CYTOTOXIC T LYMPHOCYTES FROM HIV-1 SEROPOSITIVE INDIVIDUALS RECOGNIZE IMMUNODOMINANT EPITOPIES IN Gp160 AND REVERSE TRANSCRIPTASE

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The CTL response to HIV-1 is more vigorous than for any known human pathogen and may be a significant factor in preventing the progression to symptomatic disease. T cell lines, generated by non-specific stimulation with PHA and IL-2, may be reproducibly used to identify HIV-1 isolate-invariant epitopes recognized by the CTL of infected individuals. The CTL response in each of 12 infected individuals to envelope and reverse transcriptase (RT) is dominated by the recognition of one or two viral isolate-invariant epitopes. Seven subjects respond to a single gp160 epitope; three subjects recognize 2 gp160 epitopes. There is a significant increase in recognition of epitopes in the C terminal 104 amino acids of gp41 (p < 0.002); in fact 40% of the subjects that respond to gp160 recognize the C terminal 20-mer. The CTL-mediated lysis of gp160-expressing targets is MHC restricted, but not all individuals that share the same serologically defined class I restricting element respond to the same epitope. Recognition of the terminal 20mer is restricted by both A30 and B8. The response to RT in six subjects is distributed over the RT protein. The six subjects recognize four separate regions defined by truncated RT-vaccinia recombinants, but none of the subjects' CTL demonstrate significant recognition of the RT epitope identified in H-2k mice and some humans.

Cytotoxic T cells that specifically lyse HIV-1-infected autologous target cells have been found to occur at uncommonly high frequency in the blood of HIV-infected individuals (1-13). HIV-1 and HTLV-1 are the only known human infections in which CTL isolated directly from PBL of some infected individuals show demonstrable viral-specific cytolyis without in vitro selection; the frequency of HIV-specific CTL has been shown to be as high as 1 in 1000 PBL (10, 14). This killing is mediated predominantly by CD8+ effector cells although cytotoxic CD4+ cells and NK cells also play a role (1-5). CD8+ T cells recognize antigenic peptides presented by MHC class I molecules. To be recognized by CTL, a peptide must be properly processed, be capable of binding to MHC strongly enough to compete with other peptides, and be recognized as a peptide-MHC complex by T cells in the repertoire. In murine and human influenza and murine lymphocho-riomeningitis virus, CTL specific for a small number of epitopes dominate the lytic response (15-19). Bersofsky and colleagues (13, 20-22) have shown that recognition of a few epitopes is likely to dominate the CTL response to HIV-1 in mice. In simian immunodeficiency virus-infected macaques, the CTL response to the simian immunodeficiency virus gag protein appears to have limited epitope specificity (23, 24). Culmann et al. (11) showed that the CTL response in two subjects to nef, the 206 amino acid regulatory protein, was directed predomi-

1 This work was supported in part by a gift from Immulogic Pharmaceutical Corporation, Leukemia Society of America Special Fellowship, National Institutes of Health-National Cancer Institute Clinical Investigator Award RO8 CA01449-01A1, Pew Scholar Award in the Biomedical Sciences, and National Institutes of Health Grant RO1 AI30926-01A1 (J.L.), the Life and Health Medical Research Fund (P.R.S.) and Carter Wallace Research Grant (J.L. and P.R.S.).
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Abbreviations used in this paper: HTLV-1, human T cell leukemia virus I; RT, HIV-1 reverse transcriptase; env, HIV-1 envelope; SIV, simian immunodeficiency virus; TR, total release; SR, spontaneous release.

Received for publication June 14, 1991. Accepted for publication February 18, 1992.

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epitopes recognized by T cell clones, has the added advantage of focussing on those epitopes whose recognition dominates the in vivo CTL response.

