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HIV-1-Specific Mucosal CD8$^+$ Lymphocyte Responses in the Cervix of HIV-1-Resistant Prostitutes in Nairobi[1]

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Understanding how individuals with a high degree of HIV exposure avoid persistent infection is paramount to HIV vaccine design. Evidence suggests that mucosal immunity, particularly virus-specific CTL, could be critically important in protection against sexually acquired HIV infection. Therefore, we have looked for the presence of HIV-specific CD8$^+$ T cells in cervical mononuclear cells from a subgroup of highly HIV-exposed but persistently seronegative female sex workers in Nairobi. An enzyme-linked immunospot assay was used to measure IFN-γ release in response to known class I HLA-restricted CTL epitope peptides using effector cells from the blood and cervix of HIV-1-resistant and -infected sex workers and from lower-risk uninfected controls. Eleven of 16 resistant sex workers had HIV-specific CD8$^+$ T cells in the cervix, and a similar number had detectable responses in blood. Where both blood and cervical responses were detected in the same individual, the specificity of the responses was similar. Neither cervical nor blood responses were detected in lower-risk control donors. HIV-specific CD8$^+$ T cell frequencies in the cervix of HIV-resistant sex workers were slightly higher than in blood, while in HIV-infected donor cervical response frequencies were markedly lower than blood, so that there was relative enrichment of cervical responses in HIV-resistant compared with HIV-infected donors. HIV-specific CD8$^+$ T cell responses in the absence of detectable HIV infection in the genital mucosa of HIV-1-resistant sex workers may be playing an important part in protective immunity against heterosexual HIV-1 transmission. ***The Journal of Immunology,*** **2000, 164: 1602–1611.**

It is now recognized that there is variability in susceptibility to HIV infection, and that some individuals may exhibit resistance to infection. Several mechanisms of HIV-1 resistance have been described (1). The best characterized are polymorphisms in the gene encoding the principal coreceptor for primary strains of HIV-1, CCR5; for example, individuals homozygous for a 32-bp deletion in the CCR5 gene (CCR5Δ32) are rendered relatively resistant to sexual acquisition of HIV-1 (2). However, the CCR5Δ32 mutation is largely confined to Caucasian populations (3), while over 80% of new HIV-1 infections occur in the developing world (4). Because heterosexual transmission accounts for 70–80% of infections in these populations (5), initial host-virus interactions are likely to occur at the genital mucosa.

Recent interest has focused on local HIV-specific immune responses in the genital tract of individuals who remain uninfected despite repeated sexual exposure to HIV-1 (highly exposed persistently seronegative, HEPS[3]). Mucosal HIV-1-specific IgA is found in many HEPS subjects (6–8), but it is thought that HIV-specific humoral responses alone may be insufficient to protect against infection (1). HIV-1-specific Th and CTL immune responses have been detected in the blood of HEPS individuals (8–15). In addition, a stepwise increase in the frequency of bulk CTL recognizing HIV-1 *env* has been seen with increasing levels of prior HIV exposure in HEPS prostitutes, suggesting that HIV-specific CTL may be causally associated with protection against HIV-1 infection (16). However, although CD8$^+$ lymphocytes from HEPS donors can protect against systemic HIV-1 challenge in a SCID/beige mouse model (17), other murine experiments have shown that mucosal rather than systemic (splenic) HIV-specific CTL are necessary to confer resistance to mucosal viral transmission (18). Recent work has shown that transient infection of the colonic mucosa in rhesus macaques can induce class I HLA-restricted CTL recognizing SIV *env* and that the presence of mucosal CTL correlates absolutely with protection against subsequent colonic viral challenge (19).

These studies suggest that while HIV-specific CTL are likely to be important in mediating protective immunity, these responses may need to be present in the genital tract to prevent heterosexual HIV-1 acquisition. Although HIV-1-specific CTL have been demonstrated in the genital tract of HIV-1-infected women (20), there

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[3] Abbreviations used in this paper: HEPS, highly exposed persistently seronegative; ELISPOT, enzyme-linked immunospot; STI, sexually transmitted infection; CMC, cervical mononuclear cell; SFU, spot-forming unit.

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are no previous data regarding mucosal CTL responses in the genital tract of exposed, uninfected populations. This may be partly due to the difficulties inherent in demonstrating low-frequency CTL using samples obtained from a site with a rich microbial flora and that contain relatively few T lymphocytes. The IFN-γ enzyme-linked immunospot (ELISPOT) assay, which has been previously used to document HIV-specific $CD8^+$ responses in the blood of both HIV-1-infected and HEPS donors (15, 21), provides an estimate of Ag-specific $CD8^+$ lymphocyte frequencies that correlates closely with those measured by either tetrameric MHC-peptide complexes or limiting dilution CTL assays (22). ELISPOT offers the added advantages of being an overnight assay, thereby minimizing the effect of bacterial contamination, and of requiring fewer input cells than standard bulk CTL assays (15, 22, 23). For these reasons, we have used the IFN-γ ELISPOT to estimate CTL frequencies in the cervix and blood of HEPS sex workers.

In an observational cohort study of the epidemiology and immunology of HIV-1 in sex workers in a slum area of Nairobi (24, 25), we have identified a subgroup of women who exhibit relative resistance to HIV-1 infection (26). It is estimated that these women have over 60 unprotected sexual exposures to HIV-1 per year, despite behavioral counseling and provision of condoms; their exposure is uniformly through vaginal intercourse. Resistance in this subgroup is associated with systemic HIV-1-specific Th and CTLs (7, 15), mucosal HIV-1-specific IgA responses (7), and certain MHC class I/II alleles (27), but not with polymorphisms in CCR5 or altered cellular susceptibility to HIV-1 (3). We now report the occurrence and specificity of HIV-1-specific, IFN-γ-secreting, $CD8^+$ T lymphocytes in both cervix and blood of these HIV-1-resistant sex workers.

Materials and Methods

Study populations

Women were enrolled through a dedicated sex worker clinic in the Pumwani district of Nairobi, Kenya, and were classified as HIV-1 resistant if they were seronegative on enrollment and remained both seronegative and PCR negative for at least 3 years of continuing sex work (26). We studied systemic and cervical responses to previously defined HIV-1 CTL epitopes in a subgroup of HIV-1-resistant and HIV-1-infected sex workers attending the annual clinic resurvey between October 1998 and February 1999. The influence of coexistent sexually transmitted infections (STI) on immune responses in the genital tract mucosa is unknown, and for this reason any women with clinical or laboratory evidence of STI were excluded.

Lower-risk HIV-1-uninfected control women were enrolled from a mother-child health care clinic in the Pumwani district of Nairobi and from an infertility clinic in Nairobi's Kenyatta National Hospital. A physical examination was performed, and blood was drawn for HIV-1 and syphilis (rapid plasma reagin) serology. In both groups, women with clinical or laboratory evidence of cervicitis were excluded, as were those with any history of commercial sex work.

Informed consent was obtained from all study participants, and the study conformed to ethical guidelines from the University of Manitoba and the University of Nairobi.

General laboratory methods

Molecular HLA typing was performed on all study subjects using amplification refractory mutation system-PCR with sequence-specific primers, as previously described (28). HIV-1 serological testing employed a synthetic peptide enzyme immunoassay (Detect HIV, Biochem ImmunoSystems, Montreal, Canada), and positive tests were confirmed using a recombinant Ag enzyme immunoassay (Recombigen HIV-1/2 EIA, Cambridge Biotech Corporation, Galway, Ireland). All HIV-1-seronegative sex workers were confirmed to be HIV-1-uninfected employing a PCR system that uses primers for *env*, *nef*, and *vif* HIV-1 provirus genes (26, 29), which have been specifically adapted to detect African clades.

Flow cytometry analysis

Peripheral blood T lymphocyte subset analysis was performed using anti-CD4 FITC/CD8 PE (Becton Dickinson Immunocytometry Systems, Mountain View, CA). Cervical mononuclear cell subpopulations were characterized from cryopreserved specimens using anti-CD3 FITC, anti-CD4 Tricolor, and anti-CD8 PE. After incubation with mAbs for 30 min at 4°C, 10^5 cells were washed in PBS with 1% FBS, fixed, and analyzed using a FACS flow cytometer (Becton Dickinson Immunocytometry Systems) with CellQuest software. Gating was used to select the lymphocyte fraction.

