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Antiretroviral Drug Resistance Mutations Sustain or Enhance CTL Recognition of Common HIV-1 Pol Epitopes

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Antiretroviral drug resistance and escape from CTL are major obstacles to effective control of HIV replication. To investigate the possibility of combining drug and immune-based selective pressures against HIV, we studied the effects of antiretroviral drug resistance mutations on CTL recognition of five HIV-1 Pol epitopes presented by common HLA molecules. We found that these common drug resistance mutations sustain or even enhance the antigenicity and immunogenicity of HIV-1 Pol CTL epitopes. Variable patterns of cross-reactive and selective recognition of wild-type and corresponding variant epitopes demonstrate a relatively diverse population of CD8+ T cells reactive against these epitopes. Variant peptides with multiple drug resistance mutations still sustained CTL recognition, and some HIV-infected individuals demonstrated strong CD8+ T cell responses against multiple CTL epitopes incorporating drug resistance mutations. Selective reactivity against variant peptides with drug resistance mutations reflected ongoing or previous exposure to the indicated drug, but was not dependent upon the predominance of the mutated sequence in endogenous virus. The frequency and diversity of CTL reactivity against the variant peptides incorporating drug resistance mutations and the ability of these peptides to activate and expand CTL precursors in vitro indicate a significant functional interface between the immune system and antiretroviral therapy. Thus, drug-resistant variants of HIV are susceptible to immune selective pressure that could be applied to combat transmission or emergence of antiretroviral drug-resistant HIV strains and to enhance the immune response against HIV. * The Journal of Immunology, 2004, 172: 7212–7219.

The importance of CD8+ T cells in the immune response against HIV has been clearly established. Anti-HIV CTL are often detectable in highly exposed individuals resistant to HIV infection, long term nonprogressors maintain strong, diverse anti-HIV CTL responses, and in acute infection, emergence of HIV-specific CTL precedes a sharp decline in viremia, (1–6). Selection of CTL escape mutants parallels rising virus loads in HIV and SIV infections, and when CD8+ T cell depletion before infection blunts CTL responses in SIV-infected monkeys, virus loads reach higher levels and remain high for a longer time (7–10). The immune system’s disadvantage against a rapidly mutating virus is that it can only react to mutated peptide sequences after they emerge, whereas factors such as partial reactivity with altered peptide ligands and progressive immunodeficiency mitigate against efficient serial adaptation (11). Anticipating emerging mutants could reduce the disadvantage, but CTL escape mutations can vary widely within the agretope or epitope or even within flanking residues that affect protein processing (12, 13). Therefore, HIV escape from CTL can be relatively easily accomplished by various amino acid changes at loosely defined sites.

Escape of HIV from antiretroviral drugs is a major obstacle to effective long term antiretroviral therapy. Mutations conferring resistance within one or more of the three most extensively used antiretroviral drug classes, nucleoside reverse transcriptase (RT) inhibitors (NRTIs), nonnucleoside RT inhibitors (NNRTIs), and protease (PR) inhibitors (PIs), have become increasingly common in clinical HIV isolates (www.hivresistanceweb.com). Unlike the mutations allowing escape from CTL, drug resistance mutations localize to particular genomic sites and conform to specific amino acid changes. Those conferring NRTI and NNRTI resistance clusters within RT codons 41–236, whereas those conferring PI resistance clusters within PR codons 10–90. These regions of HIV-1 Pol also encompass a number of previously identified CTL epitopes (14). If these CTL epitopes remain immunogenic after incorporating drug resistance mutations, the well-documented constraints on drug resistance mutations could potentially be exploited to prime the immune system in anticipation of emerging mutants. Furthermore, if any epitopes incorporating drug resistance mutations exhibit enhanced antigenicity or immunogenicity, this would suggest that drug treatment itself could modulate the CTL response against HIV. Therefore, we investigated how a number of common antiretroviral drug resistance mutations in HIV pol affect CTL recognition and how the relative reactivity of CTL against wild-type Pol peptides and their corresponding variants incorporating drug resistance mutations relate to treatment history and predominant endogenous viral sequences.

