

# Crippling HIV one mutation at a time

Todd M. Allen and Marcus Altfeld

**Accumulating data suggest that not all human immunodeficiency virus (HIV)-1-specific immune responses are equally effective at controlling HIV-1 replication. A new study now demonstrates that multiple immune-driven sequence polymorphisms in the highly conserved HIV-1 Gag region of transmitted viruses are associated with reduced viral replication in newly infected humans. These data suggest that targeting these and other conserved viral regions may be the key to developing an effective HIV-1 vaccine.**

Despite significant progress in our understanding of HIV-1 pathogenesis over the past two decades, the precise correlates of protective immunity against HIV-1 infection remain unknown, and the lack of this information has impaired the development of an effective HIV-1 vaccine. It is now well established that virus-specific immune responses, and in particular HIV-1-specific CD8<sup>+</sup> T cell responses, contribute to the control of viral replication in infected individuals. The virus, however, has developed several means to evade these responses, the most notable of which is its ability to rapidly acquire mutations that impair its recognition by epitope-specific CD8<sup>+</sup> T cells (1). This continuous evolution of HIV-1 has also contributed to the dramatic sequence diversity among circulating viral strains at the population level, which represents a major challenge for vaccine development (2). Fortunately, several recent studies, including a study by Goepfert et al. (3) on page 1009 of this issue, indicate that the ability of HIV-1 to escape virus-specific immunity is not limitless, but rather comes at a fitness cost to the virus, which may hold hope for the design of an effective HIV-1 vaccine.

## Balancing cytotoxic T lymphocyte escape and viral fitness

To understand the complex interplay between the immune response and the sequence evolution of HIV-1, and to understand why sustained immune control of HIV-1 is so difficult to attain, it is important to examine the lessons learned from studies of HIV-1 drug resistance (4). It is well known that HIV-1 can rapidly develop drug resistance mutations when single sites in the viral genome are under intense selective pressure, such as in patients undergoing mono- and dual-drug therapy. This observation eventually spurred the development of triple-drug therapies that target multiple highly conserved sites of the virus, making simultaneous mutations in these regions very unlikely. This multi-pronged approach has been extremely successful at containing HIV-1, limiting the development of drug resistance, and dramatically slowing disease progression. These studies also suggested that although viral escape from antiretroviral therapy through the development of drug resistance mutations may be immediately advantageous to the virus in the presence of the drug, they nonetheless result in a reduction of viral replicative fitness (4). Evidence for this fitness loss is derived in part from the observation that drug-resistance mutations quickly revert back to wild-type in the absence of the drug, either when therapy is stopped or when the virus is transmitted to a new host (5, 6). Furthermore, continuing antiretroviral

therapy even after the virus has developed resistance to a specific drug can be clinically beneficial (7).

The concept of drug-induced selection pressure can also be applied to selective pressures imparted by virus-specific CD8<sup>+</sup> T cell responses. The relationship between immune-mediated selection pressure, the emergence of viral escape mutations within targeted CD8<sup>+</sup> T cell epitopes, and the impact of these mutations on viral replicative fitness is best illustrated in the context of virus-specific CD8<sup>+</sup> T cell responses restricted by human histocompatibility leukocyte antigen (HLA)-B57. This HLA class I allele has been consistently associated with protection from HIV-1 disease progression (8–10). Individuals expressing HLA-B57 mount a strong CD8<sup>+</sup> T cell response against a highly conserved epitope within Gag called TW10 very early in acute HIV-1 infection (11, 12). The development of this TW10-specific CD8<sup>+</sup> T cell response is associated with the reduction of viral load by 1,000-fold or more. The virus eventually evades this dominant TW10-specific CD8<sup>+</sup> T cell response by selecting for escape variants within the epitope (13–15). But despite immune escape, viral replication remains well controlled in these individuals, and large numbers of individuals expressing HLA-B57 have long-term nonprogressive HIV-1 infection (16). The underlying factors responsible for this apparent paradox—efficient control of virus replication despite viral escape from CD8<sup>+</sup> T cell-mediated immune pressure—appear to be related to the reduced replicative fitness of viruses containing escape mutations in the TW10 epitope. Indeed, the rapid in vivo reversion of these mutations back to wild-type after transmission into a new HLA-B57<sup>+</sup> host (13), and the direct impact of these mutations on viral replication in vitro, confirm the

T.M.A. and M.A. are at Partners AIDS Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02129.