MATERIALS AND METHODS

Cell lines. T cell lines were generated by adding 2 \( \mu \mathrm{g} / \mathrm{ml} \) PHA-P (Difco Laboratories, Detroit, MI) and 200 U/ml-human IL2 (Cetus Corp., Emeryville, CA) to PBL obtained by Ficoll-Hypaque density centrifugation from heparinized blood. Subjects were unselected seropositive patients, either asymptomatic or with varying stages of HIV disease, in the General Medical Clinic at New England Medical Center. Informed consent was obtained from each patient, and the study was approved by the New England Medical Center Human Investigation Review Board. Cells were incubated at 5 \( \times \) 10^6/ml in RPMI 1640 supplemented with 15% FCS (JRH Biosciences, Lenexa, KS), 2 mM glutamine, 2 mM HEPES, 100 U/ml penicillin, 100 \( \mu \)g/ml streptomycin, and 50 \( \mu \)M 8-mercaptoethanol. Twice a week the cultures were adjusted to 5 \( \times \) 10^6/ml with fresh IL-2-containing media. Autologous B cell lines were generated simultaneously using B95-8 marmoset cell line supernatant, a gift from D. Thorley-Lawson, Tufts University School of Medicine, by standard methods (26).

HlA typing. PBL were typed for HLA class I by a standard complement-mediated cytotoxicity assay in the Immunology Laboratory of New England Medical Center.

Vaccinia vectors. Vaccinia vectors encoding lacZ (vSC8), gp160 of the PH13 isolate (vPE16), and all but the last 22 residues of RT of subclone HBX2.2 (vCF21) were used to screen cell lines for specific cytotoxicity against gp160 and RT-expressing targets (27-29). The vaccinia vector vD81K, encoding the HBX2 gag, was used to separate any specific lysis of gag-expressing targets (13, 25, 29) and that against lacZ-expressing targets. Peptide-specific cytotoxicity in the presence or absence of added peptide was defined as the difference between the percent specific cytotoxicity in the presence or absence of added peptide.

RESULTS

T cell lines were generated from HIV-infected individuals by nonspecific stimulation with PHA. After 2 to 3 wk of culture, the lines were screened for cytotoxicity against autologous B cell lines infected with vaccinia vectors encoding HIV-1 env and pol genes or lacZ control (vaccinia constructs vPE16, vCF21, and vSC8, respectively). Cell lines exhibited as much as 40% HIV-specific lysis in a 4 h 51Cr release assay at an E:T ratio of 12:1. In some patients, no env or RT-specific cytotoxicity was detectable above baseline in PBL but was present at significant levels in the nonspecifically generated T cell lines.

Immunodominant env epitopes. To identify whether unselected CTL recognize immunodominant epitopes in env or RT, cell lines that demonstrated significant lysis against vPE16- or vCF21-infected targets (defined as gp160 or RT-specific lysis >15% at two E:T ratio of 25:1) were studied. The target cells for the assays were autologous B cells infected with vaccinia vectors encoding nested truncations of env or RT (Fig. 1) (13, 25, 29) An epitope was defined as immunodominant in the CTL response to a particular protein if target cells infected with all constructs encoding the epitope were lysed significantly (at least 5% more specific lysis at two E:T ratios, verified in at least two separate assays) more than target cells infected with all constructs from which the epitope was deleted. When epitopes were refined further using overlapping peptides, a peptide epitope was defined as immunodominant if target cells preincubated with the peptide were lysed with at least 5% more percent specific cytotoxicity at two separate E:T ratios than target cells that had been preincubated only with media. Because the env sequence in the env-vaccinia truncations is likely to differ from that of the viral isolates of the study subjects, this method will in most instances identify only CTL epitopes encoded by relatively invariant env sequences (32). CTL from 10 of 12 subjects recognized 13 gp160 epitopes as defined by the vaccinia-gp160 recombinant truncations. In seven subjects, response to a single region of gp160 unequivocally dominated cytolyis; three individuals' T cell lines recognized two regions (Fig. 2; Tables I and II). A disproportionate number of the subjects (6 of 10) recognized epitopes in the C terminal 104 amino acids encoded by the full length vPE16, but deleted in all of the truncations. If the 13 gp160 epitopes were distributed randomly, then one would expect 1.6 epitopes to be located in the terminal 104 amino acids; the localization of 6/13 epitopes to this region is highly significant (p < 0.002). To define further the epitopes encoded in the C terminus of gp41, a set of 7 22mers with eight amino acid overlaps spanning amino acids 760–863 was synthesized and used to sensitize autologous 51Cr-labeled targets. The six T cell lines that recognized determinants in this 104 amino acid region responded to three different peptide epitopes. Four subjects recognized the C terminal 20mer, peptide 7E7 (amino acids 844–863). All of these, preferentially recognized the C terminal 12mer (peptide M288) when the epitope was further refined with three overlapping 12mers (M286, amino acids 844–855; M287, amino acids 848–859; M288, amino acids 852–863) spanning the region defined by peptide 7E7 (Fig. 3).
However, the profile for lysis of targets incubated with 7E7, M286, M287, and M288 differed somewhat among the four effector cell lines; these differences were reproducible in replicate experiments (two to four times for each cell line). Two of the T cell lines recognized the 12mer M288 better than the 20mer 7E7, but two of the T cell lines recognized 7E7 better than M288. The M286 and M287 12mers were not recognized at all by cell lines 146 and 4442 but were recognized to some extent by cell lines 113 and 142. For subject 113, a 7E7 peptide concentration of 0.2 μg/ml was needed to begin to sensitize targets and the response plateaued at 12 μg/ml (Fig. 2D).

