The T-Cell Response to HIV

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HIV is a disease in which the original clinical observations of severe opportunistic infections gave the first clues regarding the underlying pathology, namely that HIV is essentially an infection of the immune system. HIV infects and deletes $CD4^+$ T cells that normally coordinate the adaptive T- and B-cell response to defend against intracellular pathogens. The immune defect is immediate and profound: At the time of acute infection with an AIDS virus, typically more than half of the gut-associated $CD4^+$ T cells are depleted, leaving a damaged immune system to contend with a life-long infection.

The earliest studies of T-cell function among infected persons revealed aspects of HIV immunopathogenesis that are still not fully understood. Despite initial and persistent damage to $CD4^+$ T cells, and a lack of detectable HIV-specific $CD4^+$ T helper cells (Murray et al. 1984; Lane et al. 1985), the magnitude and breadth of $CD8^+$ T-cell responses to HIV in infected humans were found to be robust, with direct effector function of such a magnitude that it could be readily detected in freshly isolated lymphocytes from peripheral blood and brochoalveolar lavage in persons with AIDS (Plata et al. 1987; Walker et al. 1987; Nixon et al. 1988). HIV was already known to be an immunosuppressive disease, yet these cells were present in such robust quantity that they could be detected by assays measuring the ability of freshly isolated peripheral blood cells to lyse autologous B cells infected with recombinant vaccinia-HIV vectors or peptide pulsed targets (Walker et al. 1987; Nixon et al. 1988). Moreover, $CD8^+$ T cells from infected persons were able to inhibit HIV replication (Walker et al. 1986), clearly showing that these cells were functional at least in vitro, but despite this, persons were progressing to AIDS.

Subsequent studies using other approaches such as interferon γ Elispot (Dalod et al. 1999), intracellular cytokine (Maecker et al. 2001), and peptide MHC tetramer assays (Altman et al. 1996) confirmed and quantified these robust $CD8^+$ T-cell responses. Most chronically infected persons target more than a dozen $CD8^+$ epitopes simultaneously (Addo et al. 2003), and in some instances up to 19% of $CD8^+$ T cells are specific for HIV (Richardson 2000; Betts et al. 2001; Papagno et al. 2002), yet control of viremia is not achieved. At the same time, HIV-specific $CD4^+$ T-cell responses were found to be severely impaired, particularly as measured by the ability of these cells to proliferate to viral antigens (Wahren et al. 1987). Thus, from the early studies there was a clear

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disconnect between the lack of HIV-specific $CD4^+$ T cells and the abundance of HIV-specific $CD8^+$ T cells.

Despite these conundrums, there were early signs that $CD8^+$ T cells play a role in controlling HIV disease progression. Sequencing of autologous virus from infected persons revealed evidence of immune selection pressure mediated by these responses (Phillips et al. 1991) and an association with the initial decline in peak viremia after acute infection (Borrow et al. 1994; Koup et al. 1994). The development of HLA-class I-peptide tetramers confirmed the presence of robust induction of responses to multiple epitopes (Altman et al. 1996), and as larger numbers of patients were studied, it also became clear that among the strongest associations with disease outcome was the expression of certain HLA class I alleles (Kaslow et al. 1996; Migueles et al. 2000; Gao et al. 2001), implicating class I restricted cytotoxic T lymphocytes (CTLs) as a major modulator of disease progression. The relationship between $CD8^+$ T-cell immune function and viral control was shown by experimental depletion of $CD8^+$ T cells in animal models of AIDS virus infection (Jin et al. 1999; Schmitz et al. 1999). As sensitive viral load assays became available, it also became clear that some infected persons were able to control viremia to levels below detection by the most sensitive RNA assays, and that these persons were characterized by robust HIV-specific $CD4^+$ T-cell responses (Rosenberg et al. 1997). However, now a quarter century into the epidemic the precise role of T cells in HIV control, and the precise phenotype, specificity and function of T cells that should be induced with a vaccine, remain unclear. And from the standpoint of vaccine development, it remains controversial as to how much insight is to be gained from the study of persons who have become infected, because by definition these are not protective responses.

CTL RESPONSES IN ACUTE INFECTION

Acute HIV infection is often associated with a transient febrile illness, much like infectious mononucleosis (Ho et al. 1985), which has facilitated the identification of persons in the earliest stages of infection, who are HIV RNA positive but not yet HIV antibody positive. Important justification for the intense study of acute infection is the demonstration that early events predict subsequent disease progression (Mellors et al. 1996; Lyles et al. 2000). Acute infection is associated with a decline in peak viremia from ~1–5 million viral copies/mL to a steady state of ~30,000 copies (Lyles et al. 2000), but peak levels of viremia may be much lower in asymptomatic persons (Fiebig et al. 2003). Very few studies have actually examined $CD8^+$ T-cell responses in the earliest stages of infection (Fiebig stage I and II), but when those studies have been performed it is clear that initial immune responses are very narrowly directed (Goonetilleke et al. 2009) and remain narrowly directed as viral set point is achieved (Dalod et al. 1999; Altfeld et al. 2001). Even when responses to autologous virus are measured, the initial detectable response has usually been to only one to three epitopes (Goonetilleke et al. 2009), even though the autologous viruses may contain wild-type epitopes to which $CD8^+$ T cells are subsequently generated (Radebe et al. 2011). An important caveat is that there may be much more happening in the tissues than is sampled in the blood in these early stages. Studies comparing lymph node $CD8^+$ T-cell responses to those in peripheral blood have shown the former to be detectable well in advance of detectable responses in the periphery (Altfeld et al. 2002). Even so, there is a clear hierarchy in initial responses and immunodominance that correlates with set point viremia, which is lost during the transition to chronic infection (Streeck et al. 2009).

