JOURNAL OF VIROLOGY, Sept. 2007, p. 9061–9071 Vol. 81, No. 17
0022-538X/07/$08.00+0 doi:10.1128/JVI.00117-07

Role of Human Immunodeficiency Virus (HIV)-Specific T-Cell Immunity in Control of Dual HIV-1 and HIV-2 Infection▽

Natalie N. Zheng,[1] M. Juliana McElrath,[3,4,5] Papa-Salif Sow,[7] Stephen E. Hawes,[2] Habibatou Diallo-Agne,[7] Joshua E. Stern,[1] Fusheng Li,[6] Andrew L. Mesher,[1] Akeliah D. Robinson,[5] Geoffrey S. Gottlieb,[3] Yunda Huang,[6] and Nancy B. Kiviat[1]*

Departments of Pathology,[1] Epidemiology,[2] Medicine,[3] and Laboratory Medicine,[4] University of Washington, Seattle, Washington 98195; Program in Infectious Diseases, Clinical Research Division,[5] and Statistical Center for HIV/AIDS Research and Prevention,[6] Fred Hutchinson Cancer Research Center, Seattle, Washington 98109; and University of Dakar, Dakar, Senegal[7]

Received 17 January 2007/Accepted 25 May 2007

Progressive immune dysfunction and AIDS develop in most cases of human immunodeficiency virus type 1 (HIV-1) infection but in only 25 to 30% of persons with HIV-2 infection. However, the natural history and immunologic responses of individuals with dual HIV-1 and HIV-2 infection are largely undefined. Based on our previous findings, we hypothesized that among patients with dual infection the control of HIV-1 is associated with the ability to respond to HIV-2 Gag epitopes and to maintain HIV-specific $CD4^+$ T-cell responses. To test this, we compared the HIV-specific ex vivo IFN-γ enzyme-linked immunospot (ELISPOT) assay responses of 19 dually infected individuals to those of persons infected with HIV-1 or HIV-2 only. Further, we assessed the functional profile of HIV Gag-specific $CD4^+$ and $CD8^+$ T cells from nine HIV dually infected patients by using a multicolor intracellular cytokine staining assay. As determined by ELISPOT assay, the magnitude and frequency of IFN-γ-secreting T-cell responses to gene products of HIV-1 were higher than those to gene products of HIV-2 (2.64 versus 1.53 $\log_{10}$ IFN-γ spot-forming cells/10^6 cells [90% versus 63%, respectively].) Further, HIV-1 Env-, Gag-, and Nef- and HIV-2 Gag-specific responses were common; HIV-2 Nef-specific responses were rare. HIV-specific $CD4^+$ T helper responses were detected in nine of nine dually infected subjects, with the majority of these T cells producing gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α) and, to a lesser extent, interleukin-2. The HIV-1 plasma viral load was inversely correlated with HIV-2 Gag-specific IFN-γ-/TNF-α-secreting $CD4^+$ and HIV-2 Gag-specific IFN-γ-secreting $CD8^+$ T cells. In conclusion, the T-cell memory responses associated with containment of single HIV-1 and HIV-2 infection play a similar significant role in the immune control of dual HIV-1 and HIV-2 infection.

In West Africa both human immunodeficiency virus type 1 (HIV-1) and HIV-2 cocirculate, and a small percentage of individuals have both infections (referred to here as an "HIV-1/2" infection) (8, 11, 12, 23). The natural history of dual HIV infection has been difficult to study and is incompletely understood. Several investigations in the Ivory Coast and Gambia examined dually infected persons presenting to HIV or sexually transmitted disease (STD) clinics and reported that the HIV-1 viral loads and annual mortality rates for these patients were characteristic of individuals infected with HIV-1 alone. Further, T-cell activation was increased in dually infected persons compared to those with single HIV-1 infection, suggesting that the natural history of HIV disease in dually infected patients is similar to, or even more aggressive than, that of HIV-1 infection alone (1, 16, 18, 26). However, contrasting results were reported in the only published study to examine dually infected subjects not presenting to such clinics (2). This latter study in Guinea-Bissau examined dually infected patients initially recruited into tuberculosis and occupational cohort studies (without knowledge of HIV serostatus). These patients had lower HIV-1 plasma RNA levels than those with HIV-1 only who were identified in a similar manner within these cohorts. More than 30% of these dually infected untreated persons had HIV-1 plasma RNA levels below 10,000 copies/ml. Possibly, the contrasting findings in these studies were the result of the selection basis encountered in clinic-based studies.

Over the last 10 years we have enrolled hundreds of HIV-1-infected and more than 150 dually infected patients into various studies in Senegal, West Africa. Comparison of HIV-1 viral loads among those with dual infection versus those infected with HIV-1 alone is complicated since recruitment criteria for these two groups of patients frequently differed. However, among those recruited regardless of CD4 T-cell counts, the HIV-1 plasma RNA loads of those with dual infection tended to be lower than those with HIV-1 alone (mean plasma RNA of 3.61 versus 4.23 $\log_{10}$ copies/ml, respectively; $P = 0.08$) (8a). However, interpretation of such data is difficult, given that the date of initial infection was unknown, and most patients had little follow-up.

In our previous studies we examined the homologous and heterologous (i.e., "cross-reactive") T-cell responses of subjects with single HIV-1 or HIV-2 infection. We found that subjects with HIV-1 infection had high frequencies of cross-reactive responses to HIV-2 (and vice versa for those with

* Corresponding author. Mailing address: Department of Pathology, Harborview Medical Center, Box 359791, 325 9th Ave., Seattle, WA 98104. Phone: (206) 731-4269. Fax: (206) 731-8240. E-mail: nbk@u.washington.edu.

▽ Published ahead of print on 20 June 2007.

HIV-2 infection) (33). HIV-1-infected subjects with the ability to respond to HIV-2 Gag, as well as HIV-1 protein products had lower HIV-1 plasma viral loads than those without "cross-reactive" responses to HIV-2 Gag (33). At least in theory, the results suggested that among those infected with HIV-1 the additional presence of HIV-2 might result in increased development of HIV-2 Gag responses and perhaps provide for increased control of HIV-1 replication. Further, previous studies, including our own, have demonstrated that in contrast to most subjects with HIV-1 alone, most persons infected with HIV-2 alone maintain both $CD4^+$ T-cell responses to HIV-2 protein products (10, 21, 22, 29, 33). In addition, recent studies have suggested that the production of multiple cytokines is important in the maintenance of functional $CD4^+$ and $CD8^+$ T-cell responses in long-term nonprogressors (4). We hypothesized that if dually infected subjects did develop increased HIV-2 Gag responses, such patients might also better maintain functional $CD4^+$ T cells responses to HIV-1 and HIV-2 protein products. To determine whether HIV-1 viral load and $CD4^+$ T-cell counts are associated with the ability to respond to HIV-2 Gag protein products, as previously described among singly infected persons, and to determine whether dually infected persons are more likely to have such responses than are those with single HIV-1 infection, we characterized the HIV-1- and HIV-2-specific cellular immune responses among dually infected persons, comparing their responses to those seen with single infection.

MATERIALS AND METHODS

Study population. As previously described (33), beginning in October 2000 all patients 16 years of age and older who presented to the University of Dakar Infectious Disease Clinic (Fann Hospital, Dakar, Senegal, West Africa) were offered screening for HIV infection. Commercial sex workers, who are required to present to the STD clinic in Dakar (Institut d'Hygiene Sociale) each month for routine STD and HIV screening, were also tested. HIV testing was performed by HIV-1/HIV-2 enzyme immunoassay (Genescreen, version 2, HIV-1/HIV-2; Bio-Rad) after written informed consent was obtained, and HIV seropositivity was confirmed by synthetic peptide-based membrane immunoassay (Immunocomb II, bispot, phosphate-buffered saline). All patients who tested positive by both assays were counseled in compliance with the Senegalese AIDS National Committee and the University of Washington Human Subjects Institutional Review Board. All untreated HIV-2 and dually infected patients, as well as all untreated HIV-1-infected patients with $CD4^+$ T-cell counts of $\geq$350 cells/μl at their screening visit, were invited to enroll in an immunological study. All consenting participants underwent a physical examination, a standardized interview, venipuncture for $CD4^+$ T-cell counts, PCR confirmation of their HIV status, and immunologic assays.

By October 2004, 291 HIV-infected patients were enrolled. The T-cell responses were measured by enzyme-linked immunospot (ELISPOT) assay in 112 HIV-1-infected, 78 HIV-2-infected, and 19 HIV-1/HIV-2 dually infected treatment-naive patients. The overall HIV-1- and HIV-2-specific responses in a subset of these patients, including 68 HIV-1 and 55 HIV-2 singly infected patients, were previously reported (33). A subset of study subjects (78 HIV-1-infected, 53 HIV-2-infected, and 12 HIV-1/HIV-2 dually infected patients) was typed for their human leukocyte antigens (HLA). HLA-A23, -A33, -B49, -B78, -C02, -C04, -C07, and -C08 were common in the population. A33 and C08 alleles were somewhat more frequently detected in dually infected individuals than in HIV-1 or HIV-2 singly infected patients. However, these differences were not significant after adjustment for multiple comparisons. No significant differences were found in the frequencies in some known important HLA types such as B27, B35, B57, B58, and the homozygosis of HLA-A, -B, and -C in these groups (data not shown).

