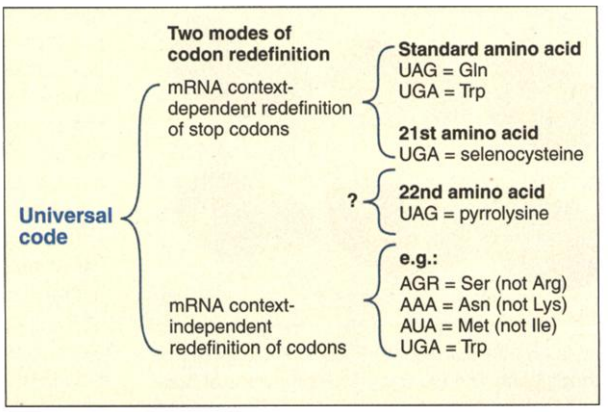
code began to unfold in the early 1960s, it might have been tempting to speculate that some of the 64 possible codons encoded the many rare amino acids found in proteins. However, it became clear that 20 is the correct number of amino acids, and that the great majority of nonstandard amino acids are created by chemical modifications of standard amino acids after translation. In 1986 came the surprise discovery that the nonstandard amino acid selenocysteine is directly specified by the genetic code and is not created by posttranslational modification (*5*, *6*). Selenocysteine is now joined by pyrrolysine, and together these two amino acids demonstrate that the genetic code can be expanded by redefining the meaning of a stop codon.



When stop means go. There are two ways in which the stop codon UAG could be redefined to specify the 22nd amino acid, pyrrolysine. In the first **(top)**, special signals in mRNAs tag a subset of stop codons that are to have their meaning redefined. In the second **(bottom)**, a codon is redefined regardless of the mRNA involved.

This redefinition requires subversion of the standard pathway for activating amino acids in readiness for protein synthesis. Instead of a tRNA being charged with the new amino acid, it receives a standard amino acid that is then enzymatically modified while still attached to the tRNA. This process is similar to the way in which some organisms modify the standard amino acids, aspartic acid and glutamic acid, while they are attached to tRNAs, in order to obtain asparagine and glutamine (*7*). In the case of selenocysteine, a selenocysteinyl tRNA is first charged with serine, which is then enzymatically modified to form selenocysteine. Similarly, pyrrolysine is likely to be produced by modifying a lysine residue attached to a special lysyl tRNA. The tRNAs involved in the production of selenocysteine and pyrrolysine are distinct from those decoding the standard amino acids, serine and lysine, but they differ from each other in certain features, for example, the pyrrolysine tRNA has a "special" anticodon arm.

In certain specialized niches, such as the mitochondrion, the meaning of a subset of codons is reassigned from that of the "universal" genetic code wherever these codons occur in all mRNAs. For example, in the starfish, the codons UGA and AGR specify Trp and Ser, respectively, when in mitochondrial mRNA, but Stop and Arg when in nuclear mRNA. In a few organisms with small genomes, rarely used codons are permanently reassigned (*8*). However, in organisms where UGA specifies selenocysteine, only a subset of UGA codons do so; the great majority specify the standard meaning: "stop translation." Special signals in mRNA help to reprogram the readout by distinguishing those codons whose meaning is to be changed. These signals can be close to the UGA codon, as in bacteria, or distant, as in the 3′-untranslated region of eukaryotic mRNAs.

Recoding UGA as selenocysteine is not fully efficient because there is direct competition from standard reading of UGA stop codons, even those that are specially "tagged." The slow-to-decode property of "stop codons" may be the reason for usurping them during the decoding of standard amino acids. The goal in recoding UGA is to specify selenocysteine, the "special" 21st amino acid. However, there are other cases of recoding where a standard amino acid is specified by a "stop codon." Here the important feature is that readthrough of the stop codon permits continued decoding of a downstream sequence producing one protein from two separated open reading frames (*9*).