Materials and Methods

Study population

Our study cohort consisted of 98 HIV-infected individuals attending the St. John’s General Hospital HIV Clinic (St. John’s, Newfoundland, Canada). HIV genotyping on selected plasma samples was conducted through a commercial service at the British Columbia Center for Excellence in HIV/
AIDS by RT-PCR amplification and automated sequencing of the entire PR-encoding and first 400 codons of the RT-encoding HIV-1 pol gene segments (www.hiv resistanceweb.com). Informed consent was obtained for blood collection and access to antiretroviral treatment records. This study was given ethical approval by the Memorial University Faculty of Medicine human investigation committee.

Lymphocyte isolation and cell culture

Whole blood was collected by venipuncture into acid-citrate-dextrose-treated Vacutainers (BD Labware, Franklin Lakes, NJ) and PBMC were isolated with Ficol-Paque Plus (Pharmacia Biotech, Uppsala, Sweden). Cells were washed twice with PBS plus 1% FCS and resuspended in medium consisting of RPMI 1640 with 10% FCS, 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 mM t-glutamine, 10 mM HEPES buffer solution, and 2 × 10−5 M 2-5 ME (all from Life Technologies Invitrogen, Grand Island, NY). HLA class I-A and B type donors were determined as previously described (15) using commercial kits (One λ, Canoga, CA). Autologous B lymphoblastoid cell lines (BLCL) were generated by EBV transformation of peripheral blood B cells. Briefly, 2.5 ml of supernatant from vaccinia virus, vSC8, expressing the entire HIV-1 HXB2 pol gene, or a control vaccinia virus, vSC8, expressing Escherichia coli β-galactosidase (17). Both viruses were from the National Institutes of Health AIDS Research Reference Reagent Program (Bethesda, MD). Infected cells were washed and incubated in 100 µl of medium with 100 µCi of Na251CrO4 (American Pharmacal Biotech, Piscataway, NJ) for 1 h at 37°C. Labeled cells were washed three times and transferred to U-bottom, 96-well plates (Corning Glass, Corning, NY) at 5 × 104 cells/well. To test peptide-specific recognition, individual peptides were added in duplicate at a final concentration of 20 µg/ml for 1 h at 37°C to 5 × 105 uninfected labeled target cells in 100 µl of medium. Effector cells were added to the desired E:T cell ratios, and volumes were adjusted to 300 µl/well. Assays ran for 5 h, after which 125 µl of supernatant/well was counted in a Wallac 1280 gamma counter (Gaithersburg, MD). Specific lysis was calculated as (experimental - spontaneous)/(maximal − spontaneous) × 100. Spontaneous 51Cr release was <15% in all assays reported.

Results

Selection of HIV-1 Pol CTL epitopes encompassing drug resistance mutations

By overlaying common, well-defined antiretroviral drug resistance mutations onto HIV CTL epitope maps, we found 11 previously known HIV Pol CTL epitopes in which drug resistance mutations occur (www.hivresistanceweb.com and Ref. 14). We focused on five epitopes restricted by the common HLA molecules A2, A3, B35, and B44 (Fig. 1). More than 90% of the subjects in our cohort expressed at least one of these common HLA class I alleles and many expressed two or more in combination. Therefore, these were the most practical epitopes with which to test the effects of the drug resistance mutations on immune recognition in our study cohort, and the results are applicable to the HIV-infected population in general. The different drug resistance mutations incorporated into the variant peptides we selected for synthesis and testing collectively reflect the impact of multiple PIs, NRTIs, and NNRTIs.

