Commentary

Upregulation of Fas Ligand by Simian Immunodeficiency Virus—A *nef*-arious Mechanism of Immune Evasion?

By R. Paul Johnson

From the Division of Immunology, New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772 and AIDS Research Center and Infectious Disease Unit, Massachusetts General Hospital Harvard Medical School, Charlestown, Massachusetts 02129

AIDS is the end result of a prolonged dynamic war of attrition between virus and host, marked by prodigious rates of viral replication opposed by host immune responses and remarkable rates of T cell regeneration (1, 2). Understanding the ultimate failure of the host immune responses to control HIV replication remains one of the central challenges of HIV research. This commentary will focus on efforts to understand why the host cytotoxic T lymphocyte (CTL) response, normally a potent effector response against viral infections, is unable to contain AIDS virus replication.

Most HIV-infected people develop a strong and broadly directed CTL response against HIV (3). This CTL response, which is mediated largely by classical $CD8^+$, MHC class I–restricted lymphocytes, can often be detected without in vitro stimulation, indicating a high frequency of circulating activated effector cells (4). Direct evidence for a high frequency of HIV-specific CTL (up to 1% of all T cells) has been provided by molecular analysis of the frequency of T cell receptors specific for a single CTL epitope (5) and by flow cytometric analysis of lymphocytes from HIV-infected subjects using fluorescently labeled HLA-A2 molecules complexed with a HIV peptide (6). In addition to their relatively high frequency, HIV-specific CTL typically recognize multiple epitopes in a single subject, generally in conserved regions of the virus (4, 7). Definitive evidence for an in vivo effect of this vigorous in vitro response has been difficult to establish, but the finding that the onset of CTL responses during primary infection with HIV-1 coincides with the control of viral replication, suggests that HIV-specific CTL are likely to suppress viral replication in vivo (8). Yet, viral replication continues to occur in HIV-infected individuals despite CTL responses and replication ultimately increases as individuals progress to AIDS, often in conjunction with a decline in CTL activity (9).

A variety of mechanisms have been proposed to account for the failure of CTL responses to effectively control HIV (Table 1) (10, 11), many of which have been previously described for other viral pathogens. Sequence variation has been the leading contender to account for the escape of HIV from CTL control, yet unequivocal evidence to document this hypothesis has proved elusive. HIV replication occurs at a rapid rate in vivo, leading to the production of 10^9 virions per day (1, 2). Coupled with the error-prone nature of reverse transcriptase, this rapid rate of replication results in a diverse population of viruses that are likely to contain a mutation within any given CTL epitope. This viral diversity rapidly results in evolution of viruses resistant to antiretroviral agents (1) and since single–amino acid changes can abort recognition by HIV-specific CTL (12), it was logical to propose that emergence of HIV strains unrecognized by CTL might be a dominant mechanism of escape. Although an early report demonstrated that evolution of HIV variants unrecognized by CTL could occur (13), it has been unclear whether these mutants emerged as the result of random variation or as a direct result of selection pressure by CTL. Other reports did not observe the emergence of escape mutations in CTL epitopes in either HIV-infected people or simian immunodeficiency virus (SIV) infected monkeys (14, 15), and a comprehensive analysis of the frequency of escape mutations in two subjects with a polyclonal CTL response has revealed dominant unrecognized sequences in only 3 of 16 epitopes examined (Johnson, R.P., S. Kalams, B.D. Walker, manuscript in preparation). However, there are now several examples that provide compelling evidence that CTL responses may select for unrecognized variant viruses in vivo (16, 17, 18), especially in instances when the dominant CTL response is directed against a single epitope. Taken together, these observations suggest that evolution of escape mutations may contribute to the ability of HIV to escape CTL surveillance, but they do not suggest that mutations in CTL epitopes are the dominant mechanism of immune escape.

Another strategy commonly employed by viral pathogens to evade CTL responses is interference with MHC class I–restricted processing of viral proteins. These mechanisms include downregulation of MHC class I molecules (19), dislocation of class I molecules from the endoplasm reticulum (20), blocking the transport of processed peptides into the endoplasmic reticulum (21), and selective inhibition of processing of specific viral proteins (22). Several reports have documented partial downregulation of MHC class I molecules in HIV-infected cells, a function ascribed to the viral proteins Tat or Nef (23, 24). Whether this downregulation was sufficient to affect recognition by HIV-specific CTL has been difficult to assess, because most investigators have not analyzed recognition of naturally infected T cells, relying instead on the more convenient system of

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Table 1. *Proposed Mechanisms Employed by HIV-1 to Evade Cytotoxic T Lymphocyte Responses*

Sequence variation
Lack of recognition
Antagonism
Variation in flanking residues
Altered antigen presentation
Downregulation of MHC class I molecules by Tat, Nef
Loss of effector cells
Clonal exhaustion
Loss of CD4 T cell help
Replicative senescence
Fas/Fas ligand–induced apoptosis
Latency
Privileged sites of viral replication

employing EBV-transformed B cell lines infected with recombinant vaccinia-expressing HIV proteins. Recent data analyzing recognition by HIV-specific CTL clones of transformed cell lines infected with laboratory-adapted HIV strains (which may be defective in viral proteins such as Nef) suggested that partial downregulation of MHC class I molecules does not appear to affect recognition by HIV-specific CTL (25), but these results need to be validated using primary HIV strains and primary T cells.

