an important mechanism of adaptation of HIV-1. In this thesis, an the adaptation of HIV-1 to HLA-B27 and HLA-B57, the two HLA-B alleles most associated with slow progression to HIV-1 disease, is reviewed. An overview of the mechanism of adaptation will be presented, after which the effects to the virus of this specific adaptation will be discussed. As the CTL response in individuals expressing HLA-B27 or HLA–B57 is mainly targeted against Gag p24, the capsid protein, the overview will focus on the effect of the mutations on capsid structure and function.

2
Escape from HLA-B27-restricted CTL response

In individuals expressing HLA-B27, the CTL response is preferentially directed against a specific region within Gag p24 known as the KK10 epitope *(35, 41)*. This highly conserved epitope spans amino acid residues 263-272 and encodes the following stretch of amino acids: KRWIIMGLNK *(67)*.

Viral escape from this highly conserved epitope occurs late in infection and is associated with a loss of control of viremia and progression to AIDS *(30, 68, 69)*. The emergence of mutations within the KK10 epitope can be observed shortly before the reemergency of the viremia, indicating that adaptation to the CTL response can cause loss of immune control *(70)*.

An overview of the mutations associated with escape from the HLA-B27 restricted CTL response is presented in figure 3.

Figure 3. Escape mutations and compensatory mutations in the HLA-B27 restricted epitope. Cleavage of the Gag polyprotein results in the production of Gag p24, which consists of amino acid residues 133-363. Within p24 Gag, one epitope is presented by HLA-B27: KK10 (residues 263-272). The CypA binding loop is also depicted. Escape mutations are shown in dark blue, the compensatory mutation is depicted in lighter colors. Arrows indicate location of mutation.

2.1.1 Escape mutation

Early in infection, viral escape initially occurs through a mutation resulting in the substitution of a leucine on position 268 for a methionine (L268M) *(35, 68, 70)*. This mutation is strongly associated with the expression of HLA-B27 *(71)*.

The initial mutation of L268 in the KK10 epitope represents an escape variant from the early recruited KK10 specific CTLs during primary infection. However, this variant is still sufficiently immunogenic to

elicit rapid development of variant-specific de novo CTL responses during chronic infection *(72)*. This host adaptation results in the selection of secondary R264K mutants *(68)*. R264K is the most commonly observed CTL escape mutation, but substitutions of the arginine with glycine *(68)*, glutamine *(73)*, or threonine *(70, 74)* are also observed.

2.1.2 Mechanism of escape

The substitution of the leucine at residue 268 does not influence HLA binding *(74, 75)*, peptide processing *(74)* or viral fitness *(76)*. Mutation of this leucine may alter the interaction with several key T-cell receptor (TCR) residues *(77)*. Indeed, in HIV-1 infected HLA-B27+ individuals, KK10 L268M variants are very poorly recognized compared to the KK10 WT sequence *(72)*, indicating that the altered interaction with the TCR could affect the CTL response to HIV-1.

The arginine (R) residue at position 2 of the KK10 epitope (residue 264) plays a crucial role in stabilizing the HLA-peptide complexes by interacting with the B pocket of HLA-B2705 *(77)*. Mutation of this arginine results in an epitope that binds poorly to HLA-B2705 *(69, 74)*. The resulting HLA-peptide complexes could be unstable, thus reducing peptide presentation to CTLs.

2.1.3 Effect of escape mutation on virus

Both the R264K single mutant, as well as the R264K/L268M double mutant, have reduced infectivity to 4% and 5%, respectively, compared to that of the WT virus. In addition, both variants failed to maintain viral replication above the level that of cell division. In contrast, the L268M single mutant showed infectivity replication rates similar to the WT virus *(71)*.

HIV-1 variants containing the R264K mutation are defective in generating late reverse transcription products, while the late stages of viral replication are not impaired *(71)*. In contrast to WT HIV-1, the R264K single mutant and R264K/L268M double mutant shows increased infectivity in the presence of CsA, the CypA binding agent. This indicates that the virus is no longer dependent on CypA for its replication, and even replicates better without CypA *(71)*.

The R264K mutation does not seem to alter capsid stability. Both the WT and RK capsids show similar disassembly kinetics, indicating that the mutation does not influence capsid uncoating *(71)*.