The T cell line from subject 120, analyzed with a set of 20mers with 10 amino acid overlaps, recognized a single peptide MRC ADP740-19 (Table I). The T cell line from subject 132 recognized two adjacent overlapping peptides (MRC ADP740-2, amino acids 49–68; and ADP740-3, amino acids 59–78) and is therefore likely to recognize an epitope in the 10 amino acid overlap region.

A number of MHC class I CTL env epitopes have now been identified by several laboratories—10 in gp120 and 4 in gp41 (22, 34–39). Eleven of the epitopes that have been defined are restricted by A2 (this predominance is due in part to the fact that some laboratories have limited their studies to A2-restricted epitopes), and one each by A3.1, B8, and B14. None of the five peptide epitopes that we have identified have been previously reported. Of the five subjects that express A2, the CTL response in all of them was dominated by the recognition of a single HIV-1\textsubscript{envelope} gp160 epitope and in four of them, the dominant response was not any of the previously reported A2-restricted epitopes. In one subject, the peptide epitope was not determined and we could not eliminate the possibility that his CTL response was dominated by the recognition of the A2-restricted epitope amino acids 295–312 (38). Furthermore, none of the four individuals who express B8 or B14 recognized the previously reported B8 and B14-restricted epitope in amino acids 582–592 (36).

**Immunodominant RT epitopes.** T cell lines from six subjects that lysed RT-vaccinia-infected targets were analyzed for immunodominant CTL epitopes in RT. Each of the T cell lines recognized predominantly a single region of RT. Of the six regions defined by the truncations, four of these regions contained epitopes recognized by at least one of the subject’s CTL (Tables I and III; Fig. 4) None of the six subjects responded to the dominant epitope (amino acids 203–219 encoded in the shortest truncation, vCF37) that is recognized by CTL from C3H/HeJ mice and by PBL from some humans (13).

**MHC-restricted cytolysis.** The determination of MHC-restricting element using human polyclonal cell lines is complicated by the presence of alloreactive T cells, which leads in some instances to high backgrounds in the absence of peptide, and by the molecular heterogeneity of MHC types defined serologically. To minimize the first difficulty, we have excluded data from target cells that are lysed to a significant extent in the absence of exogenous peptide. (In particular, we have excluded data when specific lysis of the uninfected allogeneic target in the absence of peptide is more than 7.5% above the background lysis of autologous target cells.) Because of the molecular variations in serologically defined MHC, we have defined an MHC-restricting element to be present if an Ag-presenting target that coexpresses that MHC is
CTL epitopes in HIV-1 Gp160 and reverse transcriptase

