CONCISE COMMUNICATION

Human Immunodeficiency Virus–Specific and CD3-Redirected Cytotoxic T Lymphocyte Activity in the Human Female Reproductive Tract: Lack of Correlation between Mucosa and Peripheral Blood

Hillary D. White,1 Luwy K. Musey,4 Mary-Margaret Andrews,2 Grant R. Yeaman,1 Leslie R. DeMars,3 Paul D. Manganiello,3 Alexandra L. Howell,1,6 Charles R. Wira,4 William R. Green,1 and M. Juliana McElrath4

CD8+ T cell phenotype and function were assessed in the female reproductive tracts (FRTs) of 3 human immunodeficiency virus (HIV)-positive patients who had undergone hysterectomy. FRT cytotoxic T lymphocyte (CTL) lytic activity from 1 patient (patient 872) was detected by using CD3-dependent redirected-lysis assay and HIV-specific assay, concomitant with the presence of CD8+ cells. In contrast, samples from the 2 other HIV-positive patients (patients 1536 and 1364), who also were asymptomatic for HIV-associated illnesses, demonstrated no CTL activity in any solid tissue tested by either assay, despite activity by autologous peripheral blood mononuclear cells (PBMC). This absence of CTL activity was correlated with a relative absence of CD8+ cells in the FRT, whereas CD8+ cells were present in PBMC. Thus, CTL activity in PBMC may fail to correlate with mucosal activity. The finding of CTL activity in the FRT of patient 872 represents the first description of CTL in upper and lower FRT tissues of an HIV-positive woman.

The effect of human immunodeficiency virus (HIV) infection on human female reproductive tract (FRT) immune cells is particularly important, since the AIDS epidemic is spreading increasingly to women within the United States and the rates of heterosexual infection are increasing in the rest of the world. Substantial evidence exists for the importance of cytotoxic T lymphocyte (CTL) activity in controlling virus load [1, 2] and disease progression [3, 4]. Simian immunodeficiency virus (SIV)–specific [5] and HIV-specific [6, 7] CTL activity previously have been identified within the lower FRT in macaque vaginal mucosa and human cervical secretions, respectively, but have not been studied in solid tissues of the human FRT.

We previously reported on the cytolytic function of CD8+ T lymphocytes within the benign (nontumor-bearing and HIV-negative) human FRT [8]. Results supported the hypothesis that CTL activity in the uterine endometrium is down-regulated, by ovarian hormones, in an antigen nonspecific fashion, to allow for the implantation of the embryo. CTL lytic activity is fully functional elsewhere in the FRT, particularly in the lower FRT (cervix and vagina), presumably to allow for immunologic protection from sexually transmitted pathogens.

Surgical hysterectomy samples from HIV-positive women are rare and difficult to obtain in quantities sufficient for analysis. In our study, we investigated whether CD8+ T cells in the FRT of 3 HIV-positive patients, who had undergone hysterectomy and who were asymptomatic for HIV-associated illnesses, were present and functioning in a pattern similar to that for HIV-negative patients, and to autologous peripheral blood mononuclear cells (PBMC) of the HIV-positive patients, or whether these FRT CD8+ T cells were compromised either in number or in lytic function. We hypothesized that CD8+ T cell lytic activity in the FRT may be dysfunctional independent of that for PBMC and secondary lymphoid tissues, since the FRT mucosa is a distinct immunologic compartment.