Despite the narrowness of the acute phase $CD8^+$ T-cell response, it is associated with a dramatic decline in initial viremia, suggesting that these early, presumably narrowly directed responses are at least partially effective. Although functional studies of early CTL responses are few (Ferrari et al. 2011), evidence of $CD8^+$ T-cell efficacy is suggested by detailed analysis of viral genomes during these early phases of infection, and by modeling studies. 80% of acute infections are established by a

single founder virus, which first begins to diversify at around the time of peak infection (Salazar-Gonzalez et al. 2009; Shaw and Hunter 2011). By single genome amplification one can detect viral evolution at sites of CTL pressure as early as peak viremia (Goonetilleke et al. 2009), clear indication that there is effective pressure being applied because it can lead to a wholesale turnover in the replicating virus population. This has been modeled, suggesting that 15%–35% of infected cells can be killed by CTLs of a single specificity per day in vivo during acute infection (Goonetilleke et al. 2009). This $CD8^+$ T-cell-mediated immune pressure is also apparent in population studies, which have shown clear HLA-associated signature mutations following acute infection (Brumme et al. 2008), persisting in the chronic phase of infection (Moore et al. 2002), and transmission and reversion of CTL escape variants (Goulder et al. 2001; Goepfert et al. 2008).

The characteristics and antiviral efficacy of the acute phase $CD8^+$ T-cell responses are being progressively defined. These clearly occur in the setting of acute phase proteins and proinflammatory cytokines (Stacey et al. 2009). The initial response is narrowly directed, predominantly at epitopes in Env and Nef, regions that are among the most variable in the virus (Lichterfeld et al. 2004; Goonetilleke et al. 2009; Turnbull et al. 2009). Indeed, early responses appear to be targeted to epitopes of higher entropy for reasons that are not clear (Bansal et al. 2005). The breadth of responses increases over time, as do the number of HLA alleles that are involved in recognition of infected cells (Altfeld et al. 2006; Streeck et al. 2009). Some acute phase epitopes are not those that are targeted in chronic infection and may be novel (Goonetilleke et al. 2009), as revealed by studies using peptides representing autologous virus. Often these earliest responses fade away as soon as the epitope escape has occurred. Although these detailed findings have been made in just four patients, ongoing studies in a further 12 show very similar patterns (N Goonetilleke, M Liu, and AJ McMichael, unpubl. data).

The influence of host genetics on acute phase CTL responses is readily apparent. HLA alleles associated with protection from disease progression are preferentially targeted in acute infection, whereas coexpression of risk alleles does not induce responses, a process called immunodominantion (Altfeld et al. 2006). Clinical symptoms of acute infection have been shown to be reduced in persons expressing HLA B57, and this allele is associated with improved outcome and also dominates the early response in persons who express it (Altfeld et al. 2003a). At least one study indicates the selective loss of high-avidity CTLs in acute infection, and when these are maintained they are associated with a lower viral load (Lichterfeld et al. 2007). However, the ability of $CD8^+$ T cells to produce interferon γ in primary infection is a poor predictor of viral set point and disease progression (Gray et al. 2009), suggesting that if CTLs do influence viral set point, this assay may not discern their function. In contrast to the wealth of information regarding $CD8^+$ T-cell responses in the peripheral blood, almost nothing is known about responses in tissues in acute infection, which is the major site of HIV replication.

There has been considerable interest and some controversy over whether highly exposed but HIV seronegative people, such as sex workers in high-HIV-1-prevalence communities, make CTL responses to HIV-1 and whether these are helping them avoid infection (Rowland-Jones et al. 1995). The controversy has been partly resolved by using very sensitive highly controlled and blinded assays that show anti-HIV-specific $CD4^+$ T cells in both HIV-1-exposed and -unexposed uninfected people, with stronger and more sustained T-cell responses in the former (Ritchie et al. 2011). No $CD8^+$ T-cell responses were found in that study but the level of exposure in the exposed cohort in the study was much less than in African sex workers during the early 1990s, so the earlier findings cannot be disregarded. More likely, as shown by (Ritchie et al. 2011), there are relatively frequent $CD4^+$ T-cell responses, primed by crossreacting antigens, in unexposed people and that these are amplified by HIV-1 exposure without infection, for example, in persons homozygous for the CCR5 Δ32 mutation. With very high exposure these T-cell responses could

include $CD8^+$ T cells. However, there is no evidence that either of these T-cell responses is protective.

CTL EVOLUTION FOLLOWING ACUTE INFECTION

Although a narrowly directed immune response is found at the time of maximal decline in peak viremia, responses subsequently broaden, such that during chronic infection the average person targets a median of 14 epitopes simultaneously (Addo et al. 2003), and given that these studies were performed with a reference set of peptides rather than autologous peptides, the actual number may be 20%–30% higher (Altfeld et al. 2003b; Goonetilleke et al. 2009). Immunization studies in animal models indicate that the $CD8^+$ T-cell compartment has enormous expansion capacity, without affecting the size of the naïve $CD4^+$, $CD8^+$, or B-cell populations, and while preserving memory $CD8^+$ T-cell populations to other pathogens (Vezys et al. 2009). HIV-specific $CD8^+$ T-cell responses remain detectable throughout the course of disease, and are actually broader and higher in persons with progressive infection than in those with controlled infection (Pereyra et al. 2008).