Recognizes an epitope contained in vPE16-vPE17 (data not shown), but peptide 7E7 (amino acids 844-863). C. T cell line from subject 138 also recognizes an epitope autologous targets infected with vaccinia vectors encoding envelope truncations. This cell line recognizes an epitope restricted by A30 in subject 113 and by B8 in subjects 142, 146, and 4126. T cell line 113 recognized three of four A30-expressing targets preincubated with peptide 7E7 and did not exhibit significant lysis of four other cell lines expressing other shared MHC class I Ag. T cell line 4126 lysed four of four allogeneic B8-bearing targets, but did not appreciably lyse a cell line expressing A28, its other class I molecule. The lysis of two of two B8-expressing targets by cell line 142 was comparable to lysis of the autologous targets, but the extent of lysis above that against a target expressing Bw60 was not substantial. Because this patient died, no further T cells were available to verify the assignment of B8 as the restriction element in this case. T cell line 146 lysed two of four B8-bearing targets and none of two A2 and B7 expressing targets preincubated with peptide 7E7, but the degree of lysis of the allogeneic targets was less than that of the autologous target.

More recently we have been able to select HIV-specific T cells in these cell lines by stimulation with the immunodominant peptide presented by autologous APC (J. Lieberman, J. A. Fabry, M.-C. Kuo, L. Beckett, and P. R. Skolnik, manuscript submitted). For subject 138, the restriction of recognition of gp160 peptide 788-809 by the unselected T cell line was difficult to determine, but by peptide-selected T cells was unambiguously restricted by B27 (Fig. 6). However, CTL from two other B27+ subjects, 113 and 136, did not recognize this epitope, but recognized other regions of gp160.

The gp160-specific lysis by T cell line 113 was inhibited by 63% by preincubation of the effector cells with 100 μg/ml 12F6 (an αCD3 mAb), by 17% with OKT8, and unaffected by OKT4. Depletion of CD4+ cells from the effector population had no significant effect on lysis (data not shown). These results, taken together with the class I MHC-restricted nature of the cytolysis, support the results of others that HIV cytolysis is mediated largely by CD8+ lymphocytes via the TCR (1-5).

Reproducibility of method. Epitope mapping was performed on T cell lines at varying points from 2 to 6 wk after initiating the cultures without any change in epitopes with time. For subject 113, three separate T cell lines generated over a period of 6 mo consistently recognized the same single dominant epitope, amino acids 844-863. For two subjects, quadruplicate T cell cultures, starting with 1.5 x 10^6 PBL, were set up to ascertain the reproducibility of using nonspecifically stimulated T cell lines to determine immunodominant CTL epitopes. The two studies involved subject 146, whose T cells recognize the gp160 peptide 7E7 but do not lyse RT-expressing targets, and subject 143, whose T cells recognize the RT region between truncations vCF34 and vCF35 and do not recognize gp160 epitopes. In both cases, all four cultures lysed to a comparable extent, as the autologous target. In some cases, not all targets that share the same serologically defined MHC expression are lysed equivalently. This may be attributable to micromolecular heterogeneity of serologically indistinguishable MHC Ag in their ability to either bind peptide or to present peptide and be recognized by the TCR (40-43).

Despite these difficulties, we have been able to demonstrate the MHC-restricted nature of the gp160-specific killing in the four subjects whose T cells recognize peptide 7E7 and in subject 138, whose T cells recognize peptide 7E3, amino acids 788-809 (Figs. 5 and 6). Recognition of the 7E7 peptide is restricted by A30 in subject 113 and by B8 in subjects 142, 146, and 4126. T cell line 113 recognized three of four A30-expressing targets preincubated with peptide 7E7 and did not exhibit significant lysis of four other cell lines expressing other shared MHC class I Ag. T cell line 4126 lysed four of four allogeneic B8-bearing targets, but did not appreciably lyse a cell line expressing A28, its other class I molecule. The lysis of two of two B8-expressing targets by cell line 142 was comparable to lysis of the autologous targets, but the extent of lysis above that against a target expressing Bw60 was not substantial. Because this patient died, no further T cells were available to verify the assignment of B8 as the restriction element in this case. T cell line 146 lysed two of four B8-bearing targets and none of two A2 and B7 expressing targets preincubated with peptide 7E7, but the degree of lysis of the allogeneic targets was less than that of the autologous target.

