Cohorts for the Study of HIV-1–Exposed but Uninfected Individuals: Benefits and Limitations

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Since the late 1980s, with the first identification of individuals who were exposed to human immunodeficiency virus type 1 (HIV-1) yet remained uninfected, or "HIV-1–resistant" individuals, a large number of cohorts that include HIV-exposed seronegative (HESN) subjects have been identified globally for the purpose of investigating the genetic, immunologic, and environmental factors that may help alter susceptibility to HIV-1. In this article, in light of the recent International Symposium on Natural Immunity to HIV, we review the characteristics of different groups with respect to their relative risks and briefly summarize the known cohorts that include exposed uninfected subjects worldwide.

It is now accepted that there are subsets of individuals who remain uninfected with human immunodeficiency virus (HIV) despite repeated exposure and against statistical probability. To date, only homozygosity for the *CCR5Δ32* deletion mutation [1] has been consistently replicated as a mechanism of HIV resistance. However, this mutation only accounts for a minority of cases. Clearly the identification of factor(s) affecting susceptibility to HIV would be invaluable in the quest for an effective vaccine, so research into the immunobiology, genetics, and cellular biology of these unique individuals continues apace. Particularly since the limited success of several large vaccine and microbicide trials, it is clear that we do not have a clear understanding of correlates of protection against HIV infection.

Numerous cohorts have been identified that include HIV-exposed seronegative (HESN) subjects, usually through behavioral studies. Generally they fall into 3 major groups: discordant couples; individuals with high-risk sexual behaviors, including commercial sex workers (CSWs) and men who have sex with men (MSM); and individuals exposed nonsexually, including injection drug users, infants born to HIV-infected mothers, hemophiliacs, and others exposed to contaminated blood products. The prevalence of HESN individuals, their incidence within groups, and the risks of infection in these groups vary widely.

The purpose of this review is not to summarize the research findings that have been derived from these cohorts, which have recently been reviewed [2], but to highlight the advantages and disadvantages of using different risk groups as models of HIV "resistance." We also discuss limitations and benefits and highlight the importance of greater consensus and collaboration among the HIV research community studying these subjects (Figure 1).

DISCORDANT COUPLES

Perhaps among the largest exposed seronegative groups are discordant couples, generally monogamous couples in which only 1 partner is infected. These cohorts exist

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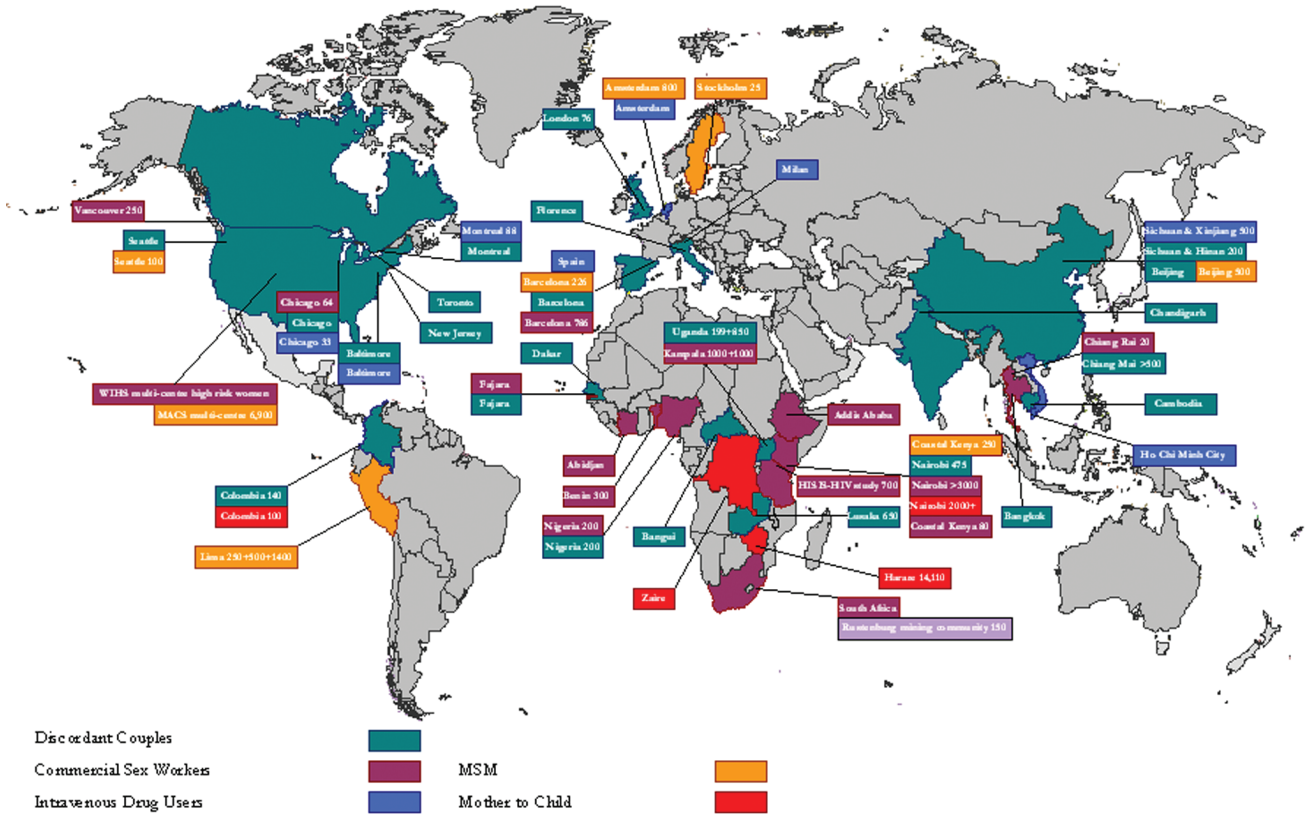

