HIV adaptation to HLA:

Loss of Protection?

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Abstract

HLA-B-restricted CD8$^+$ T cell responses exert strong pressure on HIV replication and adaptation in HIV-infected individuals and are associated with delayed progression to AIDS. Development of escape mutations result in evasion of host immunity at the cost of viral fitness, causing both wild type and escape forms to be controlled by the host. Immune pressure is lost upon transmission to HLA-mismatched recipients, allowing for reversion. However, development of additional compensatory mutations restores viral fitness and eliminates the driving force behind reversion to wild type sequence, causing fixation of escape mutations in the HIV population. This thesis assesses the question what this will mean on the long term for HLA-B protectiveness in HIV infection throughout populations. In the literature study, I conclude that HLA-B alleles are likely to remain the most influential in controlling HIV vireamia in the future as these genes display the highest binding diversity of all HLA class I clades. Adaptation of HIV to current dominant protective CTL epitopes at one end of the epitope spectrum will cause formation of a new niche at the other end, allowing present subdominant protective immune responses to fit that niche and become more dominant in controlling HIV replication. The great binding promiscuity of HLA-B alleles when compared to other HLA clades will cause HIV to adapt more readily to these other clades. HLA-B's binding diversity is therefore likely to ensure that these genes will remain the most influential in controlling HIV vireamia in the future. Obviously, the opposing forces driving escape mutations and sequence conservation will have a major influence on the direction that the adaptation of HIV will take on a population level, thereby influencing which current subdominant protective HLA-restricted immune responses will become dominant in the future.

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Epidemiology

Since the identification of the Human Immunodeficiency Virus (HIV) as the pathogen responsible for causing acquired immunodeficiency syndrome (AIDS) in 1983 (Barré-Sinoussi *et al.*, 1983; Gallo *et al.*, 1983), researchers have tried to find ways of curing, treating and preventing this disease. However, until this day only a single case is known of a patient that has been cured from HIV infection (Hütter *et al.*, 2009). In the meanwhile, millions of others still remain in very poor health all over the world.

The first patients diagnosed with AIDS were homosexuals in the city of New York (USA) in the early 1980's. However, these men were not the first to be infected with HIV. The first transmission of HIV to humans was thought to take place in Africa, where similar retroviruses are found in non-human primates, among which are chimpanzees (Hahn *et al.*, 2000). In Africa chimpanzees are hunted for food, causing the virus to enter the human body. Over several years the virus has adapted to human cells and diverted from the original virus to become HIV (Flint *et al.*, 2009).

HIV is transmitted by sexual and blood-blood contact. According to the World Health Organisation and UNAIDS (the Joint United Nations Programme on HIV/AIDS) around 34 million people worldwide are living with HIV, as of 2011. More than 20 million of these patients live in sub-Saharan Africa and of all patients, less than a quarter receive treatment. Globally, HIV has ended more than 25 million lives in the last thirty years. It thereby qualifies as one of the world's deadliest infectious diseases (WHO, 2012).

Before the development of antiretroviral treatment, there was no therapy for HIV infection. In treatment-naïve patients, the minimal release is estimated to be around 10^{10} virions every day. Combined with the high mutation rate of the virus, it is thought that there is a greater genetic diversity of HIV within a single individual than there is of the influenza virus worldwide during a pandemic. These staggering figures show us what enormous task lies ahead in the battle against HIV and AIDS (Flint *et al.*, 2009).

AIDS pathogenesis

In the first days after HIV infection the virus will infect activated T lymphocytes. Using the machinery of these cells, the virus starts replicating and will produce large quantities of virions. This stage is called the **acute phase**. Up to 5×10^3 infectious particles or 1×10^7 viral RNA molecules per mL of blood can be detected during this stage in HIV infection, causing $CD4^+$ T cell numbers to drop. The acute phase is characterised by flu-like symptoms such as fever, diarrhoea and headaches.

In the period following initial infection the immune system will have had sufficient time to mount a specific cytotoxic T lymphocyte-mediated response against virally-infected cells. The rise of HIV-specific $CD8^+$ T cells coincides with a decline of HIV titres, which subsequently results in an increase of the depleted $CD4^+$ T cell counts due to proliferation of hematopoietic stem cells. Eventually $CD4^+$ T cell numbers are restored up to a level just under normal $CD4^+$ T lymphocyte counts.

Once the immune system has generated an effective response to the virus, viral load will stay low up till several years. During the **asymptomatic phase**, $CD4^+$ T cell numbers decrease at a steady rate of

approximately 60,000 cells/mL per year. Chronic activation of the immune cells and cytopathogenicity of the virus seem plausible causes for this drop in lymphocytes.

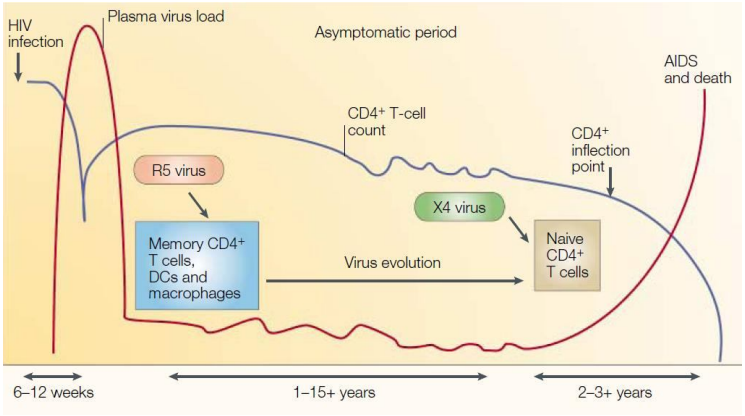

Fig. 1. Schematic presentation of AIDS pathogenesis. Viral load (red) and $CD4^+$ T cell count (blue) are presented as a function of time during the acute, asymptomatic and final stage (Rowland-Jones, 2003).

