Association between Human Immunodeficiency Virus and Herpes Simplex Virus Type 2 Seropositivity among Male Factory Workers in Zimbabwe

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To determine the seroprevalence of herpes simplex virus type 2 (HSV-2), to identify correlates of infection, and to describe the correlation with human immunodeficiency virus (HIV) seropositivity, 224 HIV-negative and 191 HIV-positive male factory workers in Zimbabwe were screened for HSV-2–specific antibodies. HSV-2 seroprevalence was 35.7% among HIV-negative subjects and 82.7% among HIV-positive subjects. The weighted estimate of HSV-2 seroprevalence in this population is 44.6%. The correlation between HIV and HSV-2 remained significant after controlling for multiple sex partners, paying for sex, and history of sexually transmitted disease (adjusted odds ratio, 8.0; 95% confidence interval, 4.8–13.1). If the association between HSV-2 and HIV is causal, then the high seroprevalence of HIV and HSV-2 suggests that suppressive HSV-2 treatment should be considered as a strategy to reduce HIV transmission in this population. HSV-2 seroconversion may be a suitable surrogate end point to evaluate HIV prevention interventions.

Epidemiologic and biologic evidence increasingly implicate sexually transmitted diseases (STDs) as causal factors in human immunodeficiency virus (HIV) transmission [1]. The relatively high prevalence of untreated STDs in sub-Saharan Africa has been proposed as a contributing factor in the higher prevalence of heterosexually transmitted HIV in that region compared with the industrialized world [2]. The hypothesis is supported by the success of an HIV prevention intervention based on community-wide enhanced STD treatment in Mwanza, Tanzania [3].

STDs that cause genital ulceration, such as syphilis, chancroid, and herpes simplex virus type 2 (HSV-2) infection, are particularly implicated in facilitating HIV transmission [1, 4]. Genital ulcer disease (GUD) is believed to increase the risk of HIV acquisition per sexual exposure by increasing the amount of HIV shedding through genital lesions and by providing an easy portal of entry of the virus into the host [5].

Although GUD is a common complaint at STD clinics in Africa, screening for HSV-2 is rarely done. Nonetheless, recent studies confirm that HSV-2 is common in adult populations on the continent. Laboratory evidence of HSV-2 was present among 36% of GUD patients in Kampala [6]. In one study that screened stored sera from Dakar, HSV-2 seroprevalence ranged from 20% of surgical patients to as high as 96% among prostitutes [7]. Considering the high prevalence of HSV-2, the increased shedding of HIV through genital herpes lesions, and the fact that persons with HSV-2 remain potentially infectious for life, HSV-2 may account for a large proportion of HIV infection in Africa.

To determine the prevalence of HIV in a population of male factory workers in Harare, Zimbabwe, we screened stored serum specimens from subjects participating in an HIV prevention intervention for HSV-1— and HSV-2—specific antibodies. The availability of HIV serostatus at enrollment in the intervention as well as demographic and sexual risk–related data permitted examination of the correlation between HIV and HSV-2 seropositivity in case-control analysis.

Methods

Study population. Serum specimens screened for HSV-2— and HSV-1—specific antibodies originated from subjects enrolled in the Zimbabwe AIDS Prevention Project, a longitudinal cohort study established to determine the prevalence, incidence, and correlates of HIV infection and to evaluate a peer education intervention. Recruitment and follow-up methods and observational findings of the cohort have been described in detail elsewhere [8, 9]. Briefly, subjects are male factory workers recruited and followed at 40 work sites in greater Harare, Zimbabwe. At enrollment and at 6-month intervals, subjects are interviewed on HIV risk–related behaviors, and blood is drawn for serologic testing for HIV, syphi-
lis, and hepatitis B. An aliquot of serum is stored. As of May 1997, >3000 subjects have been enrolled. Nineteen percent of subjects are HIV-positive at enrollment. HIV seroincidence in the cohort is ~3/100 person-years; the incidence of reported STD syndromes is 10/100 person-years.