HIV-1 CTL epitope selection and peptide synthesis

HIV-1 peptides were selected from a panel of previously defined A, B, and D clade CTL epitopes. Epitope selection was based on 1) the class I HLA haplotype of the donor; and 2) where possible, on the results of previous systemic HIV-1-specific CTL assays in this study population (15). Peptides were synthesized by F-moc chemistry using a Zinnser Analytical synthesizer (Advanced Chemtech, Louisville, KY), and purity was established by HPLC.

Sampling and transport

Blood was drawn into 12-ml tubes containing the anticoagulant acid citrate dextrose. Cervical samples were obtained using a cytobrush (Histobrush; Spectrum Labs, Dallas, TX), which was inserted into the cervical os, gently rotated through 360°, and transferred immediately into 5 ml of RPMI 1640. To avoid contamination with blood, the cytobrush specimen was obtained before other sampling (STI cultures, etc.), was not collected from women who were actively menstruating, and was rejected if it contained visible blood. All samples were transported to the laboratory within 2 h. The cytobrush was vigorously agitated and discarded, and the remaining cell suspension was agitated to loosen any mucus clumps. Both cervical mononuclear cells (CMC) and PBMC were then isolated by Ficoll-Hypaque gradient centrifugation, washed, and resuspended in RPMI 1640 with 10% FCS (R/10).

IFN-γ ELISPOT assays

A modified ELISPOT assay was used to detect peptide-specific IFN-γ release by either freshly separated or cryopreserved PBMC, and by freshly separated CMC, as previously described (15). First, 96-well nitrocellulose plates were precoated with a first layer IFN-γ mAb, 1-DIK (MABTECH, Nacka, Sweden). PBMC or CMC were then added in duplicate wells, either with predefined HIV-1 class I-restricted peptide epitopes at a concentration of 20 μM or with no peptide (negative control) or in 1:100 PHA (Murex Biotech, Dartford, U.K.; positive control). PBMC assays were run at 2×10^5 and 5×10^4/well, while CMC assays were frequently run at a single input concentration due to low cell numbers. Plates were incubated overnight at 37°C in 5% CO_2, then the cells were discarded, and the plate incubated at room temperature for 3 h with a second biotinylated anti-IFN-γ mAb (7-B6-1 biotin; MABTECH), followed by streptavidin-conjugated alkaline phosphatase (MABTECH) for 2 h. Individual IFN-γ-producing cells were detected as dark blue spots using an alkaline phosphatase-conjugate substrate kit (Bio-Rad, Hercules, CA). The spots were counted by eye by an unblinded study investigator, and the numbers were confirmed using a dissecting microscope (magnification, ×40).

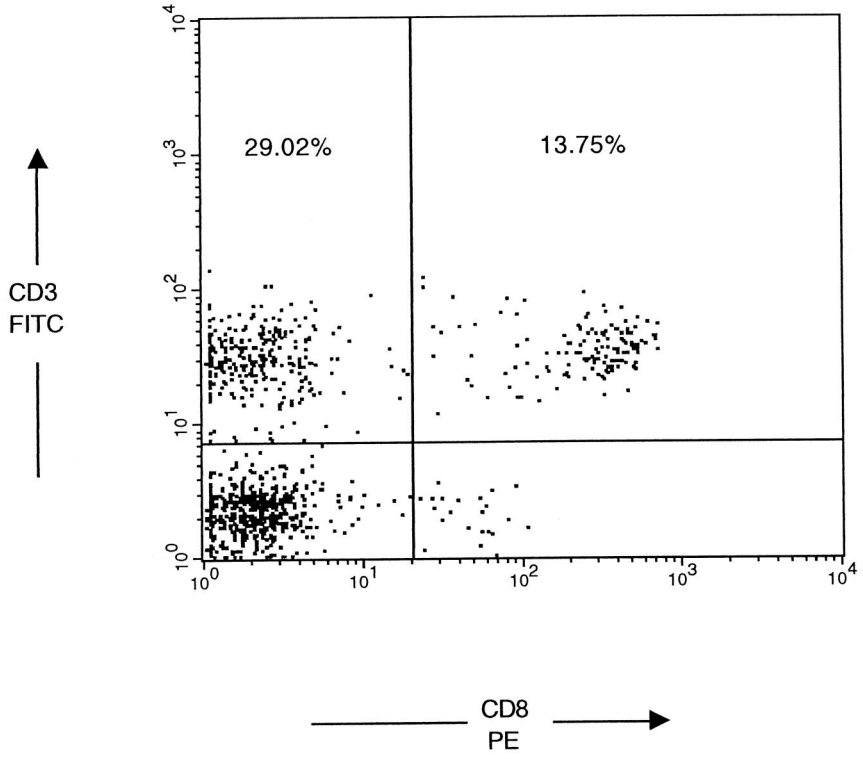
HIV-1-specific IFN-γ responses were reported as number of spot-forming units (SFU)/10^6 mononuclear cells, after subtracting background rates of spontaneous IFN-γ secretion. The background rate of IFN-γ secretion was defined as the number of SFU/10^6 mononuclear cells incubated in media alone, without peptide stimulation. Values reported generally represent the HIV-1 responses quantified at the highest cell input numbers. An HIV-1-specific ELISPOT response was defined as follows: 1) IFN-γ release seen in response to 1:100 PHA (criterion for an adequate assay); 2) ≥20 HIV-1-specific SFU/10^6 mononuclear cells; 3) IFN-γ release in HIV-1 peptide wells exceeded background (spontaneous) rates of IFN-γ release by a factor of at least 2; and 4) if serial dilutions had been established, a titratable response was required.

CD8 lymphocyte depletion assays

$CD8^+$ lymphocyte depletion was performed using anti-$CD8^+$ Ab-coated immunomagnetic beads (Dynabeads HLA cell prep I; Dynal, Lake Success, NY), according to manufacturer's instructions. Significant reduction of an ELISPOT response was defined as a ≥50% reduction in HIV-1-specific IFN-γ release after $CD8^+$ lymphocyte depletion.

Data analysis

Statistical analysis used the SPSS for Windows Rel. 9.0.0 1998 package (SPSS, Chicago, IL). Comparison of means between study groups was performed by one-way ANOVA.



File: 6/4/99.005 Sample ID: ML1072

Quad	Events	% Gated	% Total	Y Mean
UL	234	28.40	0.46	37.00
UR	97	11.77	0.19	37.69
LL	492	59.71	0.98	2.43
LR	1	0.12	0.00	1.65

File: 6/4/99.005 Sample ID: ML1072

Quad	Events	% Gated	% Total	Y Mean
UL	133	16.14	0.26	40.28
UR	199	24.15	0.39	35.02
LL	477	57.89	0.95	2.35
LR	15	1.82	0.03	4.33

FIGURE 1. CD3$^+$/CD8$^+$ lymphocytes are present in the cervix of an HIV-1-resistant sex worker. CMC were isolated from a cervical cytobrush specimen by density centrifugation and stained with PE-conjugated anti-CD8 (*x*-axis) and FITC-conjugated anti-CD3 (*y*-axis, as shown in the *lower left-hand panel*). A well-defined population of CD3$^+$/CD8$^+$ lymphocytes is demonstrated in the upper right quadrant of the *lower left-hand panel*. The *lower right-hand panel* shows similar results for CD3$^+$CD4$^+$ lymphocytes.