The relationship between the earliest CTL responses, viral set point, and disease progression remains controversial. Kinetics of immune responses in acute infection are associated with serial waves of responses (Turnbull et al. 2009), but detectable responses tend to be narrowly directed and of low magnitude (Dalod et al. 1999; Altfeld et al. 2001; Radebe 2011). This may be caused by localization of responses at inductive and effector sites within lymphoid and gut mucosa, respectively, and responses detected in lymph nodes precede those detectable in peripheral blood and are of higher magnitude (Altfeld et al. 2002). There is a high predictability of initial and subsequent epitopes targeted, based on the HLA type of the individual (Goulder et al. 2001; Moore et al. 2002; Yu et al. 2002; Draenert et al. 2006; Brumme et al. 2009). Remarkably, most responses detectable at the time a quasi-set point is achieved persist even in very late stage infection (Koibuchi et al. 2005), though function may be lost, as evidenced by up-regulation of negative immunoregulatory molecules such as PD-1 (Day et al. 2006; Petrovas et al. 2006; Trautmann et al. 2006), and up-regulation of transcription factors that inhibit cell proliferation and cytokine secretion, such as BATF-1 (Quigley et al. 2010).

Specificity of responses during the chronic phase of infection repeatedly suggests that Gag targeting is associated with lower viral load (Edwards et al. 2002; Zuniga et al. 2006; Kiepiela et al. 2007). In a large study of persons with clade C virus infection, the broader the Gag-specific response the lower the viral load, and somewhat paradoxically, the broader the Env-specific response the higher the viral load (Kiepiela et al. 2007; Ngumbela et al. 2008). A similar association between targeting of Gag and viral load is also seen in children (Huang et al. 2008). It is noteworthy that the most protective HLA molecules B57, B58, B81, B14, and B27 target epitopes in Gag, often in relatively conserved regions. It may also be relevant that Gag p24 variation may often carry a fitness cost to the virus, possibly because mutants affect the complex assembly of the viral capsid (Schneidewind et al. 2008). This may make T-cell responses to these epitopes more favorable to the host because the virus is either controlled or escapes with a loss of virulence (Martinez-Picado et al. 2006). Eventually, however, the virus may restore its virulence with compensatory mutations (Schneidewind et al. 2007, 2008, 2009; Goepfert et al. 2008; Crawford et al. 2009).

In addition to targeting epitopes within the nine expressed HIV proteins, recent studies show that antisense peptides and alternative reading frame products are also targeted by $CD8^+$ T cells, as shown first in the SIV model (Maness et al. 2007, 2009) and more recently in humans infected with HIV (Bansal et al. 2010; Berger et al. 2010). Moreover, almost all studies have used peptide-pulsed target cells to define responses, and emerging evidence suggests that processing and presentation are likely critical (Allen et al. 2004; Draenert et al. 2004; Lazaro et al. 2009). Mutations surrounding epitopes are associated with altered processing and lack of presentation (Le Gall et al. 2007), and

this is only detectable when the epitopes are processed and presented in infected cells. Even the infected cell type appears to influence this processing, based on digestion of longer peptides using cytosolic extracts—and perhaps importantly $CD4^+$ T cells have much lower proteolytic activity than monocytes, suggesting that antigen processing may be least effective in the cells that are the most critical to target (Lazaro et al. 2009).

$CD4^+$ T-CELL RESPONSES IN ACUTE AND CHRONIC HIV-1 INFECTION

Generally in those infected with HIV-1, the T-cell responses are dominated by $CD8^+$ T cells. These are much stronger than $CD4^+$ T-cell responses (Ramduth et al. 2005), which are damaged by the virus. Given the important role that $CD4^+$ T cells have in maintaining $CD8^+$ T-cell responses, it is remarkable that the latter are so strong (Kalams et al. 1999). In murine models in which $CD4^+$ T cells are depleted either with antibody infusion or genetically, $CD8^+$ T-cell responses are greatly impaired (Janssen et al. 2003; Shedlock and Shen 2003; Sun and Bevan 2003). On antigen stimulation, they expand rapidly to exhaustion and their IL-2-dependent progression to long term memory populations is abrogated (Kamimura and Bevan 2007). In HIV-1 infection $CD4^+$ T cells, though greatly depleted, are not entirely absent, but abnormalities in the development of $CD8^+$ T-cell responses could be consistent with partial loss of $CD4^+$ T-cell help, or impaired function of what cells remain (Pitcher et al. 1999).

In acute HIV-1 infection, memory $CD4^+$ T cells are massively depleted from the lymphoid system, particularly in the gut involving both direct targeting by the virus and bystander activation-induced cell death (Mattapallil et al. 2005; Douek et al. 2009). This applies to all memory $CD4^+$ T-cell populations but those specific for HIV may be preferentially infected and destroyed (Douek et al. 2002). However, the percentage of HIV-specific $CD4^+$ T cells that are infected, even in the presence of high level viremia, is typically only a few percent or less, suggesting that the majority of these cells somehow escape infection despite being activated at a time of very high viremia.

Early studies showed a lack of $CD4^+$ T-cell responses, measured by antigen stimulated proliferation assays both in early and late infection (Lane et al. 1985). However, Pitcher et al. (1999) found using antigen stimulated cytokine production, that HIV-specific T cells were present at all stages of infection, though in relatively low numbers. A lack of IL-2 production by antigen-specific $CD4^+$ T cells and $CD8^+$ T cells may account for the apparent discrepancy between results with the two assays (Zimmerli et al. 2005). In very early infection however, HIV-specific $CD4^+$ T cells may be present in larger numbers. Rosenberg et al. (2000) showed that when patients were treated very early with antiretroviral drugs, that strong $CD4^+$ T-cell responses to HIV antigens could be rescued, as measured by lymphocyte proliferation assays. Recently, Gray et al. (C Riou, VV Ganusov, C Gray, et al., submitted) have supported this by showing strong $CD4^+$ T-cell responses around the time of peak viremia, but these T cells are rapidly lost as the infection progresses. This loss may reflect the damage to the whole $CD4^+$ T-cell compartment, but it should be noted that in other acute and chronic virus infections (including responses to live vaccines) $CD4^+$ T-cell responses may peak early and then decline to a relatively low level in the blood, in the absence of damage to $CD4^+$ T cells (e.g., Amyes et al. 2003; Goonetilleke et al. 2009). At this point almost nothing is known about HIV-specific $CD4^+$ T-cell responses in lymphoid tissues and the gut, a clearly needed focus for future studies.

