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Greater CD8^+ TCR Heterogeneity and Functional Flexibility in HIV-2 Compared to HIV-1 Infection

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Virus-specific CD8^+ T cells are known to play an important role in the control of HIV infection. In this study we investigated whether there may be qualitative differences in the CD8^+ T cell response in HIV-1- and HIV-2-infected individuals that contribute to the relatively efficient control of the latter infection. A molecular comparison of global TCR heterogeneity showed a more oligoclonal pattern of CD8 cells in HIV-1 than HIV-2-infected patients. This was reflected in restricted and conserved TCR usage by CD8^+ T cells recognizing individual HLA-A2- and HLA-B57-restricted viral epitopes in HIV-1, with limited plasticity in their response to amino acid substitutions within these epitopes. The more diverse TCR usage observed for HIV-2-specific CD8^+ T cells was associated with an enhanced potential for CD8 expansion and IFN-γ production on cross-recognition of variant epitopes. Our data suggest a mechanism that could account for any possible cross-protection that may be mediated by HIV-2-specific CD8^+ T cells against HIV-1 infection. Furthermore, they have implications for HIV vaccine development, demonstrating an association between a polyclonal, virus-specific CD8^+ T cell response and an enhanced capacity to tolerate substitutions within T cell epitopes. The Journal of Immunology, 2003, 171: 307–316.

Human immunodeficiency virus-2 is a lentivirus related to HIV-1 with up to 60% sequence homology, but with markedly different epidemiological features. HIV-2 infection has a relatively limited geographical distribution and is much less transmissible than HIV-1 by both horizontal (1) and vertical (2) routes. In addition, most patients infected with HIV-2 have a more prolonged clinically asymptomatic phase, with no reduction in survival in many of those infected and an overall mortality only twice as high as that of the uninfected population (3). These differences may result from an inherent reduced pathogenicity of HIV-2 and/or from a more effective immune response to the virus. Recent data do not support the notion that HIV-2 has an attenuated phenotype with less pathogenic potential, because it has a similar in vitro cytopathicity to HIV-1 (4). Plasma RNA levels are much lower in asymptomatic HIV-2-infected patients with high CD4 counts than in similar HIV-1-infected patients, despite comparable levels of proviral DNA in the two infections (5, 6). The lack of correlation between cellular proviral DNA and plasma viremia in HIV-2 infection (6) could reflect reduced production of virions by infected cells as a result of better immune control.

The CD8^+ T cell response is one of the critical correlates of viral control in HIV infection. One mechanism increasingly recognized to be of importance in the failure of CTL control of both SIV and HIV-1 infections is viral acquisition of mutations allowing CTL escape (Refs. 7–9; N. Jones, X. Wei, D. Flower, M. Wong, F. Michor, M. Saag, B. Hahn, M. Nowak, G. Shaw, and P. Borrow, manuscript in preparation). Interestingly, such escape mutations have yet to be demonstrated in the context of HIV-2, even though CTL are often detectable directly ex vivo (10). This may be one determinant contributing to the better long-term control of viral replication in HIV-2 compared with HIV-1-infected individuals.

One factor that may limit the propensity to CTL escape mutations is the polyclonality of the virus-specific CD8^+ T cell response. A single CD8^+ T cell clone may be able to recognize a number of variations of a peptide/MHC complex due to the inherent flexibility of the TCR and the small area of contact between the TCR and MHC/peptide complex (11–13). Despite this, single amino acid mutations within critical TCR contact sites have been shown to abrogate or functionally alter T cell recognition. A CTL response composed of a broad variety of TCRs can enhance recognition of amino acid changes within the peptide/MHC complex, because different TCRs can contact different amino acid residues within the same peptide and have differing susceptibilities to mutations (13–16).

In this study, we investigated whether there is a correlation between the diversity of TCRs expressed by virus-specific responses and their capacity to tolerate substitutions within the epitope. We present the first analysis of clonality of CD8 cells in HIV-2, which is compared with that in HIV-1 infection by both molecular and functional approaches. We find that restricted TCR usage in the CD8 response to HIV-1 at both the global and epitope level is associated with limited ability to recognize amino acid changes within these epitopes. The CD8 response to HIV-2 infection appears to be...
more polyclonal, correlated with enhanced cross-recognition of epitope variants.