Virological and T-cell subset analyses. Plasma HIV-1 and HIV-2 RNA was determined by using the Amplicor Monitor HIV-1 test and the quantitative HIV-2 assay, respectively, as previously described (33). For the Amplicor Monitor HIV-1 Test, the lower level of sensitivity was 50 copies/ml, with reliable quantitation at levels greater than 400 copies/ml. The quantitative HIV-2 assay had a limit of detection of 40 HIV-2 RNA copies/ml, with reliable quantitation at levels greater than 200 HIV-2 RNA copies/ml. A quantitative in-house real-time reverse transcription-PCR assay was also used for testing plasma HIV-1 and HIV-2 RNA levels for nine individuals from whom we had adequate samples for intracellular cytokine staining (ICS) assays. Briefly, 1 ml of plasma from HIV-1/2-infected patients or diluted standards (purified HIV-1 and HIV-2 from Advanced Biotechnologies, Inc.) was centrifuged at 22,065 $\times$ *g* for 1 h at 4°C. Immediately, 860 μl of supernatant was removed carefully, and viral RNA was extracted by using a QIAGEN QIAmp viral RNA minikit (QIAGEN catalog no. 52904). The HIV RNA was then quantified via a real-time one-step RT-PCR assay using sequence-specific probes and an ABI 7900HT (for HIV-1, forward primer [GGGGACATCAAGCAGCCAT], forward primer2 [GGGGACACCA GGCAGCAAT], reverse primer [TACTAGTAGTTCCTGCTATGTCACTTC C], and probe [reverse complement; 6FAM-TCTGCAGCTTCCTCATTGAT-nfqMGB]; for HIV-2, forward primer [GCGGAGAGGCTGGCAGAT], reverse primer [GAACACCCAGGCTCTACCTGCTA], and probe [6FAM-AGAGAA CCTCCCAGG-nfqMGB]). The viral load was quantified according to the standard curve; the limit of detection was 50 copies/ml for both HIV-1 and HIV-2. Samples with a value below the detection limit were assigned a value of 50 copies/ml. Peripheral blood $CD4^+$ and $CD8^+$ T-cell counts were measured by a consensus flow cytometry method (9) and are expressed as cells/μl.

Synthetic peptides. To express HIV epitopes for use in ELISPOT and ICS assays, peptides of 15 amino acids (aa) (15-mers) were synthesized (Chiron Corp., Emeryville, CA) that overlapped by 10 aa spanning the entire HIV-1CRF-02 (HIV-1 A/G recombinant virus, accession no. AJ251056) Gag (n = 97), Env (n = 171), Nef (n = 40), and Tat (n = 19) proteins and HIV-2 ROD (accession no. M15390) Gag (n = 103), Env (n = 170), Tat (n = 24), and Nef (1 to 180 aa, n = 34) proteins. The HIV-2 Nef (181 to 256 aa, n = 15) peptides were synthesized by the Shared Resources Center at Fred Hutchinson Cancer Research Center (Seattle, WA) (as described by Zheng et al. [33]). The peptides were reconstituted in 100% dimethyl sulfoxide (Sigma Chemical Co., St. Louis, MO) and used at a final concentration of 2 μg/ml. Peptides corresponding to each protein were used in small pools—Gag (five pools), Env (nine pools), Nef (four pools), and Tat (two pools)—for both HIV-1 and HIV-2 in the ELISPOT assay. For the $CD4^+$ and $CD8^+$ T-cell subset responses and functional cytokine profile ICS assay, one large pool per gene product was used.

IFN-γ ELISPOT assay. To evaluate T-cell responses recognizing HIV-1 and -2 epitopes, peripheral blood mononuclear cells (PBMC) were isolated from anticoagulated blood and evaluated fresh in gamma interferon (IFN-γ) ELISPOT assays (33). Spots formed by IFN-γ-secreting cells were counted by using an automated ImmunoSpot plate reader (Cellular Technologies, Cleveland, OH), and the results are presented as spot-forming cells (SFC) per 10^6 PBMC. A response was considered positive when (i) the mean SFC for the experimental wells was at least twofold greater than the mean SFC for the negative control wells and (ii) the mean SFC/10^6 PBMC in the experimental wells was greater than 100 after subtraction of the mean SFC/10^6 of the control wells. To determine the magnitude of the number of IFN-γ SFC, responses with $<$100 SFC/10^6 cells were considered negative and scored as "0," whereas for positive responses ($>$100 SFC/10^6 cells), SFC were summed across all pools within a gene product and scored as the total number of SFC minus 100. To determine the breadth of responses to HIV-1 and HIV-2 gene products, the IFN-γ SFC frequencies recognizing each individual gene product were combined and expressed as a percentage of total responses. The number of HIV genes recognized was measured as the breadth of responses.

ICS. In the Senegal laboratory, one million PBMC (isolated from anticoagulated blood)/well (in 96-well round bottom plates) were stimulated with HIV peptide pools, a cytomegalovirus (CMV) peptide pool or staphylococcal enterotoxin B (for a positive control) or 2% dimethyl sulfoxide (for unstimulated negative controls) for 6 h at 37°C in the presence of 1 μg of anti-CD28 and CD49d monoclonal antibodies/ml and 10 μg of brefeldin A/ml. EDTA and ethidium monoazide were then added, and the cells were washed and fixed in fluorescence-activated cell sorting solution. The 96-well plates were stored in a −70°C freezer and dry shipped to the Seattle Fred Hutchinson Cancer Research Center laboratory.

In Seattle, intracellular staining was performed by using standard techniques (BD Biosciences, cytokine flow cytometry protocol) with previously titrated antibody reagents. Anti-CD3-PerCpCy5.5, anti-CD4-fluorescein isothiocyanate, anti-CD8-APCCy7, anti-CD14-PerCp, anti-CD19-PECy5, anti-IFN-γ-PECy7, anti-interleukin-2 (IL-2)-phycoerythrin, and anti-tumor necrosis factor alpha (TNF-α)-allopycocyanin monoclonal antibodies were supplied by BD Biosciences and BD Pharmingen. The data acquisition was performed on an LSRII flow cytometer (BD Biosciences), collecting 100,000 to 200,000 lymphocyte

TABLE 1. Demographic, clinical, and virological profiles of participants at enrollment

Parameter	Value (% or range)		
	HIV-1/2	HIV-1[a,b]	HIV-2[a]
No. of subjects	19	112	78
No. female	18 (95)	93 (85)	62 (79)
Mean age in yr	41 (26–57)	34 (16–55)	41 (26–68)
Mean $CD4^+$ T-cell count (IQR cells/μl)	329 (133–461)	461 (332–538)	543 (240–827)
Mean $CD8^+$ T-cell count (IQR cells/μl)	1,088 (649–1532)	965 (601–1235)	717 (450–840)
HIV-1 plasma RNA (log_{10} copies/ml)			
Mean RNA	4.29	3.84	NA[c]
<1,000	5 (26)	21 (19)	NA
1000–10,000	2 (11)	33 (29)	NA
10,000–100,000	2 (11)	37 (33)	NA
>100,000	10 (53)	21 (19)	NA
HIV-2 plasma RNA (log_{10} copies/ml)			
Mean RNA	2.23	NA	2.35
<100	10/14 (71)	NA	34/56 (61)
100–1,000	1/14 (7)	NA	9/56 (16)
>1,000	3/14 (21)	NA	13/56 (23)
WHO clinical stage (no. at stage/total no.)			
1	6/19 (32)	60/109 (55)	41/77 (53)
2	3/19 (16)	22/109 (20)	12/77 (16)
3	9/19 (47)	25/109 (23)	21/77 (27)
4	1/19 (5)	2/109 (2)	3/77 (4)
Study populations			
Commercial sex worker STD clinic			
No. of subjects	12	33	26
Mean $CD4^+$ T-cell count (cells/μl)	453	453	813
Mean HIV-1 plasma RNA (log_{10} copies/ml)	3.35	3.56	NA
Mean HIV-2 plasma RNA (log_{10} copies/ml)	2.52	NA	2.11
WHO clinical stage (no. at stage/total no.)			
1	6/12 (50)	25/32 (78)	20/26 (77)
2	2/12 (11)	5/32 (16)	2/26 (8)
3	4/12 (33)	2/32 (6)	4/26 (15)
4	0/12 (0)	0/32 (0)	0/26 (0)
Infectious disease clinic patients			
No. of subjects	7	79	52
Mean $CD4^+$ T-cell count (cells/μl)	116	465	408
Mean HIV-1 plasma RNA (log_{10} copies/ml)	5.57	3.96	NA
Mean HIV-2 plasma RNA (log_{10} copies/ml)	1.70	NA	2.49
WHO clinical stage (no. at stage/total no.)			
1	0/7 (0)	35/77 (45)	21/51 (41)
2	1/7 (14)	17/77 (22)	10/51 (20)
3	5/7 (71)	23/77 (30)	17/51 (33)
4	1/7 (14)	2/77 (3)	3/51 (6)

[a] In a subset of 68 HIV-1 and 55 HIV-2 singly infected patients that were described previously (33).
[b] We only enrolled HIV-1-infected patients with $CD4^+$ T-cell counts of more than 350 cells/μl.
[c] NA, not applicable.

gated. All analyses were performed by using Flowjo software from Treestar (San Carlos, CA). Responses were determined to be positive when the percentage of bright cytokine$^+$ $CD4^+$ or $CD8^+$ T cells was twice that of the negative control and >0.02% of the total $CD4^+$ or $CD8^+$ T cells. Antigen-specific responses lower than 0.01% are shown as 0.01% on the figures. All data shown are background subtracted. SPICE (for simplified presentation of incredibly complex evaluations program, version 2.8.2, created by Mario Roederer, National Institutes of Health) was used to generate the pie charts for the HIV-1 and HIV-2 Gag- and CMV-specific cytokine functional profiles.