## CORRESPONDENCE

T.M.A.: tallen2@partners.org

OR

M.A.: maltfeld@partners.org

© 2008 Allen and Altfeld

The Rockefeller University Press \$30.00

J. Exp. Med. Vol. 205 No. 5 1003–1007 www.jem.org/cgi/doi/10.1084/jem.20080569

deleterious impact of escape mutations in TW10 on viral replicative fitness (15, 17). These studies also show that the virus tries to minimize the impact of these mutations by developing secondary compensatory mutations that can partially restore the replication defects (15, 17). Furthermore, a recent population study of HIV-1 clade B- and clade C-infected individuals demonstrated an inverse correlation between the proportion of mutations within CD8<sup>+</sup> T cell epitopes and viral load (18). Collectively, these studies suggest a model in which the virus is either controlled by potent virus-specific T cell responses or evades antiviral immune pressures through sequence variations that decrease its capacity to replicate. The study by Goepfert et al. (3) provides additional evidence for this model, which may help in translating studies of HIV-1 pathogenesis into vaccine design.

---

**The ability of HIV-1 to escape virus-specific immunity is not limitless, but rather comes at a fitness cost to the virus.**

---

#### Escape mutations: the bright side

It is now well established that drug and immune escape mutations selected in an HIV-1-infected host can be transmitted to a new host (19–23). Several studies have demonstrated that the transmission of drug-resistant virus can have a significant impact on the response to antiretroviral therapy in the newly infected recipient (22, 23). But the clinical consequences of infection with viruses containing immune escape mutations are not well understood. To address this issue, Goepfert et al. studied HLA class I-associated amino acid polymorphisms of HIV-1 Gag and Nef in a cohort of 114 HIV-1 transmission pairs from Zambia. These couples were initially identified as HIV-1-discordant couples in which one partner was HIV-1 infected, and the other was HIV-1 negative. Counseling and condom provision have reduced the transmission rates in these discordant couples, but transmission of HIV-1 still occurs in ~8% of

the couples per year. These unfortunate cases provide a unique setting to study the impact of viral sequence mutations selected under immune pressure in the initial host (the donor in the partnership) on viral replication in the newly infected host (the recipient).

The authors sequenced the HIV-1 *gag* and *nef* genes from plasma samples obtained six months after the estimated date of infection, and demonstrated that accumulating transmitted amino acid mutations in Gag, but not Nef, were associated with reduced viral loads in the recipients. The protective effect of transmitted Gag mutations was largely mediated by those associated with HLA-B-restricted CD8<sup>+</sup> T cell responses. This result is in line with a previous study in HIV-1 clade C-infected individuals that demonstrated a dominant role for HLA-B-restricted T cell responses in driving viral escape and in controlling viral replication (24).

Interestingly, newly infected hosts whose HLA class I alleles were not associated with the induction of Gag mutations benefited the most from the transmission of viruses carrying these escape mutations. This later observation suggests that transmitted Gag mutations can substitute for the absence of immune responses capable of actively forcing the acquisition of Gag mutations in the new host, and that some transmitted mutations may revert sufficiently slowly to benefit the recipient early after infection. This is further supported by a recently published study of Chopera et al. (25) demonstrating that HLA-B57/5801<sup>+</sup> individuals infected with HIV-1 clade C viruses carrying mutations that indicate previous selection in HLA-B57/5801<sup>+</sup> individuals experienced lower viral loads and higher CD4<sup>+</sup> T cell counts than individuals infected with viruses without these mutations. Collectively, these studies demonstrate the potential consequences of the “immune selection history” of the transmitted virus on viral replication in the newly infected recipient, at least during the initial phase of infection. It is important to note, however, that the long-term benefits of the transmission of these mutated viruses remain to be determined. Trans-

mitted mutations may eventually revert in the new host, resulting in higher levels of viral replication. If such mutations are found to be stable over time, however, this might suggest long-term benefits of these transmitted mutations on limiting progression to AIDS.