2.1.4 Compensatory mutations

While the L268M substitution has been hypothesized to compensate for the defect in replication capacity *(68)*, the combination of L268M with R264K does not result in an increase in viral replication. Only the substitution of a serine on position 173 with either an alanine or a threonine results in the recovery of viral infectivity and replication *(71)*. There is a strong association between the mutation of S173 and the expression of HLA-B27 *(71)*. S173A has been proposed to develop simultaneously with the R264K mutation *(71)*. This compensatory mutation can rescue the defective

replication of mutant virus containing both L268M and R264K from 5% to 88% of WT levels *(71)*. The S173A/R264K mutant replicates most efficiently compared to variants containing the alternative escape mutations, providing an explanation as to why this variant is favored *in vivo* above the alternative escape mutations *(75)*.

The S173A mutation restores the infectivity of the R264K/L268M mutant to a CypA dependent phenotype as displayed by the WT virus *(71)*. Despite occupying linearly distant positions on alpha-helices 2 and 7 of the capsid NTD, R264 and S173 lie in close proximity to one another on the same planar surface of the folded p24 molecule *(71)*, as indicated in figure 4, strengthening the notion that the S173A mutation could compensate for the defect imposed by the R264K mutation.

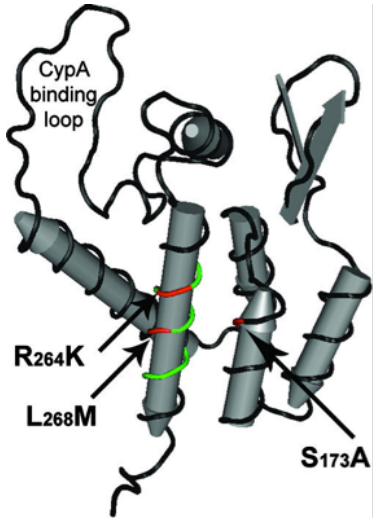

Figure 4. Location of mutations associated with escape from HLA-B27 on the capsid protein NTD *(71)*. Both L268M and R264K (in red) are both located on helix 7, as part of the KK10 epitope (green). S173A (in red), the proposed compensatory mutation for R264K, is not located on the KK10 epitope, but rather is located upstream on helix 2. However, despite the distant positions, R264 and S173 are located on the same planar surface of the folded p24 molecule.

The substantial impact of R264K on in vitro viral replication, and the requirement for compensation by S173A, likely explains the late escape from the KK10-specific CTL responses *(68)*.

2.2 Reversion upon transmission

No intrapatient evolution of HLA-B27 escape mutations can be observed in HLA-B27 positive patients, indicating that the CTL escape mutations are stable *(68, 69)*. In addition, none of the mentioned escape and compensatory mutations reverted to the WT sequence upon transmission to HLA-B27 negative hosts *(78)*. This is supported by the study of Schneidewind et al., in which the replicative capacity of the S173A/R264K/L268M variant is very similar to that of the WT virus *(71)*. In addition, both the single mutants (R264K and S173A) are severely replication deficient *(71)*. Sequential reversion of the mutations would result in a variant with severely reduced viral replicative capacity, lowering the chances of reversion taking place.

3
Escape from the HLA-B57 restricted CTL response

In contrast to mutations in the HLA-B27 restricted epitope, which are associated with disease progression, escape from HLA-B57 mediated immune pressure is associated with a lower viral load and consequent absence of disease progression. Escape mutations in the HLA-B57 restricted epitopes develop shortly, even within weeks, after HIV-1 transmission *(79)*.

There are four HLA-B57 restricted epitopes within p24 Gag, known as TW10, KF11, ISW9, and QW9. For TW10, KF11, and ISW9, mutations have been described that mediate escape from the CTL response. Within the fourth Gag epitope, QW9, two mutations have been described: a substitution of the serine residue 310 with threonine (S310T), and a substitution of glutamic acid 312 with aspartic acid (E312D) *(80, 81)*. However, no difference in prevalence of S310T can be observed between progressors and LTNPs *(81)*. Moreover, the E312D mutation tends to occur in B57-positive subjects with progressive disease *(80, 81)*. Both mutations should not result in impaired binding to HLA-molecules, and no loss of recognition is seen of peptides with the E312D substitution *(80)*. It is thus unlikely that these mutations represent true escape mutations.