Viral replication will continue in the lymph nodes, but only at slow rate due to the immunological pressure exerted by cytotoxic T lymphocytes (CTLs or $CD8^+$ T cells). Only very small numbers of infected T cells are observed during this stage and less than 0.5% of these infected cells release virus, suggesting that CTL responses are very effective at this time. The CTLs are responsible for the low vireamia, but the immunological pressure exerted by them will drive HIV adaptation to mutate and escape from the immune response. The amount of HIV-specific CTLs drops towards the end of this stage, whereas the viral heterogeneity increases as a result of immunologic selection.

During the **final phase** of HIV infection patients succumb to AIDS: acquired immunodeficiency syndrome. This phase is characterised by a drop of $CD4^+$ T cell number below 200 cells/mL or less and high viral load. Because $CD4^+$ T cells are essential for all cellular immune responses and more, HIV infection finally causes a breakdown of the immune system. In this last stage, it becomes clear that the virus has outsmarted our immune systems by rapid evolution leading up to immune evasion. The virus population again becomes relatively homogeneous. Viral properties have become more virulent than in the first stages after infection including a wider host cell range and increased viral replication kinetics (Flint *et al.*, 2009).

HIV infected people are treated with anti-viral drugs which slow down viral replication. These drugs interact with different viral proteins essential to HIV infection and replication: integrase, protease, reverse transcriptase and since recently also entry proteins. Simultaneous administration of 3 (or more) drugs to patients gives HIV not the slightest chance of generating sufficient escape mutations within the next virus generation. Being a single stranded RNA virus using a reverse transcriptase with a high mutation rate, HIV replication is accompanied by rapid development of mutations, so that administering 1 or 2 drugs would only temporarily slow down virus replication. To circumvent this problem multiple drugs are given at once. This therapy is called Highly Active Anti-Retroviral Therapy (HAART) (Flint *et al.*, 2009). Inaccurate administration of these medications often occurs, as patients find it hard to take several pills every day. Moreover, the antiretroviral medicines can have serious side-effects, which can be a reason for patients to deliberately fail administration compliance. In these cases the virus will get the chance to develop escape mutations, causing drugs to be rendered useless (Flint *et al.*, 2009). Transmission of drug-resistant HIV types might cause fixation of the escape mutation in the HIV population and cause broader resistance to a particular antiretroviral drug. Fixation of drug resistance in the HIV population can have serious consequences as it will undermine HAART efficacy.

HIV: Interaction with the Immune System

Humoral Immunity

Genome-wide association studies (GWAS) demonstrated that Human Leukocyte Antigen (HLA) class I and II genes are influential in the progression of HIV infection to AIDS (Carrington and O'Brien, 2003; de Bakker *et al.*, 2010). The extreme polymorphisms of the HLA genes fits with an advantage in battling pathogens, which themselves are highly variable. Both HLA class I and II are encoded on the most polymorphic human locus on the short arm of chromosome 6 (Carrington and O'Brien, 2003). HLA class II molecules mediate humoral immunity by presenting peptides of phagocytosed proteins on the cell surface to immune cells which results in the production of HIV-specific antibodies. The antibodies protect the host from the virus through multiple functions such as opsonisation, complement activation, ADCC and virus neutralization (Flint *et al.*, 2009). Initially, HIV vaccine research focused on broadly neutralizing antibodies, but since these antibodies are only present in a very small subset of HIV-1 patients known as 'elite controllers' that control HIV-infection naturally (Gray *et al.*, 2009; Alter and Moody, 2010), the approach was re-evaluated. Titres of these antibodies are generally low as many neutralizing epitopes of the viral surface molecules gp120 and p41 are hidden from the immune system, a sign that suggests HIV adaptation to humoral immunity (Flint *et* al., 2009). Association of class I HLA genes with delayed progression to AIDS have been observed to be stronger and more convincing than associations with class II molecules. This indicates that cellular immunity mediated by class I HLAs is more influential on disease progression than humoral HLA class II immune responses (Carrington and O'Brien, 2003). Nowadays, vaccine development focuses on the role of cytotoxic T lymphocytes in controlling viral replication and thus disease progression via cellular immunity (Phillips *et al.*, 1991; Carrington *et al.*, 1999; Moore *et al.*, 2002; de Bakker *et al.*, 2010).

Cellular Immunity

Studies concerning AIDS progression have identified patients with a strikingly different disease outcome when compared to normal prognosis. In general, after 10 years AIDS has developed in 40% of the patients. 80% of all patients show signs of progression to AIDS after this time by a decline in $CD4^+$ T cells, but 10-17% of HIV positive individuals remain AIDS free even after 20 years (Flint *et al.*, 2009). Of these cases, a very small percentage remains free of symptoms and shows no signs at all of disease progression. These particular patients are known as 'elite controllers' (sometimes termed long-term survivors or long-term non progressors) and characterized by normal $CD4^+$ T cell counts, absence of clinical manifestations (Deeks and Walker, 2007) and undetectable viral load in their blood (Lambotte *et al.*, 2005). The low viral loads prevent transmission of the virus by elite controllers, in contrast to patients in which viral replication is not controlled (Deeks and Walker, 2007).

Researchers found a significant link between the viral control of the elite controllers and the type of Human Leukocyte Antigen (HLA) class I molecules (Kaslow *et al.*, 1996; de Bakker *et al.*, 2010;

Goulder and Walker, 2012). HLA class I molecules are transmembrane proteins that present cytoplasmic peptides to $CD4^+$ T cells to detect intracellular pathogens (Flint *et al.*, 2009). In 1974, Zinkernagel and Doherty showed that HLA class I molecules are essential for virally-infected cell killing by cytotoxic T lymphocytes (Zinkernagel and Doherty, 1974).