Materials and Methods

Patients

Patients attending the Mortimer Market Center in the U.K. and the Medical Research Council clinic in The Gambia were initially tested by a combined HIV-1 and HIV-2 enzyme immunoassay (Wellcome 1 + 2; Murex Diagnostics, Dartford, Kent, U.K.). The diagnosis of HIV-2 infection was made on the basis of repeatedly negative HIV-1 and positive HIV-2 competitive ELISAs following a positive combined test (Murex Diagnostics). HIV-1- and HIV-2-infected patients were all clinically asymptomatic (with no HIV-related symptoms or signs) and had never received any antiretroviral therapy. Patients had repeated CD4 counts >250 cells/μl determined by flow cytometry using the Trestest program (BD Biosciences, San Diego, CA; data not shown). Viral loads were measured by the Chiron Quantiplex HIV RNA assay (b DNA) version 3.0 for HIV-1 and as described previously for HIV-2 (5). For HLA typing, DNA was extracted from blood samples using the Puregene kit (Flawgen, Ashby Park, Leicestershire, U.K.). Between 200 and 500 ng of DNA was used for HLA typing by a molecular PCR method that used 144 sequence-specific primer mixers (PCR-SSP). The study was approved by both local research ethics committees and all patients gave informed consent.

Heteroduplex analysis

This was performed as previously described (17), with modifications as specified below. Where required, CD4- or CD8-purified cells were obtained by positive selection using the Minimacs system (Milenyi Biotec, Auburn, CA) according to manufacturer’s instructions (≥95% purity on mAb staining). DNA was extracted from 0.5 × 10^6 PBMC (or CD4+ or CD8-purified cells) using the Dynabeads mRNA Direct Kit (Dyna Bio- tech, Oslo, Norway) according to manufacturer’s instructions. Twenty-six RT-PCRs across the β-chain complementarity-determining region 3 (CDR3)3 were conducted for each analysis, in a final volume of 50 μl and using 3% of the CDNA per reaction. Twenty microliters of the heteroduplex reactions and of the nondenatured C region control were loaded on a 12% nondenaturing polyacrylamide gel (National Diagnostics, Atlanta, GA), with a 4% stacking gel, run at 10 mA for 16 h at 4°C. Heteroduplex gels were visualized on a Fluor or Multimager (Bio-Rad, Hercules, CA) following ethidium bromide staining, and densitometrically analyzed using Quantity One software (Bio-Rad). Gels were analyzed without the usual Southern blotting and carrier hybridization (17) to reduce the resolution of the technique and limit the detection of small clones. Individual TCRβ tracks were classified as “oligoclonal” if they had three or fewer clones detectable (six or less heteroduplex bands, since each clone is usually represented by a single PCR product across the C region of the β-chain (C region control)).

Production of T cell lines and intracellular IFN-γ staining

PBMCs were seeded at 0.3 × 10^6/200 μl/well in 96-well round-bottom plates in the presence of 0.5 μM of the relevant index or analog peptide. Peptides were purchased from Chiron (Clayton, Victoria, Australia). Purity of peptides was >90% by HPLC analysis. Recombinant IL-2 (10 IU/ml; Boehringer Mannheim, Mannheim, Germany) was added on day 3. Cells were restimulated on day 10 with 0.5 μM index or substituted peptides for 5 h, the last 4 h with 10 μg/ml brefeldin A (Sigma-Aldrich, St. Louis, MO). Cells were then stained with anti-CD8 mAb (BD Pharmingen, San Diego, CA), permeabilized with Cytoperm-coxtoy (BD Pharmingen), before staining with anti-IFN-γ mAb (R&D Systems, Minneapolis, MN) and analyzing on a FACSComp (BD Biosciences) using CellQuest software. For Vβ analysis, the cells were incubated before restimulation with a panel of 17 FITC-conjugated anti-TCRβ mAb (Immunotech, Marseille, France).

Staining with HLA-A2/peptide tetrameric complexes

The HLA-class I tetramer (HLA-A2/gag 77–85) was supplied by the National Institute of Allergy and Infectious Diseases Tetramer Facility (Bethesda, MD). Tetramer staining of PBMCs was conducted for 20 min at 37°C followed by staining with anti-CD8 mAb and the panel of anti-Vβ mAbs.

Selection of IFN-γ-positive cells

Nine million PBMCs from a patient previously shown to have a response to the gag 77–85 peptide were cultured for 10 days as described above. Ag-specific cells were isolated using the IFN-γ Secretion Assay Cell Enrichment and Detection kit (Milenyi Biotec) according to manufacturer’s instructions. Briefly, day 10 cultures were restimulated with the gag 77–85 peptide (0.5 μM) for 5 h, followed by addition of the IFN-γ catch reagent (5 min, on ice). This was left for 45 min at 37°C with the cells maintained in constant motion followed by labeling with anti-IFN-γ mAb (10 min, on ice). Subsequently, anti-PE microbeads were bound to the purified cells and labeled using Minimacs MS “RS” columns.

