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Parallel Human Immunodeficiency Virus Type 1-Specific CD8⁺ T-Lymphocyte Responses in Blood and Mucosa during Chronic Infection

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Gut-associated lymphoid tissue is the major reservoir of lymphocytes and human immunodeficiency virus type 1 (HIV-1) replication in vivo, yet little is known about HIV-1-specific CD8⁺ T-lymphocyte (CTL) responses in this compartment. Here we assessed the breadth and magnitude of HIV-1-specific CTL in the peripheral blood and sigmoid colon mucosa of infected subjects not on antiretroviral therapy by enzyme-linked immunospot analysis with 53 peptide pools spanning all viral proteins. Comparisons of blood and mucosal CTL revealed that the magnitude of pool-specific responses is correlated within each individual (mean $r^2 = 0.82 \pm 0.04$) and across all individuals ($r^2 = 0.75$; $P < 0.001$). Overall, 85.1% of screened peptide pools yielded concordant negative or positive results between compartments. CTL targeting was also closely related between blood and mucosa, with Nef being the most highly targeted (mean of 2.4 spot-forming cells [SFC]/10⁶ CD8⁺ T lymphocytes/amino acid [SFC/CD8/aa]), followed by Gag (1.5 SFC/CD8/aa). Finally, comparisons of peptide pool responses seen in both blood and mucosa (concordant positives) versus those seen only in one but not the other (discordant positives) showed that most discordant results were likely an artifact of responses being near the limit of detection. Overall, these results indicate that HIV-1-specific CTL responses in the blood mirror those seen in the mucosal compartment in natural chronic infection. For protective or immunotherapeutic vaccination, it will be important to determine whether immunity is elicited in the mucosa, which is a key site of initial infection and subsequent HIV-1 replication in vivo.

The mucosa is the first line of defense and the primary portal in human immunodeficiency virus type 1 (HIV-1) infection (19). Gastrointestinal mucosa (gut-associated lymphoid tissue [GALT]) is an initial and principal site for HIV-1 replication (32). Data from acutely infected macaques indicate that gastrointestinal mucosa is the earliest and predominant tissue site of simian immunodeficiency virus (SIV) replication regardless of the route of infection (6) and is the initial site of CD4⁺ T-lymphocyte depletion (36). Thus, mucosal tissues are intimately involved in HIV-1 transmission and pathogenesis; therefore, understanding immunity within this compartment is critical for the development of vaccines and therapeutic strategies. However, little is known about HIV-1-specific immune responses in mucosal tissues; the inherent technical difficulty of studying the mucosa has limited most human studies to examining peripheral blood.

Accumulating data indicate that HIV-1-specific CD8⁺ T lymphocytes (CTL) are crucial in controlling viral replication (reviewed in reference 40). While little is known about CTL in the mucosal compartment, some evidence suggests their potential importance. In a murine model, CTL responses in the mucosa are necessary to prevent HIV-1 transmission, whereas

systemic CTL are not sufficient (5). In macaques, SIV-specific CTL in the rectal mucosa appear to be important in protection from rectal challenge with SIV (26). In humans, it is not clear what HIV-1-specific CTL responses exist in the GALT in vivo and the extent to which these responses are similar or dissimilar to those in the peripheral blood.

To address these issues, we simultaneously sampled peripheral blood and colonic mucosa in HIV-1-infected individuals with chronic infection in the absence of antiretroviral treatment. To overcome the technical barrier of small numbers of cells available from colon biopsies, we utilized polyclonal cell expansion methods that were recently developed to study T lymphocytes in GALT (33). Screening and comparison of HIV-1-specific CTL in blood and GALT revealed that these responses are highly correlated between compartments, both within and between individuals. Both the magnitude and targeting of responses are similar between compartments in these untreated, chronically infected subjects.

MATERIALS AND METHODS

Study subjects. To avoid the confounding effects of antiretroviral drug therapy, persons not on therapy were studied. Twelve HIV-1-seropositive individuals that had not been on therapy for at least 12 months and four seronegative controls were included. Written informed consent was obtained from all volunteers through a University of California, Los Angeles, institutional review board-approved study protocol. Peripheral blood and mucosal samples were collected on two consecutive visits 2 weeks apart. For comparisons of unexpanded versus expanded blood CD8⁺ T lymphocytes and expanded blood versus expanded

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TABLE 1. Characteristics of the subjects in this study

ID ^a	Birth date	Status ^b	No. of mos after infection	Viremia (HIV-1 RNA/ μ l)	No. of CD4 ⁺ T-cells/ μ l of blood
A	01/37	SP	>120	1,664	661
B	06/63	SP	>120	331	833
C	01/50	SP	>120	<50	484
D	07/57	SP	>120	49,713	738
E	10/59	SP	118	16,800	423
F	07/57	SP	24	45,399	336
H	05/58	SP	>120	67,380	245
I	06/63	SP	34	63,251	281
J	07/50	SP	>120	338,202	222
K	12/57	SP	>120	69,536	299
L	02/63	SP	>120	56,038	585
M	11/57	SP	82	98,825	41
N	09/62	SN	N/A	N/A	728
O	02/60	SN	N/A	N/A	1,418
P	11/83	SN	N/A	N/A	405
Q	01/68	SN	N/A	N/A	717