**Study design.** To assess the correlation between HIV and HSV-2 seropositivity, a case-control study without matching was used. A pre-study sample size calculation estimated that 200 cases and 200 controls would provide ample power to detect an association between the presence of HIV and HSV-2 antibodies. Stored serum specimens were consecutively retrieved starting from the first date of enrollment until the sample size was approximated for both HIV-negative and HIV-positive subjects. Although not random, the consecutive sampling method was likely to produce a representative sample of cases and controls, as no differences were observed among subjects recruited early or late in the study. Because of the size of the storage boxes and the number of tests that could be run per kit, 224 HIV-negative and 191 HIV-positive subjects were ultimately screened for HSV-1 and HSV-2 antibodies.

**Laboratory methods.** A strip recombinant immunoblot assay (RIBA HSV Type 1/Type 2 SIA; Chiron, Emeryville, CA) was used to detect and differentiate HSV-1— and HSV-2—specific antibodies. The RIBA type 1/type 2 nitrocellulose strips include recombinant antigen bands from HSV-1 (gG1 and gB1) and HSV-2 (gG2 and gD2) as well as controls for IgG. Antibodies specific for HSV-1 will react with gB1 and gG1 antigen bands but not with the gG2 band. HSV-2 antibodies will react with gG2 and gD2 bands but not with gB1 and gG1 bands. Because of homology between HSV-1 and HSV-2 in the gD glycoprotein, reactivity is expected for both viral types to the gD2 band. Compared with Western blot, the sensitivity of the RIBA assay is 95.1% for HSV-1 and 98.2% for HSV-2; specificity compared with Western blot is 99.4% for both HSV-1 and HSV-2 [10].

The presence of HIV antibodies was demonstrated by use of a third-generation EIA (HIV-1/HIV-2; Abbott, Abbott Park, IL). Specimens reactive or indeterminate in the Abbott EIA were re-tested with a second, third-generation EIA (Enzygnost Anti-HIV 1/2 Plus; Behring, Marburg, Germany). Samples were considered HIV antibody-positive when positive results were obtained from both EIAs. Indeterminate or conflicting results were resolved by Western blot (HIV Blot 2.2; Diagnostic Biotechnology, Singapore).

**Statistical methods.** We first examined associations between HSV-2 seropositivity and variables previously shown to be related to HIV infection in the study population [9]. These variables included age, education, marital status, history of STD, history of paying for sex, and number of sex partners. At baseline, subjects were asked risk information pertaining to the year prior to the interview. In the present analysis, associations between baseline serology and reported risk behaviors in the preceding year were examined. No information on previous HIV testing and knowledge of serostatus was collected. Bivariate associations were assessed by the \( \chi^2 \) test for trend or test for differences in proportions. For these analyses, HIV-negative and HIV-positive subjects were examined separately. HIV-positive subjects were then compared with HIV-negative subjects with respect to HSV-2 serostatus by use of logistic regression analysis. To control for the potential confounding effect of high-risk sexual behaviors that could result in both HSV-2 and HIV infection, we included history of STD, paying for sex, and multiple sex partners in a multiple logistic regression model.

**Results**

The prevalence of HSV-2—specific antibodies was 35.7% among HIV-negative subjects and 82.7% among HIV-positive subjects. Given that 19% of all subjects at the factories are HIV-positive at enrollment [9], a weighted estimate of the prevalence of HSV-2 in this male population is 44.6%. With the exception of a single specimen from an HSV-2—positive but HIV-negative subject, all participants were HSV-1—positive.

Among HIV-negative subjects (table 1), demographic characteristics significantly associated with HSV-2 seropositivity
The body of evidence from other studies weighs in favor of a causal relationship between HIV and HSV-2 [1, 3–5] by meeting key criteria for causation in observational studies as reasoned by Hill (cited in [11]): biologic plausibility, the correct temporal sequence, the strength of association, and consistency across different studies. In the present study, HSV-2 seropositivity was overwhelmingly the strongest correlate of HIV seropositivity, with an adjusted odds ratio of 8.0. HSV-2 seropositivity had a greater magnitude of association with HIV than did any other variable examined in the present study or in our previous analysis of risk factors for prevalent HIV infection among 2691 cohort participants [9].