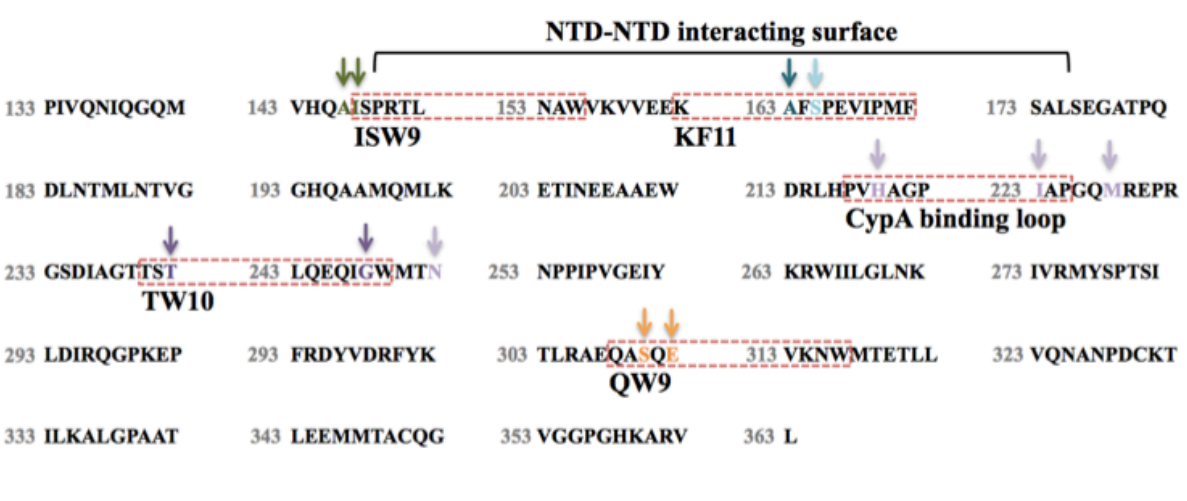

Figure 5. Escape mutations and compensatory mutations in four HLA-B57 restricted Gag epitopes. Cleavage of the Gag polyprotein results in the production of p24 capsid protein, which consists of amino acid residues 133 – 363. Within Gag p24, four epitopes are presented by HLA-B57: ISW9 (residues 147-155), KF11 (residues 162-172), TW10 (residues 240-249), and QW9 (residues 308-316). The CypA binding loop (residues 217-225) is also depicted. Escape mutations are shown in darker colors, compensatory mutations are depicted in lighter colors. Arrows indicate location of mutation. Mutations associated with each epitope are shown in different colors (green for ISW9, blue for KF11, purple for TW10, and orange for QW9).

A complete overview of escape mutations and compensatory mutations in all four epitopes is presented in figure 5.

3.1 TW10

The TW10 epitope (TSTLQEQIGW) is located to residues 240-239 of Gag p24, as indicated in figure 5. TW10 variation is seen after transmission of HIV-1 to HLA-B57/5801 positive individuals and arises because of HLA-57 mediated CTL selection pressure *(82)*.

3.1.1 Escape mutation

The most dominant escape mutation observed is the substitution of the threonine with asparagine on position 3 of the epitope, known as T242N *(82)*. This mutation is seen in approximately 84% of all HLA-B57-positive infected individuals *(82)*.

T242N is often observed together with the mutation of the glycine on position 248, resulting in substitution with alanine (G248A). Like T242N, G248A is strongly associated with the expression of HLA-B57 *(82)*.

3.1.2 Mechanism of escape

As peptides containing the T242N mutation are often still targeted in children, it is most probable that peptide presentation by HLA-B57 is not affected *(83)*. Recognition of peptides containing the T242N mutation is reduced significantly compared to WT peptides *(82)*, suggesting that recognition by the TCR could be altered.

The single G248A mutation also results in a subtle reduction of peptide-recognition, although this effect is much less pronounced as the T242N variant. However, the combination of T242N and G248A results in a complete loss of recognition of the TW10 epitope *(82)*.

3.1.3 Effect of escape mutation on virus

In competition assays, the WT virus outgrows the T242N containing variant, indicating that the T242N mutation results in a fitness cost *(84)*. In contrast, however, this fitness reducing effect of the T242N mutation cannot always be observed in single-cycle replication assays *(84)*. This implies that the T242 substitution results in a slight reduction of viral replicative capacity *(84, 85)*. In contrast to the T242N mutation, the G248A mutation does not result in a loss of fitness *(47, 86)*.

