HIV-1 Reverse Transcriptase Is a Target for Cytotoxic T Lymphocytes in Infected Individuals

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Characterization of the host immune response to human immunodeficiency virus type 1 (HIV-1) is critical to the rational design of an effective AIDS vaccine. In this study, cytotoxic T lymphocytes (CTL) specific for HIV-1 reverse transcriptase (RNA-dependent DNA polymerase) were found in blood samples from HIV-1–infected individuals. CTL targets were prepared by immortalizing B cells from ten seropositive and six seronegative individuals, and then infecting these cells with recombinant vaccinia viruses containing HIV-1 genes. CTL directed against autologous B lymphoblasts expressing HIV-1 reverse transcriptase were detected in fresh blood samples from eight HIV-1 seropositive subjects, but in no seronegative controls. The effector cells were identified as major histocompatibility complex–restricted CD3+CD8+ lymphocytes. Because the HIV-1 *pol* gene is highly conserved among different isolates and generates both humoral and cellular immune responses, it bears consideration for inclusion in a candidate AIDS vaccine.

MILLIONS OF PEOPLE WORLDWIDE are infected with the human immunodeficiency virus type 1 (HIV-1) (*1, 2*). Although it is not yet known exactly what determines progression to disease in infected individuals, a number of HIV-1–specific immune responses have been detected in vitro. These include neutralizing antibodies (*3*), antibody-dependent cellular cytotoxicity (ADCC) (*4*), cellular proliferative responses (*5*), and cytotoxic T lymphocytes (CTL) (*6, 7*). Because HIV-1 may be predominantly cell-associated and therefore not easily accessible to neutralizing antibodies, it is possible that CTL are important as a host defense against infection. CTL responses specific for the *env* and *gag* genes of HIV-1 have been reported (*6, 7*). HIV-1 also contains a *pol* gene encoding proteins of 65 and 51 kD that have been identified as having reverse transcriptase (RT) activity (*8*). We now report detection of HIV-1 RT-specific CTL in eight of ten HIV-1–infected individuals.

We evaluated the role of the HIV-1 *pol* gene in the cellular immune response to HIV-1 infection by inserting a 1.6-kb open reading frame together with a marker *Escherichia coli* β-galactosidase gene into a vaccinia virus vector. The HIV-1 open reading frame was derived by oligonucleotide-directed mutagenesis of the 2.6-kb Eco RI–Bgl II fragment of the *pol* gene of HIV-1 isolate HxB.2 (*9*). The vector so produced, designated VAC/pol, was used to infect B cells immortalized with Epstein-Barr virus. These cells then produced a protein migrating at approximately 65 kD that could be immunoprecipitated with HIV-1 antibody–positive serum (Fig. 1). In addition, a minor 51-kD protein as well as biologically active RT were detectable in cellular lysates (*10*).

Immortalized B cells from six HIV-1 seronegative and ten HIV-1 seropositive subjects were infected with the VAC/pol recombinant and used as CTL targets. B cells infected with vaccinia recombinants expressing the *E. coli* β-galactosidase gene (VAC/lac) or the HIV-1 envelope gene (VAC/env) were used as negative and positive control target cells, respectively (*6*). CTL activity of freshly isolated autologous peripheral blood mononuclear cells (PBMC) was then evaluated in a standard chromium release assay.

No HIV-1–specific CTL activity was detected in the six seronegative subjects (Fig. 2A): all three target cells were lysed equally in each case. In contrast, HIV-1 RT-specific cytotoxicity was evident in eight of ten seropositive subjects (Fig. 2B). In three asymptomatic seropositive subjects the RT-specific CTL activity was greater than the *env*-specific cytotoxicity (Fig. 2B, subjects 2, 3, and 5). On the basis of serum antibody reactivity assayed by Western blot, there was some discordance between humoral and cellular responses to RT (Fig. 2C). Whereas in one case a humoral response to p65 and p51 was observed in the absence of a detectable CTL response (subject 4), in another case CTL activity was seen in the absence of detectable antibodies to p65 and p51 (subject 5).

To identify the cell type mediating the RT-specific CTL response, we used monoclonal antibodies to the CD3 and CD8 antigens in the four subjects tested. These antibodies inhibited the response, whereas monoclonal antibodies to the CD4 antigen had no inhibitory effect (Fig. 3A). Although inhibition was only partial, incomplete inhibition of $CD8^+$ cytotoxic effector cells with monoclonal antibodies to this antigen has been previously described (*11*). Thus the cytotoxicity is mediated by a $CD3^+CD8^+$ T lymphocyte, the predominant phenotype that mediates CTL activity in most other viral systems (*12*).