Several limitations of the present study are recognized. The case-control design does not permit assessment of a causal association between HSV-2 infection and HIV acquisition. In particular, the temporal sequence or directionality of the association between HSV-2 and HIV could not be analyzed. Although the association was not confounded by the most common behavioral risk factors for HIV infection in the study population (multiple partners, history of STD, paying for sex), it is possible that unmeasured behavioral risk factors common to both HSV-2 and HIV infection could cause residual confounding. Moreover, the role of asymptomatic or subclinical HSV-2 infection on facilitating HIV transmission remains to be assessed, particularly in view of the fact that very few subjects with antibodies to HSV-2 in the present study reported history of GUD within the preceding year. In another study, only 21% of STD clinic attendees with serologic evidence of HSV-2 reported symptoms or histories of genital herpes [12].

Despite these limitations, the association between HSV-2 and HIV infection has several potential practical applications. The findings support the use of HSV-2 as a serologic end point in the assessment of HIV prevention interventions that seek to reduce high-risk sexual behaviors. The presence or acquisition of antibodies to HSV-2 is a promising, objective measure of high-risk sexual behavior [13, 14]. HSV-2 seropositivity among HIV-negative subjects may be useful in identifying a subpopulation of persons at high risk of acquiring HIV. Prevention resources and recruitment for prevention intervention studies may be targeted to such persons. If the relationship between HSV-2 infection and HIV acquisition is indeed causal, then suppressive treatment of HSV-2 has biologic plausibility as an HIV prevention intervention. Suppression of HSV-2 among HIV-positive persons may be particularly important in preventing secondary transmission.

**References**

Identification and Characterization of Herpes Simplex Virus–Specific CD4+ T Cells in Corneas of Herpetic Stromal Keratitis Patients

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Herpetic stromal keratitis (HSK) is a corneal disease initiated by a herpes simplex virus (HSV) infection with a postulated T cell–mediated immunopathology. To study the antigen specificity of corneal-infiltrating T cells in HSK patients, T cells were isolated and expanded by mitogenic stimulation from corneas of 2 patients with HSV-1–mediated HSK. A substantial number of the T cell clones (TCCs) obtained from these T cell lines were HSV-specific. All HSV-specific TCCs were of the CD3+CD4+CD8− phenotype. These TCCs responded to autologous HSV-infected corneal keratocytes, which expressed HLA class II molecules following incubation with interferon-γ. Upon HSV-specific stimulation, all TCCs secreted interleukin-4, interleukin-5, and interferon-γ. The data presented suggest that HSV-specific CD4+ T cells play a role in the immunopathogenesis of HSK in humans and that corneal keratocytes may act as antigen-presenting cells in this local T cell response.

Recurrent herpes simplex virus (HSV) infections of the cornea can lead to tissue-destructive inflammation of the corneal stroma. This disease, known as herpetic stromal keratitis (HSK), is a leading infectious cause of corneal blindness worldwide. The stromal pathology seen in HSK patients is most probably not due to the direct cytopathic effect of the virus but more likely the result of a local cellular immune response (reviewed in [1]). Studies in the mouse model of HSK have shown that CD4+ T cells, possibly HSV-specific, that secrete type 1 cytokines (i.e., Th1 cells) play a pivotal role in the immunopathology of this disease [1–4].

Studies on T cell involvement in the immunopathogenesis of HSK in humans is limited to immunohistologic analyses and phenotypic characterization of isolated intracorneal T cells [5, 6]. In the present study, a protocol was developed that enabled the expansion and functional characterization of intracorneal T cells obtained from 2 HSK patients.

Materials and Methods

Clinical material and reagents. Corneal buttons and peripheral blood mononuclear cells (PBMC) were obtained from 2 patients,