In the presence of CsA, the competitive binding agent of CypA, HIV-1 containing the T242N and G248A mutations replicated less efficiently than the WT virus, while the variant containing only the T242N mutation replicated at rates comparable to the WT virus *(85)*. This indicates that the T242N and G248A mutations within the TW10 epitope result in an increased dependency on CypA to maintain infectivity.

The T242N/G248A mutations thus could hinder the interaction of CypA with the viral capsid, resulting in an early defect in viral replication. While the mutations enable evasion from CTL response to the immunodominant TW10 epitope during primary infection, because of the impact on capsid function, the *in vivo* benefits for HIV-1 are significantly reduced *(85)*.

T242N is located in helix 6 and is part of the capping box motif, which is commonly found at the N-terminal end of alpha helices as an important factor in the stabilization of helices *(87)*. As the threonine on position 242 is thought to be important for stabilizing the electrostatic charge along helix 6 *(84)*, the T242N substitution could result in a reduction of the stabilizing effect of the capping box.

Upon binding of CypA to the CypA binding loop of the HIV-1 capsid protein, there is a conformational coupling of the N-terminal hairpin, helix 6 and the CypA binding loop, as shown in figure 6 *(88)*. The T242N mutation could thus disturb CypA binding to the CypA binding loop. This could result in the necessity of a higher concentration of CypA to allow viral replication, consistent with the observation that there is an increased dependency on CypA to maintain infectivity.

Residue 248 is located in close proximity to the CypA binding loop *(84)*. When CypA is bound, the methionine on position 228 is packed against the glycine on position 248. Changes on position 248 could thus interfere with the packing of the CypA binding loop *(84)*.

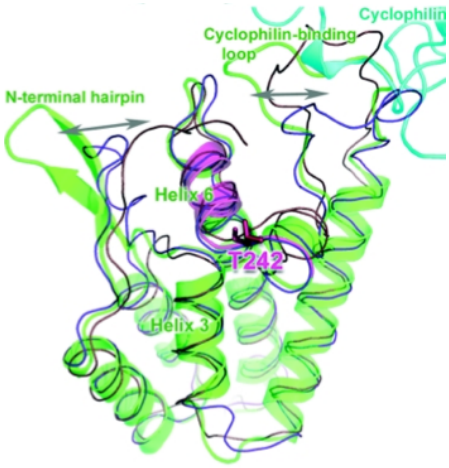

Figure 6. Conformational changes in the capsid N-terminal domain upon binding of CypA *(84)*. The immature capsid structure is shown in grey, the mature capsid is depicted in blue. Upon binding of CypA (cyan), there are conformational changes in the capsid (as shown by the green ribbon structure). Binding of CypA results in movement of the N-terminal hairpin via displacement of helix 6. Residue T242 in the TW10 epitope (shown in magenta) plays a critical role in the stabilization of the electrostatic charge along helix 6. Mutation of T242 will result in destabilization of helix 6, thereby reducing viral fitness.

3.1.4 Compensatory mutations

HLA-B57 has been shown to exert its protective effect in early stages of disease *(89)*. HLA-B57 selects for variant containing mutations in the TW10 epitope, resulting in a replication defect. As infection progresses, mutations appear that compensate for the loss of viral fitness, resulting in higher viral load and progressive disease *(85)*.

Compensatory mutations have been shown to partially compensate for the replication defect induced by T242N/G248A *(85)*. The following compensatory mutations have been proposed: H219Q, I223V, M228I, and N252H *(85)*. With exception of N252H, all of these compensatory mutations are located in the CypA binding loop (figure 7), strengthening the notion that escape from the HLA-B57 mediated immune response alters the interaction of CypA with p24. Also, mutations in the CypA binding loop are associated with increases in viremia following escape in TW10 *(85)*.

Figure 7. Escape mutations in TW10 and proposed compensatory mutations *(85)*. The TW10 epitope is shown in green. Within this epitope, two escape mutations have been described: T242N and G248A. To compensate for the loss of viral fitness imposed by these mutations, several compensatory mutations have been proposed. These compensatory mutations are located in or near the CypA binding loop (H219Q, I223V, and M228I), or in close proximity to the TW10 epitope in helix 6 (N252H).

While none of the individual mutations can be significantly linked to with disease progression, any combination of two or more of these mutations is associated with disease progression *(81)*. In addition, the plasma viral load of individuals with only the T242N mutation is significantly lower than individuals containing one or more of the compensatory mutations *(85)*.