Patients and Methods

Profiles of HIV-positive patients who had undergone hysterectomy: Three HIV-positive patients, all asymptomatic for HIV-
Figure 1. Cytotoxic T lymphocyte (CTL) activity in the reproductive tract of human immunodeficiency virus (HIV)-positive patients, determined by use of anti-CD3 monoclonal antibody (MAb)–dependent redirected lysis assay (A–C) or HIV-specific CTL assay (D–G). A–C, CTL lytic activity by anti-CD3 MAb-dependent redirected lysis assay of freshly isolated autologous peripheral blood mononuclear cells (PBMC) and unfractionated reproductive tract cells after overnight culture. Circles, Anti-CD3 MAb bound P815 target cells; squares, control P815 target cells without anti-CD3 MAb; black symbols, exogenous interleukin (IL)-2 added before the overnight culture phase; white symbols, no exogenous IL-2 added before the culture phase. C, Spontaneous release for the minus-antibody control was high (data not shown), but there was no detectable CTL activity. Effector:target ratios are the ratio of PBMC or unfractionated reproductive tract cells to target cells. CD3⁺ T cell effector:target ratios were calculated from flow cytometric data. D–G, HIV-specific percentage of specific CTL activity of reproductive tract cells or autologous PBMC from HIV-positive or HIV-negative control patients. Target cells were Epstein-Barr virus–immortalized autologous B lymphoblastoid cells infected with recombinant vaccinia virus containing either HIV env (v-Env), gag (v-Gag), or pol (v-Pol). Levels of lysis from control vaccinia target cells were subtracted to give a Δ percentage of specific lysis. Anti–vaccinia-specific CTL activity was <10%, except in the EM1 sample of patient 872 (19%), PBMC CD4 T cells in patient 1364 (42%), and undepleted (UND) PBMC from patient 1364 (43%). Data shown are at an effector:target ratio of 5:1. D–E, Responder cells were divided into replicates at the beginning of the culture phase and were designated as “EM1,” “EM2,” etc. F–G, Responder cells were either enriched after expansion by micromagnetic bead depletion (Miltenyi Biotec), using CD56/CD16/CD4-specific antibodies (“CD8,” CD8⁺-enriched cells) or CD56/CD16/CD8-specific antibodies (“CD4,” CD4⁺-enriched T cells), or cells were UND. Purity of depletion (normally 85%–95%) was not assessed, because of insufficient numbers of cells in these experiments. CX, (endo)cervix; ECX, ectocervix; EM, uterine endometrium; FT, fallopian tube; OV, ovary; VM, vaginal mucosa.
Figure 1. (Continued.)
associated illnesses and previously vaccinated for smallpox, underwent hysterectomies because of various clinical symptoms (see below). Plasma HIV RNA copy number was measured by reverse transcriptase–polymerase chain reaction or branched DNA (bDNA) analysis, as noted.

Patient 872, a 37-year-old woman, underwent hysterectomy because of pelvic pain and irregular uterine bleeding 13 years after she was tested positive for HIV. She had a past history of injection drug use. The patient had not been on antiretroviral therapy before her hysterectomy and was considered to be a long-term nonprogressor (LTNP). The patient’s earliest recorded CD4 T cell count was 1012 cells/µL 5 years before surgery; subsequent CD4 T cell counts were 868–1670 cells/µL between first report and surgery and 1035 cells/µL 4 months before surgery. The patient’s virus load was >750,000 copies/mL 7 months before surgery (earliest available record), 91,000 to >750,000 copies/mL between diagnosis and surgery, and 196,000 copies/mL at the last time point (3.5 months) before surgery. The patient continued to have a high virus load (171,000–750,000 copies/mL) for 3 years after the surgery. The patient has remained clinically stable for >3 years after the surgery. A brief 3-month trial of antiretroviral therapy initiated 1 month after surgery resulted in a lowered virus load (4000–6000 copies/mL), which rebounded to the previous high levels after therapy was stopped. The uterine endometrium was identified as being in the early-proliferative phase of the menstrual cycle, as determined by endometrial dating analysis, which identifies the endogenous levels of estrogen and progesterone associated with histologic phenotype within the uterine endometrium.

Patient 1356, a 34-year-old woman, underwent hysterectomy for abdominal pain 2.5 years after she tested positive for HIV. Patient 1356 reportedly was infected via heterosexual transmission from her HIV-positive spouse. The patient’s CD4 count was 372 cells/µL at the time of diagnosis, 259–401 cells/µL between diagnosis and surgery and 259 cells/µL 2 months before surgery. Virus load was 15,091 copies/mL at diagnosis, and <400 copies/mL thereafter with antiretroviral therapy. Patient 1356 was on antiretroviral medication (zidovudine and lamivudine) continuously for 2 years and 5 months before undergoing hysterectomy. The uterine endometrium was identified as atrophic, which was probably due to endometrial ablation surgery that the patient had undergone ~2 years before undergoing hysterectomy. Histologic analysis of the uterus after the hysterectomy indicated that the endometrial mucosa was present but not well developed.

Patient 1364, a 34-year-old woman, underwent hysterectomy for dysplasia of the labia 8.6 years after she tested positive for HIV. She had a past history of injection drug abuse. Her CD4 T cell counts were 430 cells/µL at the time of diagnosis, 248–777 cells/µL between diagnosis and surgery and 508 cells/µL 9 days before surgery. The patient’s virus load was <10,000 copies/mL (by bDNA) at diagnosis, <400–13,500 copies/mL (bDNA) between diagnosis and surgery, and 941 copies/mL 9 days before surgery. This patient was receiving antiretroviral therapy (stavudine, lamivudine, and nevirapine) continuously for ~3.5 years before undergoing hysterectomy and zidovudine discontinuously for ~5 years before that. The uterine endometrium was identified as being in the late-secretory phase of the menstrual cycle.