Results

Description of study subjects

In total, 26 HIV-1-resistant sex workers and 16 HIV-1-infected sex workers were enrolled between October 1998 and February 1999. The cervical samples from 10 HIV-1-resistant and 5 HIV-1-infected sex workers were inadequate for analysis (no response to PHA), so simultaneous cervical and systemic responses could be studied for 16 resistant and 11 HIV-1-infected sex workers. The mean cytobrush yield for adequate cervical assays was 4.3×10^5 CMC (range, 8×10^4–1.2×10^6 CMC), and for inadequate assays was 6×10^4 CMC. The cell yields of adequate cervical samples did not differ between positive and negative assays (3.7×10^5 and 6.5×10^5 cells, respectively; $p = 0.1$). HIV-1 infection status did not influence cell yield from cervical cytobrushes (4.6×10^5 CMC for HIV-1-infected and 4.1×10^5 for HIV-1-resistant sex workers; $p = 0.7$). HIV-1-infected subjects had a mean blood CD4$^+$ lymphocyte count of 416/mm^3 (160–780/mm^3) and CD8$^+$ count of 1164/mm^3 (770–2730/mm^3). HIV-1-resistant sex workers had a mean CD4$^+$ count of 975/mm^3 (660–1602/mm^3) and CD8$^+$ count of 894/mm^3 (320–1360/mm^3).

Phenotypic analysis of CMC

Phenotypic analysis was performed on CMC specimens from three HIV-1-resistant sex workers (representative example shown in Fig. 1), and two HIV-1-infected sex workers. A well-defined CD8$^+$ lymphocyte population was present in all samples, with CD8$^+$ lymphocytes comprising 11.8–24.1% of the gated lymphocyte subpopulation (0.1–0.4% of total events). No differences were noted in CD8$^+$ lymphocyte frequencies according to HIV-1 infection status.

HIV-1-specific ELISPOT responses are common in the blood and cervix of HIV-1-resistant and -infected sex workers

HIV-1-specific IFN-γ responses were found in the cervix and blood of 11 of 16 (69%) HIV-1-resistant sex workers (Table I; Fig. 2) and in the cervix and blood of 8 of 11 (73%) HIV-1-infected sex workers (Table I). HIV-1-specific responses in the cervix were associated with a systemic response to the same HIV-1 CTL epitope in 9 of 11 (82%) resistant and 7 of 8 (88%) infected sex workers. HIV-1-specific responses were localized to the blood in

Table I. *Frequency and HIV-1 epitope specificity of IFN-γ ELISPOT responses in the blood and cervix of 16 HIV-1-resistant Kenyan sex workers,*[a] 11 HIV-1-infected Kenyan sex workers, and 7 HIV-1-uninfected, lower-risk Kenyan controls[b]

	Subject	HLA Type	HIV-1 CTL Epitope	HLA Restricted	Protein (clade)	Systemic Frequency[c]	Cervical Frequency[d]
HIV-1-resistant sex workers	ML851	A*0101,6802	TPGPGVRYPL	B7(*8101)	nef(B)	**22**	**80**
		B58,*8101	SPRTLNAWV	B7(*8101)	p24(B)	**335**	**263**
	ML889	A1,*6802	DTVLEDINL	A*6802	pol(A)	**80**	**20**
		B*4901,53	ETAYFILKL	A*6802	pol(AD)	0	10
	ML1250	A*0202,*3301	SLYNTVATL	A2	p17(B)	0	0
		B58,15	AIFQSSMTK	A33	pol(B)	5	20
	ML1260	A*0214	YPLTFGWCF	B18	nef(D)	0	**50**
		B*1801,*1503	ALKHRAYEL	A2	nef(A/D)	0	0
	ML1275	A1,*3001	LSPRTLNAW	B57/58	p24(A)	**24**	0
		B57,18					
	ML1356	A29,*3001	VSFEPIPIHY	A29	gp120(B)	**24**	0
		B*1503,42					
	ML1358	A*0201,*6802	LSPRTLNAW	B57/58	p24(A)	3	0
		B42,57					
	ML1437	A*0214,*6802	DTVLEDINL	A*6802	pol(A)	**85**	**20**
		B53,72					
	ML1488	A3,*3002	KIRLRPGGK	A3	p17(B)	**40**	**80**
		B49,67					
	ML1490	A23,36	DLNMMLNIV	B14	p24(A)	**95**	**60**
		B*1402,*4901	DRFWKTLRA	B14	p24(B)	0	10
	ML1589	A1,*3002	LSPRTLNAW	B57/58	p24(A)	**50**	**25**
		B58					
	ML1601	A*0202,*6802	ALKHRAYEL	A2	nef(A/D)	40	10
		B47,58	DTVLEDINL	A*6802	pol(A)	**128**	**50**
			ETAYFILKL	A*6802	pol(A)	3	**40**
	ML1622	A1,3	LSPRTLNAW	B57/58	p24(A)	**120**	**200**
		B*44031,57	KIRLRPGGK	A3	p17(A)	**120**	**133**
	ML1643	A*6601,*3402	LSPRTLNAW	B57/58	p24(A)	0	0
		B*44031,58					
	ML1792	A3,*3303	DLNMMLNIV	B14	p24(A)	**20**	**25**
		B*1402,*1516					
	ML1803	A2	ALKHRAYEL	A2	nef(A/D)	15	0
		B18,53	ATPQDLNMML	B53	p24(A)	25	**475**
HIV-1-infected sex workers	ML646	A30,74	LSPRTLNAW	B57/58	p24(A)	355	270
		B57,58					
	ML768	A*0201,32	SLFNTVATL	A2	p17(A)	0	0
		B57,58	ILKDPVHGV	A2	pol(A)	10	3
	ML857	A*3004,*6801	VPLRPMTY	B35	nef(B)	**170**	**100**
		B35,*4501					
	ML1211	A*0214,24	SLFNTVATL	A2	p17(A)	**820**	15
		B18,*1503	SLYNTVATL	A2	p17(B)	**892**	25
	ML1387	A30,*3301	YPLTFGWCF	B18	nef(D)	**1993**	**930**
		B14,18	FRDYVDFRFK	B18	p24(B)	14	0
	ML1410	A*2301,*3001	YPLTFGWCF	B18	nef(D)	**2243**	**160**
		B18,42					
	ML1535	A*3001	YPLTFGWCF	B18	nef(D)	10	**40**
		B*1801,42	FRDYVDFRFK	B18	p24(B)	0	0
	ML1549	A*0202,24	SLYNTVATL	A2	p17(B)	**460**	**200**
		B47,*4902					
	ML1575	A*0201	SLFNTVATL	A2	p17(A)	**20**	**300**
		B42,70					
	ML1592	A*0201	SLFNTVATL	A2	p17(A)	**494**	0
		B15	SLYNTVATL	A2	p17(B)	**46**	**36**
	ML1665	A1,2	SLFNTVATL	A2	p17(A)	4	8
		B37,57	SLYNTVATL	A2	p17(B)	12	12
HIV-1-uninfected controls	MCH	A29,30	VSFEPIPIHY	A29	gp120(B)	13	15
	116413	B35,57					
	MCH	A32,*6802	LSPRTLNAW	B57/58	p24(A)	5	10
	116433	B*0801,58	DTVLEDINL	A*6802	pol(A)	10	3
			ETAYFILKL	A*6802	pol(A)	18	8
	MCH	A24,29	ATPQDLNMML	B53	p24(A)	10	20
	116434	B18,53	YLRDQQLL	A24	gp41(A)	3	10

[a] HIV-1 resistance as defined by Fowke et al. (26).

[b] Responses meeting the definition of a positive HIV-1-specific IFN-γ ELISPOT (see *Materials and Methods*) are shown in bold. In general, HIV-1-specific responses were considered positive if they exceeded background (spontaneous) rates of IFN-γ release by a factor of ≥2 and were present at a frequency of ≥20 SFU/10^6 input cells.

[c] HIV-1-specific SFU/10^6 PBMC.

[d] HIV-1-specific SFU/10^6 CMC.