Figure 1. Cohorts that include individuals who remain uninfected despite being exposed to human immunodeficiency virus (HIV). The approximate number of participants is shown for some cohorts; these values include both HIV-negative and HIV-positive individuals; where multiple numbers are shown, they represent different cohorts. MSM, men who have sex with men.

worldwide and can be heterosexual or homosexual. The risk of transmission with unprotected sex differs depending on which partner is infected, because receptive intercourse carries a higher risk per exposure than does insertive intercourse. Additionally, the incidence of positive exposure (i.e., unprotected exposure carrying a definite risk of infection) varies massively between cohorts.

HIV exposure in these groups ranges between 25 and 112 incidents per year, with study inclusion for some studies having exposure to HIV for >3 years before the study start date. Given the relative risk, the seronegative partners can rarely be classified as epidemiologically "resistant" but are still classified as being at high risk and may provide useful information. Indeed, the identification of resistance began in 1989, when T cell responses to HIV were noticed in seronegative partners of HIV-positive men [3].

Participants in discordant couple cohorts are often recruited from sexually transmitted disease clinics for specific studies rather than maintained in specific HESN cohorts. Although the clinic may possess some patient history information, a large portion of the participant's history is self-reported, which is perhaps not ideal. However, HESN subjects are well characterized in a number of comprehensive multicenter studies, such as the Multicenter AIDS Cohort Study [4] and the Partners in Prevention HSV-2/HIV-1 Transmission Trial [5].

As with all cohorts, couples enrolled in discordant-couple studies receive counseling. Though its effectiveness is dependent on social and behavioral factors, counseling inevitably leads to a decrease in risky behavior, which—thankfully—reduces infection pressure but also makes it more difficult to identify resistant individuals. A careful characterization of degree of exposure to HIV is critical in order to identify individuals who indeed are epidemiologically resistant to infection.

Discordant-couple cohorts are different from most other high-exposure cohorts in that the HIV-negative partner is repeatedly exposed to the same HIV quasispecies. One could hypothesize that repeated exposure to virus from closely related genotypes is easier for the immune response to respond to than multiple unrelated strains. However, it may limit the utility in understanding immune correlates of protection, because cross-reactive responses are likely to be what an effective HIV vaccine must mimic.

Another important confounder in discordant-couple cohorts is the antiretroviral-treatment status of the HIV-positive part-

ner. Early studies included couples in which the positive partner was untreated, but most HIV-positive individuals now are probably receiving antiretroviral therapy, which affects risk of transmission. Thus, in studies in which the infected partner is undergoing antiretroviral treatment, it may be difficult to assess infection pressure. Other concerns include the viral load and progression status of the infected partner and circumcision status, all of which will affect infection pressure [6–8]. However, one strength of discordant-couple cohorts is that these factors are known and can be appropriately included in models.

One benefit of discordant-couple cohorts is that large numbers of individuals can be enrolled from predominantly nonmobile populations. The subjects tend to be well followed-up with documented HIV exposure, and the "challenge" virus can be studied. Despite this advantage, the data can still be inaccurate—as evidenced, for example, by high pregnancy rates, which often do not reflect reported condom usage rates—but the accuracy of self-reported data on HIV exposure risk is a concern in all HIV studies. discordant couple studies may have the best epidemiologic data for assessing risk of infection and can make a strong contribution to research on HESN or HIV-resistant subjects. In summary, although infection risk can be reduced in these cohorts, and identification of truly resistant subjects difficult, discordant-couple cohorts may be among the most accessible and the most relevant for identifying correlates of protection affecting sexual transmission.