Viral antigen is recognized by $CD8^+$ CTL in the context of restricting class I major histocompatibility complex (MHC) molecule (*12*). To investigate the role of MHC in restricting the RT-specific CTL response, we compared the lysis of autologous and randomly selected allogeneic target cells; in each assay autologous target cells were compared with at least six allogeneic targets at an effector:target (E:T) ratio of 50:1. With PBMC from three HIV-1 seropositive subjects, lysis of autologous RT-expressing target cells was 38%, 11%, and 16% above values for the paired VAC/lac-infected control target cells, respectively; in these studies lysis of each of the random allogeneic RT-expressing target cells was ≤6%, ≤2%, and ≤3% above paired controls, respectively. This marked preference for the RT-expressing autologous target cells suggests that the CTL response is MHC-restricted.

We then examined the role of individual HLA antigens in restricting RT-specific cytotoxicity, using a panel of HLA-typed RT-expressing target cells. Lysis was assayed by using PBMC from a single seropositive donor (Fig. 3B). The PBMC effectively lysed two different allogeneic RT-expressing tar-

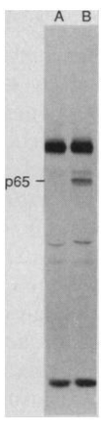

Fig. 1. Expression of HIV-1 RT in EBV-immortalized B lymphoblasts. Lymphoblasts (10×10^6) were infected (1 pfu/cell) with the control vaccinia virus, VAC/lac, or with VAC/pol (*6*), and then cultured in 5 ml of cysteine- and methionine-free RPMI medium with 10% fetal calf serum containing 50 μCi of [^{35}S]cysteine and 25 μCi of [^{35}S]methionine. Cells were harvested after 16 hours, washed, lysed, and radioimmunoprecipitated as described (*21*) with serum from an HIV-1 seropositive and an EBV and vaccinia seronegative subject. Lanes: A, VAC/lac–infected lymphoblasts; B, VAC/pol–infected lymphoblasts. The RT protein migrates at approximately 65 kD. The minor 51-kD protein is not observed here.

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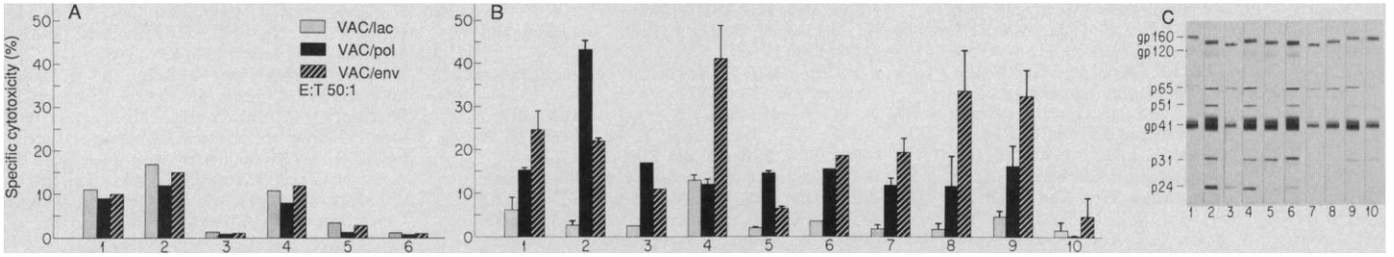

Fig. 2. HIV-1 RT-specific cytotoxicity. B cells (10×10^6) were infected with recombinant vaccinia virus (1 pfu/cell) and incubated for 16 hours. These target cells were then harvested, chromium-labeled, and subjected to lysis by autologous PBMC in a 6-hour chromium release assay performed in triplicate (*6*). Percent-specific cytotoxicity was determined from the formula: $100 \times$ [(release in assay − spontaneous release)/(maximum release − spontaneous release)]. Maximum release was determined by water lysis of labeled target cells. Results are shown at a representative E:T ratio of 50:1. Standard error bars are shown for assays performed multiple times. Average spontaneous chromium release for all assays was 18%. (**A**) Seronegative subjects. (**B**) Seropositive subjects. At the time of the study, seropositive subjects 1 through 6 were asymptomatic, 7 and 8 had AIDS-related complex, and 9 and 10 had AIDS. (**C**) Serum from each subject in (B) was analyzed by the Western blot technique (Epiblot, Epitope, Inc.) for antibody reactivity to the HIV-1 p65 and p51 polymerase antigens. Numbers correspond to the same subjects as in (B).