Table I. *Continued*

Subject	HLA Type	HIV-1 CTL Epitope	HLA Restricted	Protein (clade)	Systemic Frequency[c]	Cervical Frequency[d]
MCH 116457	A2,30 B*1503	ILKEPVHGV	A2	pol(B)	0	15
MCH 116458	A2,*31012 B35,57	ALKHRAYEL	A2	nef(A)	5	0
INF	A*6602,68	LSPRTLNAW	B57/58	p24(A)	10	0
103	B58	DTVLEDINL	A*6802	pol(A)	0	0
INF	A30,*6802	LSPRTLNAW	B57/58	p24(A)	8	0
105	B47,58	DTVLEDINL	A*6802	pol(A)	10	20

three subjects (2 of 11 HIV-resistant and 1 of 8 HIV-infected subjects) and to the cervix in three subjects (2 of 11 resistant and 1 of 8 infected subjects). The ELISPOT responses of HIV-1-infected sex workers tended to be greater in magnitude than those in resistant women, both in blood (606.4 vs 62.2 SFU/10^6 PBMC; $p = 0.01$) and cervix (189.4 vs 78.4 SFU/10^6 CMC; $p = 0.2$). Rates of background (spontaneous) IFN-γ release did not vary between subjects with or without positive HIV-1-specific responses, either in the blood (27.8 vs 22.1 SFU/10^6 PBMC, respectively; $p = 0.4$) or in the cervix (73.2 vs 67.2 SFU/10^6 CMC, respectively; $p = 0.8$).

A number of HIV-1 CTL epitopes were recognized by resistant women in both the blood and cervix, most commonly DTVLED INL (A*6802, clade A pol; three subjects) and LSPRTLNAW (B57/58 clade A p24; two subjects). The most common CTL epitopes recognized by HIV-1-infected subjects were SLYNT VATL (A2, clade B p17; three subjects); LSPRTLNAW (B57/58, clade A p24; one subject); and YPLTFGWCF (B18, clade D nef; two subjects). However, the selection of peptide epitopes for use in the ELISPOT assays differed between HIV-1-resistant and -infected study subjects and was determined by subject HLA type and previous blood ELISPOT responses (measured as part of an ongoing study of HIV-1-specific systemic immune responses in the cohort).

Follow-up cervical specimens were obtained after a positive ELISPOT assay for one HIV-1-infected and four HIV-1-resistant sex workers. Persistent HIV-1-specific responses were found in the cervix of one of one HIV-1-infected and three of four HIV-1-resistant sex workers after an interval of 5–21 wk. The intensity of cervical responses had increased from baseline in two resistant sex workers (ML889, 20–150 SFU/10^6 CMC; ML1792, 25–67 SFU/10^6 CMC), had decreased in one (ML1622, 200–50 SFU/10^6 CMC), and were no longer detectable in one (ML1589). Data were not available concerning changes in sexual behavior over the intervening period.

HIV-1-specific IFN-γ responses are enhanced in the cervix of HIV-1-resistant sex workers

To look at the relative intensity of systemic and cervical responses, a cervical-systemic differential was calculated for each peptide epitope by subtracting the peptide-specific systemic (PBMC) response from the cervical (CMC) response. This showed that the cervical responses were relatively enriched in the HIV-1-resistant group, compared with systemic (mean differential, +13.5 SFU/10^6; p = NS), while in the HIV-1-infected group systemic responses were considerably more intense than cervical (mean differential, −320.2 SFU/10^6; $p = 0.008$ between groups).

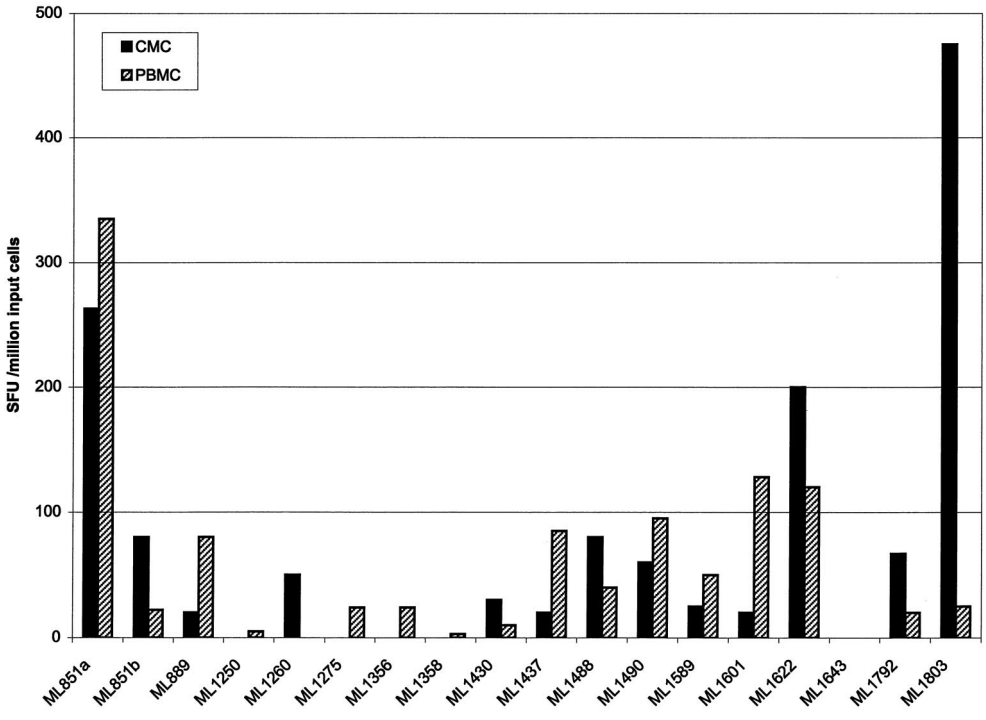


FIGURE 2. Frequency of IFN-γ ELISPOT responses in the blood and cervix of 16 HIV-1-resistant Kenyan sex workers (HIV-1 resistance as defined by Fowke et al. (26)). Responses are shown as HIV-1-specific SFU/10^6 mononuclear cells. CMC response frequencies are represented as solid bars; PBMC response frequencies are represented as cross-hatched bars. HIV-1 peptides used in the ELISPOT assays are detailed in Table I.