FIGURE 1. Schematic representation of regions of the HIV-1 pol gene encoding known CTL epitopes that overlap with common drug resistance mutation sites. The HLA restriction of each epitope is indicated above the wild-type (top) sequence. Shaded segments of the pol gene indicate regions where most of the drug resistance mutations cluster. Shaded amino acids indicate mutations commonly selected for by antiretroviral drugs. These mutations were incorporated into synthetic variant peptides and tested for recognition.

Peptide-specific CTL stimulation

Peptide-specific CTL were generated in vitro as previously described (16). Briefly, 5 × 106 PBMC were pulsed with 100 µM peptide in 100 µl of medium for 1 h at 37°C. The cells were then resuspended at 2.5 × 106 cells/ml in medium supplemented with 25 ng/ml recombinant human IL-7 (R&D Systems, Minneapolis, MN). On day 3, 10 U/ml IL-2 was added, and cells were tested between days 10 and 14 for lysis of peptide-pulsed BLCL.

CTL assay

Autologous BLCL were used as targets. To screen for anti-HIV Pol CTL, BLCL were infected for 16 h with 10 PFU/cell of a recombinant vaccinia virus, vCF21, expressing the entire HIV-1 HXB2 pol gene, or a control vaccinia virus, vSC8, expressing Escherichia coli β-galactosidase (17).
Recognition of peptides incorporating drug resistance mutations by bulk-cultured anti-HIV CTL

As the selected peptides all derive from HIV-1 Pol, study subjects were initially tested for Pol-specific CTL using vCF21, a recombinant vaccinia virus expressing the entire HIV-1 clade B HXB2 Pol protein. We detected CTL against HXB2 Pol in >60% of individuals tested (data not shown). Although recognition of epitopes containing drug resistance mutations need not correspond to recognition of this wild-type HIV laboratory strain Pol protein, peptide-specific CTL responses against our selected wild-type or variant Pol peptides were only detected in donors with Pol-specific CTL apparent using vCF21.

When bulk-stimulated anti-HIV CTL from donors with the appropriate HLA were tested against peptide-pulsed BLCL, three of five wild-type peptides and variants of four wild-type peptides triggered specific cytolysis. Wild-type peptides RT33–41 (Fig. 2a), RT107–115 (Fig. 2b), and RT203–212 (Fig. 2d) all triggered cytolysis by anti-HIV CTL. Variant peptides incorporating one of the M41L, V108I, Y115F, Y181C, or L210W mutations also triggered cytolysis (Fig. 2). Variant peptides incorporating two mutations, V108I and Y115F (Fig. 2b) or Y181C and M184V (Fig. 2c), also triggered cytolysis. Thus, common antiretroviral drug resistance mutations within the four CTL epitopes, RT33–41, RT107–115, RT179–187, and RT203–212, presented by HLA-A3, B35, A2, and B44, respectively, sustain the antigenicity of these epitopes for HIV-specific CTL. For RT107–115, recognition of wild-type and variant epitopes was cross-reactive at the bulk CTL level (Fig. 2b).

For the RT33–41 and RT203–212 epitopes, there were instances of cross-reactive recognition of wild-type and variant peptides (Fig. 2, a and d, subjects 121 and 131) and selective recognition of either the wild-type or variant peptide (Fig. 2, a and d, subjects 012, 055, 057, 077, 082, and 126). For the RT179–187 epitope, selective recognition of variant peptides incorporating the Y181C (Fig. 2c, donor 028) or Y181C and M184V (Fig. 2c, donor 001) mutations occurred. These data indicate a complex CTL repertoire capable of recognizing HIV-1 Pol epitope variants incorporating common drug resistance mutations and, in some cases, distinguishing the variants from their wild-type counterparts.