Cell preparation. FRT tissue and peripheral blood samples were obtained immediately after surgery from the patients who had undergone hysterectomy. Tissues were enzymatically digested (pancreatin/hyaluronidase/collagenase) for 2 h, and cells were dispersed through a wire screen [9]. Ficoll density gradient centrifugations were used to obtain autologous PBMC.

Anti-CD3 monoclonal antibody (MAb)–dependent redirected lysis assay. Freshly dispersed unfractionated FRT cells were cultured overnight in medium with or without exogenously added interleukin (IL)–2 (10 U/mL). Cells were assayed the next day for their ability to lyse chromium-labeled FeR-bearing P815 target cells in a 6-h antigen-independent redirected lysis assay using anti-CD3 MAb (OKT3) for CTL lytic activity or, in the absence of antibody, to measure background levels of lysis [8]. Use of an isotype control MAb W6/32, directed against a monomorphic HLA-A, HLA-B, and HLA-C determinant, demonstrated that the assay is specific, as described elsewhere [8].

HIV antigen–specific CTL assay. FRT cells were isolated and cultured overnight, as described above. Effector cells were expanded initially with irradiated autologous PBMC infected overnight with recombinant vaccinia virus (rVV) encoding HIV-1LAI env, gag, and pol (D. Panicali, Therion, Cambridge, MA) and with irradiated autologous PBMC feeder cells, followed 2 weeks later by OKT3 stimulation with fresh allogeneic feeder cells. After 12 days, effector cells then were tested in a 5-h chromium-release assay against Epstein-Barr virus (EBV)–immortalized autologous B lymphoblastoid targets infected with rVV containing either HIV env, gag, or pol or control vaccinia sequence [6]. The target cells were confirmed to be susceptible to lysis.

Fluorescence-activated cell sorter (FACS) analysis. In parallel with the redirected lysis assay, cells were analyzed by using FACS analysis, after staining with fluorescein isothiocyanate (FITC)–conjugated anti-CD3 and anti-CD8 antibodies (Caltag), and by using autolysosome (FL3; far red fluorescence) versus PE-conjugated anti-CD45 Ab (FL2; Caltag) to gate on FRT leukocytes [10]. CD4 expression was not assessed on FRT cells, because of the unstable nature of this antigen after tissue dispersal.

Results

FRT samples from HIV-positive LTNP patient 872, but not those from patients 1356 and 1364, displayed a normal profile of CTL lytic function. FRT cell cultures containing CD3+ T cells were initially tested for their ability to lyse OKT3-bound FeR+ P815 target cells, to assess their overall lytic function in an antigen-independent fashion. Patient 872 demonstrated a CTL lytic function profile (figure 1A) similar to that commonly found in the FRT of HIV-negative premenopausal patients [8]. Substantial CTL activity—that is, ~35%–40% specific lysis at CD3+ T cell effector:target (E:T) ratios (range, 4:1 to 8:1)—was found in PBMC, the fallopian tube (FT), and the cervix (endocervix and ectocervix are similar with respect to CTL function [8]), with detectable activity even after culture in the absence of exogenous IL-2. Low levels of lytic activity were found in the ovary and uterine endometrium, with the levels in the latter being consistent with the low levels of CTL activity normally associated with higher levels of ovarian steroid hormones in noninfected premenopausal endometrium [8]. However, for
Figure 2. CD3 and CD8 phenotype of leukocytes in the reproductive tract of human immunodeficiency virus-positive patients who had undergone hysterectomy. Reproductive tract cells and autologous peripheral blood mononuclear cells (PBMC) were tested for CD3 and CD8 positivity by flow cytometry. Percentage of fluorescein isothiocyanate (FITC)-positive CD45+ gated leukocytes is given for each sample. Isotype control antibody values are indicated in parentheses. FL1 indicates log_{10} fluorescence of the indicated FITC-conjugated antibody. ECX, ectocervix; EM, uterine endometrium; FT, fallopian tube; OV, ovary.
patients 1356 and 1364 (figure 1B and 1C), there was no T cell lytic function in any FRT tissue tested. Samples from the lower FRT of HIV-negative individuals normally demonstrate substantial levels of lytic activity at low CD3+ E:T ratios (1:1 to 8:1) independently of hormones [8], but, for patients 1356 and 1364, there was no evidence of lytic activity in the lower FRT at these E:T ratios, in contrast to that found for patient 872. In PBMC, CTL lytic function was detected in all 3 patients, which demonstrates discordance between the FRT and PBMC for patients 1356 and 1364.