TCR sequencing

The Vβ5.1 PCR product was cleaned-up by a PCR Purification kit (Qiagen, Valencia, CA) according to manufacturer’s instructions before sequencing. Nucleic acid was eluted in Tris-HCl and sequenced in both directions using Vβ5.1- and Cβ-specific primers (Wolfson Sequencing Unit, University College London, London, U.K.).

Generation of peptide-MHC models

The HLA-A2 crystal structure has previously been published (18). Structures were predicted for the HLA-B57 and -B58 molecules based on the published crystal structures of a number of MHC class I molecules available in the Research Collaboratory for Structural Bioinformatics protein data bank (19). Both protein models were built using the program modeller (20). Peptides were then modeled into the peptide-binding groove of each of these different HLA alleles and were energy-minimized in a solvent bath using the molecular mechanics program AMBER (University of California, San Francisco, CA).

HLA binding assay

Peptide binding to HLA-A2 was assessed using a FACS-based MHC stabilization assay (21) with modifications as described below. Briefly, T2 cells were incubated in 96-well flat-bottom plates at 1–1.2 × 10^6 cells per well in a 200 μl of volume of AIM V medium (Life Technologies, Paisley, U.K.) with human β2-microglobulin at a concentration of 100 nM (Scipac, Sittingbourne, U.K.) with and without peptides at concentrations between 200 and 0.04 μM for 16 h at 37°C. Cells were then washed and surface levels of HLA-A2 were assessed by staining with FITC-conjugated A2.1-specific mAb BB7.2 (BD Biosciences, Oxford, U.K.) or a FITC-conjugated isotype control Ab (BD Biosciences). Cells were fixed at 4°C in 1% paraformaldehyde and analyzed on a FACScalibur (BD Biosciences) using CellQuest software. Results are expressed as fluorescence index (FI) values, calculated as the test mean fluorescence intensity (MFI) minus the no peptide isotype control MFI divided by the no peptide HLA-A2-stained control MFI minus the no peptide isotype control MFI.

Results

Molecular dissection of the global ex vivo TCR repertoire shows more oligoclonal CD8+ T cell expansions in HIV-1-than HIV-2-infected individuals

Cryopreserved PBMC from 10 patients attending the outpatient clinic at the Medical Research Council (The Gambia) and 2 patients attending the outpatient clinic at University College Hospitals were used for this initial part of the study. All had asymptomatic HIV infection (six with HIV-1, six with HIV-2), with well-preserved CD4 counts and were antiretroviral naive. The RT-PCR-based heteroduplex technique (17) was used to compare the overall breadth of TCR usage of the CD8 response in HIV-1 or HIV-2 infection directly ex vivo, regardless of epitope specificity. Dissection of overall clonality within each Vβ family resulted in large expanded clones being detectable as bands with unique migration patterns, whereas polyclonal populations formed multiple heteroduplex bands resulting in a smear pattern on ethidium bromide-stained gels (Fig. 1A). As exemplified in Fig. 1A, HIV-1 patients had oligoclonal expansions represented by prominent bands in most Vβ tracks, whereas HIV-2 PBMC showed a predominant smear pattern in the majority of tracks.

To investigate whether the expansions detected represented CD8 clones, PBMC from four HIV-1 and four HIV-2 patients were fractionated into CD4+ and CD8+ subsets (purity >97%) and
FIGURE 1. Global heteroduplex analysis of TCR heterogeneity in HIV-1 and HIV-2 infection. A, Ethidium bromide-stained gels show RT-PCR heteroduplex samples Vβ1–22 from a representative HIV-1 and HIV-2 patient (with the TCRVβ primer and carrier used indicated above the tracks). In the HIV-1 patient, there are distinct heteroduplexes showing a specific migration pattern above the two dense homoduplex bands in most tracks, whereas in the HIV-2 patient polyclonal smear patterns are demonstrated above the homoduplex bands within most Vβ. The input of total TCR β-chain mRNA was similar, as suggested by the PCR control across the Cβ region (track C, arrowed). B, Heteroduplex analysis of purified CD4+ and CD8+ T cells are shown for Vβ1–8 for an HIV-1 and Vβ1–9 for an HIV-2 patient, demonstrating segregation of all heteroduplex bands with the CD8+ subset (examples arrowed in the last track). C, Densitometric profiling was applied to categorize each Vβ track into polyclonal (upper left) or oligoclonal (lower left) patterns. The bar chart (left) shows the percent of Vβ tracks with an oligoclonal pattern for six HIV-1 patients and six HIV-2 patients, with the mean and SD for each group plotted (right). Differences between the two groups of patients were analyzed using the Mann-Whitney U test.
their molecular TCR analysis was conducted in parallel. In the HIV-1 patients, the extensive CDR3 region defined TCR expansions all segregated with the CD8 fraction (Fig. 1B). Similarly, the few clones that were detectable in HIV-2 were also restricted to the CD8 subset (Fig. 1B). In both infections, the CD4 subset retained polyclonal smear patterns typical of healthy controls, as was also recently observed in the setting of acute EBV infection (17).