Statistical methods. Values of HIV-1 and HIV-2 plasma RNA viral loads, as well as counts of IFN-γ SFC, were log_{10} transformed in order to normalize the distribution of these measurements for all statistical analyses. Pearson χ^2 or the Fisher exact tests were performed to assess univariate associations between the two HIV types and categorical factors of interest, whereas *t* tests were performed to compare groups with respect to continuous factors of interest. However, continuous factors of interest that were not normally distributed were analyzed by Wilcoxon rank sum tests. McNemar's test and paired *t* tests were performed to compare paired categorical and continuous factors of interest. Multivariable logistic and linear regression analyses were performed to evaluate the independent magnitude of associations between HIV-specific IFN-γ responses (from ELISPOT assay) and HIV status and HIV plasma viral loads, after adjusting for the possible confounding effects of $CD4^+$ T-cell counts, plasma viral load, study site, and gender. A two-sided 0.05 level test determined the statistical significance for analyses concerning ELISPOT assays.

To determine the positivity of antigen-specific responses from the ICS assay, Freedom-Holton exact tests were first performed to assess the overall association between stimulation type (antigen-stimulated or negative control) and distribution of counts of $CD4^+$ or $CD8^+$ cells eliciting the intracellular cytokines IFN-γ, IL-2, and TNF-α. The Fisher exact tests were then used to conduct pairwise comparisons for each cytokine phenotype between antigen-stimulated and negative control wells. Resampling-based multiplicity adjustment using the discrete Bonferroni method (32) was used to adjust for the multiple comparisons. A two-sided nominal significance level of 0.003 was used in these positivity analyses. A lab-specified filter was also applied at the end to ensure a positive response with background-corrected responses of ≥0.02% for both $CD4^+$ and $CD8^+$ T-cell responses. The data analyses were conducted by using SAS 9.1 for Windows (SAS Institute, Cary, NC) and GraphPad Prism (Macintosh version 4.0).

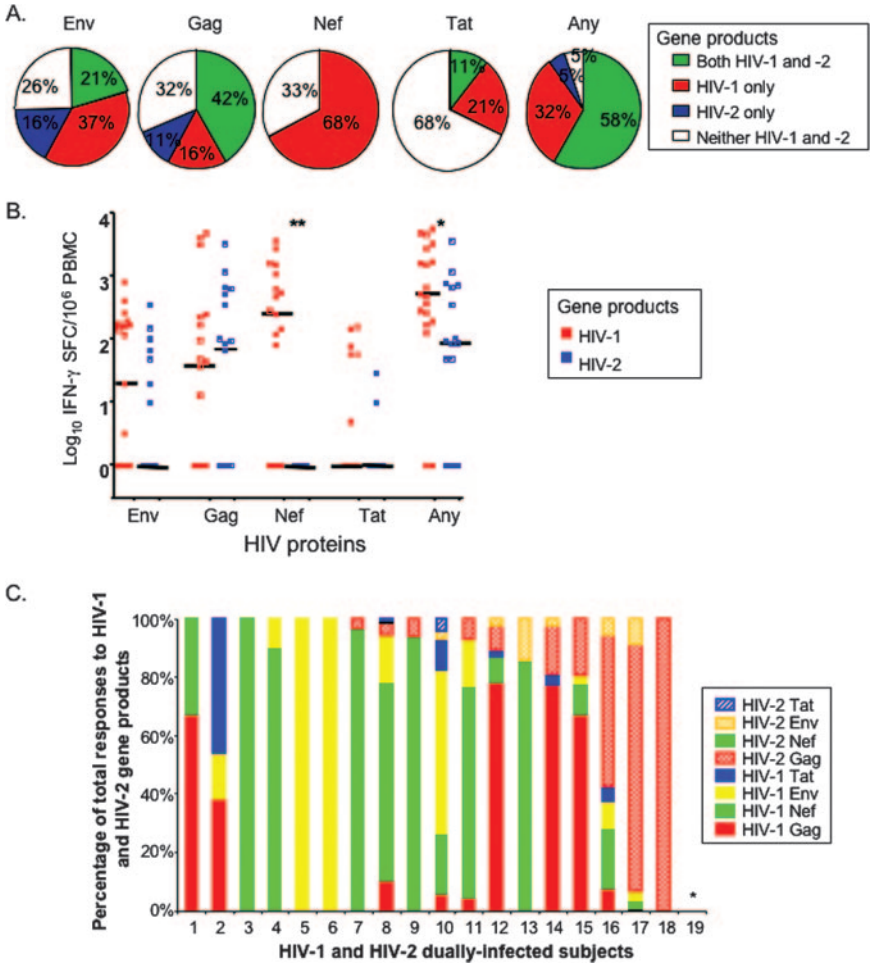


FIG. 1. Frequency, magnitude, and breadth of IFN-γ secreting T-cell recognizing HIV peptides within Gag, Env, Nef, and Tat among subjects with dual HIV-1 and HIV-2 infection ($n = 19$). (A) Frequency of IFN-γ T-cell responses recognizing Gag, Env, Nef, Tat, and any gene product of HIV-1 only, HIV-2 only, both HIV-1 and HIV-2, or neither. (B) Magnitude of IFN-γ T-cell responses to HIV-1 and HIV-2 peptides recognizing epitopes within Gag, Env, Nef, and Tat. The magnitude of total (any) HIV-1 responses was significantly greater than the median magnitude of total HIV-2 responses (2.64 $\log_{10}$ compared to 1.53 $\log_{10}$ SFC/10^6, respectively [*, $P = 0.002$]). This difference was mostly a result of significantly increased levels of Nef responses in HIV-1 compared to HIV-2 (1.88 $\log_{10}$ versus 0 $\log_{10}$ SFC/10^6, respectively [**, $P < 0.0001$]). —, Median of responses. (C) Breadth of responses to HIV-1 and HIV-2 gene products. *, Patient 19 had no detectable HIV-1- or HIV-2-specific responses.

RESULTS

Study population. Of the 209 study participants enrolled with T-cell responses measured by ELISPOT assay (Table 1), 19 had HIV-1/2, 112 had HIV-1, and 78 had HIV-2 infections. The majority of dually infected subjects (63%) were commercial sex workers who were identified through their participation in a Senegalese national program that registers female commercial sex workers, educates them about and monitors them for various STDs (including HIV), and provides condoms. The mean age of dually infected patients was similar to that of HIV-2-infected subjects but was greater (41 versus 34 years, respectively [$P = 0.004$]) than those with HIV-1 infection alone.

All stages of HIV disease, based on the World Health Organization criteria (3), were represented among those with HIV-1/2. Commercial sex workers were significantly healthier than the patients who originally presented to an infectious disease clinic, with higher $CD4^+$ T-cell counts (453 versus 116 cells/μl), lower HIV-1 plasma viral load (3.35 versus 5.57 $\log_{10}$ copies/ml [$P = 0.0045$]), and lower stage of HIV disease (33% versus 85% with WHO stage 3 or worse disease) (Table 1). In all patients infected with HIV-2, HIV-2 plasma viral loads were generally very low, with 78% of dually infected persons and 77% of those with HIV-2 alone having HIV-2 loads below 1,000 copies/ml. As noted above, since all dually infected patients but only HIV-1-only infected ones with CD4 counts above 350 were enrolled, we could not compare the HIV-1 mean RNA loads between those infected with HIV-1 alone and those with dual infection. Interestingly, however, among commercial sex workers, mean HIV-1 loads of dually infected patients were similar to those with single HIV-1 infection, despite the differences in enrollment criteria.