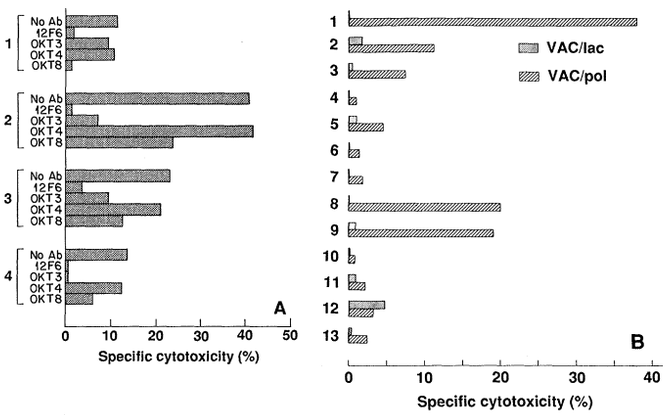

Fig. 3. (**A**) HIV-1 RT-specific cytotoxicity is inhibited by monoclonal antibodies to the CD3 and CD8 antigens. Cytotoxicity assays were performed at an E:T of 50:1 with PBMC and autologous RT-expressing target cells from four HIV-1 seropositive subjects. Prior to addition of chromium-labeled target cells, PBMC were incubated for 30 minutes with monoclonal antibodies to the CD3 antigen (12F6, OKT3), the CD4 antigen (OKT4), and the CD8 antigen (OKT8). Final antibody dilution in each case was 1:50. The reason OKT3 only minimally inhibited the response in subject 1 is not known. (**B**) HLA class I restriction of HIV-1 RT-specific CTL. PBMC cells from an asymptomatic seropositive subject (HLA A1,11; B8,Bw62; CW4,−; DR3,−; DRw52, DQw2) were tested for ability to lyse partially HLA-matched RT-expressing allogeneic target cells. Percent-specific lysis of the RT-expressing target cell (VAC/pol) and the paired control target cell (VAC/lac) is shown in each case at an E:T of 50:1. Shared class I (A,B,C) and class II (D) HLA antigens are noted: 1, autologous; 2, A1, A11; 3, A1, A11, DRw52, DQw2; 4, A1, Cw4, DRw52; 5, A1, B8, DR3, DRw52; 6, A1, DRw52; 7, A1, B8, DR3, DRw52, DQw2; 8, Bw62, DRw52; 9, Bw62; 10, DQw2; 11, DRw52; 12, DRw52; 13, DRw52.

get cells matched at the class I antigen HLA-Bw62. A lesser degree of allogeneic lysis was observed when targets and effectors were matched at other HLA class I antigens. No significant allogeneic lysis was observed when targets were matched at the HLA class II loci. Results were similar in two other subjects in whom allogeneic RT-specific lysis was predominantly restricted by the HLA class I antigens B13 and B44, respectively (*13*). No HIV-1–specific allogeneic lysis was observed with PBMC from HIV-1–seronegative individuals.

These results show that HIV-1 RT is a target for MHC-restricted $CD3^+CD8^+$ cytotoxic T lymphocytes in infected individuals. Virus-specific CTL directed against internal viral antigens have been reported in other viral infections (*14*), and presumably occur as a result of processed antigen being presented at the cell surface in conjunction with the appropriate MHC molecule (*15*). CTL directed at an influenza polymerase gene product have been noted (*16*), but CTL directed against the polymerase of retroviruses have not been described. These HIV-1 RT-specific cytotoxic T cells probably result from ongoing antigen stimulation in vivo, because they are detected in fresh blood and do not require in vitro stimulation.

It is not known whether *env*-, *gag*-, or RT-specific CTL play a role in protection from disease progression in persons infected with HIV-1. CTL are important in recovery from other viral infections, including cytomegalovirus and influenza (*17*). We have now detected envelope-specific CTL in 18 of 18 seropositive subjects, but only 3 of these 18 had CTL that recognized a recombinant HIV-1 *gag* gene product (*6, 18*). Of the ten subjects evaluated in the present study, only one had *gag*-specific CTL (Fig. 2B, subject 3), whereas eight had RT-specific CTL. Although the numbers studied are small, relatively greater CTL activity against RT-expressing target cells compared to *env*-expressing target cells was seen only in asymptomatic seropositive subjects. If it appears that RT-specific CTL have a protective role, attempts to elicit humoral and cytotoxic responses to this gene product with a vaccine should be considered, because the HIV-1 *pol* gene is more highly conserved than other HIV-1 genes among different isolates (*19*). In addition, *pol* gene sequence homology as well as *pol*-specific humoral cross-reactivity have been noted between HIV-1 and HIV-2 (*20*). Longitudinal studies with this assay system may show whether there is a correlation between CTL activity and disease progression.

