Commentary

Virus Escape from CTL Recognition

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Class I MHC–restricted cytotoxic T lymphocytes (CTL) have been demonstrated to have potent antiviral activity both in vitro and in vivo (1–3). It is therefore not surprising that viruses have evolved sophisticated mechanisms to escape the effects of CTL. Almost by definition, a persistent virus is one that has evolved some mechanism for avoiding the CTL response of the host. In most persistent virus infections, escape from CTL results in life-long infection of the host with some small fraction of the population suffering pathologic consequences from the virus infection (4). In HIV infection, however, both viral persistence and the devastating consequences of that infection are the rule, rather than the exception. Assuming that escape from CTL plays a role in the ability of HIV to maintain persistent infection, it becomes imperative to better understand this phenomenon and the mechanisms specifically employed by this virus. In this issue of the Journal, Couillin et al. (5) provide insight into how HIV may escape CTL recognition through genetic variation.

Several lines of evidence suggest that CTL are an important component of the protective immune response to HIV infection. HIV-specific CTL precursors are present at high frequency very early during infection, often being detectable before seroconversion (6). During the subsequent prolonged asymptomatic phase of infection, HIV-specific $CD8^+$ MHC class I–restricted CTL activity can routinely be detected directly from the peripheral blood in the absence of in vitro stimulation (7, 8). Limiting dilution analysis has confirmed that a high frequency of activated and memory HIV-specific CTL are present in the peripheral blood of these patients (9–11). However, despite this vigorous CTL response, the virus continues to replicate (12, 13). Progression to AIDS is marked by an increase in virus replication accompanied by a loss of the $CD8^+$ HIV-specific CTL response (11, 14). The association of the CTL response with the initial, acute decrease in viremia and the subsequent loss of that control with progression to AIDS strongly implicates the CTL response in control of HIV replication during the asymptomatic phase of infection.

How then might HIV, or any other persistent virus, evade the CTL response of the host? On first inspection one might assume that a virus would simply escape a CTL response by altering the amino acid sequence within the epitope(s) recognized by that response. While this may be the most intensively studied, it is by no means the only, or the most frequently used, viral escape mechanism. Table 1 provides a listing of defined and proposed mechanisms utilized by viruses to avoid the CTL response of the host. This commentary will not discuss all available mechanisms, but will concentrate upon the role of sequence variation in CTL escape.

Sequence variation is thought to affect CTL recognition in any one of three ways: blocking correct transport and processing of the antigen, blocking peptide binding to the MHC molecule, or blocking optimal recognition of the peptide/MHC complex by the TCR. In the last mechanism, either the peptide/MHC complex will fail to engage the TCR (15), or the TCR may be suboptimally engaged by the altered peptide/MHC complex, resulting in a decreased ability of that CTL to respond upon encountering a cell that presents the peptide/MHC complex to which the CTL was originally generated (a phenomenon referred to as "antagonism") (16–19). The exact mechanism involved in antagonism, however, is still unclear. These escape mechanisms are diagramatically shown in Fig. 1. It should be noted that this figure does not depict the recently described pathway utilized in the processing of some HIV envelope epitopes which is independent of the Tap1/Tap2 transporter complex (20).

There is evidence, in viral infections other than HIV, for all three mechanisms of sequence variation leading to CTL escape. Variation in regions surrounding an epitope has been shown to lead to nonrecognition by influenza virus–specific CTL, though it remains uncertain if proteolytic cleavage, transport, or another step in processing is affected by the sequence changes (21). In addition, lymphocytic choriomeningitis virus has been shown to alter sequences within defined epitopes as a mechanism of escape both in vitro and in vivo (22, 23). It has also recently been shown that EBV sequences recovered from a population with a high prevalence of HLA-A11–expressing individuals have an alteration in a key amino acid residue critical for binding of the peptide epitope to the HLA-A11 molecule (24). In addition to changes affecting peptide binding to MHC, there is also evidence that variation which allows altered peptide ligands to bind MHC may affect TCR recognition either directly or by the mechanism of antagonism (16–19).