For pyrrolysine, like selenocysteine, the critical feature is specification of an additional amino acid. But whether specification of pyrrolysine is due to "permanent" reassignment of UAG or to recoding of a subset of UAG codons is not yet clear (see the figure). Does "UAG" mean "pyrrolysine" wherever it occurs in these organisms, or are there signals in specific mRNAs that redefine UAG codons one at a time?

Natural selection has successfully led to a code that specifies more than the 20 standard amino acids. Meanwhile, human efforts to achieve the same goal are actively under way, particularly by Schultz and his colleagues (*10*). The challenges are formidable, but striking progress is being made. The goal is to engineer the direct encoding of additional amino acids at will. It is by no means certain, however, that all of the future excitement in this area will come from these manipulations alone. As pyrrolysine illustrates, nature may yet surprise us with more directly encoded amino acids.

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PERSPECTIVES: HIV/AIDS

HLA Leaves Its Footprints on HIV

Andrew McMichael and Paul Klenerman

The reasons for the poor control of HIV infection by the mammalian immune system are gradually being unraveled. The ability of certain HIV proteins to mutate and thus to elude immune detection is increasingly seen as crucial. On page 1439 of this issue, Moore *et al.* (*1*) provide new evidence for critical involvement of HLA proteins of the human histocompatibility complex in shaping variations in HIV proteins and possibly evolution of the virus itself.

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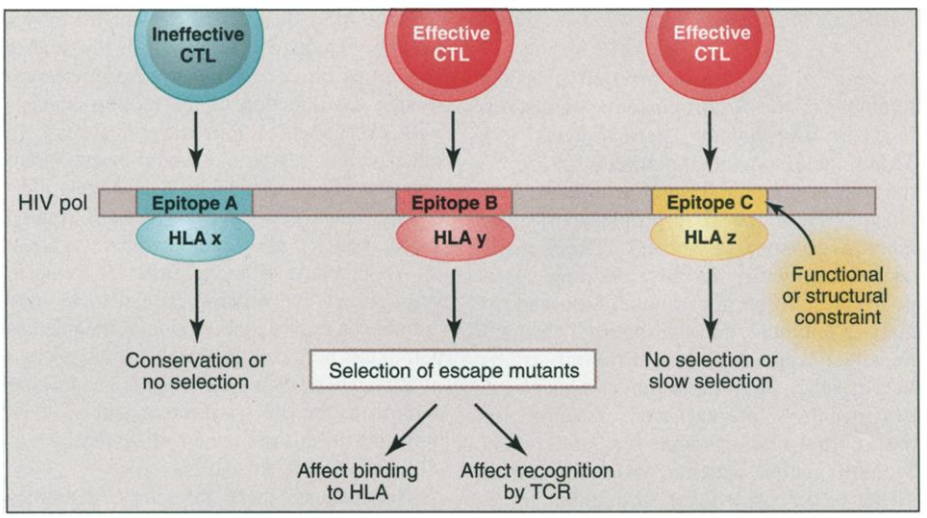
During both the acute and chronic phases of HIV infection, production of cytotoxic T lymphocytes (CTLs) by the host immune system exerts a strong inhibitory effect on HIV growth and replication. Therefore, it is not surprising that there is strong selective pressure for survival of HIV mutants that escape the CTL response (*2*–*7*). Although escape from the host antibody response is well accepted (*8*), escape from CTL responses has until recently been more controversial. Objections to the idea have centered around whether a CTL response against several HIV epitopes could be undermined by escape of only one epitope. With the host immune system trying to control a swarm of rapidly replicating viruses, a viral vari-

ant that is slightly less well controlled because it carries a mutation in one of several crucial epitopes would still have a competitive edge. Often the host immune response has an unfortunate tendency to focus on a small number of "immunodominant" HIV epitopes, sometimes only one, making viral escape even easier.