---

**Immune responses against this critical “Achilles’ heel” of HIV-1 may provide strong protection because the virus can evade this response only at significant cost to its replicative fitness.**

---

#### Specificity trumps quantity

Over the years, numerous studies have shown that neither the overall magnitude nor the breadth of HIV-1-specific CD8<sup>+</sup> T cell responses correlates with better outcome of HIV-1 infection (26–28). More recent data, however, including those in the current study by Goepfert et al., suggest that the specificity of the CD8<sup>+</sup> T cell response against HIV-1, and in particular the Gag-specific response, may be critical for immune control (24, 29–36). Many of the protective major histocompatibility complex class I alleles described for both HIV-1 and simian immunodeficiency virus (SIV) infection present CD8<sup>+</sup> T cell epitopes from the Gag protein. HLA-B57 and HLA-B27, for example, present adjacent T cell epitopes from HIV-1 Gag to CD8<sup>+</sup> T cells during the acute phase of infection at a time when control of viral replication appears to be either established or lost (12, 37). Similarly, in acutely SIV-infected macaques, CD8<sup>+</sup> T cell responses restricted by the protective allele Mamu-A01 predominantly target an epitope in Gag (38). Notably, all of these CD8<sup>+</sup> T cell epitopes are located within a short but highly conserved 100-amino acid stretch of the protein (37). These data support the hypothesis that focused CD8<sup>+</sup> T cell responses against Gag may be associated with early and sustained control of both HIV-1 and SIV. Similarly, viral escape mutations in each of these Gag epitopes have been found to impair viral replication capacity to the

**Control of HIV-1 might therefore require a combined assault on conserved regions of the virus, which can cripple HIV-1 one mutation at a time.**

extent that flanking compensatory mutations often arise that partially restore these replicative defects (15, 17, 39).

There are several reasons why Gag might be critical for raising a protective immune response. First, Gag's dominance as a target of the cellular immune response (26–28) may be due to its preferential processing and presentation by infected cells. Gag-derived epitopes can be presented very early after infection because a majority of the capsid molecules derived from the Gag p24 subunit of the infecting virion can be rapidly degraded and presented on the surface of an infected cell before the rest of the viral proteins are synthesized *de novo* (40). Furthermore, the Gag protein, and in particular the p24 subunit, is a key structural component of the virus and is thus highly conserved. Therefore, immune responses against this critical "Achilles' heel" of HIV-1 may provide strong protection because the virus can evade this response only at significant cost to its replicative fitness (1, 41). The study in transmission pairs by Goepfert et al. provides further evidence for this model by demonstrating that the transmission of multiple escape mutations in Gag, but not Nef, is associated with reduced viral loads in the new host (3).

**Broader Gag response, better protection**

A crucial observation of recent studies associating Gag-specific CD8<sup>+</sup> T cell responses with control of HIV-1 viremia was that the breadth of the Gag-specific responses appeared to be important for this control. In a large cohort study of HIV-1 clade C-infected individuals in South Africa, CD8<sup>+</sup> T cell responses against two or more epitopes in Gag were associated with markedly lower viral set points, whereas CD8<sup>+</sup> T cell responses against one or no epitope in Gag was not associated with viral con-

trol (32). In the current study, Goepfert et al. observed that the transmission of more than five sequence variations in Gag was associated with lower viral loads in the new host, whereas transmission of three or fewer mutations was not (3). These data strongly suggest that an accumulation of either multiple Gag-specific CD8<sup>+</sup> T cell responses or the resulting mutations within Gag is critical for viral containment. This observation might help to explain why the vaccine used in the recently terminated STEP trial failed to protect humans from HIV-1 infection or to improve control of viremia. The vaccine used the backbone of a common cold virus to deliver *gag*, *pol*, and *nef* genes to prime CD8<sup>+</sup> T cell responses against HIV-1 (<http://www.hvtn.org/media/pr/step.html>). Despite considerable immunogenicity in nonhuman primates, however, the vaccine elicited CD8<sup>+</sup> T cell responses against fewer than two epitopes within Gag in ~80% of vaccinated subjects in Phase I trials (slide 12; <http://www.hvtn.org/fgm/1107slides/McElrath.pdf>). Therefore, although the vaccine was capable of inducing CD8<sup>+</sup> T cell responses, the breadth of the Gag-specific response was substantially lower than that associated with protection in natural infection.

