Association of CD4 Cell Depletion and Elevated Blood and Seminal Plasma Human Immunodeficiency Virus Type 1 (HIV-1) RNA Concentrations with Genital Ulcer Disease in HIV-1–Infected Men in Malawi

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CD4 cell counts and blood plasma and seminal plasma human immunodeficiency virus type 1 (HIV-1) concentrations were compared in HIV-1 RNA–seropositive men with urethritis and with or without genital ulcer disease (GUD). GUD was associated with lower CD4 cell counts (median, 258 vs. 348/μL) and increased blood plasma HIV-1 RNA (median, 240×10^3 vs. 79.4×10^3 copies/mL). Men with nongonococcal urethritis and GUD shed significantly greater quantities of HIV-1 in semen (median, 195×10^3 vs. 4.0×10^3 copies/mL) than men with nongonococcal urethritis without GUD. These levels decreased ~4-fold following antibiotic therapy. The results indicate an association between GUD and increased blood HIV-1 RNA levels. Increased HIV-1 in semen was demonstrated in some men with GUD; such an increase could lead to increased transmission, thus complicating interpretation of the role of the genital ulcer itself in the infectiousness of HIV. Reasons for increased HIV RNA in semen in men with GUD remain to be determined.

There is epidemiologic evidence that sexually transmitted diseases (STDs), particularly those associated with genital ulceration, significantly increase the risk of acquiring human immunodeficiency virus type 1 (HIV-1) infection [1–3]. The high prevalence of such ulcerative sexually transmitted diseases (STDs) may contribute to the rapid expansion of the HIV-1 epidemic in sub-Saharan Africa. Ghys et al. [4] recently reported an independent association between genital ulcer disease (GUD) and the degree of immunosuppression (CD4 cell count) in HIV-infected female sex workers from Abidjan, Ivory Coast. They postulated that this association could result in increased transmission of HIV from women with GUD to their partners. Our group has been interested in factors that affect the concentration of HIV in semen [5]. To examine the effect of GUD on the shedding of HIV-1 in semen, we studied HIV-1–infected men attending an STD clinic in Lilongwe, Malawi [5].

Methods

The study was conducted between January and March 1996 at the STD clinic of the Lilongwe Central Hospital. A major aim of the study was to examine the effect of symptomatic urethritis on HIV-1 shedding in semen; the results have been reported elsewhere [5]. Men attending the STD clinic with symptoms of urethritis and >4 white blood cells per high-power field on microscopic examination of a urethral smear were enrolled in the study. HIV-1 serostatus was evaluated using two EIAs (HIV-1/HIV-2 EIA; Genetics Systems, Redmond, WA; and HIV-1 + 2; Murex Diagnostics Dartford, UK). If EIA results were equivocal, serostatus was evaluated by Western blot (Organon-Teknika, Durham, NC).

Semen was obtained and processed as previously described [6], without dilution. HIV-1 RNA concentrations in blood and seminal plasma were quantified by use of a nucleic acid sequence–based amplification assay (NASBA; Organon-Teknika, Boxtel, Netherlands) [7], which has been shown to reliably detect a wide variety of HIV-1 clades, including those prevalent in sub-Saharan Africa [8]. CD4 cell proportions were measured by fluorescence-activated cell sorter analysis of thawed cryopreserved peripheral blood mononuclear cells. The total number of CD4 cells was determined by multiplying the CD4 cell percent by the total lymphocyte count. Rapid plasma reagin tests were done in Malawi using the Macro-Vue Card test (Becton Dickinson Microbiology Systems, Cockeysville, MD].

All men were treated for gonorrhea with one dose of gentamicin (240 mg intramuscularly); men with Gram's-stained urethral smears negative for *Neisseria gonorrhoeae* also received 1 g of

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Informed consent was obtained from all patients participating in this study. The research was conducted according to the human experimentation guidelines of the US Department of Health and Human Services. The protocol was approved by the University of North Carolina Committee on the Protection of Human Rights and the Malawi Health Sciences Research Committee.

The results of this study do not necessarily reflect the views or policies of US Agency for International Development (USAID).

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oral azithromycin. Men with genital ulcerations were treated with one dose of benzathine penicillin and 1 g oral azithromycin [9].

Continuous data were analyzed using nonparametric tests, the Mann-Whitney *U* test for unpaired data, and the Wilcoxon signed rank test for paired comparisons. Categorical variables were compared by χ^2 analysis. The predictive value of multiple independent variables, including CD4 cell count, blood plasma HIV-1 load, and the presence or absence of gonorrhea and GUD, on HIV-1 RNA concentrations in semen was analyzed by multiple linear regression with hierarchical addition of variables. Reported *R* values are independent regression coefficients in each case. The level of significance for all analyses was set at .05.