To attempt a quantitative comparison of the CD8 clonality in HIV-1- and HIV-2-infected patients, densitometric profiling and blinded dual observer analysis were applied to categorize each track as polyclonal, giving a smooth curve pattern, or oligoclonal, producing prominent peaks (Fig. 1C, left panel). This provided a summary figure of the percent of Vβ tracks with oligoclonal patterns for each patient (arbitrarily defined as three or fewer detectable clones per track as described in Materials and Methods). This analysis confirmed significantly more oligoclonally restricted TCRVβ responses overall in the HIV-1 than the HIV-2 cohort (Fig. 1C). The variability in TCR clonality within each group of patients did not correlate with CD4 count or viral load (data not shown), suggesting it was not attributable to differences in the level of viral stimulation.

TCR usage for a single HLA-A2-restricted CD8+ T cell epitope demonstrates a highly focused response associated with poor functional flexibility in HIV-1

The differences in overall heterogeneity of responding CD8 cells highlighted by the heteroduplex analysis of HIV-1 and HIV-2 patients could be attributable to differences in the multispecificity and/or the polyclonality of individual CTL responses. To address the latter possibility, we initially studied the response to the HIV-1 p17 gag epitope 77–85 in an HLA-A2-positive patient with asymptomatic HIV-1 infection (patient 1.1). A short-term T cell line expanded for 10 days by a single round of peptide restimulation was stained with an HLA-A2/gag 77–85 tetramer (Fig. 2A). The TCR repertoire of the tetramer-positive cells was dissected by costaining with a panel of 17 anti-Vβ-specific mAbs. All the epitope-specific CD8+ T cells stained with the anti-Vβ5.1 mAb (Fig. 2A), consistent with a highly focused TCR usage.

To exclude in vitro selection for Vβ5.1 clonotypes during the limited culture period used to expand the CTL, the same analysis was repeated using the population of gag 77–85-specific CD8 cells identifiable ex vivo (around 1% of CD8 cells). Again, virtually all the tetramer-positive CD8 costained with anti-Vβ5.1, confirming restricted usage of this Vβ chain by gag 77–85-specific T cells in vivo (Fig. 2B).

To further investigate the clonality of these gag-specific T cells at the molecular level, IFN-γ-secreting CD8 cells were captured following restimulation with the specific peptide. A similar proportion of Ag-specific CD8 cells were identified as with tetramer staining. TCR usage of the captured cells was analyzed by RT-PCR heteroduplex of Vβ5.1, showing a dominant band suggestive of a single predominant clone (Fig. 2C). Direct sequencing of the Vβ5.1 PCR product confirmed oligoclonality of the gag 77–85-specific T cells in this patient because a readable sequence was obtained without the need for PCR cloning (Fig. 2C). Surprisingly, this sequence was identical (even across the highly variable CDR3 region) to that identified from a gag 77–85-specific CTL clone in a previous study on an unrelated patient (22). This would suggest the potential for a marked degree of conservation between individuals in this TCR response, as noted in the response to an HLA-B8-restricted EBV epitope (23).

The highly oligoclonal CD8 cells specific for the gag 77–85 epitope in this patient (1.1) were then tested for their ability to cross-recognize the naturally occurring HIV-2 epitope variant. T cells expanded for 10 days in vitro with the index peptide showed substantial reduction in IFN-γ production after restimulation for 6 h with the HIV-2 variant compared with that seen on restimulation with the index peptide (Fig. 3A). The functional flexibility of gag 77–85-specific CD8 from this patient and four other HLA-A2+ HIV-1-infected asymptomatic patients was further evaluated by testing cross-recognition of a series of naturally occurring variants (peptides as shown in Fig. 3B). In addition, alanine scanning mutagenesis was used to test the recognition of a series of peptides with single successive alanine substitutions in potential TCR contact sites (derived from the computer-generated model of the peptide bound to HLA-A2; Fig. 3B). There was poor cross-recognition of the peptide representing the HIV-2 variant in all cases, and in some patients even of the A clade variant with a single Y to F conservative substitution (Fig. 3C). Similarly, the alanine-substituted variants of the HIV-1 gag 77–85 epitope substantially abrogated recognition, apart from the conservative mutation V to A at position 82 (Fig. 3C). The highly restricted CD8 cells from patient