Mutation in an epitope can have a number of consequences (*2*). If mutation affects the ability of HLA molecules expressed by CTLs to bind to the viral protein, then the epitope may simply become invisible to CTLs. In other cases, mutation may alter the interaction of the T cell receptor with the viral protein, resulting in altered recognition. This could be dealt with by the immune system selecting new, more specific T cell clones—but in practice that does not always happen (*3*). This phenomenon, "original antigenic sin," could reflect the fact that a lower stringency of antigenic stimulation is required for maintaining established CTL populations than for generating new ones. A failure to make primary CTL responses to new epitopes is accentuated by HIV-mediated damage to T helper cells, dendritic cells, and the architecture of the lymphoid system. Also, in some cases, HIV variants might antagonize the original T cell response (*4*, *5*). If viral escape impairs immune control of HIV, then an increase in the number of viruses would be expected and indeed has been observed (*6*, *7*).

Striking evidence that the ability of HIV to dodge the CTL response is important in determining the outcome of infection has come from studies in macaques infected with cloned simian immunodeficiency virus (SIV) (*9*, *10*). Viral escape occurred rapidly at several different epitopes, all selected in response to pressure exerted by CTLs. Similar data from HIV-infected humans are beginning to accumulate (*7*), although it is not clear how widespread this process is. Moore *et al.* (*1*) make a timely contribution to this discussion by suggesting that HIV evasion of the CTL response is both common and important. They assessed amino acid sequence heterogeneity of the HIV pol protein in viral isolates from a large cohort of patients infected with HIV-1. The authors then analyzed the sequences according to the HLA type of the donor. They identified areas of variability in pol that were HLA class I dependent. Some of these areas map to known epitopes that engage the HLA molecules expressed by T cells; others do not, and may represent previously unidentified epitopes. Although the authors do not test whether these mutations impair CTL responses, this does seem likely.



HLA to the rescue? HLA molecules on the surface of CTLs direct the CTL response to specific epitopes within the HIV pol protein. Some of these CTL responses **(center)** exert strong selective pressure on the virus. Viral variant epitopes that escape these CTLs by failing to bind to HLA or to interact with T cell receptors have an advantage in vivo. **(Left)** Other responses are less effective and are not associated with obvious escape. **(Right)** If an effective CTL response is directed against an epitope region that is constrained functionally or structurally, there may be no selection or slow selection because a mosaic of mutations is required for escape to occur.

Intriguingly, some areas of HIV pol were less variable in patients with particular HLA types. The reason for this is not clear. One possibility is that these areas are epitope regions where common HLA types have already selected a series of "optimized" mutations by passage through many infected individuals of the same HLA type. Overall, this study supports the idea that immune escape is common in HIV-infected persons and may be an integral part of the pathogenic process, and also that it is patchy, depending largely on the epitope (see the figure).

If this view is correct, there should be regions of HIV proteins that for structural or functional reasons are harder to mutate without compromising virus survival. One might predict that persons with HLA types that select such epitopes might fare better when it comes to fighting infection. This seems to be the case for HLA B27 (*11*), which recognizes an immunodominant epitope in a conserved part of the p24 viral capsid protein. Immune escape requires at least two mutations in this region of p24 (*12*). HLA B57 also is associated with longer survival of HIV patients (*13*) and selects an epitope in this highly conserved region.

If an HLA type is common in the community, there is a good chance that an escape mutant virus could be transferred to persons of the same HLA type (*14*). This implies that people carrying common HLA types might do worse when infected with HIV, and that those with rare types might do better (frequency-dependent selection). Given that different populations have markedly different frequencies of HLA types, such selection could contribute to the generation of locally distinct viruses, although probably not the major clades, which diverged quite early in the history of HIV in central equatorial Africa (*15*).

The fact that HIV is an astounding escape artist has now been confirmed at a population level. This is worrying news for long-term immune control of HIV infection and for vaccine development. There have been clear warning signs that although CTL-inducing vaccines can offer some control of SIV infection in macaque models, such control can be undermined by these processes (*16*). HIV vaccines will have to match the relevant circulating virus, but must also elicit very broad responses to multiple epitopes in order to stay one step ahead of HIV variation.

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