Results

Two hundred six consecutive male STD clinic attendees with urethritis were studied; 113 of the men (55%) were confirmed to be HIV-1 seropositive. Thirty-five of the HIV-1 seropositive subjects (31%) had active GUD, compared with 18 of the seronegative men (19%; $P = .058$). Syphilis serology (rapid plasma reagin test RPR only) was reactive in 6 HIV-1–seronegative (6.5%) and 6 HIV-1–infected (5.3%) men, only 1 of whom had active genital ulceration: this individual did not donate semen or blood for HIV-1 RNA quantitation. The median peripheral blood CD4 lymphocyte count in HIV-1–seropositive patients with GUD (available for 23/35 men) was 258 cells/μL compared with 348/μL in those without GUD (cell counts available for 51/78) ($P = .069$).

Eighty-six HIV-1–seropositive men, 24 (28%) of whom had GUD, donated a semen sample at their initial visit. A baseline blood plasma sample was available for analysis for 69 of these 86 subjects. Differences between CD4 cell counts for men with or without GUD in the group that donated semen reflected differences in the study group as a whole ($P = .10$; table 1).

Overall, in the 24 men with urethritis and GUD, GUD was associated with increased blood plasma HIV-1 RNA concentrations ($P = .005$; table 1). In the subset of men with nongonococcal urethritis (NGU) and GUD, seminal HIV-1 RNA shedding was significantly increased compared with that for men with NGU alone ($P = .039$; table 1). In general, in individual men with GUD and NGU, the seminal HIV-1 RNA level was close to the blood plasma HIV-1 RNA level, whereas in men with NGU alone, the blood plasma level was typically much higher. The median ratio of individual blood plasma concentration to seminal plasma HIV-1 RNA concentration approached unity in men with NGU and GUD, while the median ratio was >14 in men with NGU alone (table 1). Two weeks after antibiotic therapy there was a significant decrease in seminal plasma HIV-1 RNA levels in men with NGU and GUD but not in men with NGU alone ($P = .017$ for comparison of week-2 and baseline seminal plasma HIV-1 RNA concentrations in GUD group; $P = .51$ in non-GUD group; table 1). By contrast, blood plasma HIV-1 RNA concentrations did not change significantly in any group of patients after antibiotic therapy (table 1).

In a multiple linear regression model, gonococcal infection and blood plasma HIV-1 RNA concentration independently predicted baseline seminal plasma HIV-1 levels in the group as a whole ($R = .57$, $P < .001$, and $R = .40$, $P = .001$, respectively), while GUD and CD4 cell count did not. For this model, overall R^2 was 0.33, $P = .0003$. GUD did not have a significant independent effect on baseline seminal plasma HIV-1 levels in patients with NGU when blood plasma HIV-1 RNA levels and CD4 cell counts were entered in the same linear regression model ($R = .23$; $P = .19$).

Discussion

In this study, CD4 cell counts and HIV-1 RNA levels in blood and semen of HIV-1–infected men with GUD and urethritis were compared with those for HIV-1 infected men with urethritis alone. The causes of GUD were not defined. However, our group recently demonstrated that among Malawi men with GUD and with or without HIV, 26% had chancroid, 29% had syphilis, and among those with nonhealing ulcers had who

Table 1. CD4 cell counts and seminal plasma (SP) and blood plasma (BP) HIV-1 RNA concentrations for patients with or without genital ulcer disease (GUD) who provided a baseline semen sample and for the subset of patients with nongonococcal urethritis (NGU).

		SP HIV-1 RNA ($\times 10^3$ copies/mL)		BP HIV-1 RNA ($\times 10^3$ copies/mL)		BP:SP HIV-1 RNA ratio	
	CD4 cells/μL	Baseline	Week 2	Baseline	Week 2	Baseline	Week 2
Urethritis with baseline semen							
GUD ($n = 24$)	258 ($n = 22$)	219	42.7 ($n = 19$)	240* ($n = 20$)	251 ($n = 13$)	1.32	5.96 ($n = 12$)
No GUD ($n = 62$)	348 ($n = 47$)	110	40.7 ($n = 55$)	79.4 ($n = 49$)	87.1 ($n = 41$)	0.85	3.47 ($n = 40$)
NGU with baseline semen							
GUD ($n = 12$)	212 ($n = 11$)	195†	42.7‡ ($n = 11$)	324 ($n = 12$)	216 ($n = 8$)	1.66	5.25
No GUD ($n = 17$)	359 ($n = 13$)	4	6.46§ ($n = 14$)	120 ($n = 11$)	49.0 ($n = 9$)	14.1	25.1

NOTE. Data are medians.
$P =$ *.005 or †.039, compared with values for men with urethritis but not GUD.
$P =$ ‡.017 or §.51, compared with baseline SP RNA concentration.

were tested, 23% herpes simplex virus [9]. Only 1 man in the current study had GUD and a positive rapid plasma reagin test, although some men may have had primary syphilis in an early stage prior to a positive serology.

HIV RNA levels in blood were significantly higher in HIV-1–infected men with coexisting GUD and urethritis compared with levels in men with urethritis alone. The higher blood virus burden in the presence of GUD may be because this group of men had more advanced HIV disease or had systemic immune activation caused by GUD itself, as has been demonstrated for other infection (e.g., herpes simplex virus) [10]. CD4 cell counts were lower in the men with GUD, as has been observed in female sex workers [4]. We believe many of the ulcers in this study were chancroid, which appears to develop more readily and be cured less easily in the presence of HIV-related immunodeficiency [11]. Genital herpes simplex virus infection would also be expected to be reactivated more frequently in more immunosuppressed persons. Consistent with these observations, we found GUD more frequently in HIV-1–seropositive men than seronegative subjects in this population.