Persons who progress very slowly to AIDS, in the absence of treatment, make stronger $CD4^+$ T-cell responses and there is a correlation between the strength of the response and slow progression (Rosenberg et al. 1997), which is also striking in the slow progression of HIV-2 infection (Duvall et al. 2006). In a subset of persons who control viremia spontaneously, the gene expression profile of $CD4^+$ cells is similar to that of uninfected persons (Vigneault et al. 2011). Cross-sectional data in chronically infected persons indicate a link between strong $CD4^+$ T-cell responses and effective $CD8^+$

T-cell responses (Kalams et al. 1999). Recent data implicate $CD4^+$ T cells that make IL21 as particularly important in maintaining $CD8^+$ responses (Chevalier et al. 2011; Williams et al. 2011).

The epitopes recognized by $CD4^+$ T cells occur in all viral proteins but there is a preponderance of T cells specific for Gag and Nef and several epitopes have been defined, though far fewer than the number defined for $CD8^+$ T-cell responses (Kaufmann et al. 2004) (LANL HIV Immunology database: http://www.hiv.lanl.gov/content/immunology/compendium.html). As is often the case for HLA class II restricted T-cell responses, the peptides can be presented by more than one, often several, different HLA class II molecules, in contrast to the generally tighter restriction by HLA class I molecules. In marked contrast to HLA class I presented peptides, mutational escape seems rare, or possibly absent, in HLA class II restricted T-cell responses (Rychert et al. 2007). This may reflect a lack of direct cytotoxic action on virus-infected cells as well as the relative lack of HLA class II on infected cells and also their propensity to interact primarily with dendritic cells, but additional studies are clearly warranted. And genetic studies in a number of cohorts suggest a relationship between expression of certain HLA class II alleles and HIV control/disease progression, again suggesting a possible role for these responses in active immune containment (Lacap et al. 2008; Julg et al. 2011).

Weak HIV-specific $CD4^+$ T-cell responses have been reported in HIV exposed but seronegative donors in a number of studies (Goh et al. 1999; Schenal et al. 2005). Remarkably around 25% of HIV unexposed persons make similar, though weaker and less sustained than in those exposed, $CD4^+$ T-cell responses to previously described HIV epitopes (Ritchie et al. 2011). These responses are probably cross-reactive with other pathogens and other antigens to which the person has been exposed. At least some of these epitopes are the same as those found in HIV infection (Kaufmann et al. 2004), so these pre-infection cross-reactive responses could help explain why these particular epitopes are immunodominant after infection.

Several of the vaccine candidates that have been tested in humans were more effective at stimulating $CD4^+$ T-cell responses than $CD8^+$ T cells, particularly when plasmid DNA and pox virus vectors were used (Goonetilleke et al. 2006; Graham et al. 2006; Harari et al. 2008). Similar results have been found in macaques vaccinated with DNA and pox virus vectors (Sun et al. 2010). In the latter study, the vaccine-induced $CD4^+$ T-cell response did not protect against challenge with the SHIV 89.6P virus, although an earlier study had shown that longer survival in vaccinated monkeys challenged with SIV mac251 was associated with vaccine-induced SIV-specific $CD4^+$ T-cell responses (Sun et al. 2006). Although concern has been expressed that vaccine induction of HIV-specific $CD4^+$ T-cell responses may increase the risk of HIV-1 acquisition by providing more activated $CD4^+$ T cells to the site of infection (Douek et al. 2002; Staprans et al. 2004), this has only been observed in the absence of concomitant $CD8^+$ T-cell responses (Staprans et al. 2004).

IMMUNE SELECTION PRESSURE AND VIRAL ESCAPE

The impact of CTL selection pressure is readily observed by sequencing autologous virus, which undergoes rapid immune-driven evolution in vivo following acute infection (Fig. 1). New approaches involving single genome amplification techniques have shown clear evidence of CTL escape within 25–32 days of infection, around the time of peak viremia following acute infection, and before full seroconversion (Goonetilleke et al. 2009). Indeed, deep sequencing has revealed that the virus explores multiple pathways to escape before going to fixation, indicating that there are constraints on HIV evolution that appear to be caused by imposed fitness alterations (Fischer et al. 2010). The pathways to immune escape appear limited, as shown by studies of genetically identical twins infected by the same virus as adults through shared injection drug use, in whom the earliest targeted epitopes, kinetics of escape and earliest escape variants that arose