Figure 2. Epitope mapping of T cell lines that recognize and lyse env peptides. A. ^51Cr release assay of T cell line from subject 113 against autologous targets infected with vaccinia vectors encoding envelope truncations. This cell line recognizes an epitope in the C terminus of gp41 defined by vPE16-vPE17. B. T cell line from subject 113 recognizes peptide 7E7 (amino acids 844-863). C. T cell line from subject 138 also recognizes an epitope contained in vPE16-vPE17 (data not shown), but lysed targets incubated with another peptide 7E3 (amino acids 786-809). D. Peptide dose response curve for lysis by T cell line 113 of autologous targets preincubated with peptide 7E7. Peptide concentration denotes the final concentration.

For subject 113, three separate T cell lines generated over a period of 6 mo consistently recognized the same single dominant epitope, amino acids 844-863. For two subjects, quadruplicate T cell cultures, starting with 1.5 x 10^6 PBL, were set up to ascertain the reproducibility of using nonspecifically stimulated T cell lines to determine immunodominant CTL epitopes. The two studies involved subject 146, whose T cells recognize the gp160 peptide 7E7 but do not lyse RT-expressing targets, and subject 143, whose T cells recognize the RT region between truncations vCF34 and vCF35 and do not recognize gp160 epitopes. In both cases, all four cultures
The document contains a table of HIV-1 Gp160 and reverse transcriptase CTL epitopes. The table is titled "Immunodominant env and RT CTL epitopes" and includes columns for subject, MHC class I, V\(\alpha\) epitope, restricting element, and RT epitope. The table provides detailed information on the recognition of various peptides by the immune system.

The text also discusses the identification of immunodominant epitopes using vaccinia constructs and the use of T cell lines to study the CTL response to HIV-1. It notes the recognition of specific peptides such as YRAIRHIPPR in PBL of infected individuals. The discussion section highlights the successful identification of dominant CTL epitopes in PBL with cell lines, which has implications for the stability of immunodominant epitopes and the reliability of the method used to define these epitopes.

The figures in the document illustrate the cytotoxicity assay results, with T cell lines recognized and the corresponding cytotoxicity levels.

The text is well-structured, with clear identification of key points and findings. The table and figures are essential for understanding the research outcomes.

### Table 1: Immunodominant env and RT CTL epitopes

<table>
<thead>
<tr>
<th>Subject</th>
<th>MHC Class I</th>
<th>V(\alpha) Epitope</th>
<th>Restricting Element</th>
<th>RT Epitope</th>
</tr>
</thead>
<tbody>
<tr>
<td>113</td>
<td>A2, 30; B27, w60</td>
<td>vPE16-17 844-863: YRAIRHIPPR</td>
<td>A30</td>
<td>vCF34-35</td>
</tr>
<tr>
<td>119</td>
<td>A28, ---; B17, 35</td>
<td>vPE16-17 844-863: YRAIRHIPPR</td>
<td>A30</td>
<td>vCF34-35</td>
</tr>
<tr>
<td>120</td>
<td>A2, ---; B15, 18</td>
<td>vPE21-22 219-238: PIPPHCYPAGPHAIICNKNK</td>
<td>Not done</td>
<td>vCF35-36</td>
</tr>
<tr>
<td>132</td>
<td>A9 (24); 31; B15, 40</td>
<td>vPE18-17 802-823: YWNNLGFGSSQLKSNVILLN</td>
<td>Not done</td>
<td>Insufficient lysis</td>
</tr>
<tr>
<td>136</td>
<td>A2, 29; B7, 27</td>
<td>vPE20-21 59-68: LFCSADKALF</td>
<td>Not done</td>
<td>Insufficient lysis</td>
</tr>
<tr>
<td>138</td>
<td>A2, ---; R5, 27</td>
<td>vPE16-17 788-809: IVELLRGFRWALYVVWNLQYY</td>
<td>B37</td>
<td>Insufficient lysis</td>
</tr>
<tr>
<td>139</td>
<td>A30, w34; B12, ---</td>
<td>vPE20-21 59-68: LFCSADKALF</td>
<td>Not done</td>
<td>Insufficient lysis</td>
</tr>
<tr>
<td>142</td>
<td>A1, 31; B8, w60</td>
<td>vPE16-17 844-863: YRAIRHIPPR</td>
<td>B8</td>
<td>Insufficient lysis</td>
</tr>
<tr>
<td>143</td>
<td>A9 (24); 3; B14, 16</td>
<td>vPE16-17 844-863: YRAIRHIPPR</td>
<td>B6</td>
<td>vCF34-35</td>
</tr>
<tr>
<td>147</td>
<td>A2, 32; B7, 8</td>
<td>vPE21-22 219-238: PIPPHCYPAGPHAIICNKNK</td>
<td>Not done</td>
<td>vCF36-37</td>
</tr>
<tr>
<td>4126</td>
<td>A28, ---; B8, ---</td>
<td>vPE16-17 844-863: YRAIRHIPPR</td>
<td>B8</td>
<td>Insufficient lysis</td>
</tr>
</tbody>
</table>