HIV antigen–specific CTL activity was present in hysterectomy samples from patient 872 but not in those from patients 1356 and 1364. Because the antigen-independent redirected lysis assay measures the potential of T cells to kill target cells but does not assess antigen specificity, we generated CTL lines [6] from the FRT of these patients who had undergone hysterectomy, to assess HIV-specific CTL activity. T cell lines cultured from LTNP patient 872 (figure 1E) demonstrated HIV-specific CTL activity in PBMC, the ovary, the uterine endometrium, and the cervix (endocervix). Only background levels (<10% specific lysis) of FRT HIV-specific CTL activity were found for patients 1356 and 1364 (figure 1F and 1G). In PBMC, in contrast to the FRT, anti-Gag-, anti-Env-, and/or anti-Pol–specific CTL activities were found for all 3 patients.

Phenotypically normal CD8+ T cells were present in the FRT of patient 872 but not patients 1356 and 1364. Within the FRT, CD8+ T cells are typically 50%–70% of the CD3+ T cells [11, 12], which are typically 50% of the FRT leukocytes [10]. By using FACS analysis, we wished to assess, in parallel assays, whether the absence of CTL activity in tissue samples from patients 1356 and 1364 was associated with an absence of CD3+ or CD8+ T cells. CD3+ T cells were found to be present in the FRT of all 3 patients (figure 2), although absolute numbers were lower for patient 1356 (data not shown). However, CD3+ T cells from patients 1356 and 1364 were not limiting in the lytic assays (CD3+ E:T ratios compared with those for HIV-negative individuals [8]; figure 1B and 1C), which indicates that the lack of lytic activity in the FRT of patients 1356 and 1364 was not due to an absence of CD3+ cells. In contrast, CD8 expression was mostly absent in the FRT of patients 1356 and 1364 but was normal in the FRT of patient 872. Thus, an absence of phenotypically normal CD8+ T cells correlated with an absence of CTL activity for patients 1356 and 1364. However, CD8 expression was normal in PBMC from all 3 patients.

Discussion

This study characterizes CTL activity in the FRT of 3 HIV-positive patients, which demonstrates the following. First, HIV-specific CTL activity is described for the first time in the upper and lower FRT (ovary, uterine endometrium, and cervix) of an HIV-positive LTNP (patient 872). The data confirm our previous finding of HIV-specific CTL activity in cervical cytobrush specimens from the lower FRT [6] and now extend that finding of HIV-specific CTL activity to tissues and sites throughout the FRT. Second, the presence of HIV-specific CTL activity in the FRT of patient 872 correlates with antigen-independent lytic function in the FRT of this individual, as determined by CD3-dependent redirected lysis assay. The low CD3-dependent CTL activity in the endometrium and relatively high CD3-dependent CTL activity in the lower FRT of patient 872 are consistent with the notion that CTL lytic function is down-regulated in the uterine endometrium in response to hormones in premenopausal women and is constitutively high in the lower FRT, acting as an immunologic barrier to pathogens [8]. Within the uterine endometrium, the different levels of lysis, determined via CD3-specific assay versus HIV-specific assay, may be due to release from hormone down-modulation after extended culture ex vivo before the HIV-specific assay. Third, in contrast to those from patient 872, FRT samples from patients 1356 and 1364 demonstrated an absence of HIV-specific CTL activity and an absence of lytic function (by redirected lysis assay), both of which correlated with CD8 expression that was dim or essentially absent within the FRT. Fourth, CTL activity in PBMC is not a good predictor of CTL activity in the FRT, since high CTL activity was demonstrated in the PBMC of patients 1356 and 1364. Thus, CTL activity in the FRT mucosa appears to be independent of CTL activity in the periphery. Furthermore, NK cell– and FcReceptor cell–mediated lysis was absent in the FRT, but not PBMC, of all 3 patients (data not shown), which suggests that the immune system was not totally intact within the FRT of patient 872. Last, the presence of high virus load with demonstrable mucosal CTL activity in LTNP patient 872 clearly is unusual, since the majority of LTNPs maintain undetectable or low virus loads. Thus, virus from patient 872 is replication competent but appears to be ineffective in impairing CD8+ T cell functions, because of either host and/or viral factors.

References


