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Review

Human immunodeficiency virus type 1 long-term non-progressors: the viral, genetic and immunological basis for disease non-progression

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A small subset of human immunodeficiency virus type 1 (HIV-1)-infected, therapy-naive individuals – referred to as long-term non-progressors (LTNPs) – maintain a favourable course of infection, often being asymptomatic for many years with high $CD4^+$ and $CD8^+$ T-cell counts (>500 cells μl^{-1}) and low plasma HIV-RNA levels (<10 000 copies ml^{-1}). Research in the field has undergone considerable development in recent years and LTNPs offer a piece of the puzzle in understanding the ways that persons can naturally control HIV-1 infection. Their method of control is based on viral, genetic and immunological components. With respect to virological features, genomic sequencing has shown that some LTNPs are infected with attenuated strains of HIV-1 and harbour mutant *nef*, *vpr*, *vif* or *rev* genes that contain single nuclear polymorphisms, or less frequently, large deletions, in conserved domains. Studies have also shown that some LTNPs have unique genetic advantages, including heterozygosity for the *CCR5-Δ32* polymorphism, and have been found with excitatory mutations that upregulate the production of the chemokines that competitively inhibit HIV-1 binding to CCR5 or CXCR4. Lastly, immunological factors are crucial for providing LTNPs with a natural form of control, the most important being robust HIV-specific $CD4^+$ and $CD8^+$ T-cell responses that correlate with lower viral loads. Many LTNPs carry the *HLA class I B57* allele that enhances presentation of antigenic peptides on the surface of infected $CD4^+$ cells to cytotoxic $CD8^+$ T cells. For these reasons, LTNPs serve as an ideal model for HIV-1 vaccine development due to their natural control of HIV-1 infection.

Introduction

Who are long-term non-progressors?

Years after the initiation of the 1981 AIDS pandemic, a small subset of human immunodeficiency virus type 1 (HIV-1)-infected individuals emerged with a markedly slow disease progression to AIDS. These persons, referred to as HIV-1 long-term non-progressors (LTNPs), are a group of HIV-1 seropositive individuals who have never been on antiretroviral therapy and have been infected with HIV-1 for several years – in some cases up to 20–25 years (Mikhail *et al.*, 2003). They make up between 2 and 5 % of all HIV-seropositive individuals (Okulicz *et al.*, 2009). Throughout their course of infection LTNPs maintain low levels of viraemia and elevated $CD4^+$ T-cell levels in contrast to rapid progressor HIV-1 subjects who succumb to AIDS after a few years of infection in the absence of therapy.

Normally in HIV-1 pathogenesis, chronic asymptomatic infection can span from 3 to 20 years depending on the person's rate of disease progression (Levy, 2009). The viral loads in most persons infected with HIV-1 decrease from several million RNA copies ml^{-1} in the acute phase to 11 000–50 000 copies ml^{-1} in the chronic asymptomatic phase (Gupta *et al.*, 2007). Compared with rapid progressors who typically progress to AIDS within 3–5 years, LTNPs are often asymptomatic for 10–20 years with elevated $CD4^+$ T-cell counts >500 cells μl^{-1} (Fig. 1) (Pantaleo & Fauci, 1996). In comparison, LTNPs – sometimes referred to as viraemic controllers – maintain between 5000 and 15 000 RNA copies ml^{-1} during the course of their infection with the majority having fewer than $\leqslant$10 000 copies ml^{-1} and many having 50–2000 copies ml^{-1} (Hunt, 2009; Mikhail *et al.*, 2003). Recent studies have shown that LTNPs and other controllers with undetectable viral loads control HIV-1 infection within 1–2 years of infection (Madec *et al.*, 2005; Okulicz *et al.*, 2009).

Individuals referred to as slow-progressors (SP) or long-term survivors are less conservatively defined in the literature as having lower $CD4^+$ T-cell levels (<500 cells μl^{-1}) and higher viral loads than other LTNPs (Betts *et al.*, 1999; Levy, 2009). A small subset of LTNPs ($\leqslant$1 %) referred to as elite controllers (ECs) or elite suppressors have undetectable levels of viral RNA (<50 copies ml^{-1})

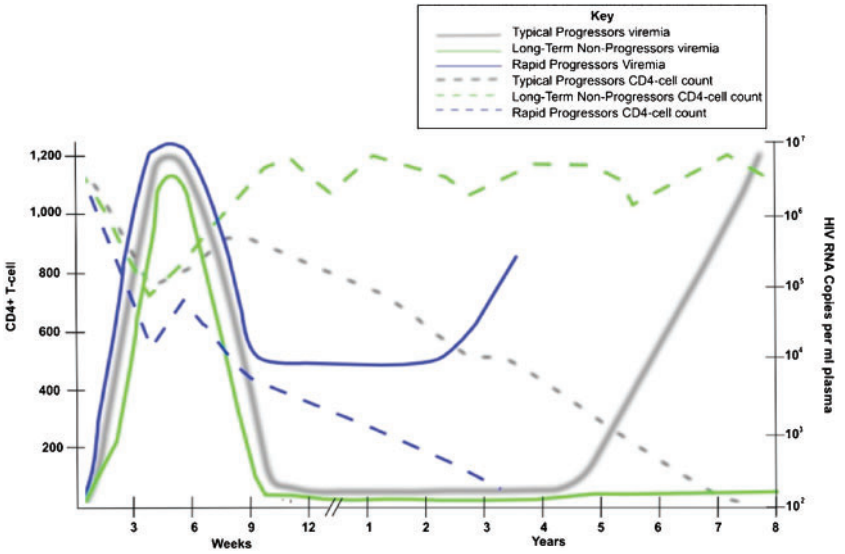

Fig. 1. Disease progression in HIV-1-infected typical progressors, LTNPs and rapid progressors according to $CD4^+$ T-cell counts and viraemia.

for an unspecified period of time that could be months rather than years (Table 1) (Piacentini *et al.*, 2009; Saksena *et al.*, 2007). The HIV Controller Consortium estimates that roughly 1 in 300 HIV-infected persons meet the criteria for being an EC and recently, in a cohort of 4586 HIV-infected subjects followed since 1986, Okulicz *et al.* (2009) showed that 0.55 % are ECs (Okulicz *et al.*, 2009; Walker, 2007). In comparison, 3.32 and 2.04 % of individuals from the same cohort are classified as LTNPs with a $CD4^+$ T-cell count >500 cells μl^{-1} during 7 and 10 years of follow-up, respectively (Okulicz *et al.*, 2009).

Though the majority of ECs and LTNPs have $CD4^+$ T-cell counts within normal ranges, several can undergo immunodeficiency and opportunistic infections as a result of $CD4^+$ T-cell depletion (Hunt, 2009). For instance, 7 % of controllers from the CASCADE cohort (Concerted Action on SeroConversion to AIDS and Death in Europe) eventually progressed to AIDS (Madec *et al.*, 2005). Similarly, 15 and 7 % of individuals from an EC cohort (n=30) infected for >16 years and with very low viral loads (<75 RNA copies ml^{-1}) had $CD4^+$ T-cell counts <350 cells μl^{-1} and met the clinical definition of AIDS (Hunt, 2009; Hunt *et al.*, 2008). This evidence shows that elite control of HIV-1 infection as defined by nearly undetectable viral loads in the absence of therapy does not correlate with a preservation of $CD4^+$ T-cell counts. Interestingly, viral load has been shown to be a likely determinant for disease progression among controllers who are defined based on levels of viraemia, but with no criteria for $CD4^+$ T-cell counts. In their recent study, Okulicz *et al.* (2009) found that individuals they classified as viraemic controllers (50–2000 RNA copies ml^{-1}) progressed to AIDS more rapidly and had low $CD4^+$ T-cell counts (<350 cells μl^{-1}) than ECs who maintained stable and more elevated $CD4^+$ T-cell counts.

Table 1. Definitions of major controller populations

	EC	LTNP
$CD4^+$ T-cell level (cells μl^{-1})	⩾500*	⩾500
Viral load (copies ml^{-1})	⩽50	⩽10 000*
Antiretroviral therapy	No	No
Years without AIDS	Months–years	⩾7–20*

*Values can vary in studies.

There is no official standard for defining the various controller subgroups and variations in clinical definitions can significantly alter clinical outcomes. For example, studies show that when comparing controller groups meeting criteria for low viral loads but not high $CD4^+$ T-cell counts with those having high $CD4^+$ T-cell counts but not low viral loads, the former are more likely to undergo continual $CD4^+$ T-cell loss and progression to AIDS (Hunt *et al.*, 2008; Madec *et al.*, 2005). For the sake of simplicity, this review will refer to controller subgroups based on criteria most commonly used by investigators (Table 1). An important caveat that should not be overlooked is that EC mechanisms of control are not necessarily the same as LTNPs because of their differences in $CD4^+$ T-cell counts (often higher in LTNPs) and viral loads (lower in ECs). Nevertheless, the two groups probably share some mechanisms of control due to their favourable clinical outcome, which will be discussed in further detail in this review.

Of interest is the pathogenesis of natural HIV-2 infection, which mirrors HIV-1 progression in LTNPs. HIV-2 is endemic to West Africa and shares the same route of

transmission as HIV-1, yet the majority of those infected with HIV-2 will not progress to AIDS (Leligdowicz & Rowland-Jones, 2008). Host HIV-2 immunology is remarkably similar to that of HIV-1: infected individuals have a slow $CD4^+$ T-cell decline and low plasma viral load, maintained NK cell functioning, broad antibody neutralization and *gag*-specific cytotoxic $CD8^+$ T cells directly correlated with lowering patient viraemia (Leligdowicz & Rowland-Jones, 2008).

This review focuses on the three central factors associated with disease non-progression – virology, genetics and immunology (Fig. 2). The principal viral factor believed to slow disease progression is HIV-1 strain attenuation arising from mutations in viral accessory genes that cause a reduction in virus infectivity and replication. In terms of host genetics, some LTNPs display polymorphisms in chemokine receptors (CCR5 and CXCR4) and chemokines (i.e. MIP-1α and RANTES). With respect to immunological factors, *HLA class I* alleles (i.e. *HLA-B57* and *-B27*), which present antigenic virus peptides to cytotoxic T cells, are found overexpressed in LTNPs. Additionally, LTNPs commonly display an HIV-specific $CD4^+$ and $CD8^+$ T-cell response capable of inducing both cytotoxic and non-cytotoxic activity against the virus.