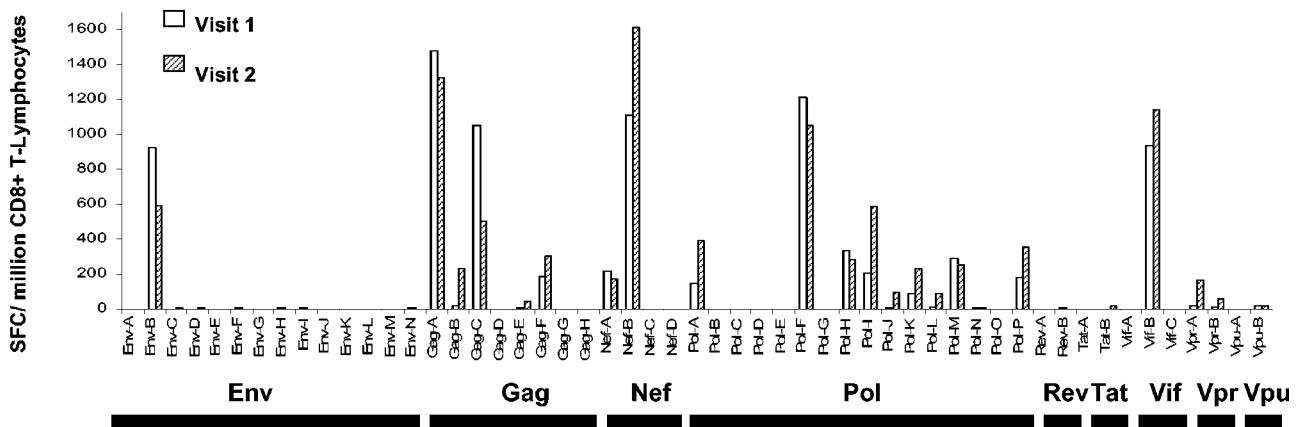
^a Identification letter.
^b SP, seropositive; SN, seronegative.

mucosal CD8⁺ T lymphocytes, only individuals from whom serial samples from both compared arms were available were included.

Isolation of mucosal mononuclear cells. Colonic mucosal mononuclear cells (MMC) were isolated as previously reported (33). Briefly, tissue was obtained by flexible sigmoidoscopy with 20 pinch biopsies at approximately 30 cm from the anal verge as previously described (4). The procedure was well tolerated and painless, without adverse clinical events. The biopsies were washed twice in RPMI 1640 medium containing L-glutamine, 2.5 mg of amphotericin B (Fungizone; GIBCO Invitrogen Corporation, Carlsbad, Calif.)/ml, and 0.1 mg of piperacillin-tazobactam (Zosyn; Lederle Piperacillin, Inc., Carolina, Puerto Rico)/ml (R medium). Samples were incubated for 30 min at 37°C in R medium plus 7.5% fetal calf serum containing 0.5 mg of collagenase Type II (Sigma)/ml. After collagenase digestion, tissue fragments were disrupted by repeated passage through a 10-ml disposable syringe attached to a 16-gauge needle. Cells were separated from undigested tissue by passage through a sterile strainer (Falcon). Cells were washed in R medium to remove collagenase and resuspended in R medium plus 15% fetal calf serum (R-15). Tissue fragments were returned to the tube, and the entire procedure was repeated two additional times, passing the tissue through an 18-gauge needle and a 21-gauge needle after the second and third digestion, respectively. The final cell suspension in R-15 was adjusted to 6 ml, and CD3⁺ T lymphocytes were quantified by flow cytometry. Typically, this procedure yielded between 2 million and 5 million viable CD3⁺ T lymphocytes per 17 biopsy samples.

Isolation of peripheral blood mononuclear cells or purified CD8⁺ T lympho-

A. Fresh CD8+ PBMC (Subject F)



B. Expanded CD8+ PBMC (Subject F)

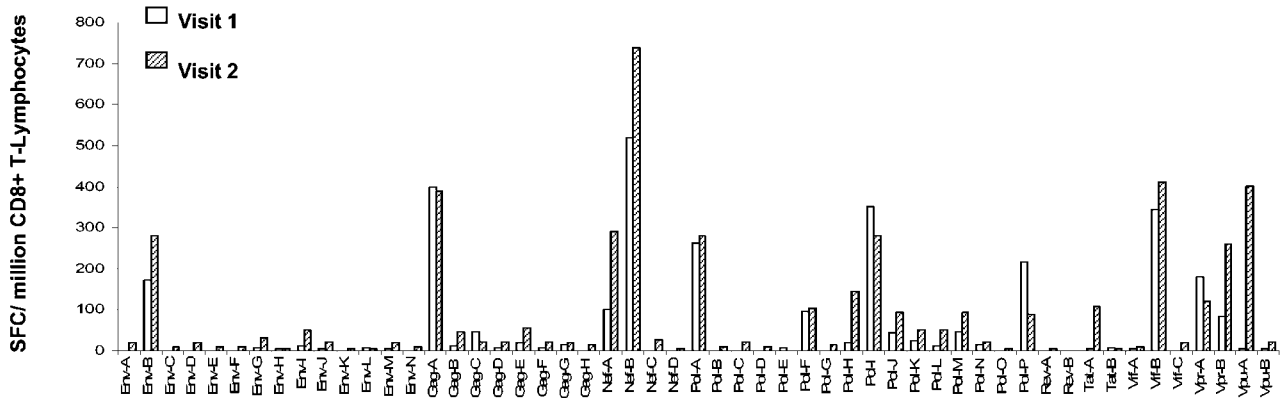


FIG. 1. Reproducibility of HIV-1-specific CTL detection in fresh and expanded CD8⁺ PBMC within individuals. CTL responses measured by IFN- γ ELISpot against pools of HIV-1 peptides are shown for a representative infected individual (subject F) over two independent assays performed with blood samples obtained 2 weeks apart. Data are shown for both fresh CD8⁺ PBMC (A) and expanded CD8⁺ PBMC (B). These results were representative of comparisons of results for each of the nine subjects tested (see Fig. 2).