FIGURE 2. Cellular and molecular TCR analysis of an HLA-A2-restricted gag response in HIV-1. A. After 10 days expansion in vitro with the gag 77–85 peptide, epitope-specific T cells from HIV-1 patient 1.1 were identified by staining with anti-CD8 and an HLA-A2/gag 77–85 tetramer. After gating on CD8+ T cells, tetramer-positive cells costained with a panel of 17 Vβ-specific mAbs showed exclusive costaining with the Vβ5.1 mAb. B. The population of cells staining with the HLA-A2/gag 77–85 tetramer after gating on CD8+ T cells directly ex vivo were also all costained by the Vβ5.1 mAb and not by any of the other Vβ mAbs. C. T cells specific for the gag 77–85 epitope were selected using the IFN-γ secretion assay cell enrichment kit and heteroduplex analysis was performed on the Vβ5.1 RT-PCR product. A prominent heteroduplex band (indicating oligoclonality, arrowed) was visualized above the carrier homoduplex. Direct PCR sequencing of the IFN-γ catch Vβ5.1 PCR product revealed a single readable Vβ5.1/β1.6 sequence with CDR3 region as illustrated.
FIGURE 3. Ability of T cells responsive to an HLA-A2-restricted epitope in HIV-1 to cross-recognize naturally occurring and alanine-substituted epitope variants. A, The percent of gag 77–85-responsive CD8 cells (from patient 1.1) producing IFN-γ on restimulation with the indicated peptides are shown in the upper right quadrants of the FACS plots. B, Computer-generated model of the HIV-1 clade B consensus gag 77–85 peptide bound to HLA-A2. The epitope peptide is in an N to C orientation, with hydrophobic aliphatic amino acids (ALA, LEU, VAL) shown in cyan, hydrophobic aromatic residues (TYR) in green, and small polar amino acids (ASN, SER, THR) in yellow. The HIV-1 gag 77–85 index, naturally occurring and alanine-substituted analog sequences are listed, with anchor residues underlined, conservative amino acid substitutions boxed, and nonconservative substitutions circled. C, Functional flexibility of HIV-1 gag 77–85-specific CD8 from five HLA-A2* asymptomatic HIV-1 patients. The ability of index peptide-responsive short-term T cell lines to produce IFN-γ in response to restimulation with the variant peptides shown in (B) are represented as a percentage of the response to the index (unmutated) peptide after subtraction of background responses with no peptide. D, Comparison of the binding of the HIV-1 gag 77–85 epitope peptide and naturally occurring and alanine-substituted analogs to HLA-A2 using a T2 cell MHC stabilization assay. T2 cells were incubated overnight with the HIV-1 gag 77–85 index peptide, indicated variants (as in B), or with an HLA-B58-restricted epitope peptide from HIV-1 Nef (Nef; KAAVDLSHF) or an HLA-A2-restricted epitope peptide from the hepatitis B virus core protein (HBc 18–27; FLPSDFPPSV) as negative and positive controls, respectively. Surface HLA-A2 expression was assessed by staining with an A2-specific mAb and nonspecific binding with an isotype control mAb (iso). The results are expressed as IFI values, calculated as described in Materials and Methods.
different Vβ chains used by HIV-1 and HIV-2 T cells (Fig. 4B) highlighted the more restricted repertoire in HIV-1-infected individuals for this shared epitope, compatible with the global data from the heteroduplex analysis.

CD8+ T cells specific for gag 240–9 were examined for their ability to cross-recognize naturally occurring variants of the index (unmutated) epitope. T cells from HIV-1-infected patient 1.2, which had been shown to have a highly restricted Vβ usage (Fig. 4), were found to have limited cross-reactive potential (Fig. 5A). The single conservative amino acid substitution at position 9 of the epitope found in the clade A HIV-1 variant resulted in substantial reduction in IFN-γ production, while the HIV-2 variant was barely recognized by the index epitope-responsive CTL (Fig. 5A). Serial peptide titrations showed that cross-recognition was not enhanced at alternative concentrations, including when using a 10-fold higher concentration (data not shown). By contrast, the T cells responding to the equivalent epitope from HIV-2 patient 2.1, expressing a broad repertoire of TCR (Fig. 4), showed efficient production of IFN-γ on restimulation with both the A and B clade HIV-1 variants, despite the presence of three mutations (one non-conservative) at potential TCR contact sites (Fig. 5A). Consistent data were obtained on testing for cross-recognition directly ex vivo (data not shown).