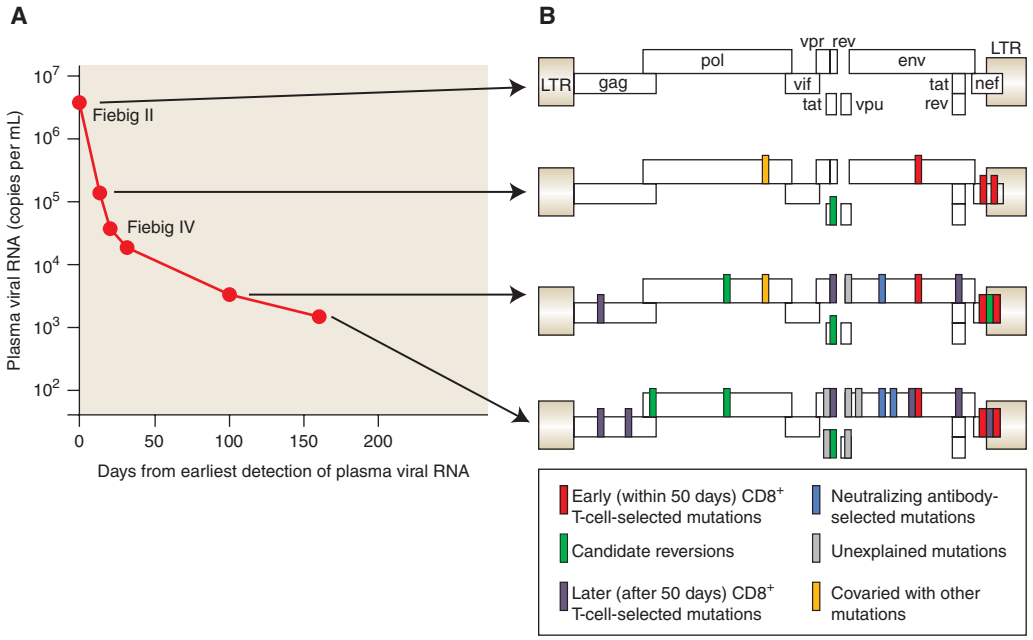

Figure 1. Evolution of the transmitted/founder virus in acute HIV-1 infection is largely driven by $CD8^+$ T-cell responses. (*A*) The graph shows the falling viral load from the time of peak viremia, about 3 weeks after infection, to the establishment of the viral set point 160 days later. Virus taken at the first sampling (the time of screening or "day 0") was characteristic of a single founder virus when sequenced; this is represented on the genetic map of the virus at the *top right* (*B*). At subsequent time points mutations appeared in all the viruses sequenced, shown by the bars on the genetic map to indicate the sites of the mutations. The bars are color coded to indicate rapid escape selected by $CD8^+$ T cells, slower or late escape selected by $CD8^+$ T cells, selection by neutralizing antibodies targeting the virus envelope, "reversions" from unusual sequences in the founder virus sequence to the sequence consensus in the whole database (the transmitted sequences may have been selected by $CD8^+$ T cells in the transmitting sexual partner of the patient). At a few sites it was not clear what was selecting the amino acid change and these are shown in gray. (Adapted from McMichael et al. 2010; reprinted, with express permission, from the author.)

were strikingly similar (Draenert et al. 2006), despite differences in T-cell receptor (TCR) usage (Yu et al. 2007).

Escape from CTL responses can occur through multiple mechanisms. Mutations in regions flanking CTL epitopes can clearly impact processing and subsequent recognition (Draenert et al. 2004; Le Gall et al. 2007; Tenzer et al. 2009), as can mutations within epitopes. Mutations in TCR contact residues have a variable impact on CTL recognition, and the ability to cross-recognize variants appears to be influenced by thymic selection (Kosmrlj et al. 2010). Those HLA alleles associated with better outcome following infection are associated with presentation of fewer self-peptides in the thymus, resulting in a repertoire in which more clones survive negative selection, and are thus are likely to be more cross-reactive to variants that arise. This may also explain the association between protective HLA alleles in HIV infection and an association between these same alleles and autoimmunity/hypersensitivity (Kosmrlj et al. 2010).

The strongest temporal relationship between CTL escape and viral load is associated with HLA B*27 restricted recognition of an epitope in the p24 Gag protein, which requires an initial mutation within the epitope followed by a second distant mutation in order for the

anchor residue to mutate and completely abrogate recognition (Schneidewind et al. 2007). However, for other mutations the effects on viral load are not as clear, likely because of competing effects of immune escape and alterations in viral fitness. Studies clearly indicate that some mutations come at the cost of a significant decrease in viral replicative capacity, suggesting that escape can be beneficial to the host (Martinez-Picado et al. 2006). In persons who control viremia without therapy, their viruses have impaired replication capacity, and this has also been shown for acute infection (Miura et al. 2010); moreover, there is a clear link between certain HLA class I alleles and viral fitness (Miura et al. 2009a), suggesting that this is immune mediated by class I restricted $CD8^+$ T cells. The evolving view is thus that CTL efficacy is not only caused by inhibiting viral replication, but is also impacted by the mutations induced by these responses. Such a view helps to reconcile the finding that the breadth of responses to Gag, a necessarily conserved protein, are associated with lower viral load, whereas breadth of responses to Env, which readily tolerates mutations without affecting viral fitness, is associated with higher viral loads (Fig. 2) (Kiepiela et al. 2007).

Some additional complexities of CTL escape have been revealed by studies of an immunodominant epitope restricted by the protective allele HLA B^*57. Population studies have shown this to be one of the earliest escape mutations to arise for HLA B57 is mutation with the dominant B57-restricted epitope TW10 in Gag (Brumme et al. 2008) that causes a clear decrease in replication capacity (Martinez-Picado et al. 2006). In elite controllers, HLA B57 can be associated with additional rare mutations at other sites within and surrounding the epitope that are even more fitness impairing (Miura et al. 2009b). In acute infection a large population study showed that viruses from individuals expressing protective HLA alleles are less fit than in persons lacking these alleles, and replication capacity in the acute stage of infection correlated with the HLA B-associated Gag polymorphisms (Goepfert et al. 2008; Brockman et al. 2010; Wright et al. 2011). However, these relationships were lost in chronic infection, but compensatory mutations that restore fitness were significantly increased. Together these data suggest that protective alleles may function in part through induction of fitness-impairing mutations. Indeed, in elite controllers, who maintain undetectable viral loads in the absence of therapy (Deeks and Walker 2007), viruses are significantly less fit (Miura et al. 2009a), and this is associated with specific HLA alleles, and more pronounced for Gag than for Env (Troyer et al. 2009). Studies of HIV controllers from the time of acute infection indicate that fitness impairing mutations are selected early or are transmitted from the donor (Miura et al. 2010), and early impairment of viral replication, including those caused by antiviral drug resistance in the donor virus, may impact long term control (Miura et al. 2010; Wright et al. 2011).