Several investigators had previously attempted to determine whether changes in amino acid sequences within defined CTL epitopes are responsible for the ability of HIV to escape the CTL response in vivo (25–29). By looking at sequences within defined epitopes, it has been shown that CTL clones or short-term lines from HIV-infected individuals will fail to recognize some, but not all, strains of HIV (23, 25–28). Some studies compared virus sequences within the patients from whom the CTL lines were derived (25, 29), while in other studies virus sequences from a national database were used

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Table 1. *Mechanisms Possibly Used by Viruses to Avoid Recognition by the Host's CTL Response*

Mechanism	Reference
Viral latency	reviewed 4
Replication in "immune-privileged" sites*	reviewed 4
Induction of immunosuppression*	(33)
Induction of CTL clonal exhaustion*	(9)
MHC class I downregulation*	(34, 35)
Adhesion molecule downregulation	(36)
Altered expression of specific viral antigens	(37)
Blockage of "lethal hit" cascade	‡
Cytokine alterations*	(38)
Amino acid sequence variation*	(5, 22, 25)
Affecting peptide transport, cleavage/processing*	(5, 21, 39)
Affecting MHC binding*	(5, 24)
Affecting TCR recognition*	(15)
Resulting in antagonism*	(16–19)

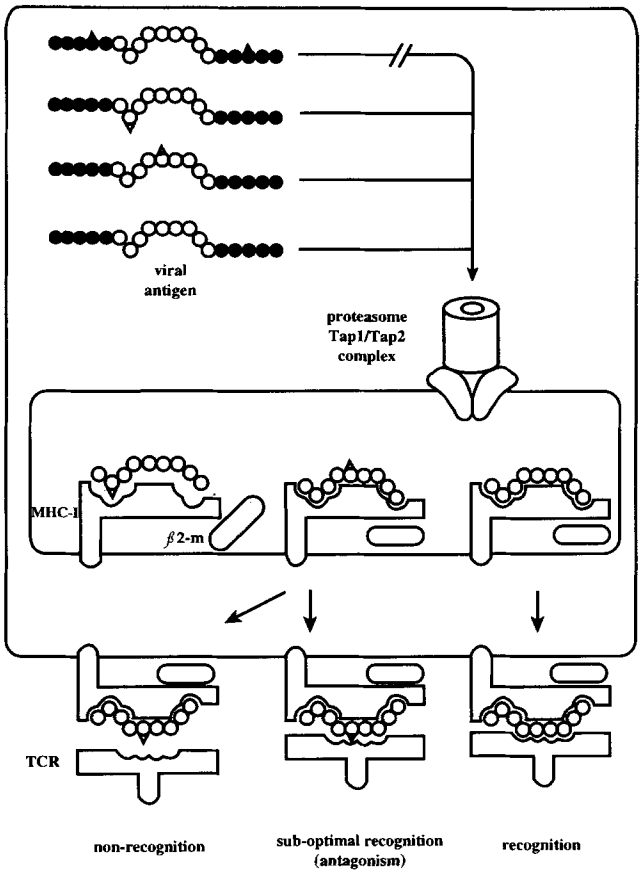
* Mechanisms for which there is evidence in HIV infection.
‡ Theoretical mechanism lacking evidence in a viral system.

(26–28). In none of these studies, however, was the impact of the changes on binding to MHC or the impact of changes in flanking sequences directly addressed.