When the prevalence of T242N is compared between progressors and LTNPs, no difference can be observed *(81)*. However, as the infection progresses, an increase in number of potential compensatory mutations can be observed in progressors, while this this increase is not seen in LTNPs *(81)*.

The histidine on position 219 (H219) in Gag p24 binds to N71 and Q111 of CypA through hydrogen bonds and hydrophobic contacts *(90)*. When this histidine is replaced with glutamine (H219Q), the interaction between Gag p24 and CypA is strongly reduced *(91)*. As a result, the HIV-1 variant containing the compensatory mutations is no longer dependent on CypA for its uncoating *(85)*. The reduced dependency on CypA has also been shown for the combination of H219Q, I223V, and M228I *(85)*.

The compensatory mutations that develop within the CypA binding loop thus alter the state of the capsid in such a way that there is no longer a need for CypA, as the fully compensated variant seems to replicate in a CypA-independent manner *(85)*.

5.2 ISW9

Viral escape from the CTL response usually results in a reduction of binding to HLA class I molecules, and/or altered TCR recognition. However, interference with intracellular antigen processing may also lead to escape, as demonstrated by escape mutations present in the ISW9 epitope. Like TW10, ISW9 is located in the N-terminal domain of the capsid protein. It is formed by residues 147-155 (ISPRTLNAW).

3.2.1 Escape mutation

In HLA-B57+ individuals, 61% of the subjects possessed variation at residue 146, involving the substitution of an alanine with a proline (A146P) *(92)*. This mutation is strongly associated with the expression of HLA-B57 *(92)*. The A146P mutation develops as a result of positive selection by immune selection pressure mediated by HLA-B57 *(92)*. In addition to the A146P mutation, 70% of HLA-B57 positive infected individuals also displays a mutation in which the isoleucine on position 147 was substituted with a leucine (I147P) *(92, 93)*.

3.2.2 Mechanism of escape

A146 is not located in the epitope itself, but rather flanks the NH2 terminal side of the epitope. During antigen processing, peptides are transported into the ER, where they undergo N-terminal trimming. This process is catalyzed by ER-aminopeptidase-1 (ERAP-1). As ERAP-1 cannot hydrolyze an X-P bond, the substitution of the alanine on position 146 with proline results in an altered peptide processing, thus escape of peptide presentation by HLA-B57 *(92)*.

3.2.3 Effect of escape mutation on virus

In contrast to the escape mutations in TW10, viral replication itself is not reduced by this mutation *(92)*. A146P is associated with an increase in viral load, thus posing an increased risk for disease progression *(92)*.

3.2.4 Compensatory mutations

As A146P does not reduce viral fitness, it seems unlikely that compensatory mutations are necessary for the virus to thrive in the viral quasi-species. Indeed, no mutations have been reported to compensate for the escape mutation in ISW9.

3.3 KF11

While TW10 and ISW9 responses dominate the HIV-1 specific response in acute infection in HLA-B57 positive individuals *(22, 94)*, KF11 is immunodominant only in chronic infection *(95)*. In a cohort of 520 HIV-1 infected South-African individuals, the previously described escape mutations in TW10 and ISW9 were present in all HLA-B57 positive individuals *(96)*. The KF11 escape mutation A163G was found in 69% of all cases, supporting the notion that KF11 mutations indeed occur subsequent to those present in TW10 and ISW9 *(96).*

3.3.1 Escape mutation

The main escape mutation in the KF11 epitope is the substitution of the alanine on position 163 with a glycine (A163G) *(97)*, as indicated in figure 5. This mutation is strongly associated with the expression of HLA-B57 *(96)*.

3.3.2 Mechanism of escape

Binding affinity of the KF11 epitope to HLA-B57 has been shown to be unaffected by mutation of A163 *(98)*. In contrast, other studies have shown that the off-rate of KF11 is increased by the substitution of alanine at position 2 *(97)*, indicating that stability of the HLA-peptide binding is greatly reduced. As the second residue of the epitope generally functions as an anchor residue *(99)*, this could very well be the cause of the escape of the CTL response.