The evidence shows that LTNPs are distinct from other HIV-infected individuals and their natural form of immunity against disease progression serves as an ideal model for the development of an HIV-1 vaccine. Much can be learned from LTNPs and their remarkable ability to overcome challenges like HIV-1's antigenic variation and immune evasion. A strong vaccine candidate should induce immune responses similar to those of LTNPs, which heavily rely on cell-mediated immunity (CMI) and Gag-specific $CD8^+$ T cells. The most realistic approach to developing an HIV-1 vaccine is one that provides partial immune control against viral replication, while preventing clinical disease and not one that prevents virus infection. Lessons have been learned from the failures of the recent STEP vaccine study (Buchbinder *et al.*, 2008), which was a phase 2 CMI-based HIV-1 vaccine trial that failed to demonstrate a protective response in vaccinees, particularly in persons with pre-existing anti-vector immunity. By better understanding the problems associated with recombinant vector-based HIV-1 vaccine design, future HIV-1 vaccine trials should use natural controllers such as LTNPs as a model, particularly for their ability to induce cross-reactive and polyfunctional CTLs that both proliferate and inhibit viral replication through granzyme B-mediated killing.

Viral features that contribute to non-progression

LTNP virus attenuation

Decreased HIV-1 strain evolution correlates with weaker viral fitness and the inability to evade the host immune system. Some studies have shown that virus strains from LTNPs are less evolved and thus less capable of evading the host immunological response when compared with progressor strains (Sandonís *et al.*, 2009; Wang *et al.*, 2003). The classic example of HIV-1 LTNP virus attenuation is the Sydney Blood Bank cohort (n=8), whose members were infected from blood transfusions from the same donor infected with an HIV-1 strain that contained a large

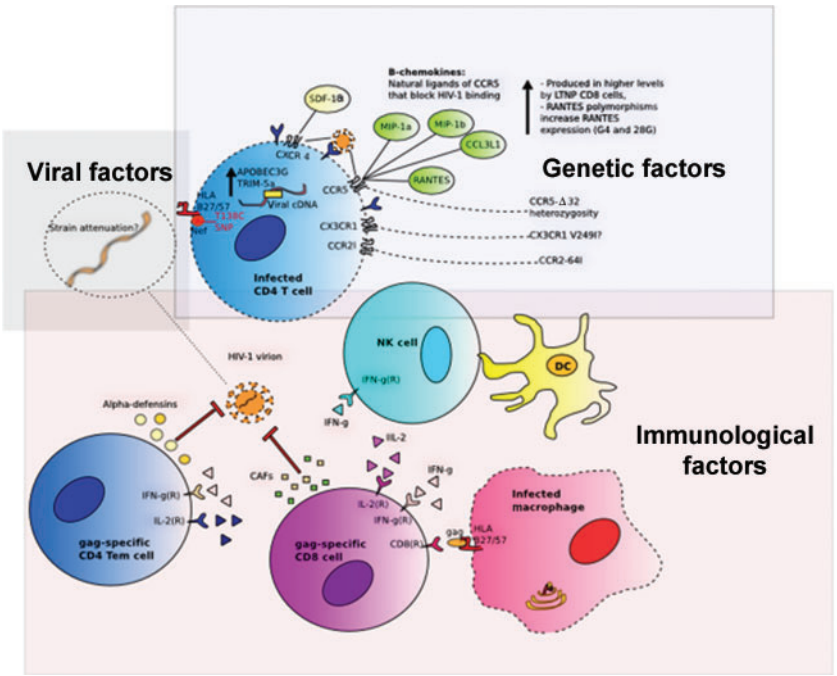

Fig. 2. Overview of the viral, genetic and immunological factors associated with long-term control of HIV-1 infection.

deletion in a long-terminal repeat from a conserved and biologically significant area in the *nef* gene (Crotti *et al.*, 2006; Learmont *et al.*, 1999). Six of the subjects went on to become LTNPs and maintained stable $CD4^+$ T-cell levels and low viral loads for over 10 years. Experimental evidence shows compared with virus clones from progressors, LTNP clones replicate more slowly and infect $CD4^+$ thymocytes less effectively in tissue culture (Choudhary *et al.*, 2005). In their study, Choudhary *et al.* (2005) found that in the absence of interleukin-1 (IL-1) and transforming growth factor beta (TGF-β) – which upregulate C-C chemokine receptor type 5 (CCR5) expression – infectivity and cytopathic events were reduced in LTNP HIV-1 CCR5 clones compared with those of progressors. These findings suggest that these LTNPs harbour attenuating virus mutations that interfere with infection. However, reductions in viral fitness such as these are unlikely to fully explain LTNP control of viral replication, especially in light of other LTNPs that have replication competent viruses (Alexander *et al.*, 2000; Blankson *et al.*, 2007).

Viral attenuation results from mutations in viral structural and non-structural genes. Several studies have reported major attenuating gene deletions in LTNPs that are associated with the control of infection (Calugi *et al.*, 2006; Deacon *et al.*, 1995; Huang *et al.*, 1998). However, this association is difficult to prove because attenuation could actually be the effect rather than the cause of long-term control. For example, following infection with a non-attenuated viral strain a strong T-cell response could induce mutations in the genome of HIV-1 that render the virus weaker. Miura *et al.* (2010) have recently shown that when constructing chimeric viruses expressing the *gag-protease* from the earliest post-infection samples from patient viruses, ECs (n=18) had significantly lower replication capacities than progressors (P=0.0003). These findings demonstrate that control of infection could be established very early and in the case of these patients could be the result of their infection with attenuated, poorly replicating viruses.

In the first study to sequence the entire genome of HIV-1 isolates from ECs (n=4), Blankson *et al.* (2007) were only able to find minor mutations that did not affect the function of HIV-1's genes and thus could not explain the subjects' elite suppression of infection. Additionally, all of the viral isolates were replication competent and were capable of growing normally in primary $CD4^+$ lymphoblasts. In a subsequent experiment (Bailey *et al.*, 2007), the investigators sequenced the viral isolates of one of the ECs before and after virologic breakthrough in order to determine whether any mutations were associated with their disease non-progression. They found that the subject retained the same detectable mutations at all time points, which demonstrates that their mutations do not explain a mechanism for the control of infection and that other mechanisms must play a part in their long-term control of infection. If the same can be shown for the remaining three ECs from the original study (Blankson *et al.*, 2007), this would potentially rule out viral attenuation as the cause of their disease non-progression, which should shift the focus to immunological and genetic modes of control.

LTNP recognition of *HLA-B57*-restricted peptides is strongly correlated with long-term control of infection and viral escape mutations in these epitopes are associated with eventual virologic breakthrough in controllers. Bailey *et al.* (2007) compared virus isolates from a therapy-naïve subject infected with a multiple drug-resistant X4-tropic virus before and after becoming viraemic and found that mutations in *B57*-restricted *gag* epitopes targeted by CTLs correlated with the viral escape in the subject, who underwent an increase in plasma viraemia after having an undetectable viral load for several months. Further research should determine whether the same is true for a wider range of *HLA class I* alleles and controller subjects.

Challenging their hypothesis that LTNPs may harbour virus isolates less capable of downregulating *HLA class I* alleles responsible for generating a robust CTL response, Nou *et al.* (2009) found that isolates from ECs (n=5) and progressors (n=8) equally downregulated *HLA-A2* and *HLA-B57* alleles. Furthermore, a recent longitudinal study (O'Connell *et al.*, 2010) that examined proviral and plasma samples from six ECs for 5 years showed that there were no significant mutations in *B57/58-* and/or *B27*-restricted *gag* epitopes. In fact, much of the observed virus evolution was due to synonymous mutations rather than mutations in *B57/58-* and/or *B27*-restricted immunodominant *gag* epitopes.

Genomic sequence analysis of virus genes from controllers

Nef. One of the normal functions of the *nef* gene is to downregulate host antigen presentation. It does so by binding to *HLA class I* receptors with its binding motif thus interfering with intracellular *HLA class I* antigen processing (Tolstrup *et al.*, 2006). Large deletions in the *nef* genome can disrupt the protein coding sequence and smaller mutations can lead to premature termination of the gene's protein synthesis. Evidence has shown that reduced inhibition of *HLA class I* antigen presentation on $CD4^+$ T cells is seen in LTNPs but not progressors, and is due to different mutations outside of the conserved region of the *nef* gene (Casartelli *et al.*, 2003). There were no patterns seen in mutations among LTNPs, making it unlikely that their *nef* genes were defective as a result of a common, non-random process.

Other studies have reported that LTNPs infected with HIV-1 from blood transfusions had very similar deletions in *nef* proviral DNA as members of the Sydney Blood Bank cohort (Brambilla *et al.*, 1999; Deacon *et al.*, 1995), which were statistically significant when compared with mutations found in progressors (Casartelli *et al.*, 2003; Geffin *et al.*, 2000). These attenuating deletions in *nef* have been shown to

inhibit *in vitro* HIV-1 replication in PBMCs when compared with virus clones lacking the mutations ($P<0.001$).

A T⟶C point mutation at position 138 (*T138Q*) in *nef* has been reported to occur more frequently in LTNPs versus progressors and is cited as being a cause of decreased viral fitness and replication (Kirchhoff *et al.*, 1999; Premkumar *et al.*, 1996; Tolstrup *et al.*, 2006). Kirchhoff *et al.* (1999) performed extensive sequence analysis to reveal the mutation was present in 8.5 % of 59 LTNPs/SPs, while it was completely absent in 32 progressors. In general, attenuating *nef* mutations such as *T138Q* are uncommon in most controllers, as has been shown in a large cohort of ECs ($n=63$), in which only three subjects had deletions in their *nef* gene. The same has been shown in other cohorts, which rules out the possibility that defective *nef* genes are a cause of long-term control of HIV-1 infection (Blankson *et al.*, 2007; Mologni *et al.*, 2006).

