Crosscurrents in HIV-1 evolution

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Substitutions in CD8+ T cell epitopes in viral proteins can alter the complex interaction between viruses and host immunity. Reduced viral fitness and recognition of altered and subdominant epitopes are possible outcomes.

The classical major histocompatibility complex (MHC) class I loci are the most polymorphic genes known. This extraordinary diversity is thought to reflect evolutionary pressure for recognition of a broad range of viral pathogens by cytotoxic T lymphocytes (CTLs). Notably, in contrast to the variable-(diversity)-joining recombination mechanism that generates a vast diversity of T cell receptors in each person, the evolutionary forces operating on the MHC generate diversity mainly at the population level. Thus, understanding of the biology of the MHC and of the host response to viral infection requires not only molecular studies of CTL responses in individual people but also population-level analyses of these responses. Such studies help to clarify the importance of virus-specific CTLs in complex chronic viral infections in which viral evolution is a substantial factor. Studies of the CTL response to human immunodeficiency virus 1 (HIV-1) infection by Iversen *et al.*[1] and by Frahm *et al.*[2] in this issue of *Nature Immunology* emphasize the power of this approach.

In people with untreated HIV-1 infection, every possible point mutation in the entire genome arises daily, making possible rapid evolution that is shaped by selective pressure from the immune system and the drive to maintain or improve fitness. It is now apparent that $CD8^+$ T cells exert considerable pressure on HIV-1 (refs. 3–6). The most notable aspect of such immune pressure is that the evolutionary forces are very different in each person because of individual differences in MHC genotype. In the average HIV-1-infected person, approximately 14 $CD8^+$ T cell epitopes, located in different regions of various viral proteins, are presented by different MHC class I molecules and are targeted by CTLs[7]. It is now apparent that the virus can 'respond' to such immune pressure by developing mutations in key epitopes, which often results in CTL escape[3–5] and in some cases disease progression[8]. It is also apparent that such 'escape mutations' sometimes come at a substantial cost to viral fitness. That fact is demonstrated by an observational study of viral transmission[9] that describes a characteristic escape mutation seen in the gene encoding an epitope of the Gag protein in HIV-1-infected people who express the MHC class I allele HLA-B*57. When viruses carrying the escape mutation are transmitted to HLA-B*57-negative people, the lack of selective pressure (because of the absence of HLA-B*57 presentation of the epitope) results in reversion of the epitope to wild-type sequence. Thus, in the absence of specific CTLs, some escape mutant viruses are less fit than wild-type virus. An elegant mutagenesis study has demonstrated the fine line between the advantage gained by CTL escape and the potential disadvantage that may result from diminished fitness[10]. Although a nonconservative substitution at any residue in a Gag epitope completely abrogates viral replication, more subtle but nevertheless substantial changes in replication are seen in half of the mutants with

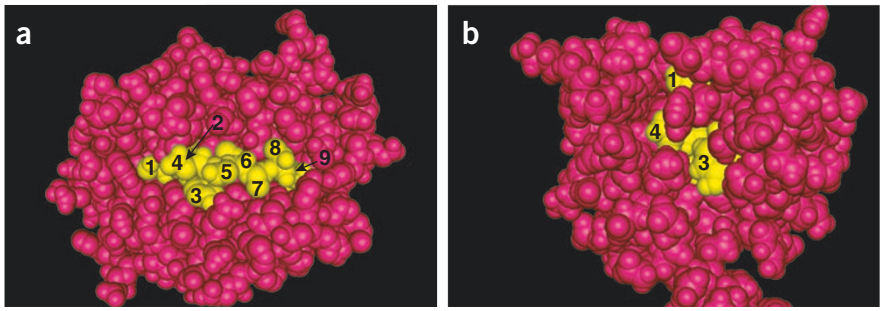

Figure 1 Opposing evolutionary pressures acting on the SLYNTVATL epitope in the HIV-1 Gag protein. (**a**) Crystal structure of the SLYNTVATL peptide bound to HLA-A*02 (E. Martinez-Hackert *et al.*, NCBI Molecular Modeling Database, 34508 (http://www.ncbi.nlm.nih.gov/structure/mmdb)). Numbers indicate residue positions in the epitope. The anchor residues at positions 2 and 9 are buried in the groove of the A2 molecule. Positions 1, 2, 4 and 9 are essentially invariant in all HIV-1 isolates. Escape mutations occur at positions 3, 6 and 8 and affect binding to the T cell receptor. (**b**) The SLYNTVATL epitope is located in a core region of the matrix subunit of Gag[11]. There is limited exposure of the residues representing positions 1, 3 and 4 of the epitope. The other residues are buried in the core of the molecule. Thus, substitutions in this region of Gag are likely to affect the overall structure of this small globular domain and to interfere with the normal function of matrix in virus assembly. As a result, there is considerable evolutionary pressure for reversion of CTL escape mutants.

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conservative single–amino acid substitutions. Notably, those mutants that maintain viral fitness are still somewhat susceptible to CTLs specific for the wild-type epitope.