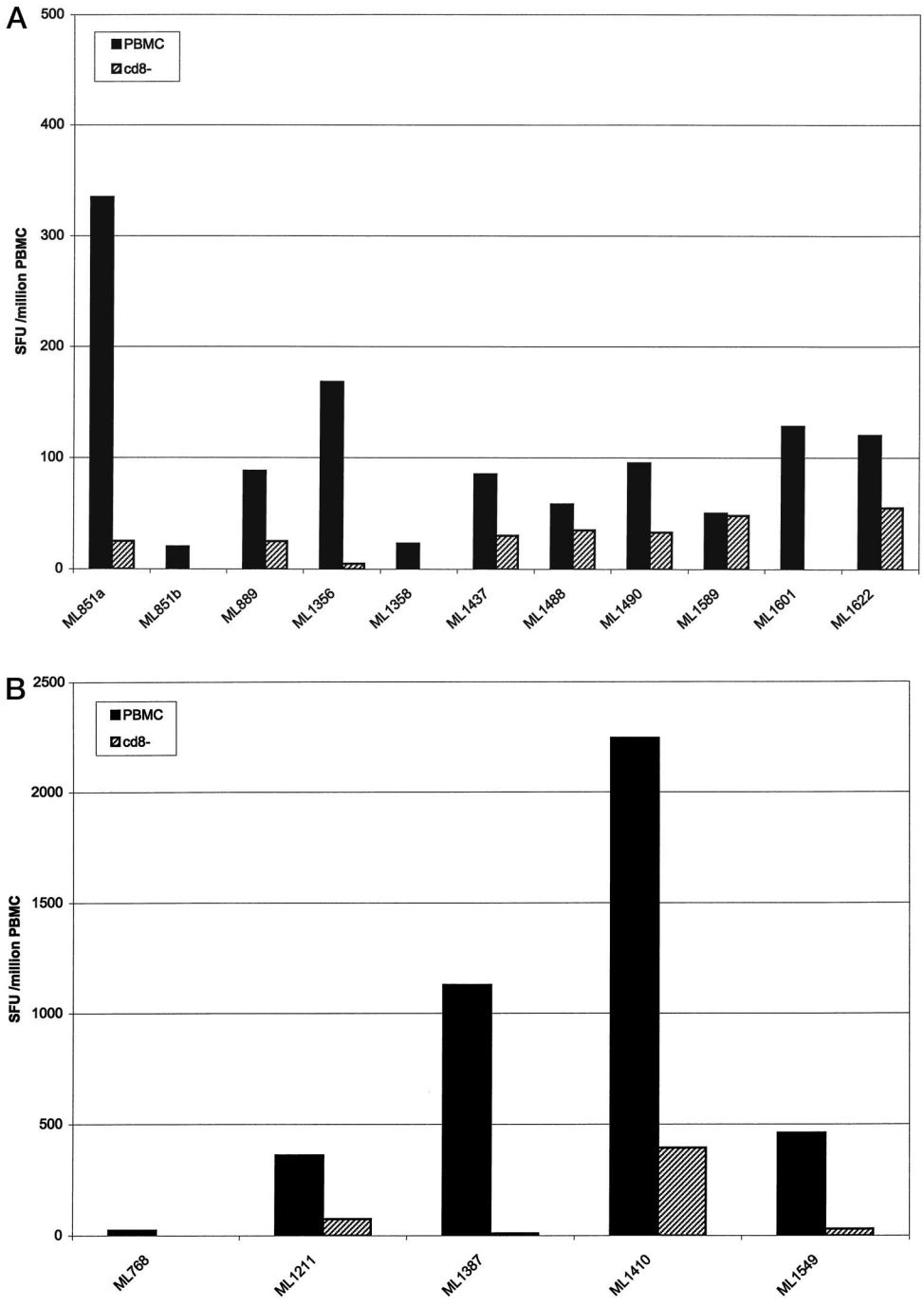


FIGURE 3. Effect of $CD8^+$ lymphocyte depletion on HIV-1-specific IFN-γ ELISPOT responses in the blood of 10 HIV-1-resistant[A] and 5 HIV-1-infected sex workers (HIV-1 resistance as defined by Fowke et al. (26)). $CD8^+$ lymphocyte depletion was performed using immunomagnetic beads coated with $CD8^+$ mAb (Dynabeads HLA cell prep I; Dynal), according to the manufacturer's instructions. Responses are shown as HIV-1-specific SFU/10^6 mononuclear cells. A response was defined as $CD8^+$ dependent if depletion resulted in ≥50% reduction in HIV-1-specific SFU. HIV-1 peptides used in the assays were as follows (with HLA restriction, HIV-1 protein, and clade specificity). *A*, HIV-1-resistant sex workers: ML851a, SPRTLNAWV (B7 p24, clade B); ML851b, TPGPGVRYPL (B7 nef, clade B); ML889, DTVLEDINL (A*6802 pol, A clade); ML1356, VSFEPIPIHY (A29 gp120, clade B); ML1358, LSPRTLNAW (B57 p24, clade A); ML1437, DTVLEDINL (A*6802 pol, A clade); ML1488, KIRLRPGGK (A3 p17, clade B); ML1490, DLNMMLNIV (B14 p24, clade A); ML1589, LSPRTLNAW (B57 p24, clade A); ML1601, DTVLEDINL (A*6802 pol, A clade); ML1622, LSPRTLNAW (B57 p24, clade A). *B*, HIV-1-infected sex workers: ML768, ILKEPVHGV (A2 pol, clade B); ML1211, SLYNTVATL (A2 p17, clade B); ML1387, YPLTFGWCF (B18 nef, clade D); ML1410, YPLTFGWCF (B18 nef, clade D); ML1549, SLYNTVATL (A2 p17, clade B).

HIV-1-specific systemic and genital tract IFN-γ responses are diminished or abrogated by $CD8^+$ lymphocyte depletion

$CD8^+$ depletion assays were performed using PBMC from 10 HIV-1-resistant and 5 HIV-1-infected sex workers and using cervical specimens from 3 resistant and 3 infected sex workers. In general, PBMC depletion assays were run using cryopreserved samples obtained at the same point in time as the original sample. Due to limited cell numbers in cryopreserved mucosal specimens, cervical $CD8^+$ depletions were run using follow-up samples obtained at a later point in time. Bead depletion resulted in a mean depletion of $CD8^+$ lymphocytes of 77% (62.2–92.1%) in PBMC assays and of 82% in cervical assays (74.9–89.5%). Systemic responses were diminished or abrogated after $CD8^+$ lymphocyte depletion in 8 of 10 (80%) resistant (Fig. 3*A*) and 5 of 5 (100%) HIV-1-infected (Fig. 3*B*) sex workers; cervical responses were diminished or abrogated in all HIV-1-resistant and HIV-1-infected individuals (Fig. 4).

HIV-1 CTL epitopes are not recognized in the blood or cervix of lower-risk control women

Responses to class I-restricted HIV-1 CTL epitopes were studied in the blood and cervix of seven HIV-1-uninfected, lower-risk Kenyan women (Table I), largely using peptides to which responses had been detected in at least one HIV-1-resistant sex worker. No HIV-1-specific cellular responses were found in the blood or cervix of these lower-risk controls (Table I; $p = 0.02$ for both comparisons).

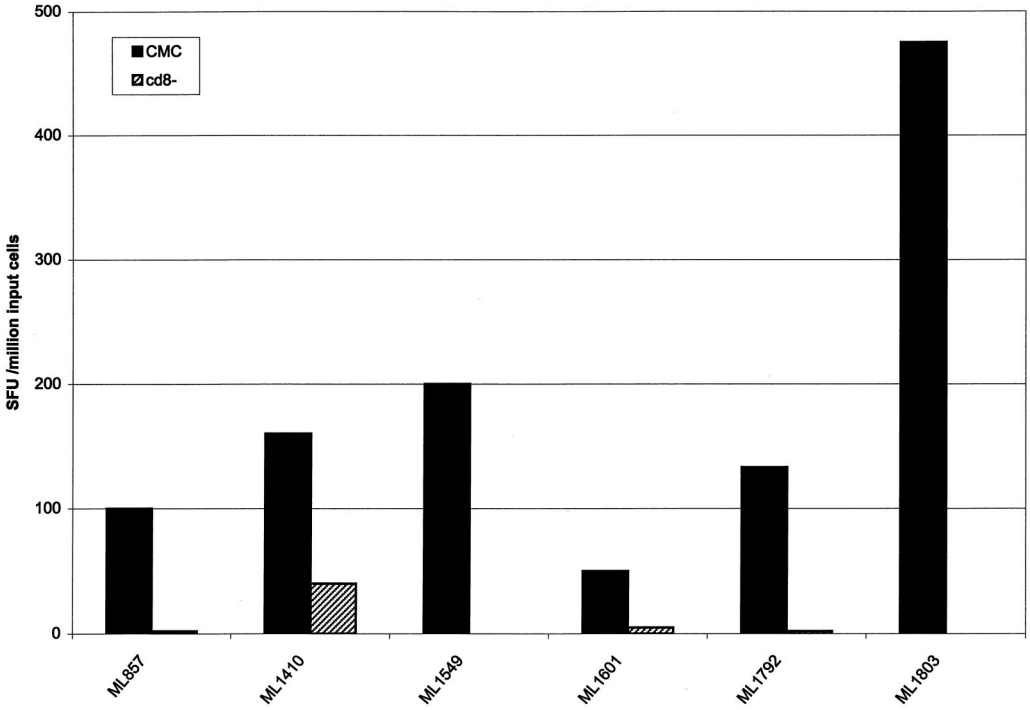


FIGURE 4. Effect of CD8$^+$ lymphocyte depletion on HIV-1-specific IFN-γ ELISPOT responses in the cervix of three HIV-1-infected and three HIV-1-resistant sex workers (HIV-1 resistance as defined by Fowke et al. (26)). CD8$^+$ lymphocyte depletion was performed using immunomagnetic beads coated with CD8$^+$ mAb (Dynabeads HLA cell prep I; Dynal), according to the manufacturer's instructions. Responses are shown as HIV-1-specific SFU/10^6 mononuclear cells. HIV-1 infection status and HIV-1 CTL peptides (with HLA restriction, HIV-1 protein, and clade restriction) used were as follows: HIV-1 infected: ML857, VPLRPMTY (B35 nef, clade B); ML1410, YPLTFGWCF (B18 nef, clade A); ML1549, SLYNTVATL (A2 p17, clade B); HIV-1 resistant: ML1601, DTVLEDINL (A*6802 pol, A clade); ML1792, DLNMMLNIV (B14 p24, clade A); ML1803, ATPQDLNMML (B53 p24, clade A).