Fig. 5B shows the summary of testing for cross-recognition by gag 240–9-specific T cells as described above for five HIV-1 and five HIV-2 patients (three and two of whom, respectively, had gag 240–9 repertoire analysis presented in Fig. 4). None of the HIV-1 HLA-B57/58+ patient samples had significant IFN-γ production on exposure to the HIV-2 variant at this epitope (Fig. 5B). However, five of six of the HLA-B58+ HIV-2 patient samples showed efficient cross-recognition of the HIV-1 variant epitope (Fig. 5B). In all these cases, responses to the variant epitope were seen at low peptide concentration (0.5 μM), indicating that the cross-reactive interaction was of a similar high avidity to the response to the HIV-2 peptide. Thus the ability to recognize the extensive amino acid changes between the HIV-1 and HIV-2 epitope correlated with the broader TCR Vβ usage by these T cells in HIV-2 patients (Fig. 4).

The HIV-1 responses exhibited restricted Vβ usage and lack of cross-reactivity even when sampled from HIV-1-infected patients with well-preserved CD4 counts and low viral loads (e.g., patient
FIGURE 5. Comparison of the ability of T cells from HIV-1- and HIV-2-infected individuals responsive to a shared gag epitope to cross-recognize peptides from different HIV types. A, Responsiveness of T cells from an HIV-1-infected patient (1.2) and an HIV-2-infected patient (2.1) to HIV-1 and -2 epitope variants. The percent of CD8 cells producing IFN-γ on restimulation with the indicated peptides are shown in the upper right quadrants of the FACS plots. The peptide sequences are shown, with anchor residues underlined, conservative amino acid substitutions boxed, and non-conservative substitutions circled. B, Cross-recognition of the HIV-2 ROD variant by HIV-1-responsive T cells (left bar chart) and of the HIV-1 consensus B clade variant by HIV-2-responsive T cells (right bar chart). The recognition on restimulation with the optimal index peptide is defined as 100%, and the proportion of these T cells able to produce IFN-γ on restimulation with the variant peptide is represented as a percentage of this (following subtraction of the background IFN-γ production on restimulation in the presence of brefeldin A without peptide). The optimal peptide response generated for HIV-1 patient 1.5 recruited from The Gambia was the A clade variant with which he was likely to have been infected. Repeat experiments on samples obtained on follow-up are shown for HIV-1 patient 1.2 (12 mo later) and HIV-2 patient 2.3 (30 mo later). C, Ability of HIV-1 gag 240–9-specific T cells and HIV-2 gag 241–50-specific T cells to expand on stimulation with the HIV-2/1 variant peptides, respectively, and cross-recognize the index peptide at day 10, expressed as a percentage of expansion induced by the index peptide (designated V/I, presented with results obtained as in B, designated I/V). D, Computer-generated models of the HIV-1 clade B consensus gag 240–9 peptide and the HIV-2 ROD gag 241–50 peptide bound to HLA-B57 (left), compared with their orientation when bound to HLA-B58 (right). The epitope peptides are in an N to C orientation, with hydrophobic aliphatic amino acids (LEU, ILE, VAL) shown in cyan, hydrophobic aromatic residues (TRP) in green, acidic residues (GLU) in red, small polar amino acids (GLN, SER, THR) in yellow, and sterically constrained residues (GLY) in orange.
1.2: CD4 count 680 cells/μl, viral load 1,500 copies/ml; and patient 1.6: CD4 count 450 cells/μl, viral load 3,100 copies/ml; HIV-specific CD8 responses were not tested. Several of the HIV-1 patients recruited from the U.K. clinic were known to have disparate durations of infection (e.g., >7 years for patient 1.6 and <2 years for patient 1.7) but exhibited the same highly focused responses (Fig. 5B). HIV-1 patient 1.2 and HIV-2 patient 2.3 were tested at two time points separated by at least 1 year, and demonstrated temporal stability of the lack or presence of cross-reactivity, respectively (Fig. 5B). This suggests stability of the clonality and resultant cross-reactive potential of responses over time, compatible with the long lifespan of HIV-1-specific CD8 clones previously demonstrated in asymptomatic infection (22, 25). Consistent with this, heteroduplex analysis showed stability of the overall clonal pattern over a year of follow-up in the two HIV-1 and two HIV-2 patients examined (data not shown).

Because variable levels of TCR triggering have been shown to be required to elicit different CD8 effector functions (26), the ability of mutated epitopes to induce CD8 proliferation was also tested. The ability of variants to expand a population of T cells directly ex vivo capable of producing IFN-γ on restimulation with the index peptide was again compared with expansion and restimulation with the index peptide. There was consistency between the results obtained with this method and that testing for cross-recognition at 10 days (indicated as V/I and I/V, respectively; Fig. 5C). Similar results of flexible cross-recognition of epitope variants using both of these approaches were observed for CD8 cells specific for another HIV-2 epitope (data not shown).