Frequency and magnitude of HIV-specific IFN-γ-secreting T-cell responses among HIV-1/2 dually infected subjects. Ninety-five percent of dually infected subjects exhibited IFN-γ-secreting T cells upon ex vivo stimulation with at least one peptide pool of each HIV gene product, and the majority (58%) recognized both

TABLE 2. Frequency of HIV-specific T-cell responses in study participants as measured by IFN-γ ELISPOT assay[a]

Peptide	Frequency of response:					
	To HIV-1			To HIV-2		
	HIV-1/2 (%) (n = 19)	HIV-1 (%) (n = 112)	OR (95% CI)	HIV-1/2 (%) (n = 19)	HIV-2 (%) (n = 78)	OR (95% CI)
Env	11 (58)	37 (33)	2.80 (0.9–8.6)	7 (37)	32 (41)	1.11 (0.4–3.4)
Gag	11 (58)	69 (62)	1.23 (0.4–3.9)	10 (53)	48 (62)	0.75 (0.2–2.4)
Nef	13 (68)	67 (60)	2.09 (0.6–7.2)	0 (0)	15 (19)	NA
Tat	6 (32)	5 (4)	11.24 (2.5–49.6)	2 (11)	11 (14)	0.76 (0.2–3.8)
Any peptide	17 (89)	87 (78)	10.26 (0.9–113.3)	12 (63)	57 (73)	0.85 (0.3–2.9)

[a] For OR determinations, multivariate logistic regression models, adjusted for $CD4^+$ T-cell count (duals versus HIV-1), were applied. NA, not applicable (could not be compared). n, Number of subjects.

HIV-1 and HIV-2 epitopic peptides (Fig. 1A). HIV Gag-specific responses were common (68%) among those with dual infection, with 42% responding to both HIV-1 and HIV-2 pools. Seventy-four percent of dually infected patients exhibited Env-specific responses, although these responses were more likely directed to epitopes of a single HIV type (53%) rather than to both HIV-1 and HIV-2 epitopes (21%). Although HIV-1 Nef-specific responses were frequent (68%), HIV-2 Nef-specific responses were not detected ($P < 0.001$). Infrequent HIV Tat-specific responses were primarily directed to HIV-1 rather than HIV-2 epitopic peptides ($P = 0.046$).

As shown in Table 3 and Fig. 1B, the total magnitude of IFN-γ-secreting T cells recognizing HIV-1 peptides (2.64 $\log_{10}$) was significantly greater than IFN-γ-secreting T cells recognizing HIV-2 peptides (1.53 $\log_{10}$) ($P = 0.002$) among patients with dual infection. This was largely attributable to the greater magnitude of responses to HIV-1 Nef (1.88 $\log_{10}$) than HIV-2 Nef (0 $\log_{10}$) ($P < 0.0001$) and, to a much lesser extent, to HIV-1 Tat in comparison to HIV-2 Tat (0.56 $\log_{10}$ compared to 0.13 $\log_{10}$ SFC/10^6, respectively [$P = 0.04$]). The IFN-γ-secreting PBMC recognizing HIV-1 and HIV-2 Gag were similar in frequencies (Table 2 and Fig. 1B). The breadth of responses to HIV-1 gene products (Env, Gag, Nef, and Tat) was significantly higher than to HIV-2 gene products ($P = 0.0005$), with dually infected patients mounting responses to an average of 2 (range, from 0 to 4) HIV-1 gene products and to 1 (range, from 0 to 3) HIV-2 gene products (Fig. 1C).

Comparison of HIV-specific T-cell responses among subjects with dual HIV-1/2 and single HIV-1 or HIV-2 infection. To determine whether the host cellular responses to one HIV type are altered by the presence of infection with another HIV type, we compared the frequency and magnitude of HIV-specific IFN-γ responses seen among dually infected patients to those with a single infection (Tables 2 and 3). The frequency of responses was evaluated by using multivariate logistic regression adjusted for $CD4^+$ T-cell counts, and the magnitude of responses was evaluated by multivariate linear regression adjusted for $CD4^+$ T-cell counts. In comparison with singly infected subjects, HIV-1 Tat-specific responses were of greater frequency (odds ratio [OR] = 11.24; 95% confidence interval [CI] = 2.5 to 49.6) and magnitude ($\beta = 0.54$; $P = 0.0004$) among dually infected patients. This finding remained after additional adjustment for HIV-1 plasma viral load, clinic site, and gender (data not shown). Compared to singly infected subjects, persons with dual infections also tended to have elevated frequencies (OR = 2.80; 95% CI = 0.9 to 8.6) and (marginally) elevated magnitudes of response ($\beta = 0.72$; $P = 0.05$) to any HIV-1 peptide, as well as an elevated frequency of responses to HIV-1 Env (OR = 10.26; 95% CI = 0.9 to 113.3). None of the results significantly changed after additional adjustment for HIV-1 plasma viral load, study sites, and gender (data not shown). T-cell recognition of HIV-2 Nef was even less common in those with dual infection than in those infected with HIV-2 alone ($P = 0.04$ [Fisher exact test]). Otherwise, no significant differences were found in the frequency or magnitude of HIV-2 responses between those with dual infection and those infected with HIV-2 alone.

Cytokine profile of HIV-specific T cells among dually infected subjects. The production of multiple cytokines is believed to be important in the maintenance of functional $CD4^+$ and $CD8^+$ T-cell responses, with recent studies reporting LTNP having an increased proportion of functional HIV-specific $CD8^+$ T-cell responses (4). To determine whether dually infected patients maintained such responses, we assessed the antiviral cyto-

TABLE 3. Magnitude of HIV-specific T-cell responses in study participants as measured by IFN-γ ELISPOT assay[a]

Peptide	Mean magnitude of response ($\log_{10}$ SFC/10^6 PBMC):					
	To HIV-1			To HIV-2		
	HIV-1/2 (n = 19)	HIV-1 (n = 112)	β (P)	HIV-1/2 (n = 19)	HIV-2 (n = 78)	β (P)
Env	1.21	0.73	0.45 (0.2)	0.66	0.94	−0.08 (0.8)
Gag	1.36	1.65	−0.06 (0.9)	1.34	1.65	−0.16 (0.6)
Nef	1.88	1.49	0.48 (0.2)	0.00	0.41	−0.22 (0.3)
Tat	0.56	0.09	0.54 (0.0004)	0.13	0.25	−0.09 (0.6)
Any peptide	2.64	2.22	0.72 (0.05)	1.53	1.99	−0.21 (0.5)

[a] β, For multivariate linear regression models, adjusted for $CD4^+$ T-cell count. n, Number of subjects.