IFN-γ production by PBMC exposed to peptides incorporating drug resistance mutations

As IFN-γ ELISPOT assays are sensitive and can estimate the frequency of peptide-specific T cells in unmanipulated PBMC, we tested freshly isolated or cryopreserved PBMC by ELISPOT for recognition of HIV-1 Pol CTL epitopes incorporating drug resistance mutations. The same three wild-type peptides and corresponding variants that triggered cytolysis by bulk-cultured anti-HIV CTL stimulated IFN-γ release from PBMC and recognition of the RT33–41 wild-type and M41L variant was detected in the context of HLA-A2 (Fig. 3, a–d). In addition, both the PR76–84 wild-type and B44 variant epitope and M41L drug resistance mutation variant stimulated IFN-γ release (Fig. 3e). ELISPOT recognition patterns with PBMC were similar to those observed in lysates with bulk-stimulated anti-HIV CTL. Recognition of RT107–115 and PR76–84 wild-type epitopes and their corresponding drug resistance variants was cross-reactive (Fig. 3, c and e), whereas RT33–41 and RT203–212 wild-type and drug resistance variant peptides showed cross-reactive (Fig. 3a, subjects 001, 007, 062, and 134; Fig. 3b, subjects 121 and 162; Fig. 3d, subjects 077 and 134) and selective recognition (Fig. 3a, subjects 043, and 131; Fig. 3b, subjects 077, 082, and 147; Fig. 3d, subjects 017, 055, 057, 118, and 131). Cross-reactivity of the RT107–115 and PR76–84 wild-type and variant peptide epitopes was confirmed across a range of peptide concentrations from 4 μM to 400 μM (Fig. 4, a and b). These ELISPOT data demonstrate a complex circulating T cell repertoire with CTL precursors capable of recognizing HIV-1 Pol epitope variants incorporating common drug resistance mutations and, in some cases, distinguishing them from their wild-type counterparts.

The ELISPOT data clearly demonstrated recognition of peptides incorporating drug resistance mutations by culturing T cells from HIV-infected individuals. The hierarchy of epitopes recognized and the pattern of cross-reactivity between wild-type and corresponding variant peptides reflected those seen with bulk-cultured anti-HIV CTL. The peptide-specific T cell frequencies observed illustrate the antigenicity and suggest in vivo immunogenicity of variant peptides incorporating drug resistance mutations. Notably, the frequency of T cells producing IFN-γ in response to the RT33–41 (M41L) variant peptide presented in the context of HLA-A3 reached ~1/200 PBMC, roughly 1% of circulating CD8+ T cells (Fig. 3b). Most responding cells in this case did not cross-react with wild-type RT33–41, suggesting selective in vivo induction of high frequency CTL against viral variants incorporating the M41L drug resistance mutation.
Relationship among CTL specificity, treatment history, and HIV genotype

HIV genotypic information was obtained from each subject with plasma virus load >1000 copies/ml to assess the relationship between predominant endogenous viral sequences at the time of testing, treatment history or current status, and specificity of anti-HIV CTL for wild-type or variant peptides. In some cases, genotypic information could not be obtained at all or could not be obtained coincident with immunological testing because of undetectable plasma virus loads, but information was always taken from the closest feasible time point. Complete antiretroviral treatment history was available for all study participants. Relevant genotypic and treatment history information is summarized in Table I. As there was CTL cross-reactivity for the PR76–84 and RT107–115 wild-type and variant peptides over a range of peptide concentrations (Fig. 4), neither the predominant endogenous sequence nor the treatment history can impact on CTL specificity. However, there could be in vivo selection for or against different endogenous variants by the CTL. Sequence information was only available for subject 082 with CTL against RT107–115 wild-type and variant peptides associated with NNRTI and abacavir (ABC) resistance. Although he was receiving the NNRTI sustiva and had a high plasma virus load, no mutations associated with NNRTI or ABC resistance were detected within RT107–115 at the time the CTL assay shown in Fig. 2 was performed.

Sequence information was available for subjects 001, 065, and 078 with CTL against PR76–84. All were receiving PIs at the time of testing, and the I84V mutation was predominant in subjects 065 and 078. This shows that the HLA-A2-restricted PR76–84 peptide with or without the drug resistance-associated mutation I84V can persist in endogenous virus in the face of a specific CTL response. The genotype for endogenous virus of subjects 001 and 065 was obtained from the same time point as the ELISPOT data, whereas the closest sample from subject 078 with a detectable plasma virus load was taken 18 mo before the ELISPOT.