HIGH-RISK SEX PRACTITIONERS: CSWs AND MSM

Highly exposed persistently seronegative cohorts, which include individuals exposed to such an extent that they can be classified as resistant according to epidemiologic models, consist almost exclusively of CSW cohorts in Africa and Asia. The first to be described, and undoubtedly the largest and most comprehensively followed, is the Pumwani CSW cohort in Nairobi, Kenya [9]. Despite counseling and provision of condoms, these women have a high frequency of unprotected sex, with as many as 15 clients per day. However, condom use is increasing [10, 11] and needs to be included in models when infection pressure is determined for this and other CSW cohorts.

These cohorts can be a key resource for research into preventing infection in women; to date, mainly female CSW cohorts have been identified. However, MSM currently bear a disproportionate burden of HIV infection in resource-rich countries, and a number of high-risk MSM cohorts have also been examined [4, 12, 13]. Mechanisms of transmission and immune responses at the site of initial contact differ between men and women, which means that correlates of protection may not always be directly relevant to both. The difference in sexual behaviors makes MSM a unique group and also adds to their risk of infection, because receptive anal sex has a relative risk of 1.43% per event [14], which is ~10 times higher than that for receptive vaginal sex.

One dilemma in immunologic studies in these cohorts is the availability of suitable negative control subjects. The control subjects available are usually low-risk (non-CSW) subjects from the same community, which does not permit control for the effects of sex work. Repeated sexual activity, allo exposure due to sperm, and genital tract infection (bacterial vaginosis and others) all are more common in these populations and probably affect baseline immunologic parameters. The alternative is to use HIV-uninfected CSWs and MSM who do not have sufficient infection pressure to be considered HIV resistant. The obvious issue is that some of these individuals will eventually go on to become resistant, but the majority will probably succumb to infection. Thus, they are not ideal negative control subjects. This concern was recently highlighted in a study looking at cervical HIV-specific immunoglobulin A in highly exposed seronegative women [15]. Perhaps the best way to address these issues is to use both populations as negative control subjects to ensure that there is no bias. It should be noted that this is not a concern for genetic studies, because they can readily use HIV-positive individuals, who are by definition susceptible to HIV, as control subjects.

Female CSWs are a useful population to study because they have high-risk behaviors and therefore are under high infection pressure. Along with MSM, they are a good model to use for vaccine development, because they are exposed to multiple viral isolates. The reduction of risk-taking behavior that accompanies ongoing counseling affects CSW and MSM cohorts, as well. Moreover, CSWs and MSM tend to be highly mobile populations from which it may be difficult to collect the detailed epidemiologic data available from discordant-couple cohorts. In summary, along with discordant couples, CSWs and MSM are probably the best groups to use as models to identify correlates of protection against sexual transmission, but they are among the most difficult cohorts to maintain and follow up, and findings in these cohorts are complicated by many potential epidemiologic or biologic confounders.

INDIVIDUALS EXPOSED NONSEXUALLY

Injection drug users are a group at high risk of infection through sharing of contaminated needles. Although exposure is difficult to estimate, the relative risk per exposure is quite high, considerably higher than through sexual contact because of the intravenous and/or subcutaneous nature of the practice; however, because a number of drugs are heated before use, injected levels of live virus are difficult to assess. Drug use often goes hand in hand with other risky behavior, such as unprotected sex, which makes the precise level of exposure difficult to estimate in this group. As with CSWs, injection drug users

can be a transient population, making accurate epidemiologic documentation and follow-up difficult.

An important factor distinguishes for nonsexually exposed individuals from others in the HESN population is that most exposures are intravenous, as opposed to mucosal, meaning that initial exposure is to the peripheral immune system, which is vastly different from the genital tract [16]. As surprising as it seems, given the high seroconversion rates after infected blood transfusions, ~6% of a group of intensively treated hemophiliacs who received HIV-positive blood during the 1980s remained uninfected [17]. This is a unique group, represented by approximately 600–1000 individuals in a multicenter study throughout North America, Europe, and South America. The cohort has quantifiable infection risks and thorough follow-up data. However, only 16% of these individuals are homozygous for the *CCR5Δ32* polymorphism, the only correlate of protection so far identified in a HESN cohort validated in animal studies and exploited for therapeutic intervention [18, 19]. Therefore, other correlates must be responsible for protecting the rest of these subjects. Another issue related to this cohort is that their last transfusion-related exposure was in 1985, and immune responses to HIV are likely to have waned. By the nature of their disease, they have also experienced repeated and sustained exposure to alloantigens throughout their lives, a confounding factor affecting baseline immune responses.

Another important group are children who were born to infected mothers and contracted HIV infection in utero or by way of breast milk