Vpr. The *vpr* gene has one of the most highly conserved sequences in the HIV-1 genome and a loss of *vpr* function significantly impairs HIV-1 infection because it impedes many of the gene's functions (i.e. G2 cell cycle arrest and apoptosis, T-cell depletion, nuclear localization of the HIV preintegration complex) (Caly *et al.*, 2008; Lum *et al.*, 2003). Whereas some studies have shown that LTNPs have increased frequencies of inhibitory mutations in the *vpr* gene compared with progressors (Caly *et al.*, 2008; Lum *et al.*, 2003; Mologni *et al.*, 2006; Wang *et al.*, 2003), others have not found genotypic differences in *vpr* functional domain sequences or HLA-binding motifs between LTNPs and progressors (Lai & Chen, 2006; Shen *et al.*, 2008).

Vpr naturally induces G2 cell cycle arrest from the activity of a highly conserved motif at aa 71–82 in the gene's C-terminal region (Macreadie *et al.*, 1995). Genomic analysis of this motif shows that some LTNPs have a mutation at position 77, called the *R77Q* mutation, which interferes with *vpr*-mediated cell cycle arrest and has been positively selected in some LTNPs (Lum *et al.*, 2003; Mologni *et al.*, 2006). For instance, Lum *et al.* (2003) found the mutation in 75 % of a large cohort of LTNPs ($n=146$), while it was present in only 36 % of progressors ($n=55$) ($P<0.001$). Caly *et al.* (2008) report a novel point mutation in an LTNP at position 72 (*F72L*) that significantly hindered Vpr's localization into the nucleus compared with wild-type (WT) Vpr. The investigators attributed the mutation to *in vivo* selective pressures that positively correlated with the LTNP's control of infection.

Vif. *Vif* is an accessory gene that normally promotes HIV-1 infectivity by enhancing viral replication and inducing the degradation of the endogenous anti-retroviral factor, apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G (*APOBEC3G*) (Zhang *et al.*, 2008). The *vif*-mediated degradation of *APOBEC3G* occurs through the recruitment of the Cullin5-ElonginB-ElonginC E3 ubiquitin ligase, which induces polyubiquitination and proteasome-mediated degradation of *APOBEC3G* (Zhang *et al.*, 2008). The relationship between *vif* mutations and disease progression is unclear due to conflicting study results; some have shown that LTNPs have fully intact *vif* genes (Huang *et al.*, 1998), while others have failed to find inhibitory mutations in the gene that could be an explanation for a reduction in LTNP virus isolate *in vitro* replicative capacities (Alexander *et al.*, 2002; Rangel *et al.*, 2009).

Rev. Defects are rarely reported, and in one study (Papathanasopoulos *et al.*, 2003) sequence analysis from two vertically infected LTNP siblings with HIV-1 subtype C virus revealed that both subjects had ORF and full-length sequences for all genes except *rev*. Instead of exon 2 having a 16 aa truncation in the 3′ end, which is typically found in subtype C viruses, the exon had a 3 aa insertion. Interestingly, the same mutation was found in subjects from the Sydney Blood Bank LTNP cohort (Oelrichs *et al.*, 1998). Another study (Iversen *et al.*, 1995) found an inhibitory mutation (*L78I*) in the activation domain of the *rev* gene from an LTNP, which was previously associated with slowing HIV-1 disease progression by impairing cell growth (Malim *et al.*, 1991).

Gag. Single and double amino acid deletions have been identified in LTNPs in the C terminus of *gag*'s p17 and p6 peptides albeit they have not been shown to correlate with lower viral loads in these subjects (Alexander *et al.*, 2000; Miura *et al.*, 2008). Miura *et al.* (2008) analysed viral sequences at the single-codon level and found only three codon differences in the *gag* sequences from more than 50 ECs and 80 progressors, which did not correlate with elite control of infection. Miura *et al.* (2010) have also recently shown that *gag-protease* chimeras constructed from acutely infected individuals (who later went on to become controllers) had significantly reduced *in vitro* replication capacities compared with chimeras constructed from acutely infected individuals that went on to become non-controllers ($n=80$) ($P=0.0003$). Controllers also had a significantly higher prevalence of antiretroviral resistance mutations ($P=0.018$) that interfered with viral replication. These results demonstrate the importance of identifying instances of reduced viral fitness in association with defective Gag proteins during early primary infection, which can be predictive of an individual's subsequent control of infection.

Env. The Env protein mediates HIV-1 entry into host $CD4^+$ T cells. By using a novel cell line that allowed for inducible surface expression of CD4 and CCR5 receptors, Lassen *et al.* (2009) found that *env* clones from ECs ($n=7$) were significantly less able to perform CD4- and CCR5-mediated entry compared with *env* clones from chronically infected progressors. The reduction in *env* fitness resulted in significantly slower infection of EC $CD4^+$ T cells compared with acutely infected subjects and progressors ($P<0.0001$).

Significance of viral factors associated with control: implications on vaccine design

There is not enough evidence to demonstrate patterns in attenuating mutations from LTNP and EC virus isolates that could contribute in any meaningful way to their control. Interesting are the studies that follow controllers at early time points during their acute phase of infection, before and after they have gone on to establish long-term control of infection. However, these studies are restricted to only a handful of controllers at a time and do not provide strong enough reason to believe that similar immune-induced mutations would be present in all controllers. While the *nef* and *vpr* mutations cause viral attenuation in some LTNPs and explain their infection with a lowly pathogenic virus, screening of larger cohorts of LTNPs has demonstrated that they are infrequently present (i.e. the *T138Q nef* mutation). Instead of being solely responsible for HIV-1 disease non-progression, inhibitory mutations in HIV-1 genes probably act in combination with other virological and immunological factors to facilitate control of infection.

Perhaps the most helpful lessons of viral mutations associated with long-term disease control have come from monkey models rather than human models. Interesting data from Mansfield *et al.* (2008) who vaccinated rhesus macaques with attenuated simian immunodeficiency virus (SIV) containing 100 aa deletions in the V1–V2 region of the *env* gene had significantly lower viral loads (<200 RNA copies ml^{-1}) after 8 weeks of infection compared with macaques infected with parental SIV. No such attenuating mutations in *env* have been detected in LTNPs. Despite exciting findings from the macaque vaccinations, there are many caveats that must be addressed before proceeding with similar experiments in humans, including whether the attenuated vaccine is capable of broad, cross-reactive humoral immunity against the frequently mutating *env* gene and whether it continues to provide near complete inhibition against virus infection for periods longer than 8 weeks.

Host factors that promote control of infection

Genetic factors

Chemokine receptor polymorphisms. *CCR5-Δ32* polymorphism. CCR5 is normally expressed at very low levels on the surface of naïve $CD4^+$ T cells and at higher levels in activated $CD4^+$ memory T cells as well as in monocytes and macrophages (Potter *et al.*, 2007). A number of studies (Table 2) show that LTNPs carry a copy of the 32 bp deletion in the gene, called the *CCR5-Δ32* polymorphism, which introduces a premature stop codon in the *CCR5* gene that causes it to be produced as a truncated protein that is trapped in the endoplasmic reticulum. The frequency of the polymorphism varies with different ethnicities and it is estimated that among populations where the polymorphism is found more frequently, such as northern Europe and western Asia, roughly 1 % of Caucasians are homozygous and between 9 and 20 % are heterozygous for the *CCR5-Δ32* polymorphism (Lucotte & Mercier, 1998; Pereyra *et al.*, 2008). The polymorphism is nearly absent among native Africans, East Asians and American Indians (Stephens *et al.*, 1998). Studies that find LTNPs are more frequently heterozygous for the polymorphism than progressors and uninfected controls have shown that possessing one copy of the polymorphism results in a reduction of CCR5 expression on $CD4^+$ T cells and is associated with slowing progression to AIDS (Dean *et al.*, 1996; Eugen-Olsen *et al.*, 1997; Romiti *et al.*, 2000). Individuals homozygous for this deletion are resistant to R5-tropic HIV-1 infection (accounting for the majority of transmitted HIV-1 strains) due to the abrogated CCR5 surface expression on $CD4^+$ T cells (Dean *et al.*, 1996).

Study results are conflicting with regard to *CCR5-Δ32*'s association with control of HIV-1 infection. Those that argue that it is associated with disease non-progression show that HIV-infected subjects heterozygous for the allele have a significantly slower progression to AIDS, whereas several more recent studies have found that the polymorphism is absent in their LTNP cohorts (Table 2). In some larger controller cohorts, LTNPs and SPs carrying the allele have a slower progression to AIDS and more stable $CD4^+$ T-cell counts than progressors or uninfected controls (Eugen-Olsen *et al.*, 1997; Misrahi *et al.*, 1998; Morawetz *et al.*, 1997; Romiti *et al.*, 2000). For instance, Romiti *et al.* (2000) demonstrated that a group of therapy-naïve HIV-1-infected children (n=229) (with at least 2 years of asymptomatic infection) that included 22 perinatally infected LTNPs had a 7.59-fold higher likelihood than progressors (n=72) for being heterozygous for *CCR5-Δ32*. Specifically, 27.3 % of LTNPs and 1.4 % of progressors were heterozygous for *CCR5-Δ32* (P=0.0288). While studies such as these strongly associate *CCR5-Δ32* with reductions in viral load and elevated $CD4^+$ T-cell counts, this does not make it an indicator of disease non-progression. Only survival studies that follow subjects over many years and measure time to AIDS can make a more substantiated association with disease non-progression.

CCR5 promoter polymorphisms. Some studies have demonstrated that excitatory mutations in the promoter region of CCR5 mRNA transcripts increase the rate of disease progression to AIDS due to HIV-1 binding to the increased cell-surface expression of CCR5 (Piacentini *et al.*, 2009). Examples include the *CCR5P1/P1* haplotype and *59029 A/G* polymorphism (Carrington *et al.*, 1999). Additionally, the *59353C* allele is found in higher frequency in some progressors compared with LTNPs (Jang *et al.*, 2008). Perhaps the best-established association with disease non-progression is the *CCR5P1/P1* haplotype, which has a frequency greater than 40 % in Caucasians, Asians and persons of African descent (An *et al.*, 2000; Carrington *et al.*, 1999). It is believed that between 10 and 17 % of persons developing AIDS within 3.5 years of infection do so as a result of having the *CCR5P1/P1* haplotype (Martin *et al.*, 1998).