As CD8+ T cells responding to RT33–41, RT179–187, and RT203–212 were only partially cross-reactive with wild-type and variant peptides, we expected to see some impact of treatment history and predominant endogenous viral sequence on relative levels of reactivity of these
CTL against wild-type and variant peptides. The RT33-41 wild-type and M41L variant peptides were recognized in the context of HLA-A2 x 6 subjects (Fig. 3a) and in the context of HLA-A3 x 5 subjects (Figs. 2a and 3b) with differential skewing toward either wild-type or mutant peptide.

Recognition of the wild-type RT33-41 peptide and M41L variant associated with azidothymidine (AZT) and stavudine (d4T) resistance was mixed for the six HLA-A2 individuals (Fig. 3a). Subject 001 was receiving ABC, and the rest were receiving d4T at the time of testing. Although ABC does not select for the M41L mutation, once acquired this mutation can contribute to ABC resistance (www.hivresistanceweb.com). Subject 001 had previously been treated with d4T and AZT and acquired the M41L mutation before ABC treatment. Sequence data coincident with the ELISPOT results were available for 001 and 062, both of whom had predominant M41L mutant endogenous virus. Sequence data from subject 007 could only be obtained from a plasma sample taken 18 mo before the ELISPOT, which showed predominantly M41 wild-type virus. These results suggest that exposure to AZT or d4T sensitizes CD8+ T cells from HLA-A2 individuals against the RT33-41 peptide epitope containing the M41L resistance-associated mutation and show that the epitope containing the M41L mutation persists in the face of this HLA-A2-restricted immune response.

Recognition of the variant peptide incorporating the M41L mutation was predominant for four of the five HLA-A3 subjects (Fig. 3b). One subject (121) was receiving AZT at the time of testing. The others were receiving d4T (077), AZT (162), or ABC (082 and 147) at the time of testing. Subject 082 had been receiving d4T until within 1 mo of the CTL assay being shown in Fig. 2a and until within 6 mo of the ELISPOT assay. Subject 147 had received AZT and d4T in the past, but not within 18 mo of the ELISPOT assay. Endogenous viral sequence data for subject 082 revealed wild-type RT33-41 as the predominant endogenous species. Subject 162, who was the only HLA-A3 subject to preferentially recognize the wild-type sequence, had started antiretroviral therapy with AZT, 3TC, and sustiva only 1 mo before testing. His predominant endogenous viral sequence was also wild type. These results suggest that exposure to AZT or d4T sensitizes HIV-infected HLA-A3 individuals against the RT33-41 peptide epitope containing the M41L resistance-associated mutation. This sensitization persists without predominance of the mutation in endogenous virus. In contrast to the HLA-A2 subjects, the M41L mutation was not seen in any of the three HLA-A3 individuals with a CD8+ T cell response against the RT33-41 M41L variant epitope for whom genotypic information was available.

With the RT179-187 epitope, we saw exclusive recognition of peptides incorporating resistance mutations by two individuals (Fig. 2c). Subject 028, who was receiving the NNRTI sustiva, recognized a peptide with NNRTI-associated mutation Y181C. Subject 001, who was receiving the NRTIs didanosine, lamivudine, and ABC, recognized a peptide with both Y181C and M184V mutations. The M184V mutation is associated with lamivudine, didanosine, zalcitabine, and ABC resistance. His predominant endogenous virus at the time incorporated the M184V mutation, but not Y181C, and he had not received NNRTIs within 4 years of testing.