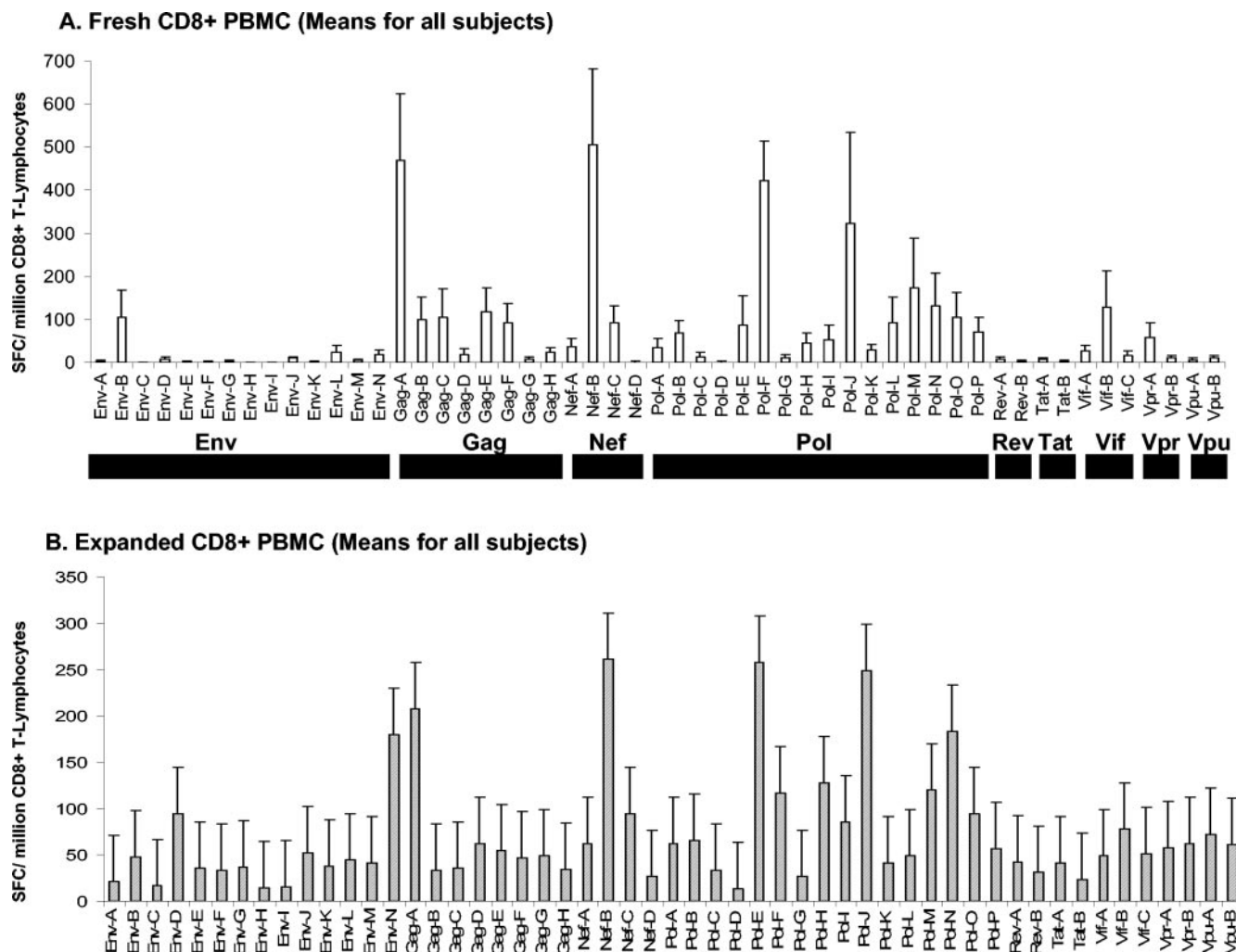


FIG. 2. Similar targeting and magnitude of HIV-1-specific CTL responses in fresh and expanded CD8⁺ PBMC. For the nine subjects from whom data were available over two consecutive visits (subjects A, B, C, D, E, F, I, J, and K), the mean \pm standard error of the mean ELISpot responses (for both visits across all individuals) to each HIV-1 peptide pool for fresh (A) and expanded (B) CD8⁺ PBMC are plotted.

cytes from whole blood. Peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation, using a Ficoll-Hypaque gradient. Cell viability was consistently >90% as assessed by trypan blue exclusion. Fresh CD8⁺ T lymphocytes (fresh CD8⁺ PBMC) from whole blood were obtained by negative selection by immunodepletion on a Ficoll-Hypaque gradient (RosetteSep; StemSep Technologies), yielding between 7 million and 20 million CD8⁺ T lymphocytes of >90% purity from 40 ml of whole blood.

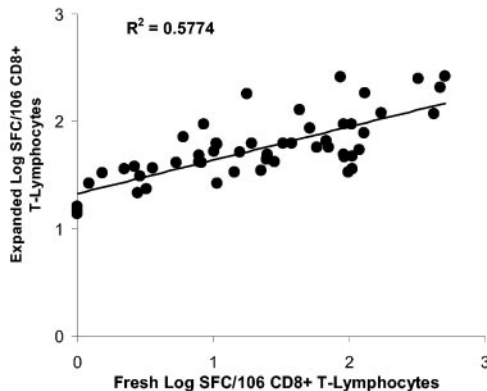
Nonspecific expansion of CD8⁺ T lymphocytes from PBMC and MMC. CD8⁺ T lymphocytes from mucosa and PBMC preparations were subjected to nonspecific polyclonal expansion using CD3:CD4-bispecific monoclonal antibodies as previously described (16, 41). Whole PBMC or CD3⁺ MMC (10⁶) were incubated in R medium containing 10% heat-inactivated fetal calf serum (Omega), 1 μ g of anti-CD3/CD4 antibody/ml (38), and 50 U of interleukin 2 (NIH AIDS Research and Reference Reagent Repository)/ml. MMC cultures also included 10⁶ autologous irradiated PBMC as feeders. Cells were fed with medium twice a week and kept in culture for 14 days. This procedure produced approximately 20 \times 10⁶ cells ("expanded" CD8⁺ PBMC and expanded CD8⁺ MMC). Cell viability was >85% by trypan blue exclusion, and the CD8⁺ purity was >75% by flow cytometry.