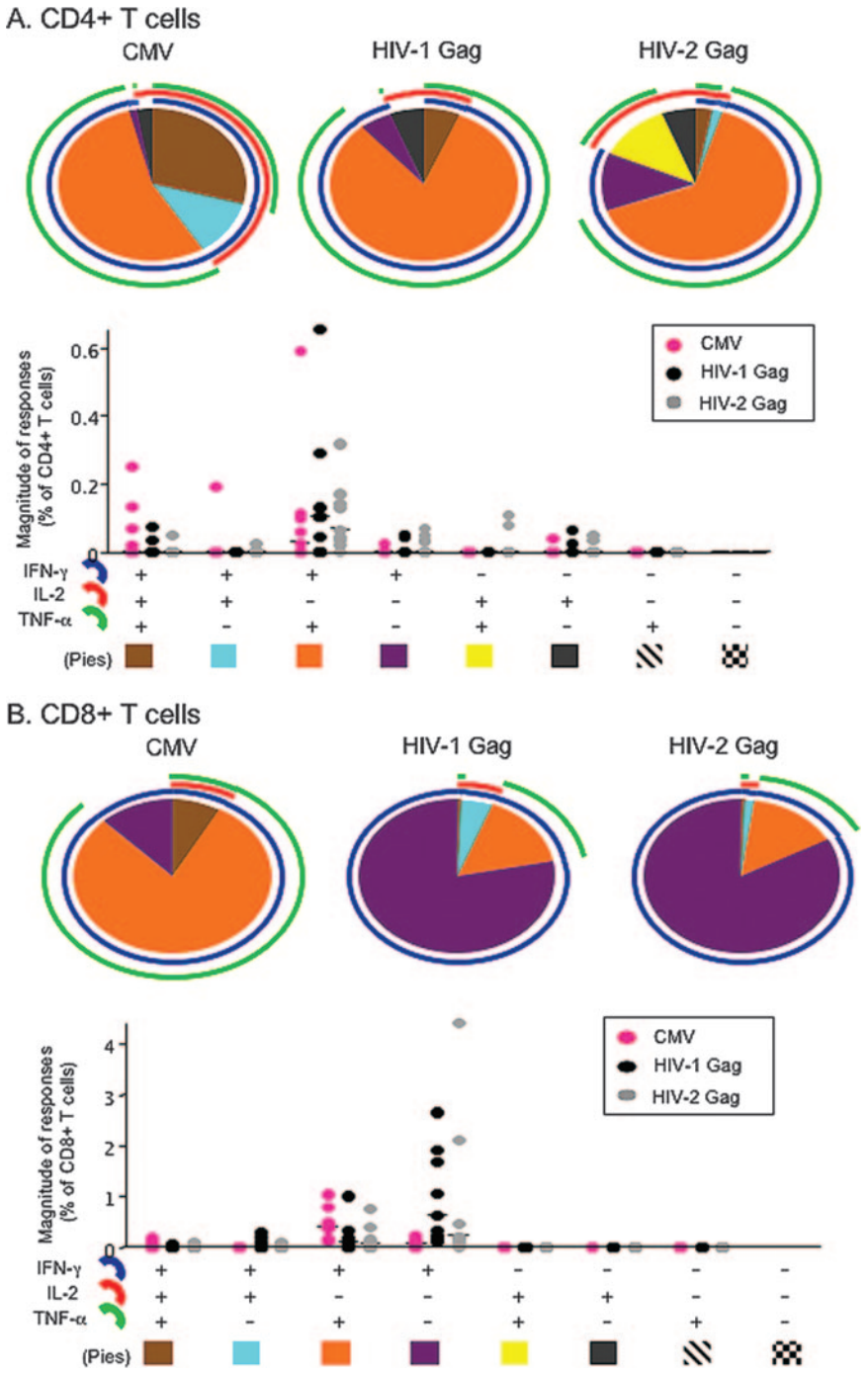


FIG. 2. Cytokine profile of $CD4^+$ (A) and $CD8^+$ (B) T-cell responses recognizing HIV Gag- and CMV pp56 in HIV-1/2 dually infected subjects ($n = 9$). Antigen-specific responses of >0.02% of the total $CD4^+$ or $CD8^+$ T cells producing each cytokine were considered positive. Each pie chart represents the mean responses of all subjects to the HIV-1, HIV-2 Gag, and CMV pp56 overlapping 15 mers. Responses are grouped by cytokines secreted and matched to the pattern in the legend. The arc outside the pie chart indicates the mean percentage of HIV-1-, HIV-2-, or CMV-specific IFN-γ (blue)-, IL-2 (red)-, or TNF-α (green)-producing cells. The dot plots represent the mean magnitude of response to CMV, HIV-1, and HIV-2 Gag with multiple functional cytokine profile indicated in the legend. Each "+" in the legend denotes IFN-γ, IL-2, and TNF-α positivity.

kine profiles of nine dually infected patients (six from the STD clinic and three from the infectious disease clinic, who had adequate material remaining), by evaluating the production of IFN-γ, IL-2, and/or TNF-α in $CD4^+$ and $CD8^+$ T cells recognizing HIV-1 Gag, HIV-2 Gag, and, as a control, CMV pp65.

Among these nine dually infected patients, seven had detectable HIV-1 Gag-specific, eight had HIV-2 Gag-specific, and seven had CMV-specific $CD4^+$ T cells (i.e., cells producing either IFN-γ, IL-2, and/or TNF-α after stimulation). More than 80% of HIV-1 or HIV-2 Gag-specific $CD4^+$ T cells pro-

TABLE 4. Association between HIV-specific IFN-γ SFC frequencies and HIV-1 plasma viral load in 19 HIV-1/2 dually infected subjects

Peptide	IFN-γ SFC frequency			
	Univariable regression		Multivariable regression[a]	
	Log_{10} plasma HIV-1 RNA	*P*	Log_{10} plasma HIV-1 RNA	*P*
HIV-1				
Env	0.04	0.92	0.02	0.94
Gag	−0.36	0.21	−0.32	0.21
Nef	−0.03	0.91	−0.06	0.79
Tat	−0.22	0.63	−0.04	0.93
Any HIV-1 peptide	−0.29	0.44	0.14	0.68
HIV-2				
Env	−0.59	0.15	−0.34	0.38
Gag	−0.52	0.06	−0.43	0.07
Nef[b]	NA[c]	NA	NA	NA
Tat	0.55	0.58	0.68	0.39
Any HIV-2 peptide	−0.50	0.09	−0.33	0.19

[a] Values were adjusted for $CD4^+$ cell counts, study site, and gender.
[b] None of the dually infected subjects had HIV-2 Nef responses.
[c] NA, not applicable.

duced both IFN-γ and TNF-α. However, only 12% of HIV-1 and 24% of HIV-2 specific $CD4^+$ cells produced IL-2. Fewer than 5% of HIV-specific $CD4^+$ cells were able to produce the three cytokines (IFN-γ, TNF-α, and IL-2) examined. Approximately 15% of HIV-2 Gag-specific $CD4^+$ T cells, but none of the HIV-1 Gag- $CD4^+$ T cells were positive for both IL-2 and TNF-α. Overall, there was no difference in the median frequency of HIV Gag- and CMV-specific cells that produced IL-2, IFN-γ, or TNF-α. However, in contrast to HIV-specific $CD4^+$ T cells, 42% of CMV-specific $CD4^+$ T cells produced IL-2, with 30% of CMV-specific $CD4^+$ T cells simultaneously producing these three cytokines (Fig. 2A).

As for CD8 T-cell function, all nine dually infected persons examined had HIV-1 Gag- and CMV-specific $CD8^+$ T-cell responses, and eight had HIV-2 Gag-specific $CD8^+$ T-cell responses. Overall, there is no difference in the cytokine profiles of HIV-1- and HIV-2-specific $CD8^+$ T cells in the HIV-1/2-infected subjects. All HIV-1- or HIV-2- specific $CD8^+$ T cells were positive for IFN-γ, with 15% positive for IFN-γ or TNF-α, (Fig. 2B). However, <10% of HIV-1- or HIV-2-specific $CD8^+$ T cells were able to secrete IL-2. In contrast, all CMV-specific $CD8^+$ T cells were able to produce IFN-γ, with 90% also producing TNF-α and 10% producing all three cytokines examined.

Association of HIV-specific responses, HIV-1 plasma viral load, and $CD4^+$ T-cell counts among dually infected patients. In our previous study among subjects with single HIV-1 infection, we found that those able to respond to both HIV-1 and HIV-2 Gag proteins had a greater ability to control HIV-1 replication (lower HIV-1 viral loads) than those without such responses (33). We hypothesized that likewise, among dually infected persons, control of HIV-1 replication was associated with the presence of responses to HIV-2 Gag. We first examined the association between the magnitudes of HIV-specific PBMC IFN-γ responses (ELISPOT) and HIV-1 plasma viral load and noted that HIV-2 Gag-specific responses were somewhat inversely associated with HIV-1 viral load ($\beta = -0.52$ log_{10} plasma RNA copies per log_{10} increase in HIV-2 Gag-specific responses [$P = 0.06$]; $\beta = -0.43$ log_{10} plasma RNA copies per log_{10} increase in HIV-2 Gag-specific responses [$P = 0.07$ adjusted for $CD4^+$ T-cell counts, study sites, and gender]) (Table 4). However, no significant associations between the magnitudes and breadth of T-cell responses and $CD4^+$ T-cell counts were found in this small sample of dually infected patients.

Since IFN-γ responses may not be an accurate measure of functional T-cell responses, we next examined the functional cytokine profiles of HIV-1 and HIV-2 Gag-specific $CD4^+$ or $CD8^+$ T cells in relationship to viral load among the nine dually infected patients. As in our previous studies, we found no association between the presence of HIV-1 Gag-specific $CD4^+$ or $CD8^+$ T-cell responses and HIV-1 plasma viral load; however, even in this very small sample, a suggestion of an inverse association was found between HIV-1 plasma viral load and the presence of HIV-2 Gag-specific $CD4^+$, TNF-α^+, and IFN-γ^+ T-cell responses ($r = -0.773$ [$P = 0.01$] and $r = -0.79$ [$P = 0.01$], respectively; Spearman test; Fig. 3A). Further, the HIV-1 viral load was also somewhat associated with the presence of HIV-2 Gag-specific $CD8^+$ T-cell responses ($r = -0.728$ [$P = 0.03$, Spearman test]; Fig. 3B). However, these

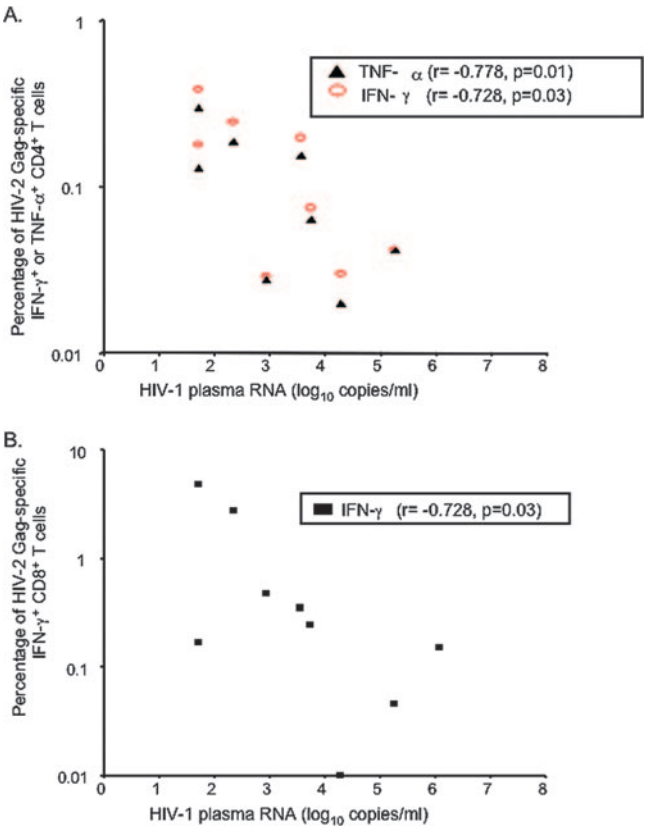
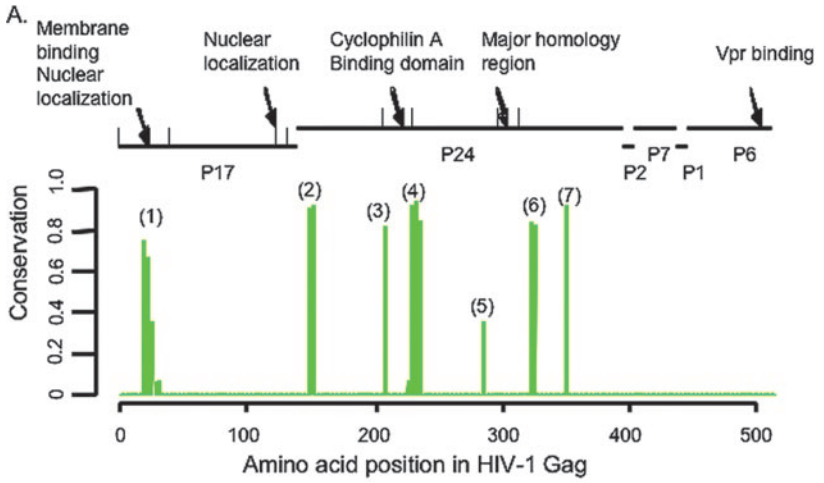