### Table 2: Gp160 CTL epitope mapping using vaccinia-env truncations to infect autologous targets

<table>
<thead>
<tr>
<th>Vacinia Re-combinant</th>
<th>T cell line</th>
<th>Percent Specific Cytotoxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>vPE16 844-863: YRAIRHIPPR</td>
<td>113, 120, 132, 136, 138, 139, 142, 146, 147, 4126</td>
<td>80.2</td>
</tr>
<tr>
<td>vPE17 844-863: YRAIRHIPPR</td>
<td>84.4</td>
<td></td>
</tr>
<tr>
<td>vPE18 844-863: YRAIRHIPPR</td>
<td>24.5</td>
<td></td>
</tr>
<tr>
<td>vPE20 844-863: YRAIRHIPPR</td>
<td>20.2</td>
<td></td>
</tr>
<tr>
<td>vPE22 844-863: YRAIRHIPPR</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>vSC8 844-863: YRAIRHIPPR</td>
<td>17.8</td>
<td></td>
</tr>
</tbody>
</table>

*E:T ratio is 50:1 for all cell lines except 6:25:1 for 120. For all cell lines at least three E:T ratios were tested but the data are not shown. Underlined values represent significantly more lysis of this infected target compared with further truncations. This identifies an epitope in the region contained in this recombinant but absent in the next truncation. An insufficient number of pfu of vPE18 virus was used in the infection of targets.*
T cells may recognize epitopes in both structural and reverse transcriptase.

of HIV-1 CTL epitopes to which an individual of a given MHC background will respond is limited. The clinical significance of the CTL response to HIV-1 may become more apparent after MHC backgrounds that cannot present CTL epitopes are identified. However, because CD8+ T cells may recognize epitopes in both structural and reverse viral targets, all MHC backgrounds may respond to some HIV-1 protein. Although only about two thirds of the patients we have studied, without a history of opportunistic infections, have a significant CTL response to either gp160 or RT, a larger number recognize the gag protein. We do not know whether CTL that recognize some of the HIV-1 proteins might be more effective than others at controlling infection.