The impact of escape at a population level is becoming recognized. Transmitted escape mutations are associated with lower viral loads (Chopera et al. 2008; Goepfert et al. 2008; Crawford et al. 2009), and reversion of these mutations can be slow. Population studies show that the frequency of specific mutations increases with the expression of the selecting HLA allele in the population, and that in some geographic areas escape mutants have become the dominant circulating viruses (Kawashima et al. 2009). Because compensatory mutations can also be transmitted, the end result can be the deletion of an epitope with no associated impairment in viral fitness (Schneidewind et al. 2009). Thus, the host $CD8^+$ T-cell response is helping to drive global HIV evolution, and the geographic differences in HIV clades relates at least in part to selection of mutations by locally prevalent HLA alleles.

IMMUNOGENETICS OF HIV-SPECIFIC T-CELL RESPONSES

Population studies have repeatedly shown dramatically different outcomes following HIV infection, with some persons succumbing to AIDS within less than a year of infection, and others surviving for more than three decades

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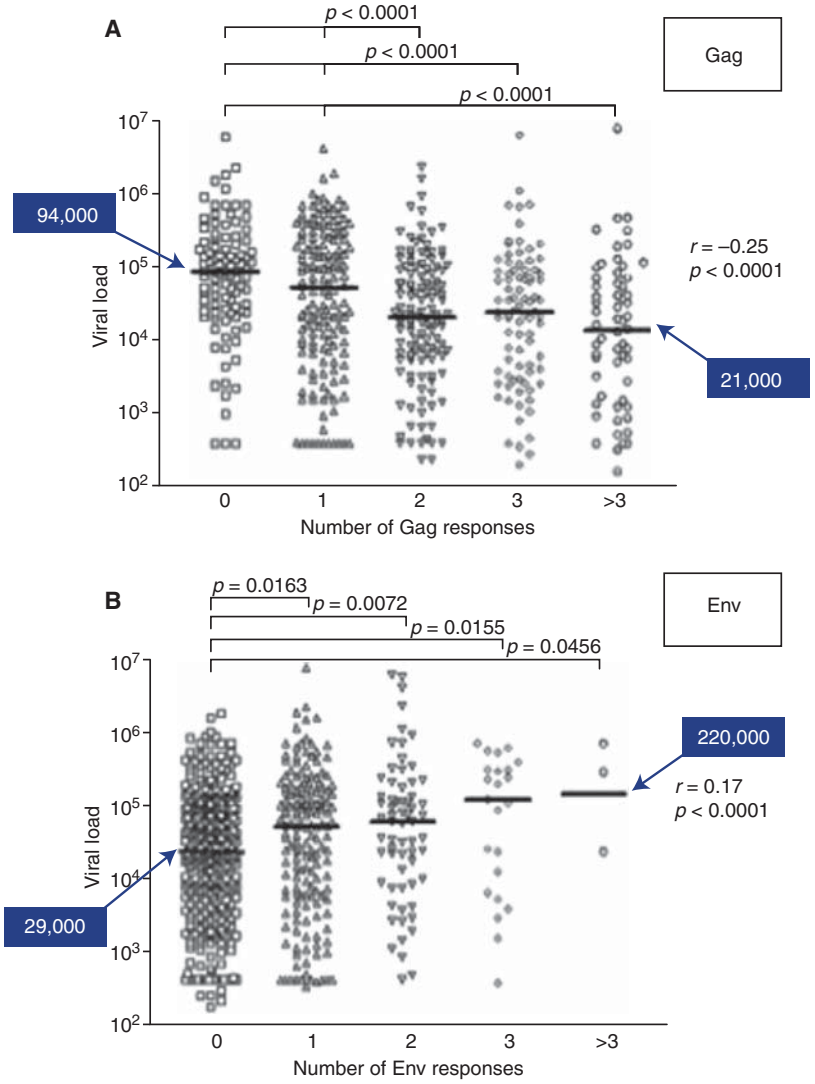

Figure 2. Breadth of protein-specific $CD8^+$ T-cell responses in relation to viral load. 578 persons infected with clade C virus were recruited, and comprehensive analysis of epitope targeting by PBMC was determined using a panel of overlapping peptides spanning all expressed viral proteins. Viral load of individuals in terms of the number of Gag (*A*)- and Env (*B*)-specific responses detected is shown, revealing that the broader the Gag-specific response, the lower the viral load, and the higher the Env-specific response, the higher the viral load, indicating that specificity impacts control. (Adapted from Kiepiela et al. 2007; reprinted, with permission, from Nature Publishers © 2007.)

without the need for antiviral therapy, and with no apparent adverse effects (Deeks and Walker 2007). Early on, it was noted that there was a strong association between expression of certain class I alleles and disease outcome, but the mechanistic basis for this was unclear (Migueles et al. 2000). Indeed, whether this was a simple association or actually causally related could not be determined. Remarkably, both the positive and negative associations between viral load and HLA class I alleles were observed to be limited to HLA B alleles (Kiepiela et al. 2004),

suggesting an active immune control mechanism. Although some associations with HLA C alleles were noted, these may be entirely explained by linkage disequilibrium (Kiepiela et al. 2004).

The first large scale attempt to define a genetic association with viral control was performed on a cohort of persons with acute HIV infection, in whom set point viral load data were available (Fellay et al. 2007). This study, and others that followed, revealed a SNP that is a proxy for HLA B57, as well as another that is associated with HLA C expression, to be the only significant associations with the level of viremia in the year following infection. This unbiased approach confirmed something that had already been recognized in cohort studies, namely that HLA B57 is in some way associated with better control, and also shed new light on HLA C, unique among class I alleles in that it is not down-regulated by the effects of Nef (Le Gall et al. 2000).