HLA class I molecules are expressed by all nucleated cells in the human body and present peptides derived from cytoplasmatic proteins on the cell surface. Proteins are typically hydrolysed to octo- or nanomeres by the proteasome and transported into the endoplasmic reticulum (ER) by the TAP protein (Flint *et al.*, 2009). Here, the peptides are mounted on HLA molecules and the protein complex is targeted to the cell surface for presentation to CTLs, therefore also referred to as CTL epitopes. During inflammation, lymphocytes release cytokines that change the composition of the proteasome to what is known as the immunoproteasome and thereby alter hydrolysis of the proteins (Driscoll *et al.*, 1993). The characteristics of the immunoproteasome differ from those of the proteasome, causing altered cleavage kinetics of proteins and thus presentation of different sets of peptides. Transformation to the immunoproteasome results in more rapid processing of certain epitopes, thereby changing the selection of epitopes presented by HLA class I molecules. In Hepatitis C Virus (HCV) infection for instance this results in more rapidly processing and presentation of HLA-B*27-restricted epitopes (Schmidt *et al.*, 2012), an HLA-B allele that is protective in HIV infection as well. Alteration of proteasome composition can hereby dramatically change HLA class I-restricted cellular immunity.

Presentation of epitopes by Antigen Presenting Cells (APCs) such as DCs leads to recognition and activation of $CD8^+$ T cells, which subsequently kill infected target cells presenting these particular peptides. $CD8^+$ T cells are highly involved in the control of HIV viremia (Balla-Jhagjhoorsingh *et al.*, 1999; Goulder *et al.*, 2001; Carrington and O'Brien, 2003; Kiepiela *et al.*, 2004; Kawashima *et al.*, 2009) and the ability of one's immune system to recognize multiple viral peptides during the acute phase and thereby mount a broad and robust immune response against the virus, is influential on future disease progression (Goulder *et al.*, 2001). In accordance with this observation, it was found that a greater breadth in targeted HIV CTL epitopes correlated with longer absence of progression to AIDS for the studied North American HLA-B*57 cohort (Brennan *et al.*, 2012). Cytotoxic T lymphocytes are therefore of vital importance in the control of HIV viremia. However, the breadth of targeted HIV CTL epitopes is limited by antigen processing. Antigen processing elements such as protein cleavage, transport to the ER and ERAAP-mediated epitope trimming all strongly influence the availability of CTL epitopes. In contrast, the ability of an epitope to bind to HLA molecules only moderately influences epitope dominance (Tenzer *et al.*, 2009). The limitation of one's CTL responses to a few epitopes is known as immunodominance. Obviously, the presence of a minor variety of epitopes will result in their dominance in immune responses.

HLA class I molecules are split up into clades A, B and C. Despite similar function, most HIV-specific CTL responses are HLA-B-restricted (Kiepiela *et al.*, 2004; Goulder and Walker, 2012). Moreover, the progression of HIV disease is strongly associated with the expression of specific HLA-B molecules, but not with HLA-A expression (Kiepiela *et al.*, 2004). Two of the best established HLA class I types associated with slow disease progression are HLA-B*27 and B*57. In 1996, Kaslow *et al.* showed that HLA-B*27 and B*57 are the polymorphisms associated with the slowest disease progression (Kaslow *et al.*, 1996). The observation that HLA-B alleles are the most important HLA genes in HIV control is

demonstrated by the fact that homozygosity of HLA-B alleles is associated with a higher relative hazard than HLA-A homozygosity in two distinct cohorts, while no effect was observed for homozygosity of HLA-C alleles (Carrington *et al.*, 1999). Moreover, a genome-wide association study (GWAS) showed that the mutations that are associated the strongest with viral control are all present in the HLA-B gene (de Bakker *et al.*, 2010). In accordance with this observation, a cohort-study demonstrated that protective CTL epitopes restricted by HLA-B molecules were lost over time, in contrast to CTL epitopes restricted by HLA-A, all suggesting that HLA-B molecules strongly influence HIV replication, whereas HLA-A alleles do not (Schellens *et al.*, 2011). The fact that HLA-B alleles are the most polymorphic alleles of HLA class I clades also supports HLA-B as the most influential gene of this protein family (Goulder and Watkins, 2008).

What can explain the association between HLA-B molecules and delayed HIV disease progression? The preferential binding and presentation of highly conserved regions of HIV proteins such as Gag by protective HLA-B molecules (Borghans *et al.*, 2007) forms part of the answer to this question. Development of escape mutations in conserved regions of a viral protein tends to cause loss of replication capacity (Martinez-Picado *et al.*, 2006; Leslie *et al.*, 2004; Peyerl *et al.*, 2004) and will only be selected for in HIV positive individuals expressing HLA-B alleles capable of targeting these conserved epitopes (Martinez-Picado *et al.*, 2006). Due to an intrinsic property of the Human Immunodeficiency Virus, mutations that decrease binding to HLA-A molecules also correlate with a decrease in viral fitness (Mostowy *et al.*, 2012). Moreover, mutations helping the virus escape from non-protective HLA-A-restricted immune responses are not associated with a higher fitness cost (Mostowy *et al.*, 2012). This would imply that HLA-A alleles are protective only when they target epitopes that escape at the cost of viral fitness. These findings suggest that, as HIV's enormous evolutionary potential would eventually cause escape mutations to arise for every targeted epitope, only those epitopes which mutate at the cost of viral fitness are rendered protective as either effective immune responses are mounted, or the virus evades immunity but does so by decreasing its own replicative capacity. Gag and Nef are the two most immunogenic HIV proteins, proteins to which the most immune responses are mounted (Kiepiela *et al.*, 2004). But in contrast to p24 Gag, the Nef protein is known to be polymorphic (Frahm *et al.*, 2004) and responses to this protein are likely to be easily evaded through HIV adaptation. It is important to note that the Mostowy *et al.* study focused on HLA-A alleles as well as other HIV proteins (Pol gene products Protease and Reverse Transcriptase) than the ones targeted by protective HLA-B alleles (p24 Gag), and therefore nothing regarding HLA-B alleles can be directly concluded from their research, even though it provides an interesting hypothesis that could apply for HLA-B alleles as well.

Another hypothesis why HLA-B-restricted Gag epitopes play a main role in protection from progression to AIDS is that the Gag protein is already abundant in immature viral particles when compared to other HIV proteins (Borghans *et al.*, 2007). The amount of processed Gag peptides is the highest within the first 2 hours after degradation and declines afterwards (Tenzer *et al.*, 2009). Since the strength of CTL immune responses is correlated with epitope abundance (Tenzer *et al.*, 2009), the abundance of the protein is probably causal for the fast Gag-directed immune response of $CD8^+$ T cells that is observed within hours after infection, even before the virus has integrated its DNA in the host cell genome (Sacha *et al.*, 2007). Taken together, the immunogenicity and abundance of the Gag protein make it an ideal target for effective CTL responses in order to delay or even prevent

progression to AIDS, as it enables cellular immunity to target the virus before a productive infection has been established.