IFN- γ ELISpot assay. Gamma Interferon (IFN- γ) enzyme-linked immunospot (ELISpot) assay was performed with fresh CD8⁺ PBMC, expanded CD8⁺ PBMC, and expanded CD8⁺ MMC as previously described (33). Briefly, 96-well nitrocellulose filter plates (Millipore, Burlington, Mass.) were precoated with a monoclonal anti-IFN- γ -antibody (PharMingen, BD Biosciences, San Diego,

Calif.). CD8⁺ T lymphocytes were plated at 2 \times 10⁵ to 3 \times 10⁵ cells/well and exposed to a library of HIV-1 peptides (consecutive 15-mers overlapping by 11 amino acids) spanning all HIV-1 proteins, obtained from the NIH AIDS Research and Reference Reagent Repository (Gag catalog no. 8116, Pol no. 6208, Env no. 9487, Nef no. 5189, Tat no. 5138, Rev no. 6445, Vpr no. 6447, Vpu no. 6444, Vif no. 6446; all clade B consensus sequences with the exception of Env). Peptides were screened in 53 pools of 12 to 16 peptides each and added to the wells at a final concentration of 5 μ g/ml for each individual peptide. Each peptide pool was screened in a single well. Each plate also included three negative control wells with cells but no peptides and three positive control wells containing 0.4 μ g of anti-CD2/CD2R and anti-CD28 monoclonal antibodies (Becton Dickinson, San Jose, Calif.)/ml. The plates were incubated for 16 h, stained with a biotinylated second IFN- γ antibody, labeled with streptavidin-peroxidase, and developed with a peroxidase color reagent. Individual IFN- γ -secreting cells (spot-forming cells [SFC]) were counted using an automated ELISpot counting system (Cellular Technologies Limited, Cleveland, Ohio). Assays with a negative control background mean of less than 100 SFC/10⁶ cells were considered valid. A positive response was defined as being higher than four times the mean of the negative controls or 60 SFC/million cells, whichever was higher.

Statistical analysis. We tested whether the proportions of positive wells arising from fresh CD8⁺ PBMC, expanded CD8⁺ PBMC, and expanded CD8⁺ MMC samples were equal by using a generalized mixed linear model based on the binomial distribution. A separate generalized mixed linear model, based on the Poisson distribution, was used to test whether the frequency of SFC per

A. Fresh versus Expanded CD8⁺ PBMC Recognition of Individual Peptide Pools (means for all subjects)



B. Mean Recognition of Individual Peptide Pools in Fresh and Expanded CD8⁺ PBMC (for all subjects)

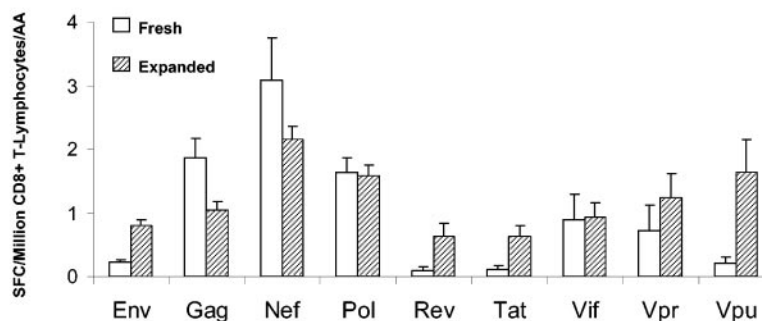


FIG. 3. Concordance of HIV-1 targeting by fresh and expanded CD8⁺ PBMC. (A) For the nine subjects in Fig. 2, the mean ELISpot responses from fresh (x axis) and expanded (y axis) CD8⁺ PBMC against individual HIV-1 peptide pools are plotted against each other. (B) The mean \pm standard error of the mean response against each protein, adjusted by its amino acid length, is plotted for fresh and expanded CD8⁺ PBMC.

million cells for those wells considered positive was the same among the different sample types. These models were fit by using the macro GLIMMIX with the MIXED procedure of SAS (SAS Institute, Research Triangle Park, S.C.).