To further examine the striking differences in cross-recognition of the HIV-1/2 variants of the shared gag 240–9 epitope in HIV-1/2-infected patients, we used computer-generated models to visualize the binding of the HIV-1 clade B and HIV-2 peptides to HLA-B57 and B58 (Fig. 5D). These models confirmed that the three amino acid substitutions between the HIV-1 and HIV-2 epitopes would be at TCR binding sites when presented by either HLA-B57 or B58. These models of the three-dimensional MHC/peptide structure also predicted that the three amino acid differences between the HIV-1 gag 240–9 epitope and the equivalent epitope from HIV-2 would result in major conformational changes at the TCR contact surface. Although it remained possible that peptide conformations altered in vivo during interaction with the TCR, these data supported the high level of flexibility required by the HIV-2-specific CD8 to efficiently cross-recognize the HIV-1 variant as demonstrated in a number of HIV-2 patients.

**Discussion**

There is mounting evidence in favor of the key role of the CD8 T cell response in successful control of HIV infection. However, loss of viral control and disease progression clearly can occur despite ongoing strong, functionally active CTL. Even though such responses are often multispecific, viral mutants still appear to be a frequent means of escape. Therefore, we addressed whether the clonality of individual CD8 responses could be one factor affecting their plasticity and hence potential to control this highly variable virus. A previous study had suggested a link between global oligoclonality and poor viral control in HIV-1 infection (27). In this study, we explored a possible mechanism for this association, suggesting a link between the clonality of a CTL response and its ability to cross-recognize variant epitopes. We found a limited capacity for cross-recognition of variant epitopes by HIV-1-specific CD8 with oligoclonal TCR usage. This contrasted with the findings in HIV-2-infected individuals, a generally well-controlled retroviral infection. A molecular analysis of TCR heterogeneity identified fewer oligoclonal expansions in the total virus-specific response to HIV-2 infection. The individual HIV-2-specific CD8 responses studied were also less restricted in terms of TCR usage than those in HIV-1 and this correlated with an enhanced functional flexibility.

In the case of two frequently recognized HIV-1 epitopes, we showed highly restricted TCR usage, contrasting with the polyclonality often demonstrated for immunodominant CTL responses in other infections (24, 28, 29). These data obtained by techniques allowing direct TCR analysis of virus-specific CD8-producing IFN-γ, are consistent with the oligoclonal populations previously noted in both the primary and chronic phases of HIV-1 infection (22, 25, 30). We found that these highly focused responses were not capable of efficient cross-recognition of a number of different variants within the epitope. Our study took advantage of intracellular cytokine staining to allow a more quantitative assessment of cross-recognition than was possible in previous studies using chromium release assays on long-term lines or clones. A number of studies have suggested some cross-clade CTL reactivity in HIV-1-infected patients, which could be due to sequence conservation across some epitopes and true cross-recognition of changes at others (31). However, previous studies of the A2-restricted gag 77–85 epitope have shown that not all patients can even cross-recognize the single amino acid mutation distinguishing the A and B clade variants (32, 33) compatible with the limited cross-recognition found at this epitope in our study. Studies of HIV-1-infected patients have also shown little or no cross-recognition of HIV-2 (34, 35). An exception to this is the response to a B27-restricted epitope (36); the ability of these CTL to recognize the HIV-2 variant despite five amino acid substitutions suggests an unusual flexibility which could contribute to the association of this HLA allele with long-term nonprogression of HIV-1 infection. A recent study showed enhanced flexibility of cross-recognition for a different HIV-1 B57-restricted epitope to that studied here (37) which is a particularly overrepresented response in long-term nonprogressors. Preliminary data suggest that the plasticity of this response may also be associated with more diverse TCR usage (A. Lopes, N. Jones, P. Newton, I. Williams, P. Borrow, and M. Maini, unpublished data). Such a link between TCR diversity and functional flexibility of CTL was also recently observed for an immunodominant epitope in a patient with chronic hepatitis B virus infection (24).