HIV-1-resistant women do not respond to HLA-mismatched HIV-1 peptides

To confirm that these responses were HLA restricted, PBMC or CMC from HIV-1-resistant sex workers were incubated with HLA class I-mismatched CTL epitopes. The majority of HIV-1 peptides selected had been recognized by at least one HIV-1-resistant woman in HLA-matched assays, including: DTVLEDINL (A*6802, clade A pol); VSFEPIPIHY (A29, clade B gp120); LSPRTLNAW (B57/58, clade A

Table II. *HIV-1-specific immune responses demonstrated to date in the 16 HIV-1-resistant[a] study subjects[b]*

Study Subject	Cervical ELISPOT[c]	PBMC ELISPOT[c]	HIV-1 *env* Th Response[d]	HIV-1 *env* Bulk CTL[e]	HIV-1 Peptide Bulk CTL[f]	HIV-1-Specific Mucosal IgA[g]	HIV-1-Neutralizing Mucosal IgA[h]
ML851	1	1		1	1		
ML889	1	1		1			
ML1250	0	0		1			1
ML1260	1	0	1	0		0	1
ML1275	0	1	1			1	
ML1356	0	1					
ML1358	0	0	0	0		0	1
ML1437	1	1		0	1		
ML1488	1	1					
ML1490	1	1		1	1		
ML1589	1	1	1			1	
ML1601	1	1					1
ML1622	1	1					
ML1643	0	0	1			1	
ML1792	1	1	1	0			
ML1803	1	1	0	1			1

[a] HIV-1 resistance as defined by Fowke et al. (26).
[b] "1" indicates a positive assay result, and "0" indicates a negative result. Where a space is left blank, the assay has not been previously performed.
[c] See text for definition of a positive cervical and PBMC ELISPOT.
[d] Defined as an IL-2 stimulation index of ≥ 4 in response to stimulation by two or more antigenic HIV-1 envelope peptides (7).
[e] Defined as >10% specific lysis by bulk CTL line of autologous BCL, infected by HIV-1 *env*/vaccinia construct (16).
[f] Defined as >10% specific lysis by bulk CTL line of autologous BCL, pulsed with class I HLA-restricted HIV-1 CTL epitope (15).
[g] Defined as levels of HIV-1-specific cervical or vaginal IgA >2 SD above mean levels found in low-risk Kenyan controls (7).
[h] Defined as ≥⅔ (67%) reduction of clade B HIV-1 p24 in culture supernatant, in the presence of purified mucosal IgA (34).

gag); ATPQDLNMML (B53, clade A p24); DLNMMLNIV (B14, clade A p24); and VPLRPMTY (B35, clade B nef). No responses were seen to these HLA-mismatched epitopes in six of six PBMC and two of two CMC specimens from HIV-1-resistant sex workers (data not shown).

Associations with HIV-1-specific ELISPOT responses

No association was found between cervical or systemic responses and total $CD4^+$ or $CD8^+$ lymphocyte counts in either HIV-1-resistant or HIV-1-infected subjects (data not shown). Neither systemic nor mucosal HIV-1-specific IFN-γ responses were associated with sexual risk behaviors as reported by study subjects at the time of study enrollment, including number of clients per day, duration of sex work, or frequency of condom use (data not shown). Because women with STI were excluded from the study, no data was available to examine the possible influence of concurrent STI on systemic or mucosal HIV-1-specific responses.

Discussion

This is the first demonstration of HIV-1-specific $CD8^+$ lymphocyte responses in the cervix of HIV-exposed, seronegative individuals. The possibility that genital tract CTL might play a key role in protection against sexually acquired HIV-1 infection was suggested by Belyakov et al., who showed that mucosal HIV-specific $CD8^+$ CTL conferred long-lasting immune resistance to mucosal viral transmission in mice (18), while systemic (splenic) CTL alone were unable to protect against mucosal transmission. More recently, Murphey-Corb et al. have shown that MHC class I-restricted CTL directed against viral *env* in the jejunal lamina propria are absolutely correlated with protection from colonic SIV challenge (19).

The exact mechanism by which these mucosal CTL could function is not yet clear. HIV-1 can cross into the submucosa by transcytosis across a tight epithelial barrier, a process that can be inhibited by HIV-specific IgA (30). Furthermore, $CD4^+$ and $CCR5^+$ cell populations, which are susceptible to productive HIV-1 infection, are present in this region (31, 32). The cervical submucosa is well-supplied with $CD8^+$ T lymphocytes, both of a memory ($CD45RO^+$) and effector (perforin$^+$) phenotype (33), and $CD3^+$ cytolytic activity is present in the genital tract throughout the menstrual cycle (31–33). Therefore, we hypothesize that HIV-1-specific cervical CTL in the submucosa may target susceptible host cells, which have undergone productive infection by HIV-1 after viral transcytosis across the epithelial tight membrane.

HIV-specific systemic (blood) responses have been previously described in our study cohort of HIV-1-resistant sex workers (15, 16); however, the vast majority of HIV-1 transmission in this population occurs across the genital mucosa during heterosexual intercourse (25). Therefore, HIV-specific CTL in the cervix might play a more direct role than do systemic responses in immune-mediated protection against sexually acquired HIV-1 infection. If these mucosal HIV-specific CTL in the genital tract play a critical role in protection against HIV-1 infection in this prostitute population, then enrichment of HIV-1-specific $CD8^+$ responses in the cervix might be predicted, because this is the site of viral exposure. In keeping with this hypothesis, we found that cervical responses were slightly enhanced relative to systemic in HIV-resistant prostitutes, while HIV-infected women showed considerable enrichment of HIV-specific responses in the blood. Although overall responses were weaker in HIV-resistant than HIV-infected prostitutes, their relative enhancement at the site of repeated viral exposure is consistent with the hypothesis that they play a major role in protection against heterosexual transmission of HIV-1.

Musey and colleagues have demonstrated that HIV-1-specific CTL (involving both $CD4^+$ and $CD8^+$ cells) could be generated from cervical specimens in HIV-1-infected women (20). In these HIV-1-infected women, comparisons of intraindividual cervical and blood CTL specificities also indicated that epitopes recognized by CTL in the cervix were commonly recognized in the blood, although relative frequencies of CTL in cervix and blood were not examined. The importance of these responses in the genital tract of HIV-1-infected individuals is not clear, although they could potentially play a part in reducing HIV transmission to sexual partners.

This study used an IFN-γ ELISPOT assay to demonstrate $CD8^+$ lymphocyte-mediated HIV-1-specific responses from cervical and blood specimens. ELISPOT has been previously used to document HIV-specific responses in both HIV-1-infected and HEPS donors (15, 21). Estimates of Ag-specific $CD8^+$ lymphocyte frequencies using ELISPOT correlate well with those measured by either tetrameric MHC-peptide complexes or limiting dilution CTL assays (22). However, the ELISPOT technique has the advantage over tetramer staining of increased sensitivity at low precursor frequencies (1/50,000 as opposed to 1/5,000 (23)), and ELISPOT also allows rapid screening of responses against a wide variety of HIV-1 CTL epitopes, many of which are not currently available as MHC-peptide tetramers. In comparison to limiting dilution assays, the ELISPOT is a relatively rapid assay and so is less subject to overgrowth by genital tract flora. In addition, ELISPOT requires fewer effector cells than limiting dilution assays, making it better suited to analysis of samples with relatively few T lymphocytes.