RESULTS

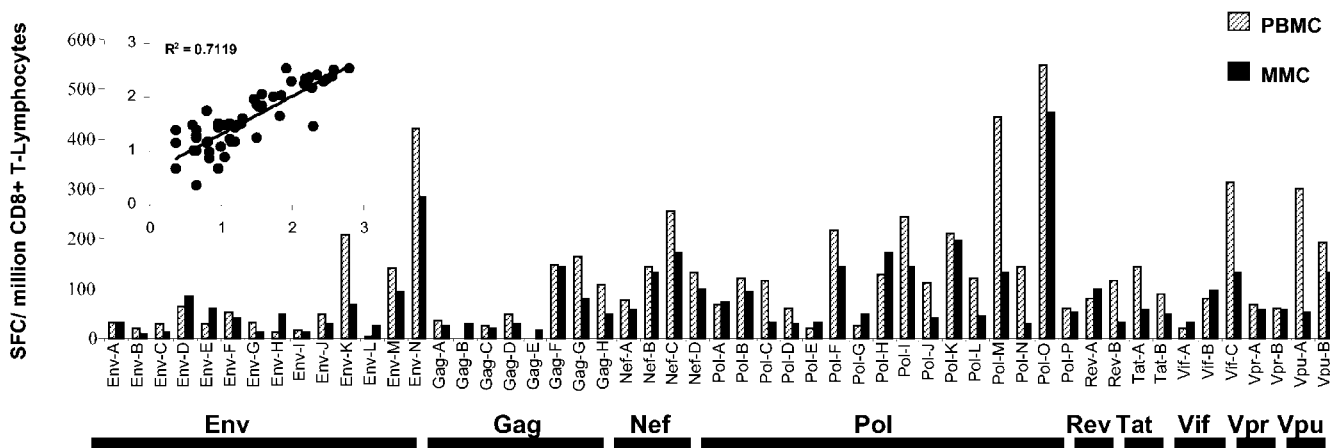
HIV-1-infected and seronegative control subjects. We examined CTL responses from fresh CD8⁺ PMBC, expanded CD8⁺ PBMC, and expanded CD8⁺ MMC in 16 individuals: 12 individuals with chronic HIV-1 infection and 4 seronegative controls (Table 1). Because antiretroviral drug therapy has been shown to affect HIV-1-specific CTL responses (2, 17, 21, 23, 28, 31, 34), the infected subjects were selected for having been untreated for at least 12 months. These infected persons had levels of viremia ranging from <50 to >300,000 genomes/ml of plasma and peripheral blood CD4⁺ T-lymphocyte absolute counts ranging from 41 to 833 and therefore spanned the clinical spectrum of disease progression.

Characterization of peripheral blood HIV-1-specific CD8⁺ T lymphocyte responses in fresh CD8⁺ PBMC. The HIV-1-specific CD8⁺ T lymphocyte (CTL) responses in fresh and expanded CD8⁺ PBMC for the nine subjects from whom data were available over two consecutive visits were assessed by standard IFN- γ ELISpot analysis of freshly isolated CD8⁺ T lymphocytes (fresh CD8⁺ PBMC), using 53 peptide pools, each containing 12 to 16 peptides (consecutive 15-mers overlapping by 11 residues) spanning Gag, Pol, Env, Nef, Tat, Rev, Vpr, Vpu, and Vif. The mean CTL response for these individuals targeted 10 ± 2 pools in 4.2 ± 1.7 proteins (range, 1 to 6)

for an average of 187 ± 9 SFC/10⁶ CD8⁺ T lymphocytes. These measurements were highly consistent within each individual over a 2-week interval (Fig. 1A). Control assays on HIV-1-seronegative controls yielded a false-positive rate of approximately 1.5% of peptide pools (data not shown), indicating specificity of CTL detection in the infected persons. In agreement with prior studies (1, 8), there were no correlations between either the magnitude or breadth of the CTL response to viremia or blood CD4⁺ T lymphocyte count (data not shown). Also in agreement with these studies, examination of the mean response to each peptide pool across all individuals revealed a pattern of recognition with regions in Gag and Nef most frequently targeted (Fig. 2A). These data demonstrated the capability to assess the targeting, breadth, and magnitude of peripheral blood HIV-1-specific CTL in an accurate and reproducible manner in this cohort of infected persons not on drug treatment.

Characterization of peripheral blood HIV-1-specific CD8⁺ T-lymphocyte responses in expanded PBMC. Because measurement of mucosal CD8⁺ T lymphocytes would require cell expansion, we first evaluated the effects of expansion on blood CD8⁺ T lymphocytes (expanded CD8⁺ PBMC), using methods previously reported to polyclonally expand these cells with minimal bias (16). Expanded CD8⁺ PBMC targeted 19 ± 3 peptide pools in 6.4 ± 1.7 proteins (range, 4 to 9) for an average of 123 ± 4 SFC/10⁶ CD8⁺ T lymphocytes. As was observed for fresh CD8⁺ PBMC, expanded CD8⁺ PBMC yielded consistent measurements over consecutive assays 2 weeks apart