**The disadvantages of too many choices**

Virus-specific CD8<sup>+</sup> T cell responses can compete with one another, with the rapid expansion of responses against one epitope impairing the subsequent development of responses against other epitopes (42). This competition might help to explain the observation that CD8<sup>+</sup> T cell responses targeting the more variable regions of the virus, such as Nef and Env, have been associated with no protection or with even higher viremia, as these responses could have suppressed protective Gag-specific responses (3, 32). This lack of protection by Nef- and Env-specific T cell responses in natural infection implies that it may be critical for vaccines not only to target highly conserved regions, such as Gag, but also to exclude variable regions to avoid competition. If ongoing vaccine studies in the nonhuman pri-

mate model substantiate this, an additional challenge will be to simultaneously prime cellular immune responses against conserved structural proteins, while eliciting neutralizing antibody responses against the envelope protein. Because antibodies recognize and eliminate virus from the blood before it has a chance to infect a cell, the combined efforts of vaccine-induced neutralizing antibodies and CD8<sup>+</sup> T cell responses will likely be needed to mount an effective immune responses against HIV-1.

To date, efforts to design an effective HIV-1 vaccine have largely focused on inducing stronger CD8<sup>+</sup> T cell responses against an array of viral proteins in the hopes of eliciting the broadest immune response possible. These attempts to recapitulate the immunity induced in natural HIV-1 infection, which fails to protect from disease progression in the vast majority of infected individuals, is prone to failure against such a highly variable pathogen that can easily evade the majority of these responses. Recent data, including the study by Goepfert et al., demonstrate a protective effect of broadly directed Gag-specific CD8<sup>+</sup> T cell responses and a cumulative effect of immune-driven mutations in Gag on viral replication capacity, suggesting that some HIV-1-specific immune responses are superior to others in mediating protective immunity. Control of HIV-1 might therefore require a combined assault on conserved regions of the virus (1, 41), which can cripple HIV-1 one mutation at a time. If so, it will be necessary to identify and target vaccine responses against the most critical regions, and only those regions, to hit HIV-1 where it hurts.

**REFERENCES**

1. Goulder, P.J., and D.I. Watkins. 2004. HIV and SIV CTL escape: implications for vaccine design. *Nat. Rev. Immunol.* 4:630–640.
2. Walker, B.D., and B.T. Korber. 2001. Immune control of HIV: the obstacles of HLA and viral diversity. *Nat. Immunol.* 2:473–475.
3. Goepfert, P.A., W. Lumm, P. Farmer, P. Matthews, A. Prendergast, J. Carlson, C.A. Derdeyn, J. Tang, R.A. Kaslow, A. Bansal, et al. 2008. Transmission of HIV-1 Gag immune escape mutations is associated with reduced viral load in linked recipients. *J. Exp. Med.* 205:1009–1017.