Other chemokine co-receptor polymorphisms. In the first-ever genome wide study (Limou *et al.*, 2010) of non-HLA-replicated association, recent data show that a polymorphism in CXCR6, known as the minor-HIV-1 co-receptor, was the strongest signal ($P=2.5\times10^{-7}$) in a large group of LTNPs ($n=186$) who were not ECs (viral load >100 RNA copies ml^{-1}), compared with uninfected controls. Investigators found that the *rs2234358* polymorphism on chromosome 3 was independent from the CCR2–CCR5 locus, and was not linked to the control of viral load (LTNPs carrying the polymorphism had a similar mean viral load), exhibiting an overall high attributable risk of LTNP status of 12 % (Limou *et al.*, 2010).

Polymorphisms in CCR2 and CX3CR1, which HIV-1 sometimes uses as co-receptors, have also been associated with slowing HIV-1 disease progression. For example, the *CCR2-64I* mutation has been shown to reduce CXCR4 expression on $CD4^+$ T cells, thereby interfering with X4-tropic virus infection (Kalinkovich *et al.*, 1999). In one study's cohort of HIV-1 positive Kenyan sex workers (Anzala *et al.*, 1998), the frequency of being positive for *CCR2-64I* was highest in LTNPs, which was three times greater than that for progressors. In a separate study, Vidal *et al.* (2005a) found the *CX3CR1 V249I* polymorphism significantly more frequently in LTNPs than progressors, but not healthy controls.

Variation in the gene encoding duffy antigen receptor for chemokines (DARC) has also been implicated in the rate of HIV-1 disease progression (He *et al.*, 2008). DARC is located on red blood cells (RBCs) and binds to numerous chemokines, including RANTES, which leads to reduced RANTES plasma levels. The *T-46C* DARC polymorphism is prevalent in persons of African descent and causes reduced DARC RBC surface expression, associated with an increased risk of acquiring HIV-1 infection. Paradoxically, *T-46C* polymorphism has also been shown to slow the rate of disease progression and the rate of $CD4^+$ T-cell loss in a cohort of HIV-infected African Americans (He *et al.*, 2008). In fact, the survival advantage conferred by *DARC T-46C* in African Americans was shown to be just as protective as carrying a copy of the *CCR5-Δ32* polymorphism in European Americans.

Chemokine variants. The natural chemokine ligands of CCR5 and CXCR4 (the two coreceptors that HIV-1 uses for infecting $CD4^+$ T cells) act as competitive inhibitors of HIV-1 infection and have been associated with control of HIV-1 infection (Mikhail *et al.*, 2003). CCR5 ligands include the β-chemokines macrophage inflammatory protein 1α and 1β (MIP-1α and MIP-1β), CCR5 ligand human CC chemokine ligand 3 like 1 gene (CCL3L1) and RANTES, and the CXCR4 ligand is SDF-1 (Kalinkovich *et al.*, 1999). $CD8^+$ T cells and macrophages secrete these soluble factors, which then inhibit HIV-1 replication in a non-cytolytic fashion (Gulzar & Copeland, 2004).

MIP-1α/CCL3 and MIP-1β/CCL4. The MIP-1α chemokine is believed to downregulate HIV-1 infection of CCR5-bearing cells by facilitating chemokine receptor desensitization and lowering CCR5 cell surface expression (Piacentini *et al.*, 2009). A few studies have shown that $CD4^+$ T cells from LTNPs produce high levels of MIP-1α in comparison with AIDS subjects who produce hardly any measurable amount of the chemokine (Saha *et al.*, 1998; Schinkel *et al.*, 1999). A caveat to consider with these studies is that AIDS patients could have generalized reduced production of chemokines as an effect rather than cause of their severe immunosuppression. In fact, some studies show no difference in T-cell production levels of the chemokines between LTNPs and progressors (Potter *et al.*, 2007).

CCL3L1/MIP-1αP. *CCL3L1* is considered the most potent agonist of CCR5 and African populations are found with greater copy numbers of the gene compared to Caucasian populations (Dolan *et al.*, 2007; Gonzalez *et al.*, 2005). *CCL3L1* gene copy number has been shown to inversely correlate with the number of $CD4^+$ T cells expressing the CCR5 coreceptor; low *CCL3L1* levels are associated with a higher risk for HIV-1 R5-tropic virus infection, increased viral loads and accelerated disease progression, while high *CCL3L1* copy numbers correlate with a slower HIV-1 disease progression (Gonzalez *et al.*, 2005; Piacentini *et al.*, 2009). High *CCL3L1* copy numbers are shown to play a greater role than CCR5 genotype in determining the rate of HIV-1 disease progression in a study of 57 global cohorts (Gonzalez *et al.*, 2005).

In one of the largest cohort studies to date looking at the relationship between *CCL3L1* and HIV-1 disease progression, Dolan *et al.* (2007) monitored HIV-seropositive patients ($n=1132$) and found that *CCLC3L1* interfered with R5-tropic virus infection of host immune cells and also enhanced CMI against HIV-1 infection. Overall, the study showed that in acute and chronic infection, the CCLC3L1-CCR5 genotype status was predictive of viral loads, $CD4^+$ T-cell depletion and strength of CMI, demonstrating that host genetics could be used to determine the rate of HIV-1 disease progression in these subjects. Research has also shown that lower *CCL3L1* copy numbers translate to an increased risk of infection without affecting rates of HIV-1 disease progression, which remains true even after stratifying according to *CCR5-Δ32* (Lee *et al.*, 2010; Nakajima *et al.*, 2007; Urban *et al.*, 2009). This could be due to differences in study population ethnicities, as it has been shown that Caucasians carry fewer *CCL3L1* copies (Gonzalez *et al.*, 2005). Urban *et al.* (2009) corrected for this confounding variable by testing for the association of *CCL3L1* copy number with HIV-1 viral load stratified according to ethnicity and found no evidence of association in either ethnicity group (European, $P=0.14$; African, $P=0.27$).

Table 2. *CCR5-Δ32*/WT genotype relationship with HIV-1 disease non-progression

HIV+, HIV-1-infected; HIV−, uninfected control; WT/WT, wild-type; WT/CCR5Δ32, heterozygotes; SP, slow progressor; RP, rapid progressor; ESN, exposed-seronegative; P, progressor; MACS, multicenter AIDS cohort studies based in Baltimore/DC, Pittsburgh, Chicago and Los Angeles; GRIV, genetics of resistance to infection by HIV-1; DC, DC gay men; SFC, San Francisco City clinic; MHCS, multicenter haemophilia cohort study; HGD, haemophilia growth and development study; IDU, injection drug user; SEROCO, HIV-1-infected volunteers; HEMOCO, HIV-1-infected haemophiliacs cohort; SEROGEST, HIV-1-infected pregnant women.

Ref	Country/cohort	Controllers (% positive)	Progressors/seroconverters/ uninfected controls (% positive)	Results
Evidence for slowing disease progression				
Romiti *et al.* (2000)	Spain, Italy	22 LTNP (27 %) 229 SP* (10 %)	72 RP (1 %) 47 ESN (6 %) 262 HIV− (8 %)	CCR5Δ32/WT in SP vs RP ($P<0.0288$)
Meyer *et al.* (1999)	France: SEROCO	1308 SEROCO (15 %)		%$CD4^+$ T cells <200μl: SEROCO: 37 % CCR5-Δ32/WT
	HEMOCO	180 HEMOCO (17 %)		HEMOCO 60 % CCR5-Δ32/WT
	SEROGEST	169 SEROGEST (15 %)		SEROGEST: 19 % CCR5-Δ32/WT
Misrahi *et al.* (1998)	French paediatric HIV cohort	276 HIV+† (10 %)		Survival study of CCR5-Δ32/WT ($n=26$) vs WT/WT ($n=126$) subjects: Risk of AIDS symptoms at 36 months: 9 vs 28 % ($P<0.004$). Incidence of AIDS symptoms at 8 years: 54 and 89 % ($P=0.003$)
Hendel *et al.* (1998)	GRIV	200 SP (28 %)	76 RP (3 %)	CCR5-Δ32/WT in SP vs RP ($P<0.0001$)
Zimmerman *et al.* (1997)	USA: MACS	614 HIV+* (23 %) 85 LTNP (34 %)		CCR5-Δ32/WT in LTNP vs HIV+ ($P=0.006$)
Michael *et al.*, 1997	San Francisco Men's Health Study	152 SP (18 %)	234 P† (30 %)	CCR5-Δ32/WT in LTNP vs P ($P=0.023$), SP vs P ($P=0.07$)
Rappaport *et al.* (1997)	France: GRIV	66 LTNP (24 %)	34 RP (3 %)	CCR5-Δ32/WT in LTNP vs RP ($P<0.05$)
Eugen-Olsen *et al.* (1997)	Denmark	9 LTNP (67 %)	99 HIV+ (22 %)‡ 9 RP (0 %) 37 HIV− (24 %)	Survival study: 55 % CCR5-Δ32/WT HIV+ subjects with AIDS in 8.4 years ($P<0.01$) 65 % WT/WT HIV+ subjects with AIDS in 5.5 years
Stewart *et al.* (1997)	Australian LTNP study	64 LTNP (35 %)	120 HIV+ (12.5 %) 95 RP (13 %)	CCR5-Δ32/WT genotype in LTNP vs RP ($P=0.0005$), LTNP vs HIV+ ($P=0.0004$)
Morawetz *et al.* (1997)	Swiss HIV cohort	61 LTNP (31 %)	235 P (10.6 %)	CCR5-Δ32/WT genotype in LTNP vs P ($P<0.0001$); CCR5-Δ32/WT genotype correlated with $CD4^+$ T-cell counts >500 cells μl^{-1} ($P=0.0006$), slower progression to AIDS ($P=0.077$) than WT/WT phenotype

Table 2. cont.