All seven individuals who recognized the RT203-212 epitope preferentially recognized the wild-type epitope over that incorporating the L210W mutation (Fig. 3f). The L210W mutation, like M41L, is selected for by AZT and d4T, but can also contribute to ABC resistance, once established. Both subjects (077 and 134) who showed some recognition of the mutant epitope were receiving d4T. Two individuals who exclusively recognized the wild-type epitope had either never received AZT or d4T (055) or had not received either of these drugs for at least 2 years (057). The endogenous viral sequence from subject 057 from a sample taken nearly 1 year after the ELISPOT assay was predominantly wild-type within RT203-212. Three other HLA-B44 individuals who exclusively recognized the wild-type epitope were nonetheless receiving d4T (017 and 131) or AZT (118). Thus, it appears that exposure to AZT or d4T can sensitize HLA-B44 individuals to a variant peptide epitope incorporating the L210W mutation, but that this variant is less immunogenic than the wild-type epitope.

### Peptide-specific CTL stimulation

The ability of wild-type and variant peptides to activate CTL in vitro was tested by peptide-specific PBMC stimulation. Although all peptides were antigenic, the pattern of in vitro activation of

<table>
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<th>Subject</th>
<th>Recent Treatment History</th>
<th>HIV Genotype</th>
</tr>
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<tbody>
<tr>
<td>001</td>
<td>DDI, 3TC, ABC, amprenavir (10/00-present), DDI, d4T, sustiva, ritonavir, indinavir (6/99–10/00)</td>
<td>RT M41L, M184V</td>
</tr>
<tr>
<td>007</td>
<td>d4T, ritonavir, indinavir (11/00-present)</td>
<td>RT M41</td>
</tr>
<tr>
<td>012</td>
<td>d4T, 3TC, sustiva (9/99-present)</td>
<td>RT M41L</td>
</tr>
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<td>017</td>
<td>d4T, 3TC (11/09-present)</td>
<td>Virus load too low</td>
</tr>
<tr>
<td>028</td>
<td>3TC, sustiva, nelﬁnavir (6/99-present)</td>
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</tr>
<tr>
<td>039</td>
<td>AZT, 3TC, ritonavir, saquinavir (11/00-present)</td>
<td>Virus load too low</td>
</tr>
<tr>
<td>043</td>
<td>d4T, 3TC, DDC (2/99-present)</td>
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<tr>
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<tr>
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<td>PR 184V</td>
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<tr>
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<td>RT M41L</td>
</tr>
<tr>
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<tr>
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<td>RT M41, L210</td>
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<tr>
<td>162</td>
<td>AZT, 3TC, sustiva (4/02-present)</td>
<td>RT M41, V108, Y115</td>
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self-reactive vs cross-reactive CTL varied for individual peptides (Fig. 5). For HLA-A3 subject 082, the RT33–41 M41L variant activated CTL specific for itself without cross-reactivity for the wild-type RT33–41 peptide, whereas the wild-type peptide activated no CTL against itself or the variant (Fig. 5a). This reflected the selective recognition of the variant peptide that we observed by bulk cytotoxicity and ELISPOT (Figs. 2 and 3b). Subject 162 showed the same response pattern as 082 after restimulation (data not shown), which did not match the ELISPOT data showing preferential recognition of the wild-type peptide, but did match the apparently enhanced in vitro immunogenicity of the M41L variant overall. In the context of HLA-A2, both the wild-type and variant peptides stimulated CTL from subjects 043 (Fig. 5b), 001, 007, and 134 (data not shown), who were cross-reactive for both peptides. This matches the generally cross-reactive recognition of both the wild-type and variant peptides seen in the ELISPOT assays. For subjects 126 (Fig. 5c) 017, 055, and 131 (data not shown), the wild-type RT203–212 peptide activated CTL cross-reactive against itself and the L210W variant peptide, whereas the L210W variant did not activate CTL against either peptide. This matched the ELISPOT data in that reactivity against the wild-type peptide was favored, but the wild-type peptide stimulated CTL against the L210 variant even in subjects who showed no significant reactivity with the L210W variant peptide by ELISPOT. For subject 039, both the wild-type PR76–84 and I84V variant peptide induced CTL cross-reactive against both peptides (Fig. 5d), matching the ELISPOT data showing complete cross-reactivity between the wild-type and I84V variant peptides. Overall, the peptide stimulation results indicate that for the HLA-A3-restricted RT33–41 and HLA-B44-restricted RT203–212 epitopes, the reactive T cell populations in HIV-infected individuals includes T cells that distinguished between wild-type and variant peptides and T cells that cross-react with both wild-type epitopes and variants incorporating drug resistance mutations. For the HLA-A2-restricted RT33–41 and PR76–84 peptides, T cells appear to predominantly cross-react with the wild-type and variant peptides.

