virus-containing fraction (e.g., by ultracentrifuging the whey). Thus, viral DNA might also have been present in a free form (i.e., not encapsidated). In our opinion, this does not rule out the hypothesized antiviral role of lactoferrin: The protein might have ruptured the virus particles in a way similar to that in which bacteria is destroyed. The lactoferrin concentrations in the examined milk samples should have been determined and correlated with the ability to culture virus from these samples.

Nevertheless, we agree with Numazaki et al. [1] that the absence of PCR-detectable virus in colostrum at first sight does not support a possible role for lactoferrin in the host's defense against HCMV. However, this does not exclude that the high lactoferrin concentrations in colostrum exert an effect at cellular sites in the mammary glands where HCMV is released from its infected host cells. Moreover, lactoferrin is capable of forming complexes with soluble immunoglobulin A (sIgA) [3, 4]. The sIgA has a virus-neutralizing effect and when complexed with lactoferrin it may obscure the detection of bound virus or virus DNA, especially in secretions with high lactoferrin concentrations [5]. In addition, lactoferrin can strongly bind viral DNA and could in principle interfere with detection by PCR.

Finally, the authors suggest the antiviral effect we observed in vitro by lactoferrin could be influenced by the presence of tumor necrosis factor (TNF)-α as a lactoferrin contaminant. It is well known that TNF-α alone or together with interferon-γ has an inhibitory effect on HCMV infections in vitro, although in vivo the presence of TNF-α may also have opposite effects [6–11]. However, by sensitive SDS-PAGE and silver staining we did not detect proteins other than lactoferrin in the preparations used. Other proteins isolated from milk, among which are β-lactoglobulin and α-lactalbumin, did not inhibit HCMV infection of fibroblasts (not shown). In addition, lactoferrin isolated from either breast milk or from colostrum had very similar inhibitory effects, while the lactoferrin isolated from these sources would very likely contain different amounts of TNF-α. However, copurification of lactoferrin and TNF-α is not a likely phenomenon, since this cytokine does not bind avidly to the protein.

Most importantly, highly purified lactoferrin prepared from a single donor that was completely free of TNF-α had inhibitory effects similar to the previously tested commercially available lactoferrins. Therefore, we feel that the apparent contradiction of transmission of HCMV by breast-feeding and the potent inhibitory effect of the milk constituent lactoferrin in vitro deserves further study.

P. J. Swart, M. C. Harmsen, J. Nuijens, D. K. F. Meijer, and T. H. The

Departments of Immunology and Pharmacology (Section of Pharmacokinetics and Drug Delivery), Groningen Institute for Drug Studies, University of Groningen, Groningen; Pharming, Leiden, Netherlands

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Genital Ulcers Associated with Human Immunodeficiency Virus–Related Immunosuppression

To the Editor—Reducing the incidence of genital ulcer disease (GUD) is an important component of any human immunodeficiency virus (HIV) disease control program in sub-Saharan Africa. Ghys et al. [1] report that among HIV-infected female sex workers in Abidjan, Ivory Coast, the number with GUD was increased among those with low CD4 cell counts, suggesting that GUD is an opportunistic disease. If the results of their study are to be utilized in strategies for improving the control of GUD, there are a number of points that require further clarification.

Ghys et al. could not determine the etiology for genital ulcers in 58%–72% of HIV-positive women from whom cultures for both *Haemophilus ducreyi* and *Herpes simplex* were done. This is far greater than the percentage of genital ulcers of unknown etiology among women with GUD attending sexually transmitted disease (STD) clinics elsewhere in Africa: In Durban, South Africa, [2], Nairobi, Kenya, [3], and Kigali, Rwanda, [4], no pathogens were identified in 20%, 33%, and 35%, respectively, of cases. What factors might account for this variation?

Possibly there was a difference in what was defined as a case of GUD. Ghys et al. did not define what they classed as an ulcer,

Reprints or correspondence: Dr. Nigel O'Farrell, Dept. of Genitourinary Medicine, Homerton Hospital, Homerton Row, London E9 6SR,UK.

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and it may be that lesions recorded in their study would not have been included as ulcers elsewhere. Furthermore, Ghys et al. made no mention of cervical ectopy and whether this was differentiated from ulceration of the cervix, which was found in 30% of their patients with genital ulcers and CD4 cell percents <14%. Paradoxically, no mention was made of cervical ulcers in a study in Nairobi in which a higher prevalence of cervical ectopy was observed among HIV-positive women than among seronegative controls [5].