4. Quinones-Mateu, M.E., D.M. Moore-Dudley, O. Jegede, J. Weber, and E.J. Arts. 2008. Viral drug resistance and fitness. *Adv. Pharmacol.* 56:257–296.
5. Gandhi, R.T., A. Wurcel, E.S. Rosenberg, M.N. Johnston, N. Hellmann, M. Bates, M.S. Hirsch, and B.D. Walker. 2003. Progressive reversion of human immunodeficiency virus type 1 resistance mutations in vivo after transmission of a multiply drug-resistant virus. *Clin. Infect. Dis.* 37:1693–1698.
6. Warden, M., C. Delaunay, A.G. Marcelin, N. Ktorza, H. Ait Mohand, S. Dominguez, L. Schneider, J. Ghosn, M. Pauchard, D. Costagliola, et al. 2004. Comparison of the dynamics of resistance-associated mutations to nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase inhibitors, and protease inhibitors after cessation of antiretroviral combination therapy. *Antimicrob. Agents Chemother.* 48:644–647.
7. Deeks, S.G., T. Wrin, T. Liegler, R. Hoh, M. Hayden, J.D. Barbour, N.S. Hellmann, C.J. Petropoulos, J.M. McCune, M.K. Hellerstein, and R.M. Grant. 2001. Virologic and immunologic consequences of discontinuing combination antiretroviral-drug therapy in HIV-infected patients with detectable viremia. *N. Engl. J. Med.* 344:472–480.
8. Kaslow, R.A., C. Rivers, J. Tang, T.J. Bender, P.A. Goepfert, R. El Habib, K. Weinhold, and M.J. Mulligan. 2001. Polymorphisms in HLA class I genes associated with both favorable prognosis of human immunodeficiency virus (HIV) type 1 infection and positive cytotoxic T-lymphocyte responses to ALVAC-HIV recombinant canarypox vaccines. *J. Virol.* 75:8681–8689.
9. Gao, X., A. Bashirova, A.K. Iversen, J. Phair, J.J. Goedert, S. Buchbinder, K. Hoots, D. Vlahov, M. Altfeld, S.J. O'Brien, and M. Carrington. 2005. AIDS restriction HLA allotypes target distinct intervals of HIV-1 pathogenesis. *Nat. Med.* 11:1290–1292.
10. Martin, M.P., Y. Qi, X. Gao, E. Yamada, J.N. Martin, F. Pereyra, S. Colombo, E.E. Brown, W.L. Shupert, J. Phair, et al. 2007. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. *Nat. Genet.* 39:733–740.
11. Altfeld, M., M.M. Addo, E.S. Rosenberg, F.M. Hecht, P.K. Lee, M. Vogel, X.G. Yu, R. Draenert, M.N. Johnston, D. Strick, et al. 2003. Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. *AIDS* 17:2581–2591.
12. Altfeld, M., E.T. Kalife, Y. Qi, H. Streeck, M. Lichterfeld, M.N. Johnston, N. Burgett, M.E. Swartz, A. Yang, G. Alter, et al. 2006. HLA alleles associated with delayed progression to AIDS contribute strongly to the initial CD8(+) T cell response against HIV-1. *PLoS Med.* 3:e403.
13. Leslie, A.J., K.J. Pfafferoth, P. Chetty, R. Draenert, M.M. Addo, M. Feeney, Y. Tang, E.C. Holmes, T. Allen, J.G. Prado, et al. 2004. HIV evolution: CTL escape mutation and reversion after transmission. *Nat. Med.* 10:282–289.
14. Allen, T.M., M. Altfeld, S.C. Geer, E.T. Kalife, C. Moore, K.M. O'Sullivan, I. Desouza, M.E. Feeney, R.L. Eldridge, E.L. Maier, et al. 2005. Selective escape from CD8+ T-cell responses represents a major driving force of human immunodeficiency virus type 1 (HIV-1) sequence diversity and reveals constraints on HIV-1 evolution. *J. Virol.* 79:13239–13249.
15. Brockman, M.A., A. Schneidewind, M. Lahaie, A. Schmidt, T. Miura, I. Desouza, F. Ryvkin, C.A. Derdeyn, S. Allen, E. Hunter, et al. 2007. Escape and compensation from early HLA-B57-mediated cytotoxic T-lymphocyte pressure on human immunodeficiency virus type 1 Gag alter capsid interactions with cyclophilin A. *J. Virol.* 81:12608–12618.
16. Deeks, S.G., and B.D. Walker. 2007. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity* 27:406–416.
17. Martinez-Picado, J., J.G. Prado, E.E. Fry, K. Pfafferoth, A. Leslie, S. Chetty, C. Thobakgale, I. Honeyborne, H. Crawford, P. Matthews, et al. 2006. Fitness cost of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1. *J. Virol.* 80:3617–3623.