Supportive evidence for the protectiveness of HLA-B alleles was found when researchers studied the origins of the Human Immunodeficiency Virus. HIV originated from a retrovirus called Simian Immunodeficiency Virus (SIV) that is endemic in chimpanzees and sooty mangabeys. Its transmission to humans is thought to have occurred on multiple occasions by hunting and eating these animals in Africa (Hahn *et al.*, 2000). However, chimpanzees do not show any pathology when infected with SIV even though high viral loads have been observed (Heeney *et al.*, 2006). For sooty mangabeys the same holds true as high viral loads and low immune activation have been observed in these monkeys, resulting in asymptomatic disease (Choudhary *et al.*, 2007). In rhesus macaques, however, SIV infection can be deadly (Bontrop *et al*, 1996). Probably, both chimpanzees and sooty mangabeys paid the ultimate price fighting the virus: only those monkeys protected by their immune system survived. These observations gave rise to a number of studies investigating the chimpanzee and sooty mangabey immunity to SIV.

In one of these studies, it was found that the $CD8^+$ T cell responses of chimps target conserved epitopes of HIV proteins, CTL epitopes that are also targeted by the immune system of human elite controllers (Balla-Jhagjhoorsingh *et al.*, 1999) expressing HLA-B*27 and B*57 (de Groot *et al.* 2010). The HLA-B*27 restricted immune response targets the p24 Gag epitope KK10 (KRWIILGLNK, Gag residues 131-140) (Goulder *et al.*, 2001), whereas HLA-B*57 targets the p24 Gag epitope TW10 (TSTLQEQIGW, Gag residues 240-249) (Altfeld *et al.*, 2006). HLA-B*57 is closely related to the protective HLA-B*5801 allele, which presents the TW10 epitope as well (Goulder *et al.*, 1996). These CTL epitopes map highly conserved regions of the viral protein Gag. The fact that the same epitopes are targeted by different species even though structural similarity between chimpanzee MHCs and HLAs is absent (Balla-Jhagjhoorsingh *et al.*, 1999), gives an insight in the importance of this viral protein in HIV immune responses. Borghans *et al.* showed that the protection facilitated by HLA-B*2705, B*5701 and B*5801 comes about by their intrinsic preference to present peptides from the conserved part of the Gag protein (Borghans *et al.*, 2007).

Viral diversity within individuals

Studies concerning general viral diversity and the presence of quasispecies within individuals have reported conflicting results. Some of these studies observed a correlation between viral evolution and delayed disease progression (Wolinsky *et al.*, 1996; Halapi *et al.*, 1997), whereas others concluded that a relationship between the absence of viral diversity and delayed disease progression exists (McNearney *et al.*, 1992; Markham *et al.*, 1998). Possibly, both observations reflect different stages of patients controlling the virus at first, but succumbing to disease when escape mutations arise. This explanation also fits with the assumption made by Nowak *et al.* that the immune system can only deal with a certain level of viral diversity. When the threshold is exceeded, disease progression will be the consequence (Nowak *et al.*, 1991). Most of the HLA-associated escape mutations resulting in disease progression are developed during acute infection (Borrow *et al.*, 1997; Goulder *et al.*, 1997; Feeney *et al.*, 2004). Correlates between high viral diversity and delayed disease progression might be a sign of things to come; while the relationship between low viral diversity and

protection from disease progression may be explained by the immune systems of elite controllers mounting very effective immune responses that are not (rapidly) evaded through development of escape mutations. Moreover, given that viral diversity within an individual is dynamic throughout phases of HIV infection (Flint *et al.*, 2009) we can explain why these contradictory observations are both equally credible.

Rachinger *et al.* investigated whether diversity of the HIV envelope is predictive of disease progression in the future. They observed that HIV envelope diversity is associated with immune activation, rather than viral replication (Rachinger *et al.*, 2012). However, it is still unclear whether viral diversity causes damage to the immune system or whether it is a result of adaption to it.

Escape mutations affecting HLA-restricted immunity

Observations that HIV adapts to host immunity by mutating CTL epitopes are widespread (Phillips *et al.*, 1991; Moore *et al.*, 2002; Kiepiela *et al.*, 2004; Kawashima *et al.*, 2009; Schellens *et al.*, 2011). Both the error-proneness of HIV's reverse transcriptase (Roberts *et al.*, 1988) and the extensive ability of the virus to replicate provide HIV with a vast genetic variation. Most of these HLA-associated escape mutations occur during the acute phase of HIV infection and can result in loss of control of viral replication and progression to AIDS (Borrow *et al.*, 1997; Goulder *et al.*, 1997; Feeney *et al.*, 2004). The genetic variation of HIV also provides an enormous challenge in the quest for an HIV vaccine. HIV-1, the most common and infectious type of HIV (Flint *et al.*, 2009), is divided into several clades, which in turn can possess around 30% in sequence variation. Sequence variation within the single clades is lower, but can still be up to 15% (Korber *et al.*, 2001).

HIV escape mutations decrease the efficacy of $CD8^+$ T cells responses to HIV-infected cells. Evasion of the immune system can be acquired through mutations in the CTL epitope itself by decreasing affinity with either the T cell receptor (TCR) or the HLA molecule by which it is presented. Prevention of stable presentation clearly reduces immune responses to the escaping epitope, while altered affinity to the TCR diminishes $CD8^+$ T cell activation. A third way of generating escape mutations takes place through mutating the flanking regions, whereby cleavage patterns are altered. Mutations in the flanking regions and within Gag p17 and p24 epitopes themselves can affect cleavage patterns, causing epitope presentation on the cell surface to diminish, subsequently influencing CTL immune responses (Draenert *et al.*, 2004; Tenzer *et al.*, 2009). Mutations disrupting antigen processing are also known of the HIV proteins Nef and Reverse Transcriptase (Milicic *et al.*, 2005; Culshaw *et al.*, 2012; Zimbwa *et al.*, 2007). Paradoxically, the ability to mount a protective immune response is associated with the rapid development of escape mutations during early infection, as these are the result of $CD8^+$ exerted immune pressure (Brumme *et al.*, 2008).