3.3.3 Effect of escape mutation on virus

Mutation of alanine 163 results in a reduction of viral replication capacity *(96, 100)*. The KF11 epitope is located in the NTD-NTD interacting surface *(101)*, as shown in figure 5. This interface consists of a small core of hydrophobic amino acids, which were shown to be crucial for proper capsid assembly and viral infectivity *(102, 103)*. Although no study has yet shown the effect of the specific A163G on capsid stability, a mutation in this fundamental region of the capsid could alter the interaction between capsid monomers, thereby destabilizing the hexamer structure of the capsid. As capsid stability is crucial for optimal viral infectivity *(86)*, escape mutations in the KF11 epitope could thus reduce viral replication.

3.3.4 Compensatory mutations

The addition of the S165N mutation partially restores the fitness defect imposed by A163G *(96)*, possibly by compensating for the structural changes that are imposed on the viral capsid by the A163G mutation *(96)*. In addition, S165N has also been shown to reduce TCR recognition even further *(98)*.

3.4 Reversion upon transmission

3.4.1 TW10

While the T242N mutation is often stable in HLA-B57 infected individuals, reversion occurs upon transmission to HLA-B57 negative individuals *(82)* and viral variants containing the N242T reversion become dominant in within a year of transmission *(82, 96)*. When the T242N mutation is accompanied by all previously described compensatory mutations, however, the T242N mutation remains stable over time *(104)*. In contrast, the T242N mutation combined with only a single compensatory mutation (I223V or N252H) is not maintained upon transmission to an HLA-B57 negative individual *(104)*. Upon transmission of virus containing the T242N mutation to HLA-B57 positive individuals, no reversion of the mutation can be observed *(104)*.

In contrast to the T242N mutation, the G248A mutation is maintained in HLA-B57 negative individuals. As the G248A mutation does not pose a fitness cost to the virus, there is no selective pressure to revert to the original amino acid *(47, 80)*.

3.4.2 ISW9

Upon transmission from an HLA-B57+ mother to an HLA-B57 negative child, no reversion of the A146P mutation could be observed *(92)*. Because the A146P mutation does not affect viral replication, there is no pressure to revert. This implies that this mutation could accumulate in the population over time.

3.4.3 KF11

In HLA-B57 negative infected individuals, S165N is observed in the absence of A163G, suggesting that the A163G mutation may undergo reversion in the absence of HLA-B57 *(96)*. When both A163 and S165 are mutated, no reversion can be seen over 17 to 26 months of follow-up. Because there is no complete restoration of the replication capacity of the double mutant *(96)*, it is likely that reversion eventually will take place but takes longer than 26 months. When only the single A163G mutation is present, reversion can be observed within 6 months of transmission to an HLA-B57 negative individual *(96)*. A163G reverses much more rapidly than T242N *(96)*.

4
Discussion

4.1 Effect of mutations on viral fitness and capsid structure and function

The in vivo benefits of escape mutations in HLA-restricted epitopes may be severely limited by the resulting loss of fitness. With the exception of the escape mutations in the HLA-B57 restricted ISW9 epitope, all described escape mutations result in a loss of viral fitness. The replication-impaired phenotype is often rescued by the development of compensatory mutations, as described for the HLA-B27 restricted KK10 epitope and the HLA-B57 restricted TW10 and KF11 epitopes. All described epitopes are located in the Gag p24 capsid protein. The escape and compensatory mutations associated with escape from the HLA-B27 and HLA-B57 restricted CTL response have diverse effects on the structure and function of the viral capsid.

4.1.1 TW10

The host protein CypA is proposed to interact with the viral capsid during early infection, thereby assisting with uncoating of the viral capsid *(55)*. The T242N and G248A mutations associated with HLA-B27 escape make the virus more dependent on CypA to sustain viral infectivity, indicating that the interaction of CypA and the capsid could be altered *(85)*. Surprisingly, compensatory mutations associated with the TW10 epitope result in complete CypA independence *(85)*. It could be that the mutations weaken the interactions between capsid monomers. As CypA has been implicated in capsid destabilization, the interactions could be too weak to allow for interference by CypA. However, if CypA would induce premature capsid uncoating, the *in vivo* rescue of the compensated viral variant in cells containing CypA poses a problem for which no solution has yet been proposed.
Acquisition of CypA by the HIV-1 capsid is suggested as a defense against host restriction factors such as Trim5α *(60)*. Alteration of the interaction of the capsid and CypA could thus also possibly modify host restriction.