A further explanation for ulcers of unknown etiology may be that some of the Abidjan patients did not have an STD. Fifty percent of the ulcers in patients with CD4 cell percents <28% were classed as "vaginal" lesions, but it was not stated whether these were intravaginal, and therefore not requiring speculum examination for diagnosis, or external and obvious on visual inspection. Intravaginal ulcers could have been caused by local application of cleansing or tightening agents, which are used in some African countries, but it is unclear whether these were used by the study population. Given the relatively high cost of drugs used in the syndromic approach for the management of GUD currently recommended by the World Health Organization, it is essential to know whether genital ulcers are of a sexually transmitted nature and justify antibiotic therapy.

Ghys et al. also found that infection with *Trichomonas vaginalis* and cervical ulceration were associated with more advanced immunosuppression. It would be interesting to know whether *T. vaginalis* was associated with cervical ulceration and, if so, whether the characteristic "strawberry cervix" was more prevalent in those with advanced immunosuppression. Although cervical abnormalities are an uncommon complication of *T. vaginalis* infection [6], it may be that HIV alters the clinical spectrum of trichomoniasis. If this is so and if *T. vaginalis* was associated with genital ulceration, syndromic algorithms for HIV-positive subjects would need to be adjusted accordingly.

A further point requiring clarification is whether Ghys et al. cultured samples from the cervix of women with external genital herpes lesions. If not, then the number of genital herpes cases was probably underdiagnosed. Genital herpes has undoubtedly been overlooked in many parts of Africa, and there are important cost implications arising therefrom [7]. In populations in which genital herpes is the commonest cause of GUD, counseling or health education rather than treatment for syphilis and chancroid may be a more appropriate management strategy than the syndromic approach [8].

It is clear that the etiology of GUD may vary significantly among countries and that relevant microbiologic tests have limited sensitivity. It is therefore vital that the STD syndromes to be treated by the syndromic approach are defined clearly and accurately so that the limited resources available for STD drugs in sub-Saharan Africa may be used more effectively.

Nigel O'Farrell
Department of Genitourinary Medicine, Homerton Hospital, London, United Kingdom

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Reply

To the Editor—O'Farrell [1] raises interesting points in his letter regarding our study of the association between genital ulcers and human immunodeficiency virus (HIV)–related immunosuppression [2].

O'Farrell contrasts the high proportion (58%–72%) of genital ulcer cases with a negative culture for both *Haemophilus ducreyi* and herpes simplex virus (HSV) in our study with lower proportions of genital ulcer cases with no identified pathogen in studies from Durban (20%) [3], Nairobi (33%) [4], and Rwanda (35%) [5]. This apparent discrepancy may be explained by a difference in laboratory tests and criteria used to elucidate the etiology of genital ulcers and by differing inclusion criteria.

While our study used cultures for *H. ducreyi* and HSV only, the latter three studies also used laboratory investigations for lymphogranuloma venereum [3, 5], donovanosis [3], and syphilis [3–5]. We did not attempt to diagnose lymphogranuloma venereum and donovanosis because both are thought to be rare in West Africa [6]. We also did not use syphilis serology results to classify genital ulcers as syphilitic, since female sex workers are highly exposed to sexually transmitted agents, including *Treponema pallidum,* and positive results for serologic syphilis tests are therefore less likely to be due to a current genital ulcer than they would be in other population groups. In fact, the proportion of women with a reactive rapid plasma reagin test and *T. pallidum*–hemagglutination assay was similar for those with (29%) and those without (24%) a genital ulcer. While our study included all women reporting to the study clinic, regardless of recent antibiotic treatment, the other three studies excluded women who had recently received antibiotic ther-

Reprints or correspondence: Dr. Peter D. Ghys, Projet RETRO-CI, 01 BP 1712, Abidjan 01, Côte d'Ivoire.

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apy. Prior antibiotic treatment may therefore have precluded identification of *H. ducreyi* for some of the ulcer cases in our study. The proportion of women with genital ulcer cultures negative for *H. ducreyi* and HSV was 67% in Durban [3], 49% in Nairobi [4], and 70% in Rwanda [5], percentages that are very similar to the 58%–72% seen in our study.