FIG. 3. Negative correlation between the frequencies of HIV-2 Gag-specific IFN-γ^+ or TNF-α^+ $CD4^+$ (A) and IFN-γ^+ $CD8^+$ (B) T cells with HIV-1 plasma viral load in dually infected subjects. A Spearman ranking test was used to analyze the correlation. Positive responses were designated when the percentage of bright cytokine$^+$ $CD4^+$ or $CD8^+$ T cells was twice that of the negative control and >0.02% of the total $CD4^+$ or $CD8^+$ T cells. Antigen-specific responses of <0.01% are shown as 0.01% on the figures.



B.

Position	HIV-1 (CRF-02) peptide sequences	HIV-2 (Rod) peptide sequences
(1) p17 17- 33	ERIRLRPGGKKKYRLKH	ERIRLRPGGKKKYRLKH
(2) p24 14- 55	PLSPRTLNAWVK	SISPRTLNAWVK
(3) p24 73- 81	INEEAAEWD	INEEAADWD
(4) p24 97-110	REPRGSDIAGTTST	REPRGSDIAGTTST
(5) p24 150-161	ILDIKQGPKEF	ILDIRQGPKEPF
(6) p24 188-199	TLLVQNANPDCK	TLLVQNSNPDCR
(7) p24 216-225	TACQGVGGPG	SACQGVGGPG

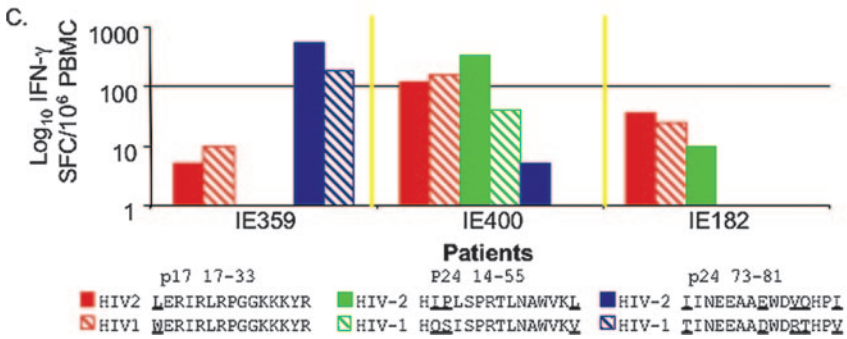


FIG. 4. IFN-γ responses to epitopes at the conserved region between HIV-1 and HIV-2 Gag. (A) Amino acid conservation between HIV-1 and HIV-2 Gag. A schematic diagram shows the functional domains in the HIV-1 and HIV-2 Gag. The sequence conservation of every nine amino acid sequence in HIV-2 Gag (Rod) was compared to all of the HIV-1 subtype CRF-02 Gag sequences available in the HIV-1 database at the same position. Amino acid sequences at seven regions in HIV-1 and HIV-2 share 36 to 94% similarity. (B) Position and amino acid sequence at the conserved regions between HIV-1 and HIV-2. Amino acids that differ between HIV-1 and HIV-2 at the conserved region are highlighted in red). (C) Detection of cross-reactive responses to epitopes at the conserved regions between HIV-1 and HIV-2 Gag. The line shows the cutoff of positive responses (at 100 SFC/10^6 PBMC).

associations did not achieve statistical significance ($P < 0.003$) after adjustments for multiple comparisons. Interestingly, HIV-2 Gag-specific, IFN-γ-secreting $CD8^+$ cells were significantly positively associated with HIV-1 Gag-specific $CD4^+$ IL-2-secreting T cells ($r = 0.923$ [$P = 0.0004$]).

Responses to epitopes at the conserved regions of HIV-1 and HIV-2. Our previous study showed that HIV-1-infected subjects with the ability to respond to HIV-2 Gag, as well as HIV-1 Gag, had lower HIV-1 plasma viral loads than those without the responses to HIV-2 Gag (33). Ongoing mapping studies of HIV-2 Gag responses among those infected with HIV-1 alone suggested that these responses are directed against epitopes in the conserved regions of HIV-1 and HIV-2 (Zheng et al., unpublished). We compared the sequence conservation of every 9-aa sequence in HIV-2 Gag (Rod) for all of the HIV-1 subtype CRF-02 Gag sequences available in HIV sequence database (www.hiv.lanl.gov)

and found that the amino acid sequences at seven regions are conserved (36 to 94%) across of all HIV-1 subtype CRF-02 Gag (Fig. 4A). All of the seven regions are located within either Gag p17 or Gag p24 regions. One region (p17 17-33) is in the nuclear localization domain, two regions (p24 73-81 and p24 97-110) are in the cyclophilin A binding domain, and one region (p24 150–161) is in the major homology region. Amino acid sequences at the p17 17-33 and p24 97-110 regions are identical for HIV-1 and HIV-2, whereas one or two amino acids show differences in sequences between HIV-1 and HIV-2 at the other five regions (Fig. 4B).

Furthermore, we mapped the HIV-1 and HIV-2 responses to the epitopes at the above seven regions in three dually infected individuals (IE359, IE400, and IE182) (Fig. 4C). Responses to epitopes at p17 17-33, p24 14-25, and p24 73-81 regions were detected in IE359 and IE400. IE400 recognized an epitope at the p17 17-33 region presented by both HIV-1 (WERIRLRPGGKKKYR) and HIV-2 (LERIRLRPGGKK KYR) peptides. IE400 also recognized an epitope at the p24 14-25 region presented only by the HIV-2 peptide (HIPLSPR TLNAWVKL), whereas IE359 recognized an epitope at the p24 73-81 region presented by both HIV-1 (TINEEAADWD RTHPV) and HIV-2 (IINEEAAEWDVQHPI) peptides. These data suggest that epitopes at the conversed functional regions were targeted by dually infected individuals.

DISCUSSION

We provide here one of the first detailed studies of the clinical and immunologic profiles of persons who are coinfected with HIV-1 and HIV-2. Generally, this type of investigation has been difficult to carry out because, although HIV infection is a global pandemic, dual infection, while relatively infrequent, is primarily concentrated in West Africa. Our findings support the premise that the parameters of clinical disease in dual infection potentially can be skewed by the source of population screening. In this case, asymptomatic subjects, identified through screening as high-risk groups (commercial sex workers), were significantly healthier than those with symptoms seeking care in an infectious disease clinic.

The supposition that the less-virulent HIV-2 infection may provide a protective role against subsequent HIV-1 infection and disease progression has received wide interest. We were unable to ascertain the order of infection with the two types because, since in other reports, these patients have chronic infection typically at the time of diagnosis. Clearly, dually infected patients can exhibit a broad spectrum of HIV disease but, in contrast to HIV-1 infection alone, our findings suggest that coinfection with HIV-2 may confer an advantage in slowing HIV-1 disease. In our original study of single-infection patients, we limited recruitment to those whose CD4 T-cell counts were $\leq$350 cell/μl, whereas recruited patients in the dual-infection study were eligible regardless of CD4 count. Interestingly, however, despite the fact that only "healthy" HIV-1-only patients were recruited, we noted no difference in the mean $CD4^+$ T-cell counts and HIV-1 plasma viral load among study patients enrolled in the STD clinic. Thus, dually infected patients (not identified as a result of being symptomatic) did no worse and, in fact, might have done better than patients infected with HIV-1 alone. While previous studies of dually infected subjects concluded that these persons have aggressive disease, this impression appears to have been the result of biased patient selection (1, 16, 18, 26).