Escape of HIV from CTL control may also occur by mechanisms that result in a loss of virus-specific CTL. Several potential mechanisms exist, including immune exhaustion (26), loss of virus-specific T helper responses (27, 28), replicative senescence (29), and apoptosis. Increased rates of apoptosis in HIV-infected individuals have been widely reported, involving both $CD4^+$ and $CD8^+$ T lymphocytes (30). Although many pathways may contribute to apoptosis in HIV infection, interactions between Fas, a member of the TNF/nerve growth factor receptor superfamily, and Fas ligand (FasL) appear to play a major role in HIV disease. In this issue of the *Journal of Experimental Medicine*, Xu et al. (31) now propose that upregulation of FasL by the SIV nef protein may lead to the loss of virus-specific CTL. The starting point for these observations was an analysis of CTL responses in cynomolgus macaques infected with an attenuated strain (pC8) of SIV which expresses a defective *nef* gene. SIV strains defective in *nef* are inhibited in their ability to replicate in vivo and cause AIDS (32). Immunization of animals infected with *nef*-deficient SIV strains has produced the most impressive results against infection with pathogenic SIV strains (33), an observation that has led to intensive efforts to define the immune responses that may be involved in mediating protection. Noting that pC8-infected animals maintained high levels of virus-specific CTL responses, while macaques infected with the pathogenic pJ5 strain have little or no CTL responses, Xu et al. went on to compare Fas expression and apoptosis in animals infected with pC8 and pJ5. Animals infected with the pathogenic pJ5 strain had increased expression of Fas on both CD4 and CD8 cells, a finding consistent with observations in HIV-infected people (34). Infection of $CD4^+$ T cells with the pathogenic strain pJ5 resulted in greater increase in FasL expression than observed in pC8-infected cells.

These findings are consistent with prior reports that HIV infection results in upregulation of FasL on infected T cells and macrophages (35, 36). Upregulation of FasL by Nef on the surface of HIV- or SIV-infected cells might therefore serve as a means to induce a local sanctuary from CTL surveillance. Expression of FasL functions in several different settings to evade or suppress cellular immune responses. Expression of FasL on Sertoli cells in the testis or on stromal cells of the anterior chamber of the eye appears to account for the privileged nature of these sites and results in the death of activated T cells expressing Fas when they enter these sites (37, 38). Similarly, expression of FasL on tumor cells may serve to kill activated lymphocytes and facilitate evasion of tumor-specific cellular immune responses (39).

Several caveats should be considered in interpreting these results. First, clarification of the molecular mechanisms involved in upregulation of FasL is necessary. Using the SIV strain pC8, which is not deleted in *nef* but expresses a mutant Nef protein, to dissect out the role that *nef* plays in upregulation of FasL may be complicated, as variation in the levels of SIV infection may affect the observed results and the effect of *nef* may be altered instead of abrogated. Expression of FasL may be upregulated after activation of T cells by several routes (40), and it is possible that Nef enhances FasL expression mediated by another viral protein (e.g., Tat) but does not directly upregulate FasL expression. This latter possibility is suggested by the failure of a recombinant vaccinia virus-expressing *nef* to upregulate FasL and the fact that even the *nef*-defective virus pC8 appears to partially upregulate FasL expression (31). HIV Tat has been shown to upregulate FasL expression independent of Nef (41). Second, generalization of these results to AIDS pathogenesis should be carried out with caution. The almost complete lack of SIV-specific CTL three months after infection with the SIV J5 strain differs with other reports of SIV-specific CTL in animals infected with pathogenic strains (42, 43) and clearly contrasts with the generally vigorous CTL responses observed in HIV-infected people (3). If HIV rapidly induced FasL expression on infected cells in vivo, one might expect a general lack of CTL responses rather that the observed pattern of persistent but only partially effective CTL. Infection of mice with a recombinant vaccinia virus expressing FasL does not lead to death of responding T cells, suggesting that expression of FasL is not sufficient to induce cell death in vivo (44) and that the factors affecting the ability of FasL to mediate immune evasion in vivo may be complex.

If confirmed, the implications of these findings for AIDS pathogenesis are multiple. First, they provide additional evidence supporting the importance of indirect mechanisms of lymphocyte death induced by HIV or SIV. Observations that the number of dying T cells far exceeds the number of

HIV or SIV-infected cells have suggested that indirect mechanisms, rather than direct virus-induced cytopathic effect, play a major role in inducing cell death in vivo (45), a conclusion bolstered by recent calculations that the percentage of actively infected $CD4^+$ T cells is rather small (less than 0.02%) (46). Since Fas expression is increased in both $CD4^+$ and $CD8^+$ T lymphocytes in HIV-infected subjects (34), FasL-mediated lymphocyte death would increase turnover of both T cell subsets. Recent measurements showing a decrease in telomere length in HIV-infected subjects support an increased turnover of $CD8^+$ T cells, a finding not previously anticipated. Second, this report adds to the expanding functions attributed to nef. Originally thought to function as a downregulator of HIV replication, *nef* is in fact necessary for efficient in vivo replication for both SIV and HIV (32, 47), although the molecular mechanism underlying this positive effect has proved elusive. Specific functions ascribed to *nef* based on in vitro experiments include downregulation of CD4 expression (48), downregulation of MHC class I expression (24), enhancement of virion infectivity (49), and activation of lymphocytes (50). With the addition of upregulation of FasL to this list, dissecting out which of these in vitro functions is responsible for the in vivo effect of *nef* represents an important goal. Finally, this report offers an alternative approach for AIDS therapy directed at interfering with the Fas–FasL interactions either by drugs that affect Fas-induced cell death (35) or by soluble Fas protein.

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Address correspondence to Dr. R.P. Johnson, New England Regional Primate Research Center, Harvard Medical School, P.O. Box 9102, Southborough, MA 01772. Phone: 508-624-8148.

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