A. Expanded CD8+ PBMC and MMC Recognition of Individual Peptide Pools (Subject F)



B. Expanded CD8+ PBMC and MMC Recognition of Individual Peptide Pools (means for all subjects)

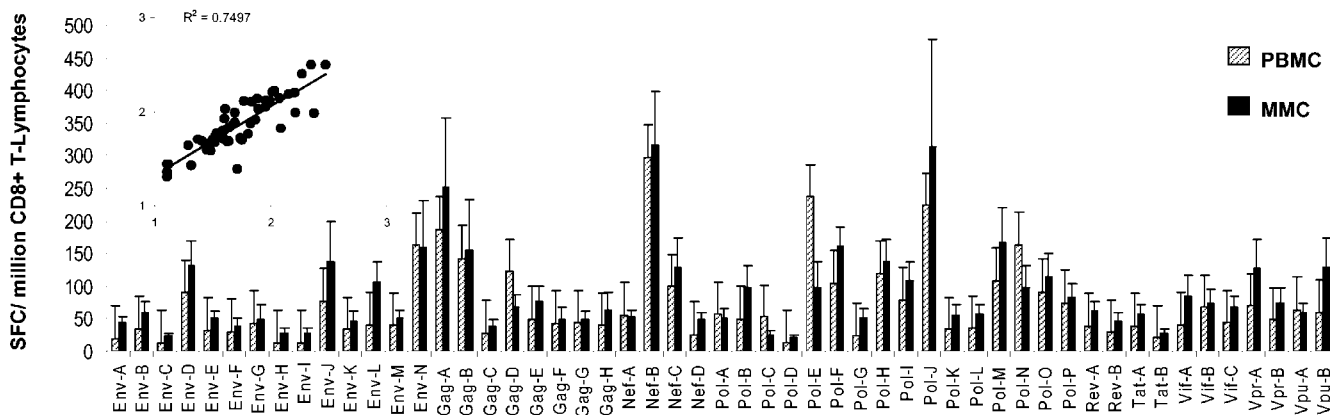


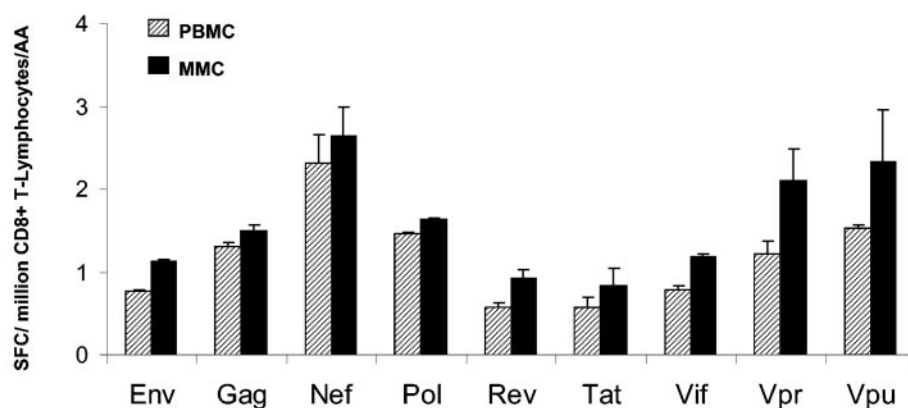
FIG. 4. HIV-1-specific CTL targeting in the blood and mucosal compartments. (A) The CTL responses (means from two assays separated by 2 weeks) against HIV-1 peptide pools from a representative individual (subject F) in expanded CD8⁺ PBMC (hatched bars) and expanded CD8⁺ MMC (solid bars) are plotted. The inset graph depicts these data plotted against each other. (B) The means \pm standard errors of the means of the same data for 10 subjects (subjects A, B, C, E, F, G, H, I, J, and K) are plotted as in panel A. Hatched bars, expanded PBMC; solid bars, expanded MMC.

(Fig. 1B). When the mean recognition of each peptide pool over two visits for all individuals was considered (Fig. 2B), fresh and expanded CD8⁺ PBMC revealed very similar patterns of targeting (Fig. 2A versus 2B). Of the 477 peptide pools compared (9 subjects \times 53 pools spanning the HIV-1 proteome), 75 pools yielded concordant positive responses (positive in both fresh and expanded CD8⁺ PBMC), 288 yielded concordant negative responses, 20 yielded responses in fresh CD8⁺ PBMC only, and 94 yielded responses in expanded CD8⁺ PBMC only, indicating a concordance rate of 76.1% (363 of 477). In agreement with prior observations that expansion increases the sensitivity of detection (3, 16), these results demonstrated detection of more responses in expanded than in fresh CD8⁺ PBMC (18.7 ± 2.6 and 10.4 ± 1.7 recognized peptide pools per individual, respectively; $P = 0.0157$). There was also a trend for lower-magnitude responses in the expanded than in the fresh CD8⁺ PBMC (187 ± 9 SFC/ 10^6 cells versus 123 ± 4 SFC/ 10^6 cells; $P = 0.1$). Overall, however, responses detected with fresh and expanded CD8⁺ PBMC

were highly correlated within individuals (Fig. 3A, $r^2 = 0.5774$). This quantitative correlation was also reflected across the group of infected subjects as a whole (compare Fig. 2A and B and Fig. 3B). In agreement with the above results with fresh CD8⁺ PBMC, neither the magnitude nor the breadth of CTL responses detected using expanded CD8⁺ PBMC correlated to viremia or blood CD4⁺ T-lymphocyte count (data not shown). Overall, these data suggest that CTL detection in expanded CD8⁺ PBMC is a reasonable approximation of responses in fresh CD8⁺ PBMC (Fig. 3) and that the bias induced by expansion is consistent in independent assays (Fig. 1). Because studies of mucosal CTL responses required expansion of MMC, expanded CD8⁺ PBMC were utilized as the baselines for comparisons of the blood and mucosal compartments.

Comparison of HIV-1-specific peripheral blood and colonic mucosal cytotoxic T-lymphocyte responses. To compare HIV-1-specific CTL responses in the peripheral blood and colonic mucosal compartments, expanded blood CD8⁺ PBMC and parallel-expanded CD8⁺ MMC from the 10 subjects from