Ref	Country/cohort	Controllers (% positive)	Progressors/seroconverters/ uninfected controls (% positive)	Results
Dean *et al.* (1996)	USA: DCG MACS, SFCC, MHCS, HGDS	137 DCG (14 %) 265 MACS (24 %) 150 SFCC (27 %) 192 MHCS (19 %) 133 HGDS (20 %)	612 HIV− (14 %)	CCR5-Δ32/WT-genotype associated with delayed progression to AIDS compared with WT/WT individuals (P=0.0045)
No evidence for slowing disease progression				
Pereyra *et al.* (2008)	Boston, USA	64 EC (14 %) 60 LTNP (22 %)		CCR5/Δ32 in ECS/LTNPs not statistically significant (P=0.44) compared with the general uninfected population
Vidal *et al.* (2005b)	Spain	60 LTNP (19 %)	102 HIV− (13 %) 109 P (19 %)	P-values not significant
Schinkel *et al.* (1999)	Amsterdam cohort of IDUs		108 HIV+ (20 %)	CCR5-Δ32/WT not significantly protective over WT/WT genotype for slowing disease progression
Wilkinson *et al.* (1998)	USA Transfusion Safety Study		543 HIV+ haemophiliacs (13 %) 88 HIV^+ transfusion recipients (11 %)	No difference in progression to AIDS or having a $CD4^+$ T-cell count <400 cells μl^{-1} between CCR5/CCR5 (n=471) and CCR5/Δ32 (n=72) haemophiliacs
Cohen *et al.* (1997)	USA: NIH	33 LTNP (38 %)		No significant difference in $CD4^+$ T-cell count (P=0.88) and viral load (P=0.99) between CCR5-Δ32/WT and WT/WT LTNPs
Huang *et al.* (1996)	USA: MACS		461 HIV+ (20 %) 446 HIV− (18 %)	No significant differences in disease progression between CCR5-Δ32/WT and WT/WT seroprevalent cases. Slower $CD4^+$ T-cell depletion for recent seroconverter cases (n=117) than WT/WT cases (P=0.04). No significant differences in time to AIDS.

*Includes LTNPs.
†Perinatal HIV-1 infection in children.
‡Includes LTNPs and RPs.

RANTES/CCL5. As one of CCR5's natural ligands, RANTES inhibits R5-tropic virus infection. Elevated RANTES levels have been associated with control of HIV-1 infection (Koning *et al.*, 2003; McDermott *et al.*, 2000). Some polymorphisms in *RANTES*' promoter region protect HIV-infected subjects against disease progression as a result of increased RANTES synthesis. *G4 RANTES* promoter region polymorphisms were studied in *CCR5-Δ32*-negative HIV-infected subjects (n=420) from the Multicenter AIDS cohort study and individuals with the *G4* genotype significantly progressed more slowly to AIDS (median time to AIDS was 7.5 years) and had a slightly reduced $CD4^+$ T-cell depletion than individuals with non-*G4* genotypes (median time to AIDS was 5.6 years) (P=0.007) (McDermott *et al.*, 2000). Kaplan–Meier survival curves showed that elevated rates of survival in these subjects were comparable to subjects heterozygous for the *CCR5-Δ32* polymorphism from the same study.

The *RANTES 28G* polymorphism found in the gene's promoter region has also been associated with protection against disease progression (Liu *et al.*, 1999; Wichukchinda *et al.*, 2006). One study's genotyping of *RANTES* from HIV-1 seroconverters (n=272) found that the mutation alone was sufficient to activate the gene's promoter region and that it correlated with increased levels of *RANTES* transcription and reduced rates of $CD4^+$ T-cell depletion (Liu *et al.*, 1999). Another study (Wichukchinda *et al.*, 2006) demonstrated the mutation's protective effect by comparing disease progression in seroconverters carrying either the *RANTES 28G* polymorphism or the *RANTES In.1.1C* polymorphism, which is located in the gene's regulatory region and inhibits RANTES synthesis. Persons carrying the *RANTES In.1.1C* allele progressed significantly faster to AIDS compared with those carrying the *RANTES 28G* allele, along with those HIV-infected subjects without either mutation (P<0.02).

In a study of HIV-seropositive individuals from the Amsterdam Cohort of Homosexual Men (Koning *et al.*, 2003), progressors (n=6) had a significant reduction in virus sensitivity to RANTES-mediated neutralization compared with LTNPs (n=7) (P<0.05). This could be the result of viral escape variants that are resistant to RANTES' antiviral effects. Results showed RANTES was the most potent inhibitor of R5-virus replication and had a considerably lower IC_{50} against virus p24 antigen than MIP-1α and MIP-1β. This finding demonstrates that RANTES is better than the other chemokines in blocking R5-strain infection of $CD4^+$ T cells.

SDF1 (CXCL12). Stromal cell-derived factor 1 (SDF1) or CXCL12 is CXCR4's only natural ligand and inhibits X4-tropic HIV-1 virus infection (Magierowska *et al.*, 1999). The *SDF1-3′A* polymorphism is a G→A mutation in the 3′ untranslated region of the *SDF1* gene that upregulates its transcription levels and has been associated with delaying disease progression in HIV-infected subjects who have been found to carry the polymorphism significantly more frequently than progressors (Hendel *et al.*, 1998; Magierowska *et al.*, 1999; Winkler *et al.*, 1998).

However, several studies show a lack of relationship between *SDF1-3′A* and HIV-1 disease non-progression (Vidal *et al.*, 2005b; Ioannidis *et al.*, 2001; Tresoldi *et al.*, 2002). For instance, in their international meta-analysis, Ioannidis *et al.* (2001) measured the effects of subjects homozygous for the *SDF1-3′A* polymorphism by reviewing studies that prospectively followed HIV-1-infected patients from seroconversion to AIDS diagnosis and death. Results showed that being homozygous for the polymorphism had no effect on disease progression and there was no significant difference in HIV-1 RNA levels among persons with and without the polymorphism.

Endogenous antiretrovirals. APOBEC3G. APOBEC3G is a cytidine deaminase that blocks HIV-1 integration into host DNA by inducing excessive G→A base mutations in the viral DNA that causes premature stop codons to form and makes HIV-1 replication-incompetent (Piacentini *et al.*, 2009). HIV-infected individuals with low levels of APOBEC3G mRNA are associated with an increased risk of progression to AIDS, while the opposite is true for those with high levels of APOBEC3G mRNA (Jin *et al.*, 2007). A recent study (Vázquez-Pérez *et al.*, 2009) that looked at APOBEC3G mRNA expression levels in PBMCs from a group of HIV-1-infected individuals at different disease stages found higher mRNA levels correlated with greater $CD4^+$ T-cell counts and lower HIV-1 viral loads (P=0.0004). Additionally, therapy-naïve subjects with lower viral loads (<10 000 copies ml^{-1}) and greater than 3 years of infection had higher APOBEC3G expression levels than both progressors and uninfected healthy controls. APOBEC3G-induced hypermutations in *vpu*/*env* proviral DNA have been correlated with higher $CD4^+$ T-cell counts (P=0.042) in a large cohort of HIV-infected Kenyan women (n=208) (Land *et al.*, 2008). Other APOBEC3G polymorphisms are associated with increased rates of disease progression, as demonstrated by An *et al.* (2004) in their study of 3073 HIV-1-infected subjects from six HIV-AIDS prospective cohorts. The study showed that the APOBEC3G exon 4 variant allele *H186R* correlated with faster progression to AIDS and a significant decline in $CD4^+$ T-cell counts when compared with subjects that did not carry the polymorphism (P=0.0009).

Tripartite motif protein 5α (Trim5α). Trim5α acts on HIV-1 in the post-cell-entry phase by attaching to the HIV-1 capsid protein to inhibit viral replication (van Manen *et al.*, 2008). Physiological levels of Trim5α have been associated with slowing down the HIV-1 disease progression and the *R136Q* polymorphism is believed to be especially effective in blocking X4-tropic HIV-1 infection (van Manen *et al.*, 2008). Javanbakht *et al.* (2006) examined the effect of 12 common Trim5α single nuclear polymorphisms (SNPs), including *R136Q*, in 939 HIV-1 seroconverters from five USA-based HIV-1 natural history cohorts and found that the SNPs were not related to patient progression to AIDS

nor to low $CD4^+$ T-cell counts (<200 cells μl^{-1}). In a subsequent study, van Manen *et al.* (2008) found that the presence of the *R136Q* polymorphism in study subjects infected with X4-tropic but not R5-tropic HIV-1 correlated with their lower viral load.

Significance of genetic factors associated with control: implications on therapeutics

The main way in which host genetics has been experimentally associated with controlling HIV-1 progression is genetic polymorphisms that interfere with virus binding to susceptible target cells, either through impaired receptor expression on the cell-surface or an increase in production of competitive inhibitors. Experimental results have hardly been concordant, and among the most highly investigated aspects of host genetics relating to HIV-1 progression is the *CCR5-Δ32* polymorphism. Nearly as many studies that relate the polymorphism to longer time to AIDS and elevated $CD4^+$ T-cell counts also fail to establish a statistically significant difference between LTNPs and progressors. Other genetic mechanisms could play a more meaningful role albeit this is not certain because they have not been as widely studied as the *CCR5-Δ32* polymorphism.

Although multiple protective effects have not been collectively observed in the same LTNP subject, the combinational effects of *CCR5-Δ32* with excitatory mutations in the promoter region of *CCL3L1* and *RANTES* would probably provide strong protection from CCR5-tropic HIV-1 infection and should be considered for HIV-1 therapy. Though preventive and prophylactive vaccines cannot rely on genetics to confer protection from HIV-1 infection, gene therapy can be administered as antiretroviral therapeutics to control infection in HIV-1 seropositive individuals. An example of gene therapy is genetically engineered $CD4^+$ T cells that are resistant to HIV-1 based on coreceptor expression or secretion of antiviral soluble factors, which are adoptively transferred to recipients (Varela-Rohena *et al.*, 2008). Genetic correlates of protection that should be targets for gene therapy include antiviral factors like APOBEC3G and TRIM5α that restrict HIV-1 replication at the post-entry, pre-integration stage. Another possible example of using adoptive immunotherapy to control HIV-1 infection involves the transfer of T-cell receptor genes from a T cell specific for *HLA-B57*-restricted antigens that are inserted into a viral vector (Varela-Rohena *et al.*, 2008). This would be especially useful in generating highly specific T-cell responses for conserved antigenic *gag* epitopes that are most strongly correlated with reducing viral load early in infection.