Seminal plasma HIV-1 RNA levels were also higher in men with GUD and urethritis than in men with urethritis alone. This difference was most marked in men with NGU. Our laboratory previously demonstrated that the presence of gonorrhea and its attendant inflammation resulted in high levels of HIV RNA in seminal plasma (158×10^3 copies/mL) [5]. In the current analysis, the presence of GUD did not significantly increase HIV-1 RNA levels in the semen of men with gonorrhea (data not shown). We have also reported that treatment of NGU significantly reduced HIV-1 RNA levels in seminal plasma [5]. Our current analysis demonstrates that this effect can be ascribed to changes observed in men with NGU and GUD whose levels of HIV RNA in semen are comparable to levels in men with gonococcal urethritis.

Increased HIV-1 RNA shedding in semen in men with GUD could be explained in part by their higher blood plasma HIV-1 levels, as suggested by the multivariate analysis and by our previous work in HIV-1–infected men in North America and Europe [12]. The fact that blood HIV-1 RNA levels in men with GUD and NGU did not change with treatment (while semen concentrations were reduced) supports the hypothesis that GUD may cause compartmentalized enhancement of HIV-1 shedding. The multivariate analysis, which was of limited power, did not conclusively demonstrate an effect of GUD on seminal virus level independent of blood plasma virus level.

Our conclusions are limited somewhat by the relatively small number of patients with GUD, the confounding effects of coexisting urethritis, and by incomplete data for CD4 cell and plasma virus load measures. Further studies of the effects of GUD on HIV-1 shedding in genital secretions in larger numbers of patients with and without urethritis are warranted.

The risk of HIV-1 transmission has been linked with the size of the virus inoculum in a variety of settings [13–15]. Sexual transmission from an infected male is likely to be directly related to the concentration of virus in genital secretions. Enhanced HIV-1 transmission in the setting of mucosal STDs could be due to increased levels of viral shedding in semen [5]. GUD increases the risk of sexual acquisition of HIV-1, presumably by directly exposing susceptible submucosal cells to infectious virus in genital secretions [1, 2]. Similarly, direct contact of HIV-1–producing cells with the mucosal surface of uninfected sex partners could increase the infectiousness of patients with HIV-1 and GUD [1]. Independent of mucosal disruption, the increase of HIV in semen, which was observed in some men with GUD, could also affect transmission and complicate interpretation of studies focused on the effects of genital ulcers. Given the high prevalence of genital ulceration in sub-Saharan Africa, our findings strongly support HIV prevention programs that provide for prompt recognition and effective treatment of STDs, including GUD.

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Horizontal and Vertical Transmission of Human Immunodeficiency Virus Type 1 Dual Infections Caused by Viruses of Subtypes B and C

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This article describes a case of horizontal (heterosexual) and subsequent vertical (mother to infant) transmission of 2 human immunodeficiency viruses type 1 (HIV-1) subtypes. Dual infection in a husband, his wife, and their child was initially detected by use of a restriction fragment length polymorphism assay of the proviral protease in peripheral blood mononuclear cells. The simultaneous presence of highly similar sets of HIV-1 subtypes B and C infecting the 3 family members was confirmed by DNA sequence analysis of *pol, gag,* and *env* genes. These data, together with available epidemiologic information, may indicate that the husband's high-risk sexual behavior was the source of dual infections. Because his wife did not report such activities, it was likely that he passed HIV-1 strains to his spouse, who subsequently transmitted them to their child.

The human immunodeficiency virus type 1 (HIV-1) pandemic is now recognized as consisting of many separate epidemics caused by 10 viral subtypes [1]. With an estimated 15 million HIV-1–infected persons, the geographic distribution of viral clades is becoming more dispersed, and the simultaneous presence of multiple subtypes in certain regions has become more common. Population migration and international travel are considered responsible for the spreading of diverse HIV-1 subtypes into regions previously affected by HIV-1 strains of 1 subtype. As a consequence, the potential for HIV-1 mixed infections and genetic recombination involving strains of distinct subtypes in an infected individual has increased [2, 3]. The development of specific genetic methods has enabled identification of dual HIV-1 infections caused by subtypes B and E in Thailand [4], as well as by subtypes D and F, and subtypes B and F in Brazil [5].

Detection of naturally occurring HIV-1 multiple infections has important implications for vaccine development because it suggests that infection with 1 HIV-1 subtype may not fully protect against subsequent superinfection with distinct HIV-1 strains. Although dual HIV-1 infections caused by viruses of distinct subtypes have been documented, the transmission of 2 HIV-1 subtypes from 1 dually infected person to another has not been reported.

Materials and Methods

Specimens. Two Brazilian specimens were from a married couple (Br19 and Br20), and 1 was from their child (Br30). The

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