4.1.2 KK10

In contrast to the escape mutations associated with the TW10 epitope, escape from the CTL response targeted on the HLA-B27 restricted epitope KK10 result in a viral variant of which replication is negatively affected by CypA, as implied by the increase in replication in the presence of CsA *(71)*. This observation suggests that the decrease in viral replication of the KK10 escape variant could be caused by capsid destabilization. The compensatory mutation associated with the KK10 epitope restore a CypA dependency that resembles the WT virus *(71)*, indicating that the possible capsid destabilization is apparently compensated for by the appearance of the S173A mutation.

Both the R264 and L268 residues and the S173 residue have their side chains at the same planar surface of the molecule *(71)*. It has been implied that charge balances in this region may be important for capsid structure and function *(75)*. Substitution of R264 with alanine results in a slower capsid assembly *(105)*. However, since both arginine and lysine are positively charged, this observation cannot explain the loss of viral fitness observed upon mutation of R264. In addition, capsid uncoating has been shown to be unaffected by the escape mutation in the KK10 epitope *(71)*. It is thus yet still unknown how this loss of viral fitness is mediated. In contrast to the R264K mutation, the less commonly observed substitutions of arginine with glycine (R264G), glutamine (R264Q), or threonine (R264T) all result in the loss of a positively charged amino acid, suggesting that charge balances could play a role in capsid structure in these viral variants.

4.2 Accumulation of mutations on the population level

The HLA footprinting effect hypothesis states that adaptation to the most commonly expressed HLA alleles in a population results in the appearance of HLA-associated footprints in the currently circulating viral strains *(97)*. Repeated selection of escape mutations that do not affect viral fitness can eventually result in fixation of epitopes in the viral population that can no longer elicit a CTL response. If a significant loss of viral fitness is induced by the escape mutations, reversion to the susceptible WT sequence is observed upon transmission in the absence of selective CTL pressure.

The TW10 escape mutation T242N reverts upon transmission to HLA-B57 negative individuals *(82)*, but in the presence of all compensatory mutations, no reversion can be observed *(104)*. This indicates that there is a nearly complete compensation of viral fitness. The KF11 associated mutations do revert upon transmission, even in the presence of compensatory mutations *(96)*, while the ISW9 mutations do not revert when transmitted to an HLA-B57 negative individual *(92)*. This indicates that the TW10 and ISW9 associated escape mutations could eventually be fixated in the population, resulting in the disappearance of these epitopes as target for CTLs in HIV-1 infection.

Mutations associated with escape from the HLA-B27 restricted immune response do not revert to the WT sequence when transmitted to HLA-B27 negative individuals *(78)*. Surprisingly, however, there is still a low frequency of these escape mutations observed in the HIV-1 infected population *(78)*. The expression of HLA-B27 is, in general, relatively low, implying that development of the KK10 escape mutations is only observed in a small percentage of all HIV-1 infected individuals. In addition, risk of transmission is particularly high early in infection *(106)*, KK10 escape occurs late in infection, thus lowering the risk of transmission of this specific escape mutation. Moreover, as escape from KK10 is associated with disease progression *(69, 70)*, individuals carrying these mutations may be less likely to infect new hosts because of their clinical status *(68, 69)*. The low prevalence of the escape mutations accompanied by the compensatory S173A mutation might represent the slow rate of accumulation of this variant in the population *(78)*. However, mutations associated with escape

from more frequent HLA alleles such as HLA-B57, e.g. mutations in the TW10 epitope, may have more ability to accumulate on the population level if there is no significant loss of fitness.

In addition, it has been shown that HIV-1 adaptation is not driven by the most common HLA alleles expressed in a population, but more surprisingly, by HLA-alleles that are most protective against progression of HIV-1 infection to AIDS, such as HLA-B27 and HLA-B57 *(107)*.

The HLA footprinting effect could, over time, result in loss of epitopes able to elicit a CTL response. The appearance of less immunogenic HIV-1 variants could pose difficulties in developing new therapy options.

4.3 Consequences for vaccine development

The mutational capacity is one of the major challenges to be overcome in the development of vaccines and therapies. HIV-1 has undergone dramatic diversification since its introduction into humans less than 100 years ago *(108)*. The immune response is an important factor in evolution of HIV-1. If CTL escape mutations are transmissible and stable after transmission, they will spread through the population and the epitope will eventually become extinct. Conversely, if the escape mutant reverts, the epitope will remain useful for CTL-based vaccine design.

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