One caveat for studies using peptide sets to screen T-cell responses is that the detection of responses is partly dependent on the degree of mismatch between peptides and autologous viral sequences in the patients. Studies by us and others have shown that, in Senegal, the majority of circulating HIV-1 viruses are HIV-1 CRF-02 (45%) and subtype A (20 to 30%) (12, 31) and nearly all HIV-2 viruses are subtype A (24, 25). Furthermore, we and others have shown that no significant difference in HIV-1 and HIV-2 subtype distribution exists between individuals with dual HIV-1/2 infections and individuals with single HIV-1 or HIV-2 infections (12, 25). Thus, we chose to screen HIV-1 responses with the CRF-02 peptides and HIV-2 responses with HIV-2 subtype A Rod peptides. As determined by phylogenetic analysis, CRF-02 Gag is subtype A, gp120 is predominantly subtype A, gp41 is predominantly subtype G, Nef is predominantly subtype G, and Tat is both subtypes A and G (www.hiv.lanl.gov). We compared the amino acid differences of Gag, Env, Tat, and Nef proteins between consensus HIV-1 CRF-02 and consensus subtype A. The difference in Gag, Env, Nef, and Tat is 6.9, 10.3, 13.7, and 21.8%, respectively. This suggests that, although there was some HIV-1 peptide subtype mismatching, the overall effect was no different than if we had used consensus or ancestral sequences commonly used for HIV-1 analyses. Ideally, we would have preferred to use autologous peptides for each subject, or subtype-specific peptides; however, the expense of such an approach (both in viral sequencing costs and peptide production) made it infeasible in our population-based study.

Guided by our previous studies in the same clinic population with HIV-1 or HIV-2 (33), we focused on the specificities of HIV-specific T-cell responses that distinguish HIV-1 from HIV-2 infection. Importantly, we found that the dominant T-cell responses in the dually infected patients are specific for HIV-1 rather than HIV-2. We postulate that this finding relates to the >100-fold higher levels of HIV-1 plasma viremia relative to HIV-2 in these patients. Interestingly, upon examination of representative HIV-specific T cells in dually infected patients, the responses associated with enhanced control of HIV-1 replication were indeed the same as those with HIV-1 infection alone (33). While Gag-specific T cells recognizing both HIV-1 and HIV-2 predominated, the presence of HIV-2 Gag-specific responses was somewhat associated with lower HIV-1 viral loads. Such responses likely represent "cross-reactive" responses, directed at epitopes conserved between HIV-1 and HIV-2 and particularly at those located within functional domains essential to the replication competency of both viruses (13, 19, 20).

Our sequence conservation analysis between HIV-1 and HIV-2 revealed seven conserved regions in Gag. Four of these seven regions are located at the nuclear localization and cyclophilin A binding domains of Gag (Fig. 4). Responses to epitopes at these regions presented by both HIV-1 and HIV-2 peptides were detected in some dually infected patients. Thus, the fact that such epitopes might be important for control of both HIV-1 and HIV-2 replication is not surprising. Screening for T-cell responses to HIV-2 peptides may have facilitated the ability to distinguish the many HIV-1 Gag-specific T cells that do not contribute to the control of viral load from the few that

are important in control of HIV-1 replication. Finally, however, we concede that the use of exogenous small peptides to induce T cells ex vivo to secrete antiviral cytokines may not necessarily correspond to the ability of these T cells in vivo to recognize naturally processed HIV proteins that are expressed with MHC molecules on the cell surface and clear HIV-infected cells of either subtype.

We observed that T-cell responses to HIV-1 were somewhat different in specificity among those with dual infections than in those with a single HIV-1 infection. Responses to HIV-1 Tat-, Env-, and overall to any HIV-1-specific responses were more frequently detected in the dually infected patients than in HIV-1 singly infected individuals, even after we took into account differences in $CD4^+$ T-cell count and HIV-1 plasma viral load. Since HIV-1 Tat- and Env-specific responses are commonly seen in acute rather than late HIV infection (6, 7), it is possible that dually infected patients are better able to maintain such responses long-term than are those with single HIV-1 infection. In contrast, few differences were noted between the HIV-2-specific T-cell responses seen in dually infected subjects compared to those of HIV-2 singly infected subjects. Thus, while the presence of HIV-2 might alter the response to HIV-1, we found nothing to suggest that HIV-1 alters the HIV-2-specific response. However, the order of infection may possibly be important in determining the presence and magnitude of specific responses present. Unfortunately, we have no data on this issue.

We still do not fully understand why there was lack of HIV-2 Nef responses in HIV-1 and HIV-2 dually infected individuals. We previously reported this observation in HIV-2 singly infected individuals (33). One possibility is that HIV-2 Nef is more variable, leading to greater sequence mismatch between the screening peptides and the HIV-2 Nef viral sequences in the patients. We tested this hypothesis by using an HIV-2 Nef peptide set whose sequences were constructed according to the potential T-cell epitope approach (17) to screen HIV-2 Nef responses in cryopreserved PBMC of six dually infected individuals, which provides substantially more coverage of HIV-2 Nef variants. Surprisingly, no additional Nef-specific responses were detected with the potential T-cell epitope versus Rod peptide panel, providing additional support that dually infected individuals naturally mount low levels of HIV-2 Nef responses (data not shown).

Although we previously observed that only 8% of HIV-1-infected subjects had detectable IFN-γ-secreting HIV-1 specific $CD4^+$ T cells (33), we identified these helper cells in seven of the nine dually infected individuals examined here. This pattern of response in the dually infected individuals is thus more typical of HIV-2 infection than HIV-1. Previous studies have shown induction and preservation of proliferating HIV-1-specific $CD4^+$ T cells are essential for the maintenance of effective antiviral $CD8^+$ T-cell responses and the generation of functional $CD8^+$ T memory cells and for efficient neutralizing-antibody-mediated viral clearance (5, 15, 27, 30). Whereas $CD4^+$ T cells of HIV-1-infected patients have been shown to have a selective defect in IL-2 production, frequently producing IFN-γ alone (14, 28), the frequency of those that can produce IL-2- or IL-2/IFN-γ has been shown to be negatively correlated with the levels of viral load (14). Of note, IL-2-producing HIV-specific $CD4^+$ T cells were uncommonly detected in the patients with dual infections. However, the production of IFN-γ- and TNF-α-secreting HIV-2 Gag-specific CD4-T cells correlated with lower HIV-1 plasma viremia.

In summary, we have demonstrated that, although dually infected patients mounted higher frequencies, magnitude, and breadth of HIV-1- than HIV-2-specific responses, the responses to epitopes in the HIV-2 Gag or in the conserved region between HIV-1 and HIV-2 Gag was inversely correlated with the HIV-1 plasma viral load. Although the functional cytokine profiles of HIV Gag-specific $CD4^+$ and $CD8^+$ T cells from individuals with dual infections were partially skewed, the majority of dually infected individuals still have functional HIV Gag-specific $CD4^+$ and $CD8^+$ T-cell responses.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health grants R01-AI48470, R01-AI47086, and U01-AI48017 and a Burroughs Wellcome Clinical Scientist Award in Translational Research (M.J.M.).

We thank Macoumba Toure for his invaluable coordination of study procedures in Senegal; Mame Dieumbe Mbengue-Ly, Marie Pierre Sy, and Pierre Ndiaye for patient care; Alison Starling for data management; Donna Kenny for sample and reagent supply management; Aaron Wilson and Devin Adams for technical support; Stephen DeRosa for the guidance in flow analysis; Stephen Cherne for the HIV RNA measurement testing; Michelle Moerbe for data entry; and Phyllis Stegall for preparation of the manuscript. We thank the study subjects for their ongoing participation.

REFERENCES

1. **Alabi, A. S., S. Jaffar, K. Ariyoshi, T. Blanchard, M. Schim van der Loeff, A. A. Awasana, T. Corrah, S. Sabally, R. Sarge-Njie, F. Cham-Jallow, A. Jaye, N. Berry, and H. Whittle.** 2003. Plasma viral load, CD4 cell percentage, HLA and survival of HIV-1, HIV-2, and dually infected Gambian patients. AIDS **17:**1513–1520.
2. **Andersson, S., H. Norrgren, Z. da Silva, A. Biague, S. Bamba, S. Kwok, C. Christopherson, G. Biberfeld, and J. Albert.** 2000. Plasma viral load in HIV-1 and HIV-2 singly and dually infected individuals in Guinea-Bissau, West Africa: significantly lower plasma virus set point in HIV-2 infection than in HIV-1 infection. Arch. Intern. Med. **160:**3286–3293.
3. **Anonymous.** 1993. Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. Morb. Mortal. Wkly. Rep. Recomm. Rep. **41:**1–19.
4. **Betts, M. R., M. C. Nason, S. M. West, S. C. De Rosa, S. A. Migueles, J. Abraham, M. M. Lederman, J. M. Benito, P. A. Goepfert, M. Connors, M. Roederer, and R. A. Koup.** 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific $CD8^+$ T cells. Blood **107:**4781–4789.
5. **Bourgeois, C., B. Rocha, and C. Tanchot.** 2002. A role for CD40 expression on $CD8^+$ T cells in the generation of $CD8^+$ T-cell memory. Science **297:** 2060–2063.
6. **Cao, J., J. McNevin, S. Holte, L. Fink, L. Corey, and M. J. McElrath.** 2003. Comprehensive analysis of human immunodeficiency virus type 1 (HIV-1)-specific gamma interferon-secreting $CD8^+$ T cells in primary HIV-1 infection. J. Virol. **77:**6867–6878.
7. **Cao, J., J. McNevin, U. Malhotra, and M. J. McElrath.** 2003. Evolution of $CD8^+$ T-cell immunity and viral escape following acute HIV-1 infection. J. Immunol. **171:**3837–3846.
8. **Cot, M. C., M. Poulain, J. F. Delagneau, M. Peeters, and F. Brun-Vezinet.** 1988. Dual HIV-1 and HIV-2 infection in West Africa supported by synthetic peptide analysis. AIDS Res. Hum. Retrovir. **4:**239–241.