When HAART is given to HIV-positive patients, cellular immune responses remain important in controlling viral replication. Cytotoxic T lymphocyte-mediated responses continue to shape HIV adaptation. Poor viral response to the therapy is connected to occurrence of HLA class I-associated escape mutations (Knapp *et al.*, 2012). The study of Knapp *et al.* showed that the rate of HIV adaptation to HLAs in HAART treated patients is decreased by 4- to 7-fold when compared to untreated HIV patients. Even though the study of Knapp *et al.* only characterized adaptation of the

HIV proteins Protease and Reverse transcriptase, the finding is likely representable of other HIV-1 proteins. However, besides decreasing HLA class I influence on viral adaptation HAART is not capable of inducing reversion of HLA-associated escape mutations (Knapp *et al.*, 2012), probably because replication rates are very low during treatment.

Escape mutations evading protective HLA-B-restricted immune responses generally coincide with a loss of viral fitness (Martinez-Picado *et al.*, 2006; Peyerl *et al.*, 2004). The epitopes targeted by these HLA alleles are located in conserved regions of HIV proteins such as Gag (Borghans *et al.*, 2007). Mutations in these regions will therefore bring about functional constraints that result in a reduction of the viral replication capacity. To overcome this decrease, compensatory mutations can arise that partly restore viral fitness (Carrington and O'Brien, 2003; Martinez-Picado *et al.*, 2006; Schneidewind *et al.*, 2007).

An example of an escape mutation evading an HLA-B allele has been observed in the case of the protective HLA-B*27 molecule. HLA-B*27 targets the highly conserved p24 Gag CTL epitope KK10 (Goulder *et al.*, 2001). The dominant escape mutation observed for this epitope consists of the single amino acid substitution R264K (Kelleher *et al.*, 2001). Arise of this escape mutation is associated with progression to AIDS (Goulder *et al.*, 1997; Feeney *et al.*, 2004). However, the R264K mutation severely decreases viral replicative capacity due to weakened interaction between the capsid and host protein cyclophilin A, an interaction required for efficient infection of host cells (Braaten *et al.*, 1996). The compensatory mutation S173A is needed for the virus to remain wt viral fitness and restore interaction with cyclophilin A (Schneidewind *et al.*, 2007).

For the other prominent protective HLA-B*57 allele escape mutations have been observed as well. Both HLA-B*57 and B*5801 are known to target the TW10 epitope of the Gag protein (Altfeld *et al.*,

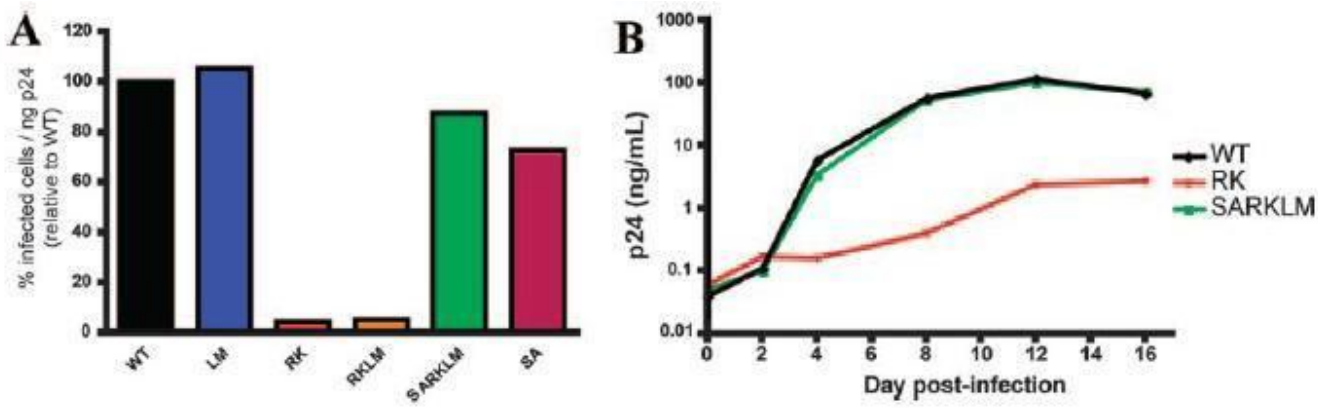

Fig. 2. Escape mutant R264K in protective HLA-B*27-restricted epitope decreases viral fitness *in vitro* which is restored near wild type level by development of compensatory mutations L268M and S173A. (**A**) CEM-GXR cells were infected with wild type or variant viruses and the percentage of infected cells/ng p24 was determined at 48 h by flow cytometry. Values were normalized to wild type values (black). Variant RK (red) and RKLM (orange) decrease viral fitness, while LM (blue), SA (pink) and SARKLM (green) variants displayed only minor variation from wild type virus. Results shown are representative of three or more independent experiments. (**B**) The replicative defect of the RK variant was confirmed using primary cells. PBMC were inoculated with virus, viral spread was measured by p24 ELISA over 16 days. Wild type virus and variant containing all compensatory mutations produced equal amounts of p24 protein, whereas the escape variant produced substantially less p24 protein. Results shown are representative of three independent experiments using different donors (adapted from Schneidewind et al., 2007).

2003). A dominant escape mutant of this epitope involves a substitution of threonine 242 to an asparagine, which reduces viral replication capacity (Martinez-Picado *et al.*, 2006). The T242 residue is important for the stability of one of the helices of the highly conserved p24 Gag protein. Mutation of this part of the protein therefore causes a 10-fold loss of viral fitness.