Recent population genetic studies comparing persons who progress rapidly to disease with controllers who maintain low viral loads in the absence of antiviral therapy have now shed additional light on these associations (Pereyra et al. 2010). At a viral load below 2000 copies the chances of disease progression drop dramatically, as does the likelihood of transmission (Quinn et al. 2000; Wawer et al. 2005). Through an international collaboration (the International HIV Controllers Study 2010) involving nearly 300 collaborators, a genome wide association study was performed on a cohort of nearly 974 HIV controllers, who maintain viral loads of less than 2000 copies/mL in the absence of therapy, and 2648 HIV progressors with viral loads in excess of 50,000 copies. Analysis of more than one million SNPs in each person revealed over 300 that were statistically associated with influencing viral load, and remarkably all of these lay within the HLA region of chromosome 6. However, after correction for multiple comparisons, only four SNPs remained significant, two of which were the same as those identified to be associated with lower set point viral load after acute infection (Fellay et al. 2007), as well as a SNP associated with psoriasis and with a noncoding SNP in the *MICA* gene.

The above findings were still unable to reconcile whether these were simply associations, and not causal, or whether this reflected an HLA-mediated impact on HIV control. Subsequent sequence analysis within the HLA region, and stepwise regression analysis, revealed that 5 amino acids within HLA B, all of which are involved in binding of viral peptide in the HLA binding groove (Fig. 3), along with a minor effect of a SNP associated with HLA C expression, explained the entire genetic signal being detected, and revealed the major genetic influence on HIV control (Pereyra et al. 2010). The specific amino acids at these positions reconcile both the protective and risk HLA alleles, and thus provide a parsimonious explanation for the long-recognized HLA associations with HIV control, namely that it is the nature of viral peptide presentation that is the major genetic determinant of viral control, likely caused by differences in the relative efficacy of $CD8^+$ T-cell responses induced in the context of slightly altered T-cell-infected cell interactions. Indeed, the site with the strongest association with control is position 97 in HLA B, which sits at the base of the C pocket and depending on its allelic form, has the most impact on the nature of the binding groove and the way in which peptides are bound. Extension of these studies has now shown that the HLA C-associated SNP actually marks a polymorphism that affects microRNA binding, and thereby expression of HLA C (Kulkarni et al. 2011).

The overall impact of $CD8^+$ T-cell-mediated immune pressure on the virus is readily apparent from population analysis of expressed HLA types and sequencing of autologous viruses. Such selection pressure, first shown associated with mother to child transmission (Goulder et al. 2001) and revealed through examination of RT sequences and HLA alleles (Moore et al. 2002), has not only shown clear adaptation of HIV to host HLA genes, but also that some immunodominant epitopes are being eliminated over time (Kawashima et al. 2009). The extent to which $CD4^+$ T-cell responses are driving HIV evolution appears to

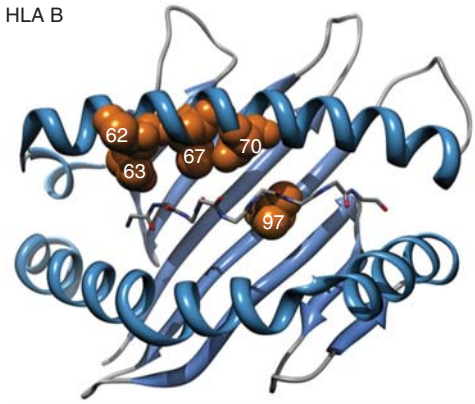

Figure 3. Three-dimensional ribbon representation of the HLA B protein, highlighting amino acid positions 62, 63, 67, 70, and 97 lining the peptide binding pocket that are significantly associated with HIV control. The peptide backbone of the epitope is also displayed. (Adapted from the International HIV Controllers Study 2010; reprinted, with permission, from Science © 2010.)

be negligible, although mutations within $CD4^+$ T-cell epitopes have been documented in early infection (Rychert et al. 2007). And the impact of transmitted $CD8^+$ T-cell-selected mutations on viral set point and disease progression, likely caused by fitness impairing mutations, is clear (Goepfert et al. 2008; Matthews et al. 2008), and consistent with defects in viral fitness as described above.

FUNCTION OF $CD8^+$ T CELLS IN HIV INFECTION

Given the strong evidence that HIV-1-specific $CD8^+$ T cells contribute to the control of HIV-1 in the acute and chronic stages of infection, it is important to know what T-cell functions are responsible for this. Antiviral $CD8^+$ T cells were first identified as T cells that mediate lysis of virus-infected cells and are often referred to as cytotoxic T lymphocytes (Plata et al. 1975). Although most antigen-specific $CD8^+$ T cells have this activity, they can use other effector mechanisms in addition. These include production of interferon-γ, IL-2, TNF-α, MIP-1α (renamed CCL3), MIP-1β (CCL4), and RANTES (CCL5) (Betts et al. 2006; Streeck et al. 2008). This list is probably not complete as revealed by transcriptional profiling, which also reveals subtle interplay between multiple pathways linking function, proliferation, and survival (Quigley et al. 2010). It is also clear that all $CD8^+$ T cells do not display all functions at all times (Ferrari et al. 2011). Naive T cells are quiescent and require days of antigen stimulation to show these functions (Veiga-Fernandes et al. 2000; Peixoto et al. 2007), in contrast memory $CD8^+$ T cells respond rapidly producing interferon-γ within a few hours (Lalvani et al. 1997). Production of lytic granules requires a bit longer but once activated, effector memory $CD8^+$ T cells can release perforin and granzymes within minutes (Barber et al. 2003). The delay in activating lytic functions in memory T cells probably protects the body from autoimmune attack when the TCR encounters weakly binding self antigens, but has the disadvantage that it may take many hours or days before these T cells can be recruited into lytic antipathogen activity. This may be a disadvantage for vaccines that are unlikely to maintain effector $CD8^+$ T cells in a fully primed state and therefore only kill infected cells after some hours.