Our sample size is too small to determine whether there may be a small number of HIV-1 epitopes that most individuals in a genetically diverse population will recognize. The large number of subjects in our study whose T cells recognize the 7E7 epitope may be due to the fact that one quarter of our study group are B8+, whereas the B8 gene frequency is approximately 12% in Caucasian populations and lower in other racial groups (45). If recognition of a small number of epitopes dominates the CTL response at the population level, it might be possible to design a vaccine to enlist CTL immunity that is based on a small number of peptide epitopes. Peptide 7E7, or a truncated version of it, which is recognized by one-third of our seropositive subjects, might be a candidate for inclusion in such a vaccine. Achour et al. (46) have reported a 30mer peptide in HIV-1 p17 that is recognized by short term HIV-1-stimulated T cell lines from seropositive, but not seronegative, donors. Hosmalin et al. (13) have also reported the recognition of RT peptide 205-219 by unselected PBL from seropositive donors. However, none of our 12 subjects recognized that epitope. Apart from peptide 7E7, no region of IIIe gp160 and RT was recognized by CTL from more than 2 of the 12 seropositive subjects in our study. It is perhaps significant that 6 of 10 gp160-responding lines recognize epitopes in the terminal 76 amino acids of gp41 because a region of this size could be incorporated into vaccines designed to elicit CTL immunity. A response to gp41 would not present the same hypothetical problem as cytolytic activity against gp120 epitopes (i.e., the lysis of uninfected CD4+ cells that have bound serum gp120).

The diversity of recognized CTL epitopes is striking in light of the significant overlap in the study population of serologically defined MHC class I expression: five individuals express A2, three express B8 and B27, and two each express A9, A28, B5, B7, and Bw60. T cells from all of the B8+ subjects recognized peptide 7E7. However, for the two other restricting element-Ag pairs we have defined and for the A2-restricted gp120 epitope, we have shown that expressing a particular serologically defined MHC Ag in no way guarantees that a subject's T cells will recognize the epitope it can restrict. Although the recognition of the gp160 peptide 788-809 by CTL from subject 138 is restricted by B27, T cells from the other two B27-expressing subjects in this study did not recognize that peptide.
CTL EPITOPES IN HIV-1 Gpl60 AND REVERSE TRANSCRIPTASE

Figure 5. MHC restriction of anti-gpl60 cytotoxicity by four T cell lines that recognize the peptide 7E7 epitope (amino acids 844-863). Peptide 7E7-specific cytotoxicity by T cell line 113 [A] is restricted by the MHC class I determinant A30 and by T cell lines 4126 [B], 142 [C], and 146 [D] is restricted by B8. Autologous and heterologous B cell lines that share MHC class I determinants with the effector T cell line were preincubated with 50 ng/ml peptide 7E7 or media and used as targets in a 4-h 

\[^{3}H\]Cr release assay at E:T ratios of 20:1-50:1. The difference between the percent specific cytotoxicity against the gp160- and lacZ-expressing targets is depicted. Only the B27-expressing target was lysed to the same extent as the autologous target. YJ is a homozygous A2.B7 expressing B cell line and 9005 is a homozygous A3.B27 expressing B cell line. Cell lines 109 and 132 are described in the legend to Figure 5.

Figure 6. T cell line 138 was selected with gp160 peptide 788-809 and used as effector at an E:T ratio of 5:1 in a \[^{3}H\]Cr release assay against autologous or heterologous targets that share a single MHC class I determinant, infected with vaccinia-gp160 (vPE16) or vaccinia-lacZ (vSC8). The difference between the percent specific cytotoxicity against the gp160- and lacZ-expressing targets is depicted. Only the B27-expressing target was lysed to the same extent as the autologous target. YJ is a homozygous A2.B7 expressing B cell line and 9005 is a homozygous A3.B27 expressing B cell line. Cell lines 109 and 132 are described in the legend to Figure 5.

The fact that three different T cell lines generated over 6 mo from one subject and that quadruplicate T cell cultures from two other subjects set up at one time consistently recognized the same single dominant epitope suggests that our results are not an artifact of the outgrowth of an unrepresentative clone in tissue culture. Inasmuch as our method was designed to determine CTL epitopes that are not isolate specific, it is also not surprising that all of the epitopes defined at the peptide level are encoded by sequences that are not highly variable. It is of course possible that these cell lines recognize additional isolate-specific epitopes that are not detectable with the IIIB-encoding vaccinia used in our screening procedure.