In this work, we took advantage of a common CTL response in B57/8+ individuals which represents a shared epitope between HIV-1 and HIV-2 to compare clonality and cross-reactivity. The HIV-2 responses studied were less restricted in terms of TCR usage and this correlated with enhanced functional flexibility. The efficient cross-reactivity of T cells specific for the B58-restricted gag 241–50 epitope in HIV-2 extends the data from a previous study in HIV-2-infected patients, the majority of whom had gag-specific CTL lines capable of cross-recognizing HIV-1 gag, with some having lytic responses to the gag 240–252 region on peptide mapping (38). We observed some variability between individuals in the amount of cross-recognition as suggested from previous studies (38, 39). Although the differences we identified may have a degree of epitope selectivity (40, 41), the global molecular analysis points to an overall reduction in large oligoclonal expansions in the total virus-specific response to HIV-2 compared with HIV-1 infection. The HIV-1 and HIV-2 patients tested were all clinically HIV asymptomatic and had similar ages and CD4 counts at recruitment, but seroconversion dates were often not known; therefore it is possible that the HIV-2 sample was biased toward a longer duration of infection because they typically have a much slower decline in CD4 numbers. This would be an unlikely explanation for their broader responses because we found no effect of
duration of infection in the cases examined, and the existing literature points to a maintenance or narrowing of CD8 repertoires on prolonged or repeated pathogen exposure (16, 17, 23, 42, 43).

Perhaps the most plausible explanation for the differences in breadth and functional flexibility of CD8+ T cell responses observed in HIV-1- and HIV-2-infected individuals is a difference in the availability of HIV-specific CD4 help in the two infections. Limited availability of CD4 help during CTL priming could result in expansion of a narrower CD8 response. This might be restricted to T cell clones bearing high affinity TCR, which would be consistent with the tendency to select highly conserved TCRs in the HIV-1 responses we studied and in a recent analysis of an immunodominant SIV response (43). CD4 help has been shown to be critical for the persistence of functionally active CTL (reviewed in Ref. 7), so it is possible that paucity of CD4 help may also limit the maintenance of polyclonal, broadly cross-reactive CTL. HIV-2-specific CD4 proliferative responses are well-preserved and broadly cross-reactive (44), whereas HIV-1-specific CD4 responses are depleted early in infection (45), even when CD4 numbers remain well-preserved. Thus it will be important to test whether greater availability of HIV-specific CD4 help can enhance the breadth and plasticity of individual CTL responses. In this context, a recent study identified several epitope-specific clonotypes able to recognize a variant containing two amino acid substitutions in an HIV-1 patient undergoing intermittent antiretroviral therapy (16).

We demonstrated efficient cross-recognition of the HIV-1 epitope by HIV-2-specific CD8 for a frequently recognized epitope (38) in individuals expressing a common Gambian HLA allele. Recent data confirm that this type of broad response capable of cross-recognizing mutated epitopes can be generated de novo after exposure to the wild-type sequence alone (46). This ability of HIV-2-specific CTL to cross-recognize HIV-1 variants could contribute to a degree of cross-protection to HIV-1 proposed to result from pre-existing HIV-2 infection or exposure (reviewed in Ref. 44). Although the epidemiological evidence for protection against HIV-1 by prior HIV-2 infection has been disputed (47), it is supported by animal studies showing long-term protection against SIV-induced disease in macaques vaccinated with a live, attenuated HIV-2 vaccine (48). Cross-reactive virus-specific CTL have been proposed to account for the apparent resistance to HIV-1 infection of seronegative sex workers who may have been initially exposed to HIV-2 (41), and for the protection against mucosal SIV infection in some HIV-2-exposed seronegative macaques (49).

The greater flexibility of HIV-2-specific CTL to tolerate variations within the epitope could also play a role in enhancing viral control by limiting the successful development of escape mutants. Many studies confirm the important role of mutational escape in the HIV and SIV models (7), but thorough prospective studies are now required to investigate whether such escape mutations are less likely to be selected in HIV-2 infection. Our data indicate that escape by loss of TCR interaction (rather than by inhibition of processing or MHC binding) may be reduced in HIV-2 infection, as many amino acid changes arising within the HIV-2 CTL epitope studied might not affect the capacity of the epitope both to expand and be recognized by the original population of CTL. By contrast, in HIV-1 infection, similar mutations would create a window with loss of viral recognition while switching to a new response; such new responses to variant epitopes are proposed to be unlikely because of the phenomenon of “original antigenic sin” (50). Although the multispecificity of the CTL response should allow viral control to be exerted through the response to an entirely different epitope, this is likely to weaken the overall response through forcing constant shifts to potentially sub-dominant epitopes and allowing temporary bursts of viral replication (51).

In summary, we show for the first time that there can be greater flexibility of recognition associated with the broader TCR usage seen in the CD8 responses in HIV-2-infected patients than the limited cross-recognition possible with a highly focused TCR usage in HIV-1 infection. Recent data have highlighted how a single gag CTL escape mutation arising in the SIV/HIV model can limit the protective efficacy of an otherwise promising env/gag plasmid DNA vaccine approach (52). This work suggests that future HIV-1 vaccine strategies may need to address the polyclonality in addition to the multispecificity of CTL elicited.

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References

the CD8 and not the CD4 subset and persist with a variable CD45 phenotype. 