8a. **Critchlow, C. W., S. E. Hawes, J. M. Kuypers, S. Kwok, and N. B. Kiviat.** 2000. HIV-1 and HIV-2 co-infection is associated with reduced HIV-1 plasma RNA and DNA levels among Senegalese men and women. Am. J. Epidemiol. **151**(Suppl.):S11.

9. **Dewar, R. L., H. C. Highbarger, M. D. Sarmiento, J. A. Todd, M. B. Vasudevachari, R. T. Davey, Jr., J. A. Kovacs, N. P. Salzman, H. C. Lane, and M. S. Urdea.** 1994. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. J. Infect. Dis. **170:**1172–1179.
10. **Duvall, M. G., A. Jaye, T. Dong, J. M. Brenchley, A. S. Alabi, D. J. Jeffries, M. van der Sande, T. O. Togun, S. J. McConkey, D. C. Douek, A. J. McMichael, H. C. Whittle, R. A. Koup, and S. L. Rowland-Jones.** 2006. Maintenance of HIV-specific $CD4^+$ T cell help distinguishes HIV-2 from HIV-1 infection. J. Immunol. **176:**6973–6981.
11. **Evans, L. A., J. Moreau, K. Odehouri, D. Seto, G. Thomson-Honnebier, H.**

Legg, A. Barboza, C. Cheng-Mayer, and J. A. Levy. 1988. Simultaneous isolation of HIV-1 and HIV-2 from an AIDS patient. Lancet **ii:**1389–1391.
12. **Gottlieb, G. S., P. S. Sow, S. E. Hawes, I. Ndoye, A. M. Coll-Seck, M. E. Curlin, C. W. Critchlow, N. B. Kiviat, and J. I. Mullins.** 2003. Molecular epidemiology of dual HIV-1/HIV-2 seropositive adults from Senegal, West Africa. AIDS Res. Hum. Retrovir. **19:**575–584.
13. **Goulder, P. J., and D. I. Watkins.** 2004. HIV and SIV CTL escape: implications for vaccine design. Nat. Rev. Immunol. **4:**630–640.
14. **Harari, A., S. Petitpierre, F. Vallelian, and G. Pantaleo.** 2004. Skewed representation of functionally distinct populations of virus-specific CD4 T cells in HIV-1-infected subjects with progressive disease: changes after antiretroviral therapy. Blood **103:**966–972.
15. **Janssen, E. M., E. E. Lemmens, T. Wolfe, U. Christen, M. G. von Herrath, and S. P. Schoenberger.** 2003. $CD4^+$ T cells are required for secondary expansion and memory in $CD8^+$ T lymphocytes. Nature **421:**852–856.
16. **Koblavi-Deme, S., L. Kestens, D. Hanson, R. A. Otten, M. Y. Borget, C. Bile, S. Z. Wiktor, T. H. Roels, T. Chorba, and J. N. Nkengasong.** 2004. Differences in HIV-2 plasma viral load and immune activation in HIV-1 and HIV-2 dually infected persons and those infected with HIV-2 only in Abidjan, Cote D'Ivoire. AIDS **18:**413–419.
17. **Li, F., U. Malhotra, P. B. Gilbert, N. R. Hawkins, A. C. Duerr, J. M. McElrath, L. Corey, and S. G. Self.** 2006. Peptide selection for human immunodeficiency virus type 1 CTL-based vaccine evaluation. Vaccine **24:** 6893–6904.
18. **Nkengasong, J. N., L. Kestens, P. D. Ghys, S. Koblavi-Deme, R. A. Otten, C. Bile, C. Maurice, M. Kalou, M. Laga, S. Z. Wiktor, and A. E. Greenberg.** 2000. Dual infection with human immunodeficiency virus type 1 and type 2: impact on HIV type 1 viral load and immune activation markers in HIV-seropositive female sex workers in Abidjan, Ivory Coast. AIDS Res. Hum. Retrovir. **16:**1371–1378.
19. **Peyerl, F. W., D. H. Barouch, W. W. Yeh, H. S. Bazick, J. Kunstman, K. J. Kunstman, S. M. Wolinsky, and N. L. Letvin.** 2003. Simian-human immunodeficiency virus escape from cytotoxic T-lymphocyte recognition at a structurally constrained epitope. J. Virol. **77:**12572–12578.
20. **Peyerl, F. W., H. S. Bazick, M. H. Newberg, D. H. Barouch, J. Sodroski, and N. L. Letvin.** 2004. Fitness costs limit viral escape from cytotoxic T lymphocytes at a structurally constrained epitope. J. Virol. **78:**13901–13910.
21. **Pinto, L. A., M. J. Covas, and R. M. Victorino.** 1993. T-helper cross reactivity to viral recombinant proteins in HIV-2-infected patients. Aids **7:**1389–1391.
22. **Pinto, L. A., M. J. Covas, and R. M. Victorino.** 1995. T-helper reactivity to simian immunodeficiency virus gag synthetic peptides in human immunodeficiency virus type 2 infected individuals. J. Med. Virol. **47:**139–144.
23. **Rayfield, M., K. De Cock, W. Heyward, L. Goldstein, J. Krebs, S. Kwok, S. Lee, J. McCormick, J. M. Moreau, K. Odehouri, et al.** 1988. Mixed human immunodeficiency virus (HIV) infection in an individual: demonstration of both HIV type 1 and type 2 proviral sequences by using polymerase chain reaction. J. Infect. Dis. **158:**1170–1176.
24. **Sankale, J. L., S. Mboup, M. E. Essex, and P. J. Kanki.** 1998. Genetic characterization of viral quasispecies in blood and cervical secretions of HIV-1- and HIV-2-infected women. AIDS Res. Hum. Retrovir. **14:**1473–1481.
25. **Sarr, A. D., J. L. Sankale, A. Gueye-Ndiaye, M. Essex, S. Mboup, and P. J. Kanki.** 2000. Genetic analysis of HIV type 2 in monotypic and dual HIV infections. AIDS Res. Hum. Retrovir. **16:**295–298.
26. **Schim van der Loeff, M. F., S. Jaffar, A. A. Aveika, S. Sabally, T. Corrah, E. Harding, A. Alabi, A. Bayang, K. Ariyoshi, and H. C. Whittle.** 2002. Mortality of HIV-1, HIV-2, and HIV-1/HIV-2 dually infected patients in a clinic-based cohort in The Gambia. AIDS **16:**1775–1783.
27. **Shedlock, D. J., and H. Shen.** 2003. Requirement for CD4 T-cell help in generating functional CD8 T-cell memory. Science **300:**337–339.
28. **Sieg, S. F., D. A. Bazdar, C. V. Harding, and M. M. Lederman.** 2001. Differential expression of interleukin-2 and gamma interferon in human immunodeficiency virus disease. J. Virol. **75:**9983–9985.
29. **Sousa, A. E., A. F. Chaves, A. Loureiro, and R. M. Victorino.** 2001. Comparison of the frequency of interleukin (IL)-2-, interferon-gamma-, and IL-4-producing T cells in 2 diseases, human immunodeficiency virus types 1 and 2, with distinct clinical outcomes. J. Infect. Dis. **184:**552–559.
30. **Sun, J. C., and M. J. Bevan.** 2003. Defective CD8 T cell memory following acute infection without CD4 T cell help. Science **300:**339–342.
31. **Toure-Kane, C., C. Montavon, M. A. Faye, P. M. Gueye, P. S. Sow, I. Ndoye, A. Gaye-Diallo, E. Delaporte, M. Peeters, and S. Mboup.** 2000. Identification of all HIV type 1 group M subtypes in Senegal, a country with low and stable seroprevalence. AIDS Res. Hum. Retrovir. **16:**603–609.
32. **Westfall, P. H., and R. D. Wolfinger.** 1997. Multiple tests with discrete distributions. Am. Statistician **51:**3–8.
33. **Zheng, N. N., N. B. Kiviat, P. S. Sow, S. E. Hawes, A. Wilson, H. Diallo-Agne, C. W. Critchlow, G. S. Gottlieb, L. Musey, and M. J. McElrath.** 2004. Comparison of human immunodeficiency virus (HIV)-specific T-cell responses in HIV-1- and HIV-2-infected individuals in Senegal. J. Virol. **78:** 13934–13942.