A. Mean Recognition of HIV-1 Proteins in Expanded CD8⁺ PBMC and MMC (for all subjects)



B. Expanded CD8⁺ PBMC Versus MMC Recognition of HIV-1 Proteins (means for all subjects)

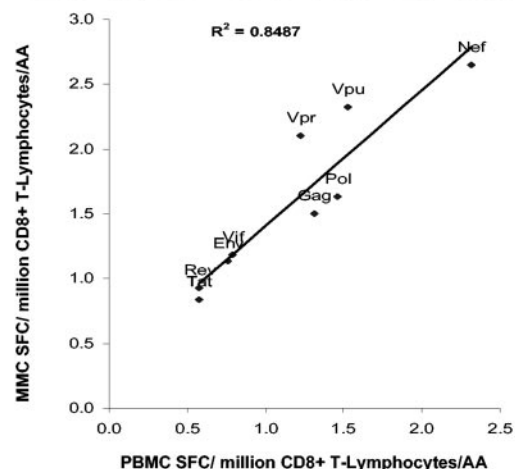


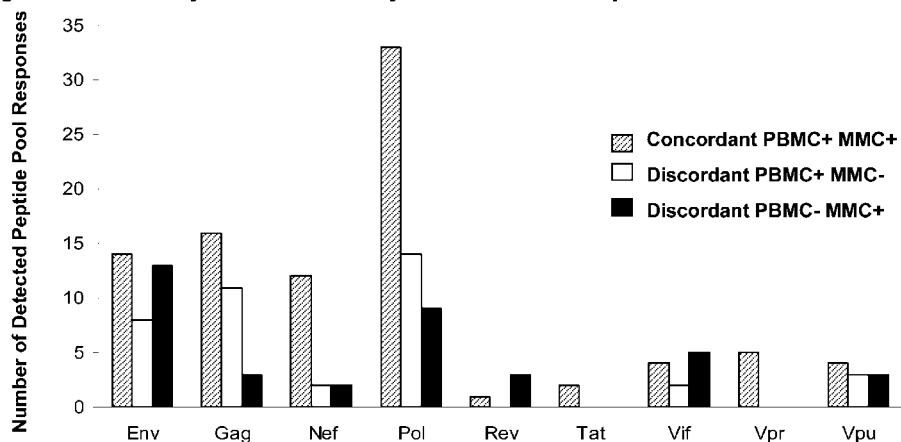
FIG. 5. Concordance of HIV-1 protein CTL recognition in the blood and mucosal compartments. (A) The mean CTL targeting \pm standard error of the mean for each HIV-1 protein (from the 10 individuals from Fig. 4), adjusted by amino acid length of each protein, is plotted for expanded CD8⁺ PBMC and expanded CD8⁺ MMC. (B) The size-adjusted recognition of each protein is plotted for expanded CD8⁺ PBMC (x axis) versus expanded CD8⁺ MMC (y axis). Hatched bars, expanded PBMC; solid bars, expanded MMC.

whom data were available over two consecutive visits were analyzed. Of the 530 pools screened (10 subjects \times 53 pools), there was 85.1% concordance: 92 positives and 359 negatives consistent between compartments, 41 positives in blood only, and 38 positives in mucosa only. The mean number of recognized peptide pools per person and the magnitude of recognition of these pools were similar for blood and mucosa (10.2 ± 2.2 and 10.3 ± 1.8 recognized pools per person with mean magnitudes of 156 ± 3 versus 175 ± 4 SFC/ 10^6 in blood and mucosa, respectively). Individual responses against peptide pools were well correlated between the blood and the mucosa (Fig. 4), indicating similarity of fine targeting between the two compartments. Further characterization of responses in subject F revealed recognition of the same individual peptides by both compartments (data not shown), suggesting that the similarity in targeting of peptide pools is reflective of similarity of targeting at the epitope level. As with the above measurements of CTL in blood, there were no correlations of the magnitude and breadth of CTL responses to viremia and blood CD4⁺ lymphocyte levels (data not shown).

When CTL targeting of whole proteins across all individuals was examined, the frequencies of recognition of each protein were very similar between blood and mucosal CD8⁺ lymphocytes. Responses in both compartments were detected against all viral proteins and were highly correlated (Fig. 5). with the magnitude of responses normalized by the size of the targeted proteins (to reflect the size-adjusted immunogenicity), both blood and mucosal CTL preferentially targeted Nef (means of 2.3 and 2.6 SFC/million cells per amino acid, respectively), followed by Gag (1.3 and 1.5), Vpu (1.5 and 2.3), and Vpr (1.2 and 2.1), similar to previously reported results with PBMC alone (1). When compared, targeting in the blood and mucosal compartments was highly correlated (Fig. 5B). As a whole, these data demonstrated that targeting of HIV-1-specific CTL responses in the peripheral blood and that in mucosal compartments during chronic infection are highly similar.

Characteristics of CTL responses found to be discordant between blood and mucosa. Despite this overall similarity in the blood and mucosa, some detected responses were discordant (79 of 530 of peptide pools; 14.9%). The characteristics of

A. Targeting of Concordantly and Discordantly Detected CTL Responses in PBMC and MMC



B. Magnitudes of Concordantly and Discordantly Detected CTL Responses in PBMC and MMC

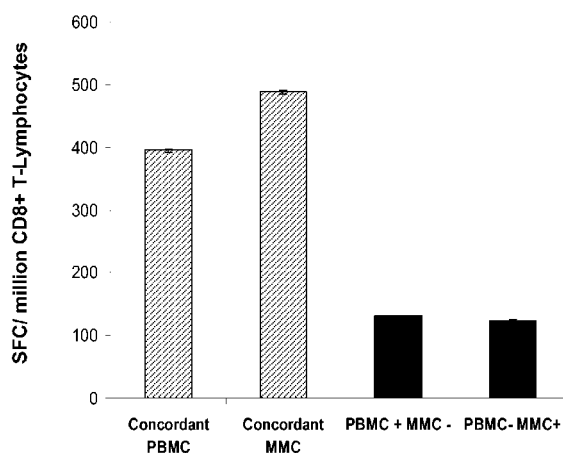


FIG. 6. Characteristics of concordant and discordant HIV-1-specific CTL responses in blood versus mucosa. HIV-1-specific CTL responses in blood and mucosa compartments were assessed as described in the legends to Fig. 4 and 5. Responses to peptide pools scored as positive (at least four times the mean for the negative control and 60 SFC/million cells) were classified as concordant if positive in both compartments and discordant if positive in only one compartment. (A) The mean numbers of recognized peptide pools for each HIV-1 protein are plotted for concordant and discordant responses between PBMC and MMC. (B) The mean magnitudes \pm standard errors of the means are plotted for all concordant and discordant peptide pool responses between PBMC and MMC. Discordant PBMC+ MMC- represents the pools that are positive in PBMC but negative in MMC. Discordant PBMC- MMC+ represents pools negative in PBMC and positive in MMC.