The relative importance of lytic mechanisms versus cytokine or chemokine production in anti-HIV activity is much discussed. In very early HIV-1 infection $CD8^+$ T cells rapidly select virus escape mutants, often selecting a new mutant to replace all of the previous virus population in a few days (Goonetilleke et al. 2009). It is easiest to explain this by effector $CD8^+$ T cells killing HIV-infected target cells they recognize and thereby reducing their life span and capacity to generate new virus particles. However, new virus production could also be lowered by, for instance, β-chemokines (CCL3, 4, and 5) secreted by the $CD8^+$ T cells reducing infection of new target cells by direct competition for binding to CCR5 or by reducing its expression on the target cells. However, this would not shorten the life of the infected cells, which is what the mathematical model, described by Goonetilleke et al. (2009), implied. These studies suggest that lysis of infected cells is the most important antiviral function of the

$CD8^+$ T cells in acute infection. This is consistent with the finding that the T cells that select early virus escape mutants are high perforin expressors (Hersperger et al. 2010) whereas these early T cells are rather limited in their expression of cytokines and chemokines (Ferrari et al. 2011). This importance of cytolysis in HIV-1 infection contrasts with some other virus infections such as hepatitis B virus infection where, in animal models, perforin-deficient T cells are highly effective because the main antiviral activity is mediated nonlytically by interferon-γ (Yang et al. 2010). And it is important to note that there are data from in vivo analysis of SIV-infected macaques indicating that depletion of $CD8^+$ T cells 2 and 6 months after infection does not result in a measureable change in the lifespan of infected cells, suggesting that direct killing may not be not be the main mechanism of control, at least in this model.

During chronic infection, once viral set point is established, the other functions of $CD8^+$ T cells may become more important, although lytic potential may still be essential (Betts and Harari 2008). In patients who control virus well, the T cells are more quiescent than in acute infection. Many studies have shown that T cells in those who control HIV-1 well are polyfunctional, showing not only cytolytic potential but also have the capacity to produce cytokines and chemokines (Betts and Harari 2008), although it is not clear whether this is cause or effect. Prolonged antigen stimulation in the absence of excessive activation and exhaustion, as occurs in slow progressors, could favor expression of multiple functions. Production of IL-2 may be important in the long term persistence of $CD8^+$ T cells and can be provided by the $CD8^+$ T cell itself or by $CD4^+$ T cells, which survive much better in those whose disease progresses slowly (Rosenberg et al. 1997; Zimmerli et al. 2005). Similar observations have been made in HIV-2 infection in which elite controllers are relatively common (Duvall et al. 2008). These findings are entirely consistent with data in $CD4^+$ T-cell-depleted mice that show the importance of IL-2 in the maintenance of long-term $CD8^+$ T-cell memory (Williams et al. 2006).

THE INTERSECTION OF VIRAL FITNESS AND IMMUNE CONTROL

Although most of the focus on immune control and lack of control has been on $CD8^+$ T-cell function and differential induction of negative immunoregulatory molecules, an increasing body of data suggests that immune-mediated mutations within $CD8^+$ T-cell epitopes lead to reduced viral fitness. These data include assays in which replication of virus containing a B57-selected mutation is out-competed by wild-type virus (Martinez-Picado et al. 2006), evidence of reduced viral fitness in Gag-PR in persons who control virus spontaneously (Miura et al. 2009a), and evidence of compensatory mutations leading to restoration of fitness (Schneidewind et al. 2007, 2009). Importantly, mutations in Env do not lead to reduced fitness, suggesting that structural constraints are likely key to this effect (Troyer et al. 2009). More recent studies have shed further light on this, by demonstrating that there are multidimensional constraints on HIV evolution because certain combinations of mutations must occur in a coordinated manner to maintain virus viability, and thus constrain immune escape pathways (Dahirel et al. 2011). Persons who spontaneously control HIV without medications preferentially target sites that are most constrained, providing further evidence that the specific sites targeted by the immune system may have a major impact on overall control (Dahirel et al. 2011).

FUTURE DIRECTIONS: TOWARD A UNIFIED EXPLANATION FOR HIV PATHOGENESIS

A unified view of HIV pathogenesis is emerging, but there remain many unknowns, and a better understanding will undoubtedly require an integrated analysis of innate and adaptive immune responses, host genetics, and viral genetics. Although both viral load and $CD4^+$ cell count predict disease progression, we remain convinced that the adaptive immunologic response is also predictive of the subsequent course. Clear signals are there: $CD8^+$ T cells are associated with initial control, depletion of

these cells in animal models of AIDS leads to an increase in viremia, virus is evolving to escape detection by CTLs, specific functions mediated by $CD8^+$ T cells have demonstrable antiviral effects in vivo, the effect of HLA far outweighs any other genetic factors, $CD8^+$ and $CD4^+$ T-cell dysfunction is associated with lack of viral control, and immune-induced mutations reduce viral fitness and likely contribute to the antiviral efficacy of the $CD8^+$ T-cell response. But important questions remain, and in particular questions regarding the actual functional profile of $CD8^+$ T cells that might lead to long-term control of HIV, or prevention of disseminated infection. And the extent to which such data will be important to vaccine design remains unclear—although animal models suggest that $CD8^+$ T cells may be able to prevent progressive systemic dissemination of infection (Hansen et al. 2009) and even reduce the level of virus to barely detectable levels (Hansen et al. 2011).

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