Previous studies of HIV-specific immune responses in the genital tract of HEPS subjects have been confined to the detection of HIV-1-specific IgA, both in this cohort of HIV-1-resistant Kenyan sex workers (7) and other exposed uninfected groups (6, 8). Eight of the 16 (50%) HIV-1-resistant subjects in the present study have been enrolled in previous studies of genital tract IgA (7, 34); either HIV-1-specific IgA or HIV-neutralizing mucosal IgA was found in eight of eight (Table II), including three subjects who had no demonstrable $CD8^+$ lymphocyte responses in our study. Several study subjects have also been tested for systemic HIV-1 *env*-specific Th responses (detected in five of seven) (7); for systemic CTL responses against an HIV-1 *env*/vaccinia construct (detected in five of nine) (16); and for systemic CTL responses against class I HLA-restricted HIV-1 peptide epitopes (detected in three of three) (15) (Table II). However, many of these data were only available for a small subset of study subjects, and the data were not collected at the same time as those in the current study. Because HIV-specific immune response frequencies may increase or wane over time (10), analysis of the association between various responses based on these data may therefore not be warranted.

An alternate explanation for the presence of HIV-1-specific immune responses in HEPS individuals is that these persons are infected with HIV-1, and that the infection is locally contained. This hypothesis could perhaps account for the persistence of both mucosal and systemic HIV-1-specific humoral and cellular immune responses. This possibility has recently been given credence by work from Corey and colleagues, who have amplified HIV-1 viral sequences from bulk $CD4^+$ lymphocytes in a minority of HEPS subjects (35). While this hypothesis cannot be addressed by the current study, it should be noted that those HIV-1-resistant women enrolled in the current study have remained HIV-1 seronegative and PCR negative, in the face of normal CD4/$CD8^+$ lymphocyte numbers, for many years. For instance, subject ML851 has been followed in the Pumwani clinic for 11 years, with CD4/8^+ lymphocyte counts in 1998 of 880/mm^3 and 320/mm^3, respectively. Indeed, if these women have managed to contain HIV-1 infection

for this considerable period of time, while the average time for progression to HIV-1 stage IVC disease in the Pumwani cohort is only 4.4 years (36), then further elucidation of immune responses in this group are still likely to be crucial to effective vaccine design.

The B57-restricted HIV-1 CTL epitope LSPRTLNAW, derived from the p24 region of A clade HIV-1, was recognized in several B58$^+$/B57$^-$ women from both HIV-1-infected and -resistant sex worker groups. This is not unexpected, given that cross-presentation of B57-restricted HIV-1 epitopes by the structurally related B*5801 molecule (which is more common in African populations) has been well described (37, 38). Similarly, the B7 restricted epitopes TPGPGVRYPL (HIV-1 nef) and SPRTLNAWV (HIV-1 p24) were recognized in resistant sex worker ML851, who has B*8101 rather than B7; this is also likely to be due to cross-presentation by the closely related African B*8101 molecule (39, 40).

In summary, we have demonstrated CD8$^+$ lymphocyte-mediated IFN-γ responses to HIV-1 CTL peptide epitopes in the cervix of highly exposed, uninfected Kenyan sex workers. The specificity of these responses was generally mirrored by systemic (PBMC) responses. These HIV-1-specific responses were enhanced in the genital tract at the likely site of repeated viral exposure and persisted in some subjects for up to 5 mo. These responses were shown to be mediated by CD8$^+$ T cells using depletion experiments in both HIV-resistant and HIV-infected subjects and were not found in lower-risk control donors. These data suggest that HIV-1-specific CD8$^+$ responses in the genital tract may play a role in protection against heterosexual HIV-1 infection in exposed, uninfected individuals.