Genital ulcers were diagnosed clinically and macroscopically. They were defined as an interruption of the genital epithelium. We do not believe that instances of cervical ectopy were mistaken for genital ulcers, since the examining physicians (M.O.D. and P.D.G.) were specifically made aware of this possibility for misclassification. Furthermore, only 4% of genital ulcer cases had an exclusively cervical localization.

O'Farrell further suggests that some of the genital ulcers in our study patients may have been caused by the local application of cleansing or tightening agents rather than by a sexually transmitted disease. However, anecdotal evidence from this population suggests that neither tightening agents nor toxic cleansing agents are used.

Among HIV-infected women, *Trichomonas vaginalis* was not detected significantly more frequently in women with than without a cervical ulcer (1/4 [25%] vs. 10/38 [26%], 4/13 [31%] vs. 37/113 [33%], and 10/19 [53%] vs. 19/45 [42%] for women with CD4 cell percents >28%, 14%–28%, and <14%, respectively). The presence of "strawberry cervix" was not specifically recorded in our study.

Samples for HSV culture were not systematically obtained from the cervix of women with external genital herpes lesions. Genital herpes cases may therefore have been underdiagnosed. However, we do not agree with O'Farrell's statement that "In populations in which genital herpes is the commonest cause of GUD [genital ulcer disease], counseling or health education rather than treatment for syphilis and chancroid may be a more appropriate management strategy than the syndromic approach (his [8])." Counseling and health education should always be part of the disease management plan for patients with genital ulcers [7]. In addition, since there is a cost associated with not treating true cases of chancroid and syphilis, omitting treatment for these conditions from a management strategy for genital ulcers should only be considered when the ratio of genital ulcers due to herpes versus those due to chancroid or syphilis (whichever is the next commonest cause) is quite large and not merely when this ratio exceeds 1, as O'Farrell suggests.

We do agree with O'Farrell that "it is essential to know whether genital ulcers are of a sexually transmitted nature and justify antibiotic therapy" and that "relevant microbiologic tests have limited sensitivity." To help address both of these issues, we are carrying out a study incorporating polymerase chain reaction for the diagnosis of *T. pallidum, H. ducreyi,* and HSV. It is our hope that this and other studies will provide the necessary guidance for updating recommendations for the management of genital ulcers in the Ivory Coast and elsewhere.

Peter D. Ghys, Mamadou O. Diallo, and Marie Laga
Projet RETRO-CI, Abidjan, Côte d'Ivoire, Institute of Tropical Medicine, Antwerp, Belgium

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Human Immunodeficiency Virus (HIV) Type 1–Specific B Cell Response in Seronegative Subjects at Risk for HIV Exposure

To the Editor—We read with interest the recent article of Beretta et al. [1] in which the detection of human immunodeficiency virus type 1 (HIV-1) envelope-specific T cell responses and antibodies cross-reactive for HIV-1 gp120 and HLA epitopes, in seronegative uninfected drug users at high risk for HIV-1 infection, is described. The observation is noteworthy: In fact, the finding of T and B cell responses to the HIV envelope in seronegative subjects supports the possibility that exposure to the virus may lead to an immunologic response without the development of productive infection. Moreover, even though the protective potential of the Th1 response needs to be further investigated, the findings of these authors agree with the supposed role of Th1 response in preventing HIV-1 infection [2].

We feel that additional observations confirming the data of Beretta et al. [1] would give evidence of the importance of an HIV-1–specific immune response in exposed but persistently uninfected subjects.

With a different approach, we previously demonstrated a B cell response in seronegative subjects exposed to HIV-1. In fact, we [3] and others [4] found that, after polyclonal activation with pokeweed mitogen (PWM), peripheral blood mononuclear cells from high-risk seronegative subjects (drug users with a history of repeated needle-sharing and steady heterosexual partners of HIV-1–positive subjects) produced in vitro HIV-1–specific antibodies as detected by means of a PWM in vitro antibody production (PWM-IVAP) test. In our series, the antibodies were detected in 4 of 27 patients studied and in none of the controls and were mainly di-

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Reprints or correspondence: Dr. Gioacchino Angarano, Clinic of Infectious Diseases, University of Bari, Policlinico, p.zza G. Cesare 11, 70124 Bari, Italy.

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