Escape mutations arise through immune pressure exerted by HLA-restricted host immunity. However, when virus containing the escape mutation is transmitted to an HLA-mismatched recipient, the selective force on the concerning epitope is lost. Transmission therefore allows HIV to revert to wild type sequence to restore viral fitness (Leslie *et al.*, 2004). Reversion is complicated when compensatory mutations have arisen, as these additional mutations have already restored viral fitness, but in a different manner. Transmission to an HLA-mismatched recipient will not result in reversion as there is no force driving it. Generation of compensatory mutations and subsequent transmission will therefore cause fixation of escape mutations which eventually may become present in the consensus sequence of HIV. This observation of viral adaptation to HLA-restricted immunity brings several questions to mind. How does this influence future protection from the virus? Will elite controllers disappear as a whole or will other HLA alleles take the place of current protective HLA types? Will the amount of epitopes targeted by cellular immunity decrease with time as HIV evolves? This thesis will try to answer what this means on the long term for HLA protectiveness in HIV infection throughout populations, through study of up to date research literature.

Discussion

Reversion and transmission

Genome-wide association studies point out that HLA-B alleles are strongly associated with delayed progression to AIDS after HIV infection (Kiepiela *et al.*, 2004; Goulder and Walker, 2012). HLA-B protectiveness will therefore be subjected to change in the strongest manner compared to the other HLA clades. However, the mechanism also works the other way around: HIV was shown to have an influence on HLA-B frequencies at small population levels (native tribes in South America) and causes HLA-B to be the most rapidly adapting HLA class I clade (Belich *et al.*, 1992; Watkins *et al.*, 1992). Since HIV transmission is dependent on viral load, transmission is also dependent on the HLA-B genes expressed within infected individuals and populations, thereby influencing the presence of the virus within a population.

The protective character of HLA-B alleles results from the intrinsic preference of these molecules to present peptides of highly conserved regions of the HIV p24 Gag protein (Borghans *et al.*, 2007). Targeting of the p24 Gag protein is also thought to be beneficial for host immunity in controlling viral replication due to the abundance of the protein in immature virions (Borghans *et al.*, 2007), allowing for fast processing of the protein and quick presentation of peptides by HLA-B molecules (Tenzer *et al.*, 2009), before productive infection has been established (Sacha *et al.*, 2007). HIV adapts to these HLA-restricted immune responses by development of escape mutations (Phillips *et al.*, 1991; Moore *et al.*, 2002; Kiepiela *et al.*, 2004; Kawashima *et al.*, 2009; Schellens *et al.*, 2011). These escape mutations can be positioned within the epitope itself, causing either decreased affinity with the HLA molecule or the TCR, or they can be positioned in the cleavage sites whereby epitope processing is affected in a way that epitope availability is negatively influenced (Draenert *et al.*, 2004; Tenzer *et al.*, 2009).

Development of escape mutations in HLA-B restricted CTL epitopes coincides with a decrease in viral fitness (Martinez-Picado *et al.*, 2006; Peyerl *et al.*, 2004). Upon transmission to an HLA-mismatched host, viral fitness will favour reversion of the mutation (Leslie *et al.*, 2004). Generation of compensatory mutations that partly restore viral replicative capacity eliminate the driving force of reversion (Carrington and O'Brien, 2003; Martinez-Picado *et al.*, 2006; Schneidewind *et al.*, 2007), causing fixation of escape mutations (van Maarseveen *et al.*, 2007). Fixation of such mutations can result in overall adaptation to particular protective HLA-B restricted CTL epitopes in HIV populations.

Whether HIV adapts to HLA-restricted immune responses or reverts after transmission to a mismatched recipient appears to differ from protein to protein as exemplified by the two most immunogenic HIV proteins: Gag and Nef (Kiepiela *et al.*, 2004). Where escape mutations in the Nef protein are observed to revert at a fast rate, Gag escape mutations are less readily reverted upon transmission (Brumme *et al.*, 2008). These observations may be explained by overall conservation of the protein sequences: the Gag amino acid sequence is highly conserved (Frahm *et al.*, 2004) whereas Nef displays a high level of variability (Frahm *et al.*, 2004), suggesting low impact of mutations on Nef functionality. Escape mutations within Gag will therefore be more strongly associated with decrease of viral fitness, in contrast to Nef escape mutations. Compensatory mutations will restore viral replicative capacity for Gag, complicating reversion upon transmission

later on. As Nef escape mutations are less likely to influence viral fitness, compensatory mutations will not be generated, allowing for reversion in the future.

Besides the immune pressure exerted by HLA-restricted immunity, escape mutations can also be induced by HAART antiretroviral drugs including reverse transcriptase, integrase, protease and entry inhibitors (Falkensammer *et al.*, 2002; Quashie *et al.*, 2013; van Maarseveen *et al*, 2007; de Feo and Weiss, 2012). A recent study assessed the prevalence of drug-related resistance among HIV infected individuals in 7 western European countries between 1997 and 2008 (de Luca *et al.*, 2013). The study demonstrated that, as of 2008, 70% of patients exposed to antiretroviral therapy carried at least one drug–related resistance mutation. Resistance mutations to protease inhibitors were observed to remain present after transmission due to development of compensatory mutations (van Maarseveen *et al*, 2007), while mutations in reverse transcriptase were observed to revert after switching therapy (Falkensammer *et al.*, 2002).

HIV adaptation to HLA alleles on population level

Observations of virus adaptation to modern day protective HLAs gives rise to the idea that HIV has already adapted to the most common HLA types in humans (Moore *et al.*, 2002), which has been shown to be the case in variants of Epstein-Barr virus (de Campos-Lima *et al.*, 1993; de Campos-Lima *et al.*, 1997). HLA class I alleles associated with either fast disease progression or viral control seem to differ between racially distinct/geographical populations. Common HLA types differ between these populations, causing the virus to adapt to other HLA alleles and leaving space for effective immune responses restricted by HLAs that fit in the gaps left by the virus in this population. Consensus sequences can therefore differ between populations in which HLA class I allele frequencies are different. Moore *et al.* presented a model for HIV evolution in their *Science* article of 2002, in which viral variability (CTL escape mutations) is determined within the functional limits of the virus to the immune repertoire of a patient. An important factor that was missed by this study, however, are the founder effects present in relatively closely related viruses when assessing polymorphism associations with HLA alleles (Bhattacharya *et al.*, 2007). The Bhattacharya study showed that phylogenetic effects need to be taken into account when trying to detect escape mutations associated with HLA allele expression, since false-positive associations might be the result of immune selection in a population with a high frequency of a particular HLA allele, which may not be present in the studied individual. The selection within an individual patient and subsequent transmission to others will result in the wild type consensus viral sequence for a population (Moore *et al.*, 2002). In addition, it has been shown that HIV-1 adaptation to HLA-restricted host immunity has gradually resulted in an increased replicative ability of HIV over time (Gali *et al.*, 2007).

On population level, HLA-B dominance in immune responses to HIV might be explained by the greater binding promiscuity of HLA-B when compared to the other HLA class I clades (Marsh *et al.*, 2000). The greater epitope binding diversity of HLA-B might cause HIV to adapt more easily to other HLA clades, thereby causing HLA-B to have a bigger influence on HIV viral load. As the virus evolves and escapes from modern day protective HLA-B restricted responses, it is likely that the characteristics of HLA-B alleles ensure that these genes will remain the most influential on AIDS pathogenesis (Kiepiela *et al.*, 2004). Obviously, the opposing forces driving escape mutations and

sequence conservation will have a major influence on the direction that the adaptation of HIV-1 will take on a population level (Martinez-Picado *et al.*, 2006), thereby influencing which current subdominant protective HLA-restricted immune responses will become dominant in the future.

In accordance with this idea, Kawashima *et al.* hypothesize that loss of protection via HLA-B*27 and/or B*57 could promote responses restricted by present day subdominant HLA types. The HLA-B*51 allele is an example of an HLA allele that has lost its protection over time. Development of escape mutations in the concerning CTL epitope arises during acute infection. Upon transmission to a HLA-B*51-negative recipients, the escape mutation does not revert, causing the HIV adaptation on a population level in Japan. This has led to elimination of its protectiveness there, where it was still associated with low vireamia and relatively high $CD4^+$ counts in 1983 (Kawashima *et al.*, 2009). Other HLA-B alleles are likely to fit the niche that has arisen from HIV adaptation towards HLA-B*51, since they display the greatest binding diversity. As the virus has already fully adapted to protective HLA-B*51-restricted immune responses in Japan, HIV adaptation to present-day protective HLA alleles is under construction.

Escape mutants are known for both HLA-B*27 and HLA-B*57 (Goulder *et al.*, 2001; Kelleher *et al.*, 2001; Martinez-Picado *et al.*, 2006), the two best established HLA class I types associated with slow disease progression (Kaslow *et al.*, 1996). Escape mutations in the respective epitopes KK10 and TW10 arise at cost of viral fitness, but when they are accompanied by additional compensatory mutations viral fitness is restored (Schneidewind *et al.*, 2007; Schneidewind *et al.*, 2008; Martinez-Picado *et al.*, 2006). Generation of compensatory mutations that restore viral fitness can result in fixation of escape mutations that evade these protective HLA-B alleles at population level.

The hypothesis that HIV adaptation to current protective HLA-B-restricted immune responses will promote protective subdominant HLA-B restricted responses in the future was confirmed by the results of a mother-child transmission study with HLA-B*27-positive patients (Goulder *et al.*, 2001). HLA-B*27 restricted KK10 escape mutant arising in mothers were transmitted to their HLA-B*27-positive children. This resulted in failing immune responses of these children to the KK10 epitope. However, they did develop HLA-B*27-restricted responses to an otherwise subdominant Gag epitope IK9. As escape from present day dominant epitopes occurs, subdominant epitopes such as IK9 may become dominant in the future. These results also suggest that viruses can accumulate escape mutations as the epidemic progresses eventually causing population level HIV adaptation to protective CTL epitopes, which was confirmed by other studies (Schellens *et al.*, 2011).

Furthermore, HIV adaptation to HLA-restricted immune responses targeting conserved regions of p24 Gag may be replaced by responses targeting other viral proteins. As shown by AIDS vaccine research, rhesus macaques can be elite controllers of AIDS, even though their $CD8^+$ T cells do not target the Gag protein. Instead, they target the viral proteins Vif and Nef (de Groot *et al.*, 2010). This shows that immunity has effectively evolved in a different way in other primates; a way that at someday may result in control of viral replication in humans as well.

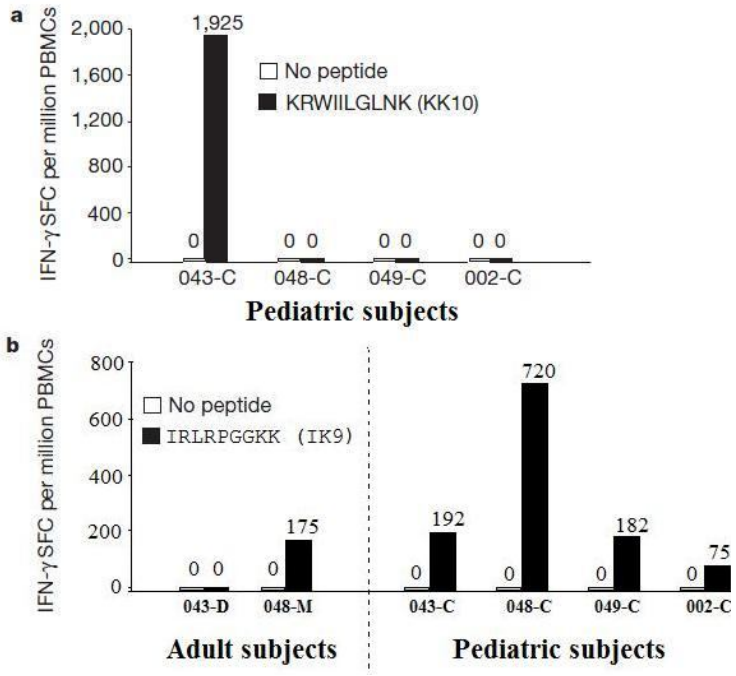

Fig. 3. Recognition of HLA-B*27-restricted epitopes KK10 and IK9 in HIV-infected, HLA-B*27-positive family members. (**A**) Recognition of the B27-restricted epitope KK10 in measured by means of IFN-γ in Elispot assays by HLA-B27-positive children. All three children with no response to KK10 had mothers who expressed HLA-B27 in which the virus had adapted to this response and was subsequently perinatally transmitted to these children; the child with a KK10 response (043-C) received the HLA-B*27 allele from the father. (**B**) Recognition of the B27-restricted epitope IK9 in measured by means of IFN-γ in Elispot assays by HLA-B27-positive family members. One of the children that is unable of recognizing the HLA-B*27-restricted KK10 epitope, as the virus has adapted to this protective response in the mother, mounts a dominant immune response against the otherwise subdominant IK9 epitope, which is also HLA-B*27-restricted, demonstrating that HIV adaptation promotes rise of otherwise subdominant immune responses (adapted from Goulder et al., 2001).

Innate immunity and HIV

HIV-infected individuals resistant to progression to AIDS are known as elite controllers. However, part of these elite controllers lacks effective adaptive T cell responses to the virus (Saez-Cirion *et al.*, 2009). Therefore, innate immunity is likely to be involved in controlling HIV replication in these patients (Tomescu *et al.*, 2012). Besides providing a first line of defence that might be essential in innate elite controllers, innate immunity can also modulate the type of adaptive response as it represents the first step in immune activation (Ploquin *et al.*, 2012), which may be particularly important to targets for vaccine development.

Innate effector mechanisms such as Killer cell immunoglobulin-like receptor (KIR) recognition might be influential in the progression of HIV disease (de Bakker *et al.*, 2010). HLA class I molecules interact with these receptors to modulate the activity of innate Natural Killer cells, which can kill virally infected cells in a similar manner to CTLs. The Bw4 motif that acts as a ligand for interaction with KIRs is expressed by HLA-B*27, amongst others. Homozygosity of Bw4 motif bearing HLA-B alleles has been reported to have a positive effect on HIV disease progression (Flores-Villanueva *et al.*, 2001). However, the dominant role of HLA-B alleles in HIV infection is unlikely to be the result of NK cell activation as the HLA-B Bw4 motif implicated in NK cell interaction is expressed by HLA-B types associated with both slow and rapid disease progression (Kiepiela *et al.*, 2004).

Other factors involved in HIV adaptation

HLA genes are not the only force driving HIV evolution, as the immune system comprises many different kinds of immune responses likely to be involved in controlling HIV replication. Mutations in the viral genome that are not recognised as escape mutations can therefore actually be escape

mutations from other factors influencing viral evolution. Factors implicated in restriction of progression to AIDS by genome-wide association studies include the chemokine receptors CCR5 (Dean *et al.*, 1996; Samson *et al.*, 1996) and CXCR6 (Limou *et al.*, 2010), CYP7B1, an ER-localized enzyme involved in regulating IgA levels (Limou *et al.*, 2012) and the tyrosine kinase DYRK1A (Bol *et al.*, 2011). $CD4^+$ T cell activity, humoral responses and innate effector mechanisms such as Killer cell immunoglobulin-like receptor (KIR) recognition are also thought to influence disease progression in HIV infected individuals (de Bakker *et al.*, 2010). Studying genes other than HLA class I is critical to improve our understanding of AIDS pathogenesis and reveal new opportunities for therapeutic or preventive interventions.

Concluding remarks

The error-proneness of HIV's reverse transcriptase (Roberts *et al.*, 1988) and the extensive ability of the virus to replicate, provide the virus with a vast genetic diversity that allows for immune evasion. Development and accumulation of HLA-B driven escape mutations results in overall evasion of current protective immune responses and implies loss of protection from HIV over time. However, as the virus adapts to immune responses targeted to a specific part of viral proteins, it will become less well adapted to other epitopes that protect and initiate CTL responses (Kawashima *et al.*, 2009). When HIV adapts away from present day subdominant protective HLA-restricted immune responses, these will become dominant protective responses in the future (Goulder *et al.*, 2001). The direction of this adaptation to our immune system and the shifting of protective HLA alleles will be largely influenced by the opposing forces driving escape mutations and sequence conservation (Martinez-Picado *et al.*, 2006). However, the great binding promiscuity of HLA-B might cause HIV to remain least well adapted to this HLA clade when compared to the others. As the virus evolves and escapes from modern day protective HLA-B restricted responses, it is likely that the characteristics of HLA-B alleles ensure that these genes will remain the most influential on AIDS pathogenesis (Kiepiela *et al.*, 2004).

As of 2011, the HIV/AIDS pandemic affected 34 million people globally and qualifies as one of the world's deadliest infectious diseases due to the high mortality rates (WHO, 2012). Studying the loss of protective HLA-B-restricted CTL epitopes through HIV adaptation helps us understand interactions between HIV and cellular immune evolution, as well as HIV evolution. Clearly, HIV adaptation is also relevant to the development of an HIV vaccine. The direction and rate at which the virus will adapt to host immunity and change its consensus sequence will be of vital importance to studies regarding vaccine development either focusing on T cell responses or neutralizing antibodies. It is conceivable that future vaccines can only be used for a limited amount of time, as HIV will evade the responses elicited by the drug when sufficient mutations have been developed.

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List of abbreviations

ADCC		Antibody-Dependent Cell-mediated Cytotoxicity
AIDS		Acquired Immunodeficiency Syndrome
APC		Antigen Presenting Cell
CD		Cluster of Differentiation
CTL	$CD8^+$ T cell	Cytotoxic T Lymphocyte
DC		Dendritic Cell
ER		Endoplasmic Reticulum
ERAAP		ER Aminopeptidase associated with Antigen Processing
GWAS		Genome-Wide Association Studies
HAART		Highly Active Antiretroviral Therapy
HIV		Human Immunodeficiency Virus
HLA		Human Leukocyte Antigen
KIR		Killer-cell Immunoglobulin-like Receptor
MHC		Major Histocompatibility Complex
SIV		Simian Immunodeficiency Virus
TAP		Transporter associated with Antigen Processing
TCR		T Cell Receptor
WHO		World Health Organisation