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References

1. Rowland-Jones, S. L., and A. McMichael. 1995. Immune responses in HIV-exposed seronegatives: have they repelled the virus? *Curr. Opin. Immunol. 7:448.*
2. Paxton, W. A., R. Liu, S. Kang, L. Wu, T. R. Gingeras, N. R. Landau, C. R. Mackay, and R. A. Koup. 1998. Reduced HIV-1 infectability of CD4$^+$ lymphocytes from exposed-uninfected individuals: association with low expression of CCR5 and high production of β-chemokines. *Virology 244:66.*
3. Fowke, K. R., T. Dong, S. L. Rowland-Jones, J. Oyugi, W. J. Rutherford, J. Kimani, P. Krausa, J. Bwayo, J. N. Simonsen, G. M. Shearer, and F. A. Plummer. 1998. HIV type 1 resistance in Kenyan sex workers is not associated with altered cellular susceptibility to HIV type 1 infection or enhanced β-chemokine production. *AIDS Res. Hum. Retroviruses 14:1521.*
4. 1995. World Health Organization: the current global situation of the HIV/AIDS pandemic. *Wkly. Epidemiol. Rec. 70:7.*
5. d'Cruz-Grote, D. 1996. Prevention of HIV infection in developing countries. *Lancet 348:1071.*
6. Beyrer, C., A. W. Artenstein, S. Rugpao, H. Stephens, T. C. VanCott, M. L. Robb, M. Rinkaew, D. L. Birx, C. Khamboonruang, P. A. Zimmerman, et al. 1999. Epidemiologic and biologic characterization of a cohort of human immunodeficiency virus type 1 highly exposed, persistently seronegative female sex workers in northern Thailand: Chiang Mai HEPS Working Group. *J. Infect. Dis. 179:59.*
7. Kaul, R., D. Trabattoni, J. J. Bwayo, D. Arienti, A. Zagliani, F. M. Mwangi, C. Kariuki, E. N. Ngugi, K. S. MacDonald, T. B. Ball, et a. 1999. HIV-1-specific mucosal IgA in a cohort of HIV-1-resistant Kenyan sex workers. *AIDS 13:23.*
8. Mazzoli, S., D. Trabattoni, S. Lo Caputo, S. Piconi, C. Ble, F. Meacci, S. Ruzzante, A. Salvi, F. Semplici, R. Longhi, et al. 1997. HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. *Nat. Med. 3:1250.*
9. Clerici, M., J. V. Giorgi, C. C. Chou, V. K. Gudeman, J. A. Zack, P. Gupta, H. N. Ho, P. G. Nishanian, J. A. Berzofsky, and G. M. Shearer. 1992. Cell-mediated immune response to human immunodeficiency virus (HIV) type 1 in seronegative homosexual men with recent sexual exposure to HIV-1. *J. Infect. Dis. 165:1012.*
10. Goh, W. C., J. Markee, R. E. Akridge, M. Meldorf, L. Musey, T. Karchmer, M. Krone, A. Collier, L. Corey, M. Emerman, and M. J. McElrath. 1999. Protection against human immunodeficiency virus type 1 infection in persons with repeated exposure: evidence for T cell immunity in the absence of inherited CCR5 coreceptor defects. *J. Infect. Dis. 179:548.*
11. Langlade-Demoyen, P., N. Ngo-Giang-Huong, F. Ferchal, and E. Oksenhendler. 1994. Human immunodeficiency virus (HIV) nef-specific cytotoxic T lymphocytes in noninfected heterosexual contact of HIV-infected patients. *J. Clin. Invest. 93:1293.*
12. Pinto, L. A., J. Sullivan, J. A. Berzofsky, M. Clerici, H. A. Kessler, A. L. Landay, and G. M. Shearer. 1995. ENV-specific cytotoxic T lymphocyte responses in HIV seronegative health care workers occupationally exposed to HIV-contaminated body fluids. *J. Clin. Invest. 96:867.*
13. Rowland-Jones, S. L., D. F. Nixon, M. C. Aldhous, F. Gotch, K. Ariyoshi, N. Hallam, J. S. Kroll, K. Froebel, and A. McMichael. 1993. HIV-specific cytotoxic T-cell activity in an HIV-exposed but uninfected infant. *Lancet 341:860.*
14. Rowland-Jones, S., J. Sutton, K. Ariyoshi, T. Dong, F. Gotch, S. McAdam, D. Whitby, S. Sabally, A. Gallimore, T. Corrah, et al. 1995. HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women. *Nat. Med. 1:59.*
15. Rowland-Jones, S. L., T. Dong, K. R. Fowke, J. Kimani, P. Krausa, H. Newell, T. Blanchard, K. Ariyoshi, J. Oyugi, E. Ngugi, et al. 1998. Cytotoxic T cell responses to multiple conserved HIV epitopes in HIV-resistant prostitutes in Nairobi. *J. Clin. Invest. 102:1758.*
16. Kaul, R., K. Fowke, E. Njagi, J. Rutherford, K. S. MacDonald, I. Onyango, J. J. Bwayo, and F. A. Plummer. 1998. HIV-1 specific cytotoxic T-lymphocyte responses in uninfected Kenyan prostitutes: behavioural and immunologic correlates (Abstr. 600/31123). In *Twelfth International Conference on AIDS*, vol. 1. International AIDS Society, Geneva, Switzerland, p. 519.
17. Zhang, C., Y. Cui, S. Houston, and L. J. Chang. 1996. Protective immunity to HIV-1 in SCID/beige mice reconstituted with peripheral blood lymphocytes of exposed but uninfected individuals. *Proc. Natl. Acad. Sci. USA 93:14720.*
18. Belyakov, I. M., J. D. Ahlers, B. Y. Brandwein, P. Earl, B. L. Kelsall, B. Moss, W. Strober, and J. A. Berzofsky. 1998. The importance of local mucosal HIV-specific CD8(+) cytotoxic T lymphocytes for resistance to mucosal viral transmission in mice and enhancement of resistance by local administration of IL-12. *J. Clin. Invest. 102:2072.*
19. Murphy-Corb, M., L. A. Wilson, A. M. Trichel, D. E. Roberts, K. Xu, S. Ohkawa, B. Woodson, R. Bohm, and J. Blanchard. 1999. Selective induction of protective MHC class I-restricted CTL in the intestinal lamina propria of rhesus monkeys by transient SIV infection of the colonic mucosa. *J. Immunol. 162:540.*
20. Musey, L., Y. Hu, L. Eckert, M. Christensen, T. Karchmer, and M. J. McElrath. 1997. HIV-1 induces cytotoxic T lymphocytes in the cervix of infected women. *J. Exp. Med. 185:293.*
21. Herr, W., U. Protzer, A. W. Lohse, G. Gerken, K. H. Meyer zum Buschenfelde, and T. Wolfel. 1998. Quantification of CD8$^+$ T lymphocytes responsive to human immunodeficiency virus (HIV) peptide antigens in HIV-infected patients and seronegative persons at high risk for recent HIV exposure. *J. Infect. Dis. 178:260.*
22. Tan, L. C., N. Gudgeon, N. E. Annels, P. Hansasuta, C. A. O'Callaghan, S. Rowland-Jones, A. J. McMichael, A. B. Rickinson, and M. F. Callan. 1999. A re-evaluation of the frequency of CD8$^+$ T cells specific for EBV in healthy virus carriers. *J. Immunol. 162:1827.*
23. Lalvani, A., R. Brookes, S. Hambleton, W. J. Britton, A. V. Hill, and A. J. McMichael. 1997. Rapid effector function in CD8$^+$ memory T cells. *J. Exp. Med. 186:859.*
24. Ngugi, E. N., F. A. Plummer, J. N. Simonsen, D. W. Cameron, M. Bosire, P. Waiyaki, A. R. Ronald, and J. O. Ndinya-Achola. 1988. Prevention of transmission of human immunodeficiency virus in Africa: effectiveness of condom promotion and health education among prostitutes. *Lancet 2:887.*
25. Simonsen, J. N., F. A. Plummer, E. N. Ngugi, C. Black, J. K. Kreiss, M. N. Gakinya, P. Waiyaki, L. J. D'Costa, J. O. Ndinya-Achola, P. Piot, et al. 1990. HIV infection among lower socioeconomic strata prostitutes in Nairobi. *AIDS 4:139.*
26. Fowke, K. R., N. J. Nagelkerke, J. Kimani, J. N. Simonsen, A. O. Anzala, J. J. Bwayo, K. S. MacDonald, E. N. Ngugi, and F. A. Plummer. 1996. Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. *Lancet 348:1347.*
27. Plummer, F. A., T. B. Ball, J. Kimani, and K. R. Fowke. 1999. Resistance to HIV-1 infection among highly exposed sex workers in Nairobi: what mediates protection and why does it develop? *Immunol. Lett. 66:27.*
28. Bunce, M., C. M. O'Neill, M. C. Barnardo, P. Krausa, M. J. Browning, P. J. Morris, and K. I. Welsh. 1995. Phototyping: comprehensive DNA typing for HLA-A, B, C, DRB1, DRB3, DRB4, DRB5 & DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (PCR-SSP). *Tissue Antigens 46:355.*
29. Dawood, M. R., R. Allan, K. Fowke, J. Embree, and G. W. Hammond. 1992. Development of oligonucleotide primers and probes against structural and regulatory genes of human immunodeficiency virus type 1 (HIV-1) and their use for amplification of HIV-1 provirus by using polymerase chain reaction. *J. Clin. Microbiol. 30:2279.*
30. Bomsel, M., M. Heyman, H. Hocini, S. Lagaye, L. Belec, C. Dupont, and C. Desgranges. 1998. Intracellular neutralization of HIV transcytosis across tight epithelial barriers by anti-HIV envelope protein dIgA or IgM. *Immunity 9:277.*
31. Yeaman, G. R., H. D. White, A. Howell, R. Prabhala, and C. R. Wira. 1998. The mucosal immune system in the human female reproductive tract: potential insights into the heterosexual transmission of HIV. *AIDS Res. Hum. Retroviruses 14(Suppl. 1):S57.*
32. Patterson, B. K., A. Landay, J. Andersson, C. Brown, H. Behbahani, D. Jiyamapa, Z. Burki, D. Stanislawski, M. A. Czerniewski, and P. Garcia. 1998. Repertoire of chemokine receptor expression in the female genital tract: implications for human immunodeficiency virus transmission. *Am. J. Pathol. 153:481.*

33. Olaitan, A., M. A. Johnson, A. MacLean, and L. W. Poulter. 1996. The distribution of immunocompetent cells in the genital tract of HIV-positive women. *AIDS 10:759.*
34. Broliden, K., J. Hinkula, C. Devito, S. Frankel, R. Kaul, and M. Clerici. 1999. HIV specific IgA: neutralizing responses in mucosal samples from HIV-exposed, seronegative individuals (Abstr. 105). In *HIV Vaccine Development: Opportunities and Challenges*, Keystone, CO, p. 34.
35. Zhu, T., L. Corey, R. Akridge, Y. Change, F. Feng, J. Kim, C. Alef, A. McElroy, J. Mullins, and J. McElrath. 1999. Evidence for HIV-1 latent infection in exposed seronegative individuals (Abstr. 8). In *6th Conference on Retroviruses and Opportunistic Infections*, Chicago.
36. Anzala, O. A., N. J. D. Nagelkerke, J. J. Bwayo, D. Holton, S. Moses, E. N. Ngugi, J. O. Ndinya-Achola, and F. A. Plummer. 1995. Rapid progression to disease in African sex workers with human immunodeficiency virus type 1 infection. *J. Inf. Dis. 171:686.*
37. Goulder, P. J., M. Bunce, P. Krausa, K. McIntyre, S. Crowley, B. Morgan, A. Edwards, P. Giangrande, R. E. Phillips, and A. J. McMichael. 1996. Novel, cross-restricted, conserved, and immunodominant cytotoxic T lymphocyte epitopes in slow progressors in HIV type 1 infection. *AIDS Res. Hum. Retroviruses 12:1691.*
38. Klein, M. R., S. H. van der Burg, E. Hovenkamp, A. M. Holwerda, J. W. Drijfhout, C. J. Melief, and F. Miedema. 1998. Characterization of HLA-B57-restricted human immunodeficiency virus type 1 Gag- and RT-specific cytotoxic T lymphocyte responses. *J. Gen. Virol. 79:2191.*
39. Skerrett, D., O. Rosina, O. Gudzowaty, L. Isola, E. Scigliano, and S. Fruchtman. 1999. Comparative analysis of HLA-B 8101 by serology, isoelectric focusing, and sequence-based typing. *Hum. Immunol. 60:168.*
40. Vilches, C., L. Sanz, R. de Pablo, M. E. Moreno, S. Puente, and M. Kreisler. 1996. Molecular characterization of the new alleles HLA-B*8101 and B*4407. *Tissue Antigens 47:139.*