18. Frater, A.J., H. Brown, A. Oxenius, H.F. Gunthard, B. Hirschel, N. Robinson, A.J. Leslie, R. Payne, H. Crawford, A. Prendergast, et al. 2007. Effective T-cell responses select human immunodeficiency virus mutants and slow disease progression. *J. Virol.* 81:6742–6751.
19. Leslie, A., D. Kavanagh, I. Honeyborne, K. Pfafferoth, C. Edwards, T. Pillay, L. Hilton, C. Thobakgale, D. Ramduth, R. Draenert, et al. 2005. Transmission and accumulation of CTL escape variants drive negative associations between HIV polymorphisms and HLA. *J. Exp. Med.* 201:891–902.
20. Goulder, P.J., C. Brander, Y. Tang, C. Tremblay, R.A. Colbert, M.M. Addo, E.S. Rosenberg, T. Nguyen, R. Allen, A. Trocha, et al. 2001. Evolution and transmission of stable CTL escape mutations in HIV infection. *Nature* 412:334–338.
21. Allen, T.M., M. Altfeld, X.G. Yu, K.M. O'Sullivan, M. Lichterfeld, S. Le Gall, M. John, B.R. Mothe, P.K. Lee, E.T. Kalife, et al. 2004. Selection, transmission, and reversion of an antigen-processing cytotoxic T-lymphocyte escape mutation in human immunodeficiency virus type 1 infection. *J. Virol.* 78:7069–7078.
22. Little, S.J., S. Holte, J.P. Routy, E.S. Daar, M. Markowitz, A.C. Collier, R.A. Koup, J.W. Mellors, E. Connick, B. Conway, et al. 2002. Antiretroviral-drug resistance among patients recently infected with HIV. *N. Engl. J. Med.* 347:385–394.
23. Little, S.J., E.S. Daar, R.T. D'Aquila, P.H. Keiser, E. Connick, J.M. Whitcomb, N.S. Hellmann, C.J. Petropoulos, L. Sutton, J.A. Pitt, et al. 1999. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. *JAMA* 282:1142–1149.
24. Kiepiela, P., A.J. Leslie, I. Honeyborne, D. Ramduth, C. Thobakgale, S. Chetty, P. Rathnavalu, C. Moore, K.J. Pfafferoth, L. Hilton, et al. 2004. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. *Nature* 432:769–775.
25. Chopera, D.R., Z. Woodman, K. Mlisana, M. Mlotshwa, D.P. Martin, C. Seoighe, F. Treurnicht, D.A. de Rosa, W. Hide, S.A. Karim, et al. 2008. Transmission of HIV-1 CTL escape variants provides HLA-mismatched recipients with a survival advantage. *PLoS Pathog.* 4:e1000033.
26. Addo, M.M., X.G. Yu, A. Rathod, D. Cohen, R.L. Eldridge, D. Strick, M.N. Johnston, C. Corcoran, A.G. Wurcel, C.A. Fitzpatrick, et al. 2003. Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load. *J. Virol.* 77:2081–2092.
27. Frahm, N., B.T. Korber, C.M. Adams, J.J. Szinger, R. Draenert, M.M. Addo, M.E. Feeney, K. Yusim, K. Sango, N.V. Brown, et al. 2004. Consistent cytotoxic-T-lymphocyte targeting of immunodominant regions in human immunodeficiency virus across multiple ethnicities. *J. Virol.* 78:2187–2200.
28. Betts, M.R., D.R. Ambrozak, D.C. Douek, S. Bonhoeffer, J.M. Brenchley, J.P. Casazza, R.A. Koup, and L.J. Picker. 2001. Analysis of total human immunodeficiency virus (HIV)-specific CD4(+) and CD8(+) T-cell responses: relationship to viral load in untreated HIV infection. *J. Virol.* 75:11983–11991.
29. Barouch, D.H., J. Kunstman, M.J. Kuroda, J.E. Schmitz, S. Santra, F.W. Peyerl, G.R. Krivulka, K. Beaudry, M.A. Lifton, D.A. Gorgone, et al. 2002. Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. *Nature* 415:335–339.
30. Klein, M.R., C.A. van Baalen, A.M. Holwerda, S.R. Kerkhof Garde, R.J. Bende, I.P. Keet, J.K. Eeffinck-Schattenkerk, A.D. Osterhaus, H. Schuitemaker, and F. Miedema. 1995. Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. *J. Exp. Med.* 181:1365–1372.
31. Edwards, B.H., A. Bansal, S. Sabbaj, J. Bakari, M.J. Mulligan, and P.A. Goepfert. 2002. Magnitude of functional CD8+ T-cell responses to the gag protein of human immunodeficiency virus type 1 correlates inversely with viral load in plasma. *J. Virol.* 76:2298–2305.
32. Kiepiela, P., K. Ngumbela, C. Thobakgale, D. Ramduth, I. Honeyborne, E. Moodley, S. Reddy, C. de Pierres, Z. Mncube, N. Mkhwanazi, et al. 2007. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. *Nat. Med.* 13:46–53.