**Discussion**

In this study we examined the effect of antiretroviral drug resistance-associated mutations on recognition of HIV-1 Pol CTL epitopes presented by common HLA molecules. Although cross-reactivity patterns differed, variant peptides incorporating common mutations associated with the three major antiretroviral drug classes all sustained and in some cases even enhanced T cell recognition. Recognition of the wild-type and variant peptides encompassing sites of common drug resistance mutations was quite prevalent in our study cohort with relatively high frequencies of reactive T cells. It was not uncommon for individuals to show T cell reactivity against multiple epitopes potentially or actually incorporating drug resistance mutations, indicating a strong immunological focus on functional determinants of the HIV Pol proteins.

We saw several patterns in terms of relative recognition and in vitro immunogenicity levels of wild-type and variant peptides that in most cases reflected antiretroviral treatment history. In the case of the HLA-A2-restricted PR76–84 and HLA-B35-restricted RT107–115 epitopes, recognition and immunogenicity patterns were independent of treatment history due to the complete cross-reactivity of the wild-type and variant peptides. The HLA-B44-restricted RT203–212 wild-type epitope was always preferentially recognized relative to the L210W variant, and the variant was only recognized by individuals who had been treated at some point with antiretrovirals known to select for the L210W mutation. Despite the apparent in vivo immunogenicity of the L210W variant peptide, it failed to expand CTL against itself in vitro, whereas with PBMC from individuals sensitized in vivo against the L210W peptide, the wild-type peptide expanded CTL against itself and the L210W variant. Although this might suggest immunological selection against the wild-type sequence in HLA-B44 individuals, we did not observe the L210W mutation in any of three HLA-B44 individuals for whom viral sequence data were available regardless of their ongoing or previous antiretroviral treatment.

The HLA-A3-restricted RT33–41 epitope exhibited the most interesting pattern of recognition and immunogenicity. Although there was some cross-reactivity, the M41L variant peptide was nearly always preferentially recognized by CTL or PBMC from HIV-infected individuals currently or previously receiving antiretrovirals selecting for the mutation. This preferential recognition pattern, sustained even in the absence of in vivo M41L predominance, suggests that the M41L mutation increases the immunogenicity of this epitope in vivo. In vitro activation and expansion of CTL reinforced this suggestion, as the M41L variant peptide activated CTL against itself, but not the wild-type peptide, whereas the wild-type peptide was nonimmunogenic in vitro, even with