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The Nasotemporal Division in Primate Retina: The Neural Bases of Macular Sparing and Splitting

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In primates, each hemisphere contains a representation of the contralateral visual hemifield; unilateral damage to the visual pathways results in loss of vision in half of the visual field. Apparently similar severe, unilateral lesions to the central visual pathways can result in two qualitatively different central visual field defects termed macular sparing and macular splitting. In macular sparing a 2° to 3° region around the fovea is spared from the effects of unilateral damage to the visual pathways. In macular splitting there is no such spared region and the scotoma produced by unilateral brain damage bisects the fovea. The patterns of decussation of the different classes of retinal ganglion cells in both New World (*Saimiri sciureus*) and Old World (*Macaca fascicularis*) monkeys have been determined by horseradish peroxidase injection. In both species the distributions of ipsilaterally and contralaterally projecting ganglion cells in the central retina are different from those in other mammals and suggest neural bases for macular sparing and splitting, respectively.

IN PRIMATES, THE CELLS IN THE NASAL retina project contralaterally, and those in the temporal retina project ipsilaterally. As a result, the postchiasmal visual pathways in each hemisphere represent the contralateral visual hemifield. There is very little bilateral representation, and thus unilateral damage to the visual pathways in man results in a profound loss of vision in the contralateral visual hemifield. In their paper describing the visual field defects produced by penetrating wounds of the brain, Koerner and Teuber (*1*) note "the age-old problem of macular sparing and splitting" or, more correctly, as they point out "foveal sparing" and "foveal splitting." In macular (foveal) sparing a 2° to 3° region around the fovea is spared from the effects of unilateral damage to the visual pathways. In less commonly observed macular (foveal) splitting there is no such spared region, and the scotoma produced by unilateral brain damage bisects the fovea. In advanced primates, the fovea comprises the central 3° of retina and is a roughly circular region devoid of ganglion cells (the foveal pit) surrounded by a multilayered, annular region of densely packed ganglion cells (the foveal slope).

We have studied the central projections of retinal ganglion cells in both New World (*Saimiri sciureus*) and Old World (*Macaca fascicularis*) monkeys after electrophoretic injection of horseradish peroxidase (HRP) into the lateral geniculate nucleus and superior colliculus. A total of eight animals were studied; the results for all animals were similar.

The procedures used for the surgery, extracellular single unit recordings, electrophoretic injection of HRP, histology, histochemistry, and computer-aided morphometric analysis are standard and have been described (*2–4*). All animals were deeply anesthetized.

The retinas of New World and Old World monkeys contain classes of ganglion cells with similar morphologies and patterns of central projection (*5*). Retinal ganglion cells in *S. sciureus* and *M. fascicularis* can be classified as A cells (p α), B cells (p β), C cells (p γ), or E cells (p ϵ) (*2, 6*). In both species, A cells project heavily to the magnocellular laminae of the lateral geniculate nucleus (LGNd) and have large cell bodies, large dendritic trees, and coarse axons. B cells project to the parvocellular laminae of the LGNd and have small cell bodies, very small dendritic fields, and medium-gauge axons. Within central retina, B cells are "midget" ganglion cells (*5–7*). C cells project to the superior colliculus and pretectum and constitute a heterogenous group of cells with small- to medium-sized cell bodies, large dendritic fields, and fine axons. E cells also project to the superior colliculus and pretectum. They have medium-sized cell bodies, large dendritic fields, and rather fine axons.

We determined the patterns of decussation of the different classes of primate retinal ganglion cells that were labeled as a result of large, unilateral injections of HRP into the LGNd of adult monkeys (Fig. 1). In the adult, the nasotemporal overlap is smallest (about 1° wide) adjacent to (but not within) the fovea (*8, 9*). As in the cat (*4, 10*), the region of overlap was wider in peripheral than in paracentral regions of retina. For example, 6 mm from the fovea, the region of overlap increased in width to about 2° because contralaterally projecting A and C cells extended farther into the temporal retina than they did more centrally. Occasionally, isolated, contralaterally projecting cells were observed up to 8° into temporal retina at far peripheral elevations (*11*).

Injections of HRP into the LGNd of both *S. sciureus* and *M. fascicularis* revealed that most of the foveal pit was located in temporal retina and that a 0.5° (125 μm) wide "ring" of densely packed, ipsilaterally projecting cells circled the nasal side of the foveal pit (Fig. 1, A and C). Some ipsilaterally projecting midget cells were always found throughout the foveal pit. Such a ring of cells was not observed after injections into the superior colliculus, and there was no ring of contralaterally projecting cells around the foveal pit in temporal retina after LGNd injections (Fig. 1, B and D). Only 40% of the foveal pit was surrounded by contralaterally projecting cells and they were in the nasal retina. In addition, the foveal pit ipsilateral to LGNd injections contained

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