33. Zuniga, R., A. Lucchetti, P. Galvan, S. Sanchez, C. Sanchez, A. Hernandez, H. Sanchez, N. Frahm, C.H. Linde, H.S. Hewitt, et al. 2006. Relative dominance of Gag p24-specific cytotoxic T lymphocytes is associated with human immunodeficiency virus control. *J. Virol.* 80:3122–3125.
34. Masemola, A., T. Mashishi, G. Houry, P. Mohube, P. Mokgotho, E. Vardas, M. Colvin, L. Zijenah, D. Katzenstein, R. Musonda, et al. 2004. Hierarchical targeting of subtype C human immunodeficiency virus type 1 proteins by CD8+ T cells: correlation with viral load. *J. Virol.* 78:3233–3243.
35. Geldmacher, C., C. Gray, M. Nason, J.R. Currier, A. Haule, L. Njovu, S. Geis, O. Hoffmann, L. Maboko, A. Meyerhans, et al. 2007. A high viral burden predicts the loss of CD8 T-cell responses specific for subdominant gag epitopes during chronic human immunodeficiency virus infection. *J. Virol.* 81:13809–13815.
36. Riviere, Y., M.B. McChesney, F. Porrot, F. Tanneau-Salvadori, P. Sansonetti, O. Lopez, G. Pialoux, V. Feuille, M. Mollereau, S. Chamaret, et al. 1995. Gag-specific cytotoxic responses to HIV type 1 are associated with a decreased risk of progression to AIDS-related complex or AIDS. *AIDS Res. Hum. Retroviruses.* 11:903–907.
37. Streeck, H., M. Lichterfeld, G. Alter, A. Meier, N. Teigen, B. Yassine-Diab, H.K. Sidhu, S. Little, A. Kelleher, J.P. Routy, et al. 2007. Recognition of a defined region within p24 gag by CD8+ T cells during primary human immunodeficiency virus type 1 infection in individuals expressing protective HLA class I alleles. *J. Virol.* 81:7725–7731.
38. Allen, T.M., D.H. O'Connor, P. Jing, J.L. Dzuris, B.R. Mothe, T.U. Vogel, E. Dunphy, M.E. Liebl, C. Emerson, N. Wilson, et al. 2000. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. *Nature.* 407:386–390.
39. Schneidewind, A., M.A. Brockman, R. Yang, R.I. Adam, B. Li, S. Le Gall, C.R. Rinaldo, S.L. Craggs, R.L. Allgaier, K.A. Power, et al. 2007. Escape from the dominant HLA-B27-restricted cytotoxic T-lymphocyte response in Gag is associated with a dramatic reduction in human immunodeficiency virus type 1 replication. *J. Virol.* 81:12382–12393.
40. Sacha, J.B., C. Chung, E.G. Rakasz, S.P. Spencer, A.K. Jonas, A.T. Bean, W. Lee, B.J. Burwitz, J.J. Stephany, J.T. Loffredo, et al. 2007. Gag-specific CD8+ T lymphocytes recognize infected cells before AIDS-virus integration and viral protein expression. *J. Immunol.* 178:2746–2754.
41. Altfield, M., and T.M. Allen. 2006. Hitting HIV where it hurts: an alternative approach to HIV vaccine design. *Trends Immunol.* 27:504–510.
42. Yewdell, J.W. 2006. Confronting complexity: real-world immunodominance in antiviral CD8+ T cell responses. *Immunity.* 25:533–543.