Immunological factors

Innate immunity

Toll-like receptor (*TLR*) 9. Two studies (Bochud *et al.*, 2007; Soriano-Sarabia *et al.*, 2008) have found that SNPs in the *TLR9* gene are associated with a rapid HIV-1 disease progression. The *1635A/G TLR9* and *1174G/A* polymorphisms positively correlated with rapid disease progression, higher viral loads and low $CD4^+$ T-cell counts in the studies' large cohorts of therapy-naïve HIV-infected individuals (Bochud *et al.*, 2007; Soriano-Sarabia *et al.*, 2008).

Mannose-binding lectin (*MBL*). Elevated MBL serum levels correlate with better survival in patients with AIDS and homozygosity for *MBL* variant alleles *B*, *C* and *D* have been associated with lower MBL serum levels and an increased susceptibility to HIV-1 infection (Garred *et al.*, 1997; Piacentini *et al.*, 2009). Studies that have looked at the effects of MBL genotype on the clinical outcome of HIV-1 infection in therapy-naïve subjects were unable to make a definitive correlation between MBL genotype (homozygous versus WT for MBL variants) and HIV-1 disease non-progression (Garred *et al.*, 1997; Maas *et al.*, 1998). For instance, in a cohort study (Garred *et al.*, 1997) of HIV-infected homosexual seroconverters (n=96) who were followed for 10 years, the correlation between MBL genotype and the time to AIDS was not statistically significant (P=0.8). For subjects that went on to develop AIDS (n=61), those who were carriers of the *MBL* variant alleles had a significantly shorter survival time after being diagnosed with AIDS versus those who were not carriers (11 versus 18 months, P=0.007). While this suggests that higher *MBL* copy levels reduces AIDS mortality, it does not demonstrate a protective effect against the actual progression to AIDS.

NK cells. NK cells have many functions in innate and CMI due to their vast expression of surface receptors that enables them to both inhibit and activate target cells (Vieillard *et al.*, 2010). NK cells from both viraemic and non-viraemic LTNPs have been shown to produce significantly higher levels of gamma interferon (IFN-γ) than progressors and healthy uninfected subjects, which is the main mechanism by which NK cells mediate control of infection (O'Connor *et al.*, 2007; Vieillard *et al.*, 2010). NK cells are certainly important for controlling viral infection in HIV-infected subjects, albeit $CD8^+$ T cells have been shown to exhibit superior control of HIV-1 replication in EC subjects (O'Connell *et al.*, 2009).

Evidence shows that NK cells from LTNPs have an abnormal phenotype compared with both HIV-1-infected viraemic subjects and uninfected subjects (O'Connor *et al.*, 2007). Recently, Vieillard *et al.* (2010) performed large-scale analysis of 21 different phenotypic markers on NK cells to show that markers from LTNPs with undetectable viral loads were significantly different from viraemic controllers (50 000 RNA copies ml^{-1}) and progressors. This indicates that for these subjects viral load influences receptor expression on NK cells.

As recent data have shown, polymorphic killer cell immunoglobulin-like receptors (KIR) in the presence of

specific *HLA-B* alleles play a positive role in HIV-1 disease pathogenesis, particularly the inhibitory *KIR3DL1* allotypes co-expressed with *HLA class I* alleles of the *HLA-Bw4* family (Alter & Altfeld, 2009). Early in infection, strong inhibitory reactions between *HLA-Bw480I* alleles (i.e. *HLA-B57*) and *KIR3DL1* have been postulated to activate developing NK cells and promote a robust cytotoxic cell response (Alter & Altfeld, 2009). In more than 1500 HIV-1-infected individuals Martin *et al.* (2007) found that *KIR3DL1*$^+$ *Bw4* genotypes were protective against HIV-1 disease progression, with the *KIR3DL1*004* allele being the most protective. Martin *et al.* (2002) were the first to show that HIV-infected individuals with co-expression of the *KIR3DS1* allele – which is frequently found in Caucasians but not African-Americans – with *HLA-Bw480I* progressed significantly slower to AIDS than individuals with either one or neither of these two alleles. Slower progression to AIDS was related to rapid degranulation by *KIR3DS1*$^+$ NK cells in response to HIV-infected *HLA-Bw480I*$^+$ CD4$^+$ T cells (Alter & Altfeld, 2009). In a recent study, O'Connell *et al.* (2009) did not find either *KIR3DS1* or *KIR3DL1* overexpressed in a cohort of 20 ECs who were mostly African-American, and their KIR frequencies were similar to that seen in uninfected African-Americans. For functional analysis of NK cells for eight of the ECs, NK cell inhibition of viral replication did not correlate with *KIR3DS1* or *KIR3DL1* genotypes.

Other findings have implicated specific binding of *KIR2DL2* to *HLA-C* alleles as being protective against HIV-1 progression, though *KIR2DL2* has been shown to bind non-specifically to *HLA-C1* and *HLA-C2* molecules, indicating their mode of protection could be independent from a specific *HLA-C* subtype (Alter & Altfeld, 2009).

Adaptive immunity

HLA class I alleles. *HLA class I* alleles (*A*, *B* and *C*) present antigens from intracellular pathogens to CD8$^+$ T cells. A heterozygous *HLA class I* genotype has been correlated with slowing HIV-1 disease progression and lowering HIV-1 viral load by enabling the presentation of a wider range of peptides and decreasing the risk of viral escape variants (Piacentini *et al.*, 2009). In particular, *HLA-B57* variants – predominantly *B5701* and *B5703* – strongly correlate with the control of HIV-1 infection by inducing a cross-reactive response against immunodominant Gag epitopes (Klein *et al.*, 1998; Piacentini *et al.*, 2009). *HLA-B27* is also a well-studied allele with respect to HIV-1 disease non-progression though the same relationship is not as statistically significant in large cohort studies (Bello *et al.*, 2005; Klein *et al.*, 1998). Interestingly, an analogous relationship has been found between *HLA-B27* and the *MHC class I* allele, *Mamu-B08*, in terms of disease non-progression; elite control of viral load in rhesus macaque monkeys infected with SIV is correlated with their carriage of the *Mamu-B08* allele (Loffredo *et al.*, 2009). Research shows that up to 50 % of *Mamu-B08*-positive Indian rhesus macaques become elite controllers and that *Mamu-B08*-restricted epitopes nearly match the binding profile for *HLA-B27*-positive LTNPs (Loffredo *et al.*, 2009).

Between 1 and 10 % of Caucasian, African and Asian populations carry a copy of the *HLA-B57* allele and 1.4–8 % of persons in major continental populations carry a copy of the *B27* allele (Di Lorenzo, 2008; Middleton *et al.*, 2003). To date, at least 15 studies have measured the frequency of EC/LTNP/SP subjects carrying either *HLA-B27* or *-B57* in comparison to viraemic HIV-1 progressors or healthy uninfected controls (Table 3). *B57*'s frequency in HIV-1 controller study groups varies considerably. In studies with cohorts of at least 30 subjects, between 8 and 63 % of either LTNPs or ECs have been found to carry the *B57* allele with frequencies being the highest in ECs. These allelic frequencies are statistically significant when compared to frequencies in subjects with progressive HIV-1 infection that range from 2 to 24 %. In comparison, the *B27* allelic frequencies in study cohorts larger than 30 subjects range from 2 to 20 % in controllers and 3 to 4 % in progressors, though comparisons of the two do not reach statistical significance. For this reason, there is no strong enough evidence to suggest that *HLA-B27* provides any appreciable protection against HIV-1 disease progression.

Though the majority of studies find that the rates in controllers compared with progressors are statistically significant, there is a considerable amount of variation in study cohort size and duration of follow-up period for survival studies. One factor that is important to take into account when considering the significance of the *B27* and *B57* alleles is the viral load parameters applied to different LTNP cohorts. Studies that adhere to the most conservative definition of what constitutes an LTNP (viral load of $<$75 RNA copies ml^{-1}, CD4$^+$ T-cell counts $>$850 cells μl^{-1}) have high frequencies of subjects positive for *B57* but negative for *B27* (Migueles & Connors, 2010). Even more revealing and clinically important are studies that compare rates of *B57* and *B27* positivity in LTNPs to HIV-infected progressors – more studies report that *B57* rather than *B27* is present in LTNPs at statistically significant higher frequencies in both progressors and healthy seronegative controls.

Other protective *HLA class I* alleles include *B13*, *B15*, *B44*, *B51* and *B58* – in some cohorts as many as 90–95 % of LTNPs carry at least one of these alleles (Migueles & Connors, 2010). While individually *B57* plays a role with HIV-1 disease non-progression, it is unclear what the protective effects of multi-allelic polymorphisms are in individuals that carry more than one of these protective alleles. Leslie *et al.* (2010) have recently demonstrated the power of additive effects of *HLA-B class I* alleles in a large cohort of HIV C-clade-infected individuals from South Africa (n=1211). Even after excluding the alleles identified to have the strongest positive (*B57* and *B5801*) and negative influence (*B5802* and *B18*) on subject viral load and CD4$^+$ T-cell count, *HLA-B* remained the strongest predictor of

Table 3. *HLA-B27* and *-B57* haplotype relationship to HIV-1 non-progression

ART+, on antiretroviral therapy; ART−, antiretroviral therapy naïve; HCV, hepatitis C virus; NS, not significant.