Although we have been able to define MHC-restricting elements for all of the epitopes that we attempted, it is
Figure 7. Four replicate T cell lines initiated at the same time from 1.5 x 10^6 PBL from subject 143 recognize gag-expressing targets and the same region of RT encoded by vaccinia construct vCF34 but truncated in VCF35. None of the cell lines demonstrated lysis of gp160-expressing targets. A, ^51Cr release assay of T cell lines from subject 143 tested for cytotoxicity against autologous targets expressing lac2 control (vSC8), gp160 (vPE16), RT (vCF21), and gag (vDK1). B, ^51Cr release assay of replicate T cell lines from subject 143 against autologous targets infected with vaccinia constructs that encode nested truncations of RT. E:T ratio = 25:1. All four cell lines recognize an epitope encoded in the region defined by vCF34-vCF35.

Our results are also in apparent disagreement with the work of Clerici et al. (35) who detected cytotoxicity in the PBL of seropositive subjects directed against at least one of four HIV-immunotype gp160 peptides, chosen for their predicted amphipathic helical structure. Although they showed that the recognition of these peptides could be restricted by A2, none of our five A2-expressing cell lines appear to recognize these peptides. One possible explanation for this apparent discrepancy is that our definition of an immunodominant epitope is somewhat more stringent than theirs; we have used a shorter cytotoxicity assay, lower E:T ratios, and the requirement that recognition be verified at several E:T ratios in at least two experiments. Moreover they were unable to detect any substantial cytotoxicity of cells presenting these peptide epitopes at later time points. It is of course possible that our subjects recognize other epitopes than the ones that we have defined as immunodominant but that they are recognized less often than the dominant epitopes. The possibility of eliciting a strong CTL response to nondominant epitopes with immunization or at different time points during the natural infection remains.

In most of the subjects in this study, freshly isolated PBL do not demonstrate significant cytolysis against env or RT-bearing targets (data not shown). Although the culture conditions for generating the T cell lines used in this study involve nonspecific stimulation with lectin and IL-2, the resultant T cell lines have enhanced HIV-specific cytotoxicity. This effect is not due to loss of TS cells from the PBL because mixing studies show no evidence of suppression by PBL of cytolysis by T cell lines (data...
not shown). One possibility is that because the T cell culture conditions designed to stimulate T cell growth also enhance viral replication, viral Ag present in the culture lead to selection of HIV-specific T cell clones in the polyclonal population (47). Another possible explanation is that the culture conditions activate precursor CTL, that are not cytotoxic, to develop cytolytic granules.

Recently we have been able to select HIV-1-specific CTL in these T cell lines by selection with immunodominant CTL epitope peptides4. These selected T cell lines have HIV-specific cytolytic activity comparable to that of HIV-specific clones and, because they are easier to generate and grow to large numbers than clones, will be a useful tool to study the interaction of epitope-specific CTL with HIV. Moreover, because they become free of recoverable virus despite the persistence of a small percent of CD4+ cells, they could be considered for use in clinical trials of adoptive immunotherapy for patients infected with HIV-1.

Acknowledgments. We thank B. Ardman, H. N. Eisen, and R. S. Schwartz for helpful discussions and for critical review of the manuscript. J. Mazzullo for providing clinical subjects, A. Rabson for MHC typing study subjects, J. Greenstein for MHC-typed cell lines, the MRC AIDS Reagent Project for env peptides, the AIDS Research and Reference Program for the vaccinia vector vDK1 contributed by D. Kouritzkes, and D. Thorley-Lawson for B95-8 EBV supernatants used to generate B cell lines.

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Figure 8. Four replicate T cell lines initiated at the same time from 1.5 x 10^6 PBL from subject 146 recognize gag- and gp160-expressing autologous targets. In gp160, the four cell lines all recognize peptide 7E7. A. ¹¹⁵⁴⁶Cr release assay of T cell lines from 146 tested for lysis of autologous targets expressing lacZ (vSCB), gp160 (vPE16), RT (vCF21), and gag (vDK1). B. To delineate the region of gp160 recognized, the four T cell lines were assayed for lysis of targets infected with the vaccinia-gp160 truncations and all were found to recognize only the C terminal region between the end of vPE17 and the C terminus (data not shown). Using peptides spanning vPE16-vPE17, all the cell lines recognized targets preincubated with peptide 7E7. E:T = 50:1.
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