the responses that were concordant and discordant between mucosa and blood were compared (Fig. 6). Of the 91 concordant positive peptide pool responses, most were in Pol (33 of 91), Gag (16 of 91), Env (14 of 91), and Nef (12 of 91). Of the 40 responses detected in blood but not mucosa, most were in Pol (14 of 40), Gag (11 of 40), and Env (8 of 40), suggesting a similar distribution of the shared responses. Of the 38 responses detected in mucosa but not blood, most were in Pol (9 of 38) and Env (13 of 38). Thus, the targeting of discordant responses was similar to that of concordant responses. The magnitudes of concordant responses in blood and mucosa were similar (Fig. 6B), with values of 396 and 488 SFC/ 10^6 , respectively. However, the magnitudes of the discordant responses found only in the blood or mucosa were lower, with values of 130 and 124 SFC/ 10^6 , respectively (Fig. 6B). The findings that discordant measurements were primarily those of lower magnitude suggested that the discordance was either a result of measurement artifact from values near the negative cutoff or

biologic variability in the frequency of CTL before or after expansion. The generally high correlation of responses between these compartments (Fig. 3, 4, and 5) favored the former possibility.

DISCUSSION

The mucosa is the first barrier against many pathogens, including HIV-1. Furthermore, GALT is the largest lymphoid organ in the body (25) and is a major reservoir of HIV-1 replication during chronic infection (32), due to constitutive activation of gut lymphocytes (4). Results from studies of infected humans and nonhuman primates indicate an important role for mucosal immune responses, particularly mucosal CTL, in the prevention of HIV-1 and SIV infection (12, 13, 22, 37). However, the technical barrier of obtaining sufficient mucosal lymphocytes has limited the study of these responses in HIV-1-infected persons. Our previous work has shown that 20 sig-

moid colon biopsies yield between 1 million and 3 million total CD3⁺ T lymphocytes (33), a quantity insufficient to perform comprehensive mapping of HIV-1-specific CD8⁺ T-lymphocyte responses even with cell-sparing assays such as ELISpot.

Here we apply recently developed methods (33) to expand mucosal CD8⁺ T lymphocytes to evaluate the HIV-1-specific CTL responses of several chronically infected individuals. In agreement with a prior study (16), we found that there is a good correlation between CTL responses detected in fresh CD8⁺ PBMC and expanded CD8⁺ PBMC with this expansion protocol, suggesting that bias in PBMC expansion is minor. We therefore applied this method in parallel to MMC to allow comparisons of the blood and mucosal compartments. While we cannot entirely exclude that this nonspecific expansion induced bias in the MMC, the consistency of detected CTL responses between independent visits in both expanded PBMC and expanded MMC provides evidence that any bias is consistent. In addition, targeting of HIV-1 by CTL in our subjects is similar to that previously described in other studies of blood (1, 8, 9) for both blood and mucosa. A further caveat is the use of clade B consensus sequence peptides for CTL detection and not autologous sequences. We cannot exclude that these peptides preferentially detect shared responses in both compartments while other CTL specific for autologous sequences differ between compartments; however, such compartment-specific bias would seem unlikely. Thus, overall, the high degree of qualitative and quantitative similarity between the blood and mucosa suggest that HIV-1-specific lymphocytes may traffic freely between these compartments.

Despite the observed similarity, it remains to be determined whether the interactions between HIV-1 and CTL reflected in the peripheral blood are identical to those in the gut. Studies addressing the effects of CTL on HIV-1 sequence have documented viral escape in the blood (10, 11, 14, 15, 18, 20, 24, 29, 30), but the extent to which this interaction reflects that in the gut, the major reservoir of viral replication (32), remains undefined. It is known that replication in GALT can persist even when virus is undetectable in blood during chronic infection (35), indicating that the compartments are not always equivalent. While this probably reflects that the virus in blood is spilled over from inadequate containment in tissues, it remains to be determined whether immune pressure on HIV-1 in GALT and other tissues is reflected in blood.

Further supporting the concept that peripheral blood HIV-1 reflects spillover from tissues, studies of acute SIV infection in macaques have shown that there is a period of early local mucosal replication preceding systemic dissemination (6, 12), which is not observable in blood until days after initial exposure (42). There is also strong evidence for asymmetrical trafficking of CTL from the mucosa to the peripheral blood with little traffic from the blood to the mucosa (reviewed in reference 6). These observations suggest that efficient generation of HIV-1-specific CTL responses in both blood and mucosa may require antigenic stimulation within the GALT.

This principle may have pivotal implications in HIV-1 vaccine design. The SIV macaque model suggests that local mucosal CTL responses are important for protective immunity, and their absence correlates with uncontrolled infection after mucosal challenge (12, 26, 37). Peripheral immunization induces virus-specific CTL in blood (6, 7) but does not protect

against mucosal transmission (5). In humans, intramuscular delivery of canarypox-based vectors induces HIV-1-specific immunoglobulin G in serum, while mucosal delivery is inefficient for inducing this serum response (27). However, intrarectal but not specific intramuscular vaccination is required to provoke HIV-1-specific immunoglobulin A in the gut (39). These data indicate the complexity of immunity elicited by vaccines and underscore the importance of understanding the role of various immune responses in protection from infection and/or disease by HIV-1.

In summary, this study provides a comprehensive analysis of the HIV-1-specific CTL responses in GALT, finding that they are mirrored by responses in the peripheral blood during chronic infection. The pivotal role of this compartment as a portal of entry in acute infection and reservoir for replication in chronic infection underscores the importance of understanding the relationship of immune responses in peripheral blood to those in gut mucosa. Elucidating such mechanisms may be important to efforts to optimize vaccine efficacy by eliciting HIV-1-specific immunity in the GALT.

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