Ref	Controllers	Progressors/ uninfected controls	% *HLA-27*	% *HLA-57*	*P*-value for study population versus progressors
Jagannathan *et al.* (2009)	15 LTNP 10 SP*	10 P (ART−) 15 P (ART+)	13 % LTNP 20 % SP 20 % P (ART−) 13 % P (ART+)	73 % LTNP 60 % SP 60 % P (ART−) 66 % P (ART+)	–
Emu *et al.* (2008)	30 EC 31 LTNP	293 P		40 % EC 23 % LTNP 9 % P	<0.001
Pereyra *et al.* (2008)	64 EC 60 LTNP	30 P	15 % EC 20 % LTNP 3 % P	44 % EC 33 % LTNP 10 % P	B57 : 0.01
Chakraborty *et al.* (2006)	7 LTNP 5 SP†	17 P	0 % LTNP 20 % SP 0 % P	0 % LTNP 0 % SP 12 % P	–
Bello *et al.* (2005)	16 LTNP	97 HIV−	50 % LTNP 26 % HIV−		NS (>0.05)
Munkanta *et al.* (2005)	42 LTNP	38 HIV (ART+)	2 % LTNP 18 % HIV (ART+)		0.044
Lopez-Larrea *et al.* (2005)	54 SP†	34 RP		63 % SP 24 % RP	0.002
Migueles *et al.* (2002)	15 LTNP	8 P	13 % LTNP 0 % P	87 % LTNP 38 % P	–
Valdez *et al.* (2002)	6 HCV–HIV coinfected LTNP	26 HCV–HIV P 7 HIV P		50 % HCV-HIV LTNP 0 % HCV-HIV P 0 % HIV P	–
Migueles & Connors (2001)	10 EC 4 LTNP	7 P (ART−) 6 P (ART+)	20 % EC 25 % LTNP 0 % P (ART−) 0 % (ART+)	80 % EC 25 % LTNP 43 % P (ART−) 83 % P (ART+)	–
Migueles *et al.* (2000)	13 LTNP	200 P	85 % LTNP 10 % P	15 % LTNP 0 % P	<0.001
Hendel *et al.* (1999)	200 SP‡	75 P	8 % LTNP 3 % P	8 % LTNP 2 % P	B27 : 0.024 B57 : 0.01
Magierowska *et al.* (1999)	70 LTNP	83 P	20 % LTNP 4 % P		0.001
Klein *et al.* (1998)	23 LTNP	86 P 804 HIV−	13 % LTNP 4 % P 8 % HIV−	26 % LTNP 2 % P 5 % HIV−	B27 : NS B57 : 0.0006
Goulder *et al.* (1996)	30 LTNP	29 P		30 % LTNP 7 % P	<0.013

*$CD4^+$ T-cell count between 394 and 1307.
†$CD4^+$ T-cell count <500.
‡$CD4^+$ T-cell count >500.

viral load (P=0.0007) and $CD4^+$ T-cell count (P<0.0001). This demonstrates that the role that *HLA-B* plays in controlling HIV-1 infection could be the additive effect of some or all of the *HLA B* alleles.

There are also *HLA class I* alleles associated with accelerating HIV-1 disease progression, which include *A24*, *A29*, *B35*, *C4* and *Cω4* (Piacentini *et al.*, 2009; Saksena *et al.*, 2007). Specifically, the *B35-Px* subtype allele has been linked to accelerated disease progression due to its reduced ability to bind HIV-1 peptides and activate a CTL response (Carrington & O'Brien, 2003).

$CD4^+$ T cells. The role of $CD4^+$ T cells in long-term control of HIV-1 infection is unclear, especially in terms of their ability to induce and maintain an HIV-specific $CD8^+$

T-cell response. Some LTNPs have been found to have a robust, polyclonal and Gag-specific $CD4^+$ T-cell response, which unlike progressors, is maintained at elevated levels for the duration of their infection and directly correlated with a lower viral load (Boaz *et al.*, 2002; Migueles *et al.*, 2000; Rosenberg *et al.*, 1997). Compared with progressors and viraemic controllers, $CD4^+$ T-cell responses from LTNPs have been shown to have the $CD4^+IL\text{-}2^+IFN\text{-}\gamma^+$ phenotype with a large production of IL-2, indicative of a robust type 1 helper T-cell response (Migueles *et al.*, 2000; Potter *et al.*, 2007). However, it is difficult to assess the causal relationship that HIV-specific $CD4^+$ T cells have with disease non-progression in these individuals because low levels of virus replication could in fact be inducing the production of virus-specific $CD4^+$ T cells in LTNPs. So far, studies have not established causality of HIV-specific $CD4^+$ T-cell levels and disease non-progression beyond reasonable doubt.

Some evidence suggests that LTNPs have reduced $CD4^+$ T-cell activation in comparison with subjects with rapid disease progression (Bello *et al.*, 2009; Marchetti *et al.*, 2009). Less $CD4^+$ T-cell immune activation reduces the number of cells susceptible to HIV-1 infection and can potentially lead to a better disease prognosis. In one study, ECs (n=4) that were examined longitudinally for 4 years had low levels of immune activation (measured as $HLA\text{-}DR^+CD38^+$ cells) and spontaneous $CD4^+$ T-cell apoptosis similar to uninfected controls and less than progressors (Bello *et al.*, 2009).

The same has been shown in primate models. Sootey mangabeys infected with SIV rarely progress to AIDS, maintaining low $CD4^+$ T-cell counts despite the presence of highly replicating virus (Estes *et al.*, 2008). Sootey mangabeys have been found to have lower rates of immune activation than their primate counterparts, rhesus macaques, who suffer a poorer clinical outcome. In one study, both primate species underwent initial immune activation, which eventually declined in sootey mangabeys due to increased PD-1 expression on their $CD4^+$ T cells (Estes *et al.*, 2008). Analogous findings have been shown for HIV-1 controller human subjects. When followed for shorter periods (i.e. 1.5 years) they have elevated levels of immune activation and spontaneous T-cell apoptosis similar to treated HIV-infected subjects or untreated rapid progressors (Ronquillo *et al.*, 2010; Goicoechea *et al.*, 2009). Low levels of virus replication probably explain for the presence of T-cell activation in controllers and progressors earlier in infection and which has been shown to decline over time in controllers, but not in progressors (Bello *et al.*, 2009).

Contrary to these results, there is evidence that has shown LTNP $CD4^+$ T effector cells are in a chronic activated state based on their increased *HLA-DR* cell-surface expression, decreased IL-7α production, and increased IFN-γ production (Potter *et al.*, 2007). These LTNP $CD4^+$ T cells do not show signs of the same kind of generalized immune activation seen in rapid progressors, but rather specific immune activation restricted to effector memory T cells. In an antibody microarray analysis of 135 T-cell surface antigens, Wu *et al.* (2007) showed that LTNP $CD4^+$ T cells had an upregulated cell-surface expression of *HLA-DR*, CD71 and CD38 compared with viraemic patients on highly active antiretroviral therapy (HAART).

IL-7/IL-7 receptor (IL-7R) balance is important for the maintenance of $CD4^+$ T-cell levels and disrupting this balance increases the risk of progressing to AIDS (Marchetti *et al.*, 2009). Studies have found that LTNPs have a significantly elevated IL-7R expression on $CD4^+$ T cells compared with progressors. These results support an IL-7-mediated model of $CD4^+$ T-cell thymic output used to replenish low levels of peripheral $CD4^+$ T cells (Marchetti *et al.*, 2009; Potter *et al.*, 2007). This raises the question of whether there is a correlation between reduced T-cell activation with elevated IL-7R-expressing $CD4^+$ T cells. LTNP $CD4^+$ memory T cells have also been found to express elevated levels of chemokine receptor CCR7 on their cell surface compared with progressors and uninfected controls, which may reflect a difference in their $CD4^+$ central memory cell homing patterns into secondary lymphoid organs (Marchetti *et al.*, 2009).

$CD8^+$ T cells. $CD8^+$ T cells and their conversion into CTLs – the effector cytotoxic cells responsible for killing HIV-infected host cells – are the most important mediators of lowering viral load in HIV-1 controllers and are most strongly correlated with facilitating immune control in LTNPs (Migueles & Connors, 2001). The $CD8^+$ T-cell responses of many LTNPs have been shown to have a high CTL precursor frequency that gives rise to broadly reactive CTLs specific for conserved sequences in the *env*, *gag* and *pol* genes (Betts *et al.*, 1999; Geldmacher *et al.*, 2007; Migueles & Connors, 2001). Gag-specific CTL responses, which are activated after binding to antigenic p24 epitopes presented by *HLA class I* alleles, positively select for viral escape variants that are unable to replicate as effectively (Geldmacher *et al.*, 2007). Geldmacher *et al.* (2007) isolated specific epitopes from the N- and C-terminal regions of Gag that most strongly correlated with low viral loads and elevated $CD4^+$ T-cell counts in LTNPs, identifying epitopes from these regions as important immunological targets. Recently, it has been shown (Ferre *et al.*, 2010) that polyfunctional HIV-specific $CD8^+$ T cells responding to Gag p24 were more abundant in rectal mucosa of HIV controllers than in non-controllers or HAART-treated individuals. The same $CD8^+$ T-cell response was seen in the sera from these individuals, which was directed against conserved regions of Gag that included *HLA-B27-* and *-B57*-restricted epitopes.

However, the same caveat that applies to LTNP $CD4^+$ T cells also applies to their $CD8^+$ T cells – it is unknown if high levels of virus-specific $CD8^+$ T cells are the cause or effect of their low viral loads. Before major advances in a cell-mediated HIV-1 vaccine can be made, it is important to understand what particular aspects of a robust and

virus-specific HIV-1 CTL response (qualitative versus quantitative) most strongly correlate with disease non-progression. Quantitative factors (the frequency of an HIV-specific CTL response) are not found to differ significantly between LTNPs and progressor and have been ruled out as playing a strong role in disease non-progression (Betts *et al.*, 2006). In contrast, studies have found that LTNP $CD8^+$ T-cell responses differ significantly qualitatively (i.e. proliferation, production of cytotoxic molecules like IFN-γ, IL-2 and granzyme B) (Betts *et al.*, 2006; Migueles *et al.*, 2002). Betts *et al.* (2006) showed that progressors had much lower $CD8^+$ T-cell polyfunctional responses (based on cytokine and chemokine secretion) than LTNPs. Specifically, MIP-1β secretion was the most predominant HIV-specific $CD8^+$ T-cell secretory phenotype to each of HIV's antigens. The total frequency of HIV-specific $CD8^+$ T cells did not correlate with viral load in study subjects, whereas qualitative factors like the frequency and per cent of Gag-specific $CD8^+$ T cells expressing all five functions (degranulation, IFN-γ, MIP-1β, tumour necrosis factor-α and IL-2) correlated inversely with viral load in LTNPs ($P<0.001$). While $CD8^+$ T cells from progressors were found to produce no more than three functions at once, LTNP $CD8^+$ T cells produced four and five responses simultaneously, demonstrating their enhanced polyfunctional response.