**FIGURE 5.** Specificity of CTL induced by PBMC stimulation with wild-type Pol peptides or variants incorporating drug resistance mutations for wild-type Pol peptides or variants. The left graph shows results for stimulation with wild-type peptides with variant peptide stimulation on the right. Peptides tested for recognition are shown in the right graph of each series. Representative specificity or cross-reactivity patterns for the RT33–41 (a and b), RT203–212 (c), and PR76–84 (d) wild-type and variant peptides are shown.
PBMC from individuals preferentially recognizing the wild-type peptide. Preferential recognition and enhanced immunogenicity of RT33–41 incorporating the M41L mutation raise the possibility of immunological selection against this drug resistance-associated mutation in vivo in HLA-A3 individuals. Despite an overall high frequency of the M41L mutation, this mutation was only seen in two HLA-A3 individuals in our cohort, both of whom were severely immunodeficient with no residual CD8+ T cell response against HIV detectable. This contrasts with the RT33–41 epitope presented in the context of HLA-A2, where recognition of both the wild-type peptide and the M41L variant is common, with no general preference observed, and both peptides activate and expand CTL against themselves and the corresponding variant in vitro. Consistent with this apparent relatively equal immunogenicity, the M41L mutation was seen in two of the HLA-A2 individuals with CD8+ T cells against this epitope, suggesting no immunological selection against either variant. Longitudinal studies of a larger number of HLA-A2 and A3 individuals with CD8+ T cell responses against RT33–41 could best evaluate immunological selection against the M41L mutation associated with AZT and d4T resistance. Of note in this regard was the endogenous viral section against the M41L mutation associated with AZT and d4T in the available CD8+ associated variant peptides offer opportunities for exploitation of fitness mutations that in at least some cases was specific for the variant incorporating the drug resistance mutation (18–20). Both cross-reactive activity and specific recognition of drug resistance-associated peptides offer opportunities for exploitation of the available CD8+ T cell repertoire, especially when the variant peptides have enhanced immunogenicity. For example, the general cross-reactivity of the HLA-A2-restricted RT33–41 and PR76–84 and the HLA-B35-restricted RT107–115 variants implies that these peptides could be used therapeutically to boost immunity against both wild-type virus and drug-resistant variants. Inclusion of such epitopes in prophylactic vaccines would potentially offer coverage against both wild-type HIV and the drug-resistant HIV strains now being transmitted with increasing frequency (21, 22). The partially selective recognition of other drug resistance-associated CTL epitopes offers different opportunities. Firstly, as the T cell populations recognizing the wild-type and variant peptides are not completely overlapping, it implies the variants could be used to both strengthen and diversify the CTL response against HIV. Diversification to include CTL responses selective for drug-resistant HIV might be especially effective if evoked before using antiretroviral drugs selecting mutations represented in the variant epitopes used for immunization. Pre-existing immunity against drug-resistant viruses present at low frequencies, if present at all, should delay the emergence of drug resistance. Another implication of selective recognition of M41L and L210W mutation-incorporating variants is an opportunity to strengthen and diversify the anti-HIV CTL response through novel forms of autoimmunization without treatment interruption. Both mutations reflect the selective impact of AZT and d4T; therefore, manipulating the relative dominance of endogenous viral variants by cyclical administration and withdrawal of one or both of these drugs could augment immunity against both wild-type and drug-resistant HIV.

Documenting the antigenicity and immunogenicity of CTL epitopes incorporating antiretroviral drug resistance mutations is a first step toward developing immune strategies against the emergence and transmission of drug-resistant HIV. Although the immunogenicity of these epitopes is fundamental, a number of additional factors will determine the antiviral efficacy of CTL they might induce. Pol is a major T cell Ag in human HIV infection, and importantly, HLA-associated selection for sequence variation, within Pol CTL epitopes demonstrates selective pressure of anti-HIV Pol CTL in vivo (23–25). In humans and macaques, CTL activity against just one epitope can sustain relative viral quiescence and slow disease progression (9, 10). The five CTL epitopes and corresponding drug resistance variants we tested are presented by HLA-A2, -A3, -B35, and -B44, four alleles that together cover 90% of people of western European descent and a majority of most other ethnic populations. Other CTL epitopes with drug resistance mutations are known, and more almost certainly remain to be identified (18–20). Thus, broadly applicable multiprotein vaccines against drug-resistant HIV are feasible.

In summary, a number of common drug resistance-associated mutations sustain or enhance immunogenicity of HIV-1 Pol CTL epitopes in individuals with common HLA types. Knowledge of the particular patterns of selective and cross-reactive recognition of drug-resistant variants under different circumstances could be used to develop novel strategies engaging immune-based and antiretroviral selection synergistically against HIV.

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References