The study by Iversen and colleagues shows how viral evolution reflects the balance between CTL escape and viral fitness. They have analyzed Gag sequences from many HIV-1-infected people expressing HLA-A*02, the most prevalent MHC class I molecule world-wide. Focusing on the immunodominant, HLA-A*02-restricted Gag peptide SLYNTVATL, they define two distinct evolutionary pathways affecting the T cell receptor contact residues of this epitope. The first pathway involves acquisition of CTL escape mutations. Selective pressure from CTLs drives substitutions at residues 3, 6 and 8, usually resulting in Y3F, V6I, and T8V substitutions in HLA-A*02$^+$ people; these appear in a sequential way, with the first mutation seen an average of 3–6 years after infection and mutants with all three substitutions (SLFNTIAVL) appearing 5–10 years after infection.

The pressure to accumulate multiple escape mutations in the SLYNTVATL epitope can be partially explained by the finding that some people maintain strong CD8$^+$ T cell responses to single mutants. CTLs specific for the index peptide recognize the triple-mutant epitope less well than any single or double mutant. Such poor recognition could not be explained by decreased binding of the mutant peptide to HLA-A*02. In fact, a crystal structure suggests that the conformation adopted by this peptide bound to HLA-A*02 is much closer to that of the wild-type SLYNTVATL peptide bound to HLA-A*02 (**Fig. 1**) than to that of the single-mutant SLFNTVATL bound to HLA-A*02. However, surface plasmon resonance shows that binding of the HLA-A*02–SLFNTIAVL complex by a T cell receptor specific for the index peptide results in a less favorable equilibrium enthalpy change, suggesting that fewer bonds are formed between the variant epitope and the T cell receptor.

The finding that the same substitutions occur at the same three residues in different patients strongly suggests that considerable constraints exist for this epitope. The epitope is located near the core of the small globular matrix domain of Gag (**Fig. 1**). Mutations might affect the overall conformation of the matrix domain, which could alter its ability to form multimers, associate with the plasma membrane and facilitate virion assembly (all normal functions of the matrix domain). Indeed, the other evolutionary pathway identified by Iversen and colleagues in population studies favors the retention of, or reversion to, the index residues at the same three positions. The most likely explanation is the greater fitness of the wild-type virus. The balance between the two evolutionary pathways may have important clinical consequences. The viral loads of patients with escape mutations in SLYNTVATL are much lower than those of patients who have no evidence of selective pressure at this epitope; the lower viral loads are probably because of reduced fitness of the escape mutants. An alternative explanation is that escape mutations indicate a more effective CTL response. In other words, CTL responses to HIV-1 epitopes other than the immunodominant SLYNTVATL peptide may be more common in these patients and may be involved in the control of viral replication.

These so-called subdominant responses are the focus of the paper by Frahm and colleagues, who have examined the HIV-1-specific CD8$^+$ T cell responses in patients expressing HLA-B*1503. This MHC class I molecule is found in only about 1% of the US population, in which clade B HIV-1 predominates, but is more prevalent in South Africa (14% of the cohort studied), where clade C predominates. The authors use overlapping peptides spanning the entire HIV-1 proteome to screen clade B and clade C cohorts and have identified ten HLA-B*1503-restricted epitopes. In HLA-B*1503 patients from both cohorts, there is a very high frequency of recognition (more than 80%) of one of these epitopes, an immunodominant peptide in the integrase protein. The other peptides, located in six different HIV-1 proteins, are not recognized as frequently as the immunodominant peptide and thus are thus subdominant epitopes. Seven of the subdominant epitopes are much more likely to be targeted by HLA-B*1503-positive patients in the clade B cohort. Sequence analysis shows that whereas the immunodominant epitope is conserved, six of seven subdominant epitopes have at least one amino acid substitution in clade C consensus virus compared with the clade B consensus virus. Functional analysis demonstrates that HLA-B*1503-positive patients in the clade B cohort make considerably lower responses to the clade C versions of the subdominant peptides.

The differences in the responses to the subdominant HLA-B*1503 restricted epitopes seem to affect viral replication. In the clade B cohort, untreated HLA-B*1503-positive patients have viral loads approximately one log lower than those of HLA-B*1503-negative patients. This difference is not found in the clade C cohort. It is possible that escape mutations occur in the subdominant HLA-B*1503-restricted epitopes of clade C virus and that these escape mutations either do not result in reduced fitness or are accompanied by compensatory mutations that improve viral replication. In either case there would be no pressure for the virus to revert to wild-type sequence even if it were transmitted to HLA-B*1503-negative people. Because the HLA-B*1503 allele is relatively prevalent, viruses with these escape mutations would enjoy a selective advantage at the population level and thus could eventually replace wild-type virus; conversely, the low prevalence of the HLA allele in the clade B cohort may have prevented replacement. In any case, the data directly demonstrate that subdominant epitopes can be key in the control of viral replication.

The choice of epitopes to use in an AIDS vaccine is a daunting task. In addition to the sequence differences among different HIV-1 clades, the polymorphism of MHC and the different frequencies of various MHC alleles complicate the selection. The studies described here are important in that they analyze viruses from different clades and address the ways in which immune pressure can lead to viral evolution. The results have considerable implications for vaccine development. The choice of an epitope must be influenced not only by immunogenicity but also by the likelihood and 'fitness cost' of escape mutations. The study by Iversen and colleagues and other studies suggest that immune responses can be made to escape mutants. Thus, it may be possible to immunize with both wild-type epitopes and common escape variants. Epitopes that can sustain mutations without a substantial cost to fitness should be avoided. However, targets that have tight constraints, as determined by the lack of sequence variation, may represent ideal targets even if they are not overtly immunogenic. Subdominant epitopes can elicit functionally important responses and should not be ignored.

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