In the study published in this issue of the Journal, Couillin et al. (5) provide evidence suggesting in vivo escape from CTL occurs as a result of alterations in anchor residues (affecting peptide binding to MHC), nonanchor residues (affecting TCR recognition) and flanking residues (affecting transport/processing) of epitopes within HIV *nef*. The unique aspect of this study is that the ability of autologous virus sequences to be recognized by CTL was compared with the ability of the peptide epitopes to bind to and stabilize the MHC/β2 microglobulin complex. Peptides altered at predicted anchor residues were unable to bind MHC and were also not recognized by the CTL response. In addition, it was shown that there are multiple variable regions of HIV *nef* that are potential CTL epitopes and that can escape the CTL response as a result of that variability. These studies therefore suggest that sequence variation within the genome of HIV may lead to nonrecognition by the CTL response through a variety of mechanisms.

Having documented that variants of HIV which escape a defined CTL response do exist within patients, it becomes logical to ask if these variants then predominate within the viral quasispecies of the patients. In one study, CTL escape variants of HIV were identified in patients, yet these viruses did not go on to dominate the viral quasispecies within those patients (25). In a separate study of four patients, no escape variants to an HLA-B27–restricted response were identified over a 14-mo period (30). In addition, it has also been shown that simian immunodeficiency virus (SIV)-infected rhesus monkeys can progress from initial infection through to AIDS without ever generating a virus that escapes a MaMu-A1–restricted response to *gag* (31), indicating that escape from CTL is not necessary for either the persistent replication of HIV/SIV, nor for the pathologic consequences thereof. On closer inspection, however, it is apparent that these results do not necessarily rule out the potential importance of HIV sequence variation in escape from CTL and viral persistence.

Most of the studies published to date have investigated a small number of CTL epitopes present within conserved regions of the genome, a result of a reliance on laboratory strains of HIV in the definition of CTL epitopes (25–31). In reality, multiple epitopes within conserved and variable regions of the HIV genome are probably recognized by the CTL response of any given individual (32). The fact that escape is not observed or does not predominate at a single epitope does not rule out the possibility that escape is occurring in multiple other epitopes. HIV replication in the face of a vigorous CTL response is evidence that the CTL response is not 100% efficient at controlling HIV. A virus which escapes part of the CTL response may therefore have a significant survival advantage over one that does not. In the studies cited above, it is possible that sequence variation leading to CTL escape occurred within other nonidentified epitopes, or was mediated by sequence variation outside of the defined epitopes and led to improper processing. In addition, the recent description of variant CTL epitope antagonism in HIV suggests that the presence, within a patient, of a small percentage of viruses containing altered epitope sequences may be adequate to block CTL lysis of cells infected with viruses containing wild-type sequences (18). This would suggest that escape variants may not necessarily become the predominant viral species since they will also confer a survival advantage



to viruses that do not contain the escape variant sequences.

Based upon the work published in this issue of the Journal, and that of others, it is safe to say that sequence variation resulting in escape from certain CTL responses does occur in HIV infection, and that multiple mechanisms are probably involved. The relevance of these findings, however, remains to be determined. Further work along the lines of Couillin et al. (5), in which both MHC binding and CTL recognition of autologous virus sequences are assessed, may help provide insight into the dynamic interplay which exists between HIV and the CTL response to this pathogen.

Figure 1. Diagrammatic representation of how amino acid sequence variation can affect antigen processing, presentation, and recognition. Open circles represent a nine amino acid CTL epitope which, when processed and presented, is recognized by a TCR. Flanking amino acids are shown as darkened circles. Altered amino acids are shown as pointed circles. If the flanking amino acids are altered, the epitope is not cleaved/transported/processed into the endoplasmic reticulum/golgi complex. If an anchor residue is altered (shown as an amino acid that binds into a deep pocket in the MHC molecule), transport and processing may occur, but binding to the MHC molecule does not occur and a stable peptide/MHC/β2 microglobulin (β2-*m*) complex is not formed. If a nonanchor residue is altered, binding to, and stabilization of, the MHC/β2-m complex may occur, followed by transport of the complex to the cell surface. The TCR may then either not engage the peptide/MHC complex or it may suboptimally engage the complex, leading to the phenomenon of antagonism.

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