In their experiments, Migueles *et al.* have shown that HIV-specific proliferating $CD8^+$ T cells from LTNPs had greater secretion of effector molecules like perforin and granzyme B, required for CTL-mediated lysis of infected $CD4^+$ T cells (Migueles *et al.*, 2002, 2008). Perforin secretion is normally tightly regulated and is not coordinately secreted with granzyme B. Other phenotypic differences between LTNP and progressor $CD8^+$ T cells include high secretion levels of IFN-γ and IL-2 and low levels of IL-4 seen in LTNPs, with the converse seen in progressors. The anti-inflammatory IL-4 secretion probably provided some advantages to virus survival in progressor $CD4^+$ T cells. Additionally, Wu *et al.* (2007) used antibody microarrays to show that LTNP, but not progressor $CD8^+$ T cells, had upregulated CD16 and CD56 cell surface expression, which activated NK cells.

Humoral immunity. Broadly acting neutralizing antibodies (NAbs) are rarely found in HIV-1-infected individuals with conflicting consensus regarding their frequency in LTNP populations. Some studies have shown they are more frequently found in LTNPs than progressors (Cecilia *et al.*, 1999; Pilgrim *et al.*, 1997), while others have shown they are not (Doria-Rose *et al.*, 2010; Pereyra *et al.*, 2008). Several studies have demonstrated, albeit with small sample sizes, that LTNPs have a stronger virus-specific NAb response than progressors against both autologous and heterologous viral strains (Braibant *et al.*, 2008; Cecilia *et al.*, 1999; Mahalanabis *et al.*, 2009; Pilgrim *et al.*, 1997; Sreepian *et al.*, 2004). Interestingly, studies have shown that NAb levels peak in LTNPs late in their infection as a result of low levels of viral replication and more heterogeneous virus populations. In contrast, progressors rapidly lost their NAb response over the course of their infection due to their steady decline in $CD4^+$ T cells (Cecilia *et al.*, 1999; Lopalco, 2004).

HIV-specific NAbs predominantly target epitopes within the Gag and Env structural proteins, though these epitopes are poorly immunogenic (Sreepian *et al.*, 2004). The strongest NAb-mediated response found in LTNPs has been against Env's gp120 and gp41 proteins (Lopalco, 2004). The C1 and C2 region of gp120, the V3 loop of gp120 (GPGRAF and NNNT motifs) and the KLIC motif of the gp41 are examples of immunogenic peptides LTNP NAbs target (Sreepian *et al.*, 2004). In a large LTNP cohort ($n=67$), mutations in gp120 induced a broad LTNP NAb response, defined as at least 90 % NAb-mediated reductions in infectivity of four heterologous virus primary isolates from different clades (Mahalanabis *et al.*, 2009). Similar results have been found in other studies that looked at the breadth of NAb responses from LTNPs and progressors to over 20 viral isolates (Deeks *et al.*, 2006; Doria-Rose *et al.*, 2010).

For the first time ever, Simek *et al.* (2009) have utilized a novel high-throughput screening technique to identify a group of 'elite neutralizers', who make up a subset of HIV-infected individuals with broadly acting neutralizing antibodies against multiple HIV-1 isolates representing several clade groups. These elite neutralizers, who make up roughly 1 % of approximately 1800 HIV-1-infected individuals from five continents, were found to neutralize more than one pseudovirus at an IC_{50} titre of 300 within a clade group and across at least four clade groups. These newly discovered antibodies, which include the VRC01 antibody that targets the CD4-binding site in gp120, offer renewed hope that a vaccine can elicit HIV-1-specific NAbs. Wu *et al.* (2010) found that this antibody neutralizes roughly 90 % of nearly 200 HIV isolates from multiple clades and is both broad and potent in its neutralization – a dual response that researchers have feared would not be found simultaneously in HIV-specific neutralizing antibodies. The next step forward is to determine whether these specific antibodies are found in LTNP and EC subjects and whether they directly correlate with reducing their viral loads.

Significance of immunological factors associated with control: implications on vaccine design

When taking into consideration all the immunological responses to HIV-1 (i.e. innate, humoral and cell-mediated), Gag-specific $CD8^+$ T cells are most positively associated with a lower viral load and promoting long-term control of infection (Walker, 2007). In comparison to Env antigens that undergo frequent mutations in their genome in order to evade the host's immune response, mutations in the *gag* gene do not occur as frequently because they render the virus inactive and unable to

replicate. Furthermore, Env-specific $CD8^+$ T cells have a shorter-lasting response compared with Gag-specific $CD8^+$ T cells (Walker, 2007). In light of past HIV-1 vaccine trial failures, the most realistic approach to developing an HIV-1 vaccine is one that provides partial immune control against viral replication, while preventing clinical disease rather than developing a vaccine that prevents virus infection. An ideal vaccine would utilize a combination of immunological responses, most importantly being robust and cross-reactive T-cell responses against highly heterologous viruses in unison with broad NAb activity. Importantly, the T cell must have a wide breadth of coverage against HIV-1 epitopes in order to prevent viral escape. This will prove particularly challenging with $CD8^+$ T cells that have the tendency to focus on specific epitopes.

Important lessons should be learned from the failure of recent efforts in developing a CMI vaccine that relied on generating a strong CTL response in order to protect vacinees against viral replication and disease progression. Merck's phase IIb STEP vaccine trial (Buchbinder *et al.*, 2008), which failed to demonstrate efficacy in protection of HIV-1 infection in the vaccinated versus placebo groups, used a recombinant adenovirus type 5 (rAd5) vector presenting HIV-1's *gag*, *pol* and *nef* genes. The trial resulted in a higher HIV-1 incidence in vacinees versus placebo recipients among Ad5 seropositive men and uncircumsized men, and an incidence that was similar for vacinees versus placebo recipients among Ad5 seronegative men and circumsized men. The risk of becoming infected was the same between Ad5 seronegative vacinees and the placebo group and the risk was over two times greater for Ad5 seropositive vacinees compared with the placebo group. Male vacinees that became infected had a similar viral load set point as infected individuals from the placebo group (Buchbinder *et al.*, 2008).

As subsequent analysis has shown (O'Brien *et al.*, 2009), the vaccine's failure to show efficacy in the vaccinated group is probably not related to T-cell activation and enhanced HIV-1 infection susceptibility, but rather elevated baseline Ad5 NAb titres in vacinees, which interfered with the rAd5 vector and caused a lower effective dose of the vaccine in vacinees. In fact, vaccination with the rAd5 vector resulted in a lower vector-specific T-cell response in Ad5 seropositive vacinees compared with seronegative vacinees and this is highly suggestive of the fact that baseline levels of Ad5-specific NAbs in vaccine recipients are not surrogate markers for Ad5-specific T-cell responses (O'Brien *et al.*, 2009). For this reason, inducing a strong HIV-1 CMI response should still be considered the major goal of any future vaccine candidates and that failures in the STEP study were not due to the absence of an HIV-specific CTL response – the vaccine elicited IFN-γ ELISPOT responses in 75 % of vacinees (Buchbinder *et al.*, 2008) – but rather the partial neutralization of the vaccine vector by baseline Ad5 vector-specific NAbs.

An HIV-1 vaccine must induce a strong HIV-specific and polyfunctional Th1 $CD4^+$ cell response capable of secreting high levels of IL-2 and IFN-γ and activating $CD8^+$ T cells. However, a caveat to such a response is the potential for increased HIV-1 infectivity due to proliferation of CCR5-expressing $CD4^+$ T cells. O'Brien *et al.* (2009) found no increase in Ad5-specific $CD4^+$ T-cell activation among STEP trial vacinee recipients, though other research (Benlahrech *et al.*, 2009) has shown that vaccination of human sera with an Ad5 vector results in expansion of $CCR5^+$ Ad5-specific $CD4^+$ T cells that have a mucosal-homing phenotype, which are especially susceptible to HIV-1 infection. Future research should determine if it is possible to minimize activation of vector-specific CCR5-expressing $CD4^+$ T cells, and if it were possible, these vectors would be attractive candidates for use in HIV-1 vaccine trials. Another important target for HIV-1 vaccine design is the induction of a cytotoxic virus-specific NK-cells response, which can be achieved by enhancing the binding of inhibitory KIRs to *HLA class I* molecules. A robust NK response would be important for connecting innate immunity to the CMI component of HIV-1 vaccine candidates.

Producing an HIV-1 vaccine that stimulates virus-specific NAbs is difficult because of the sequence diversity of the *env* gene and the *N*-glycosylations that hide many of its immunogenic and conserved regions (Baker *et al.*, 2009). Nevertheless, an important component of vaccines is to stimulate a long-lasting humoral response. Immunodominant peptides of the *env* gene that are recognized by LTNP NAbs are potential vaccine targets as long as they have been strongly correlated with lowering viral load and are capable of broad cross-neutralizing activity.

Immunogens targeted by elite neutralizers and efforts to identify broadly neutralizing mAbs capable of protection against HIV-1 infection are a valuable asset to HIV-1 vaccine development. Additionally, recent studies have discovered broad and potent neutralizing antibodies in separate donors with broad NAb responses to HIV-1 (Burton & Weiss, 2010). These broadly acting antibodies are ideal targets for vaccine design because when given in high doses they could potentially provide sterilizing immunity. However, developing an HIV-1 vaccine capable of sterilizing immunity has proven unsuccessful in the past and continued effort should be placed on inducing a strong CMI response with minimal pre-existing immunity against the recombinant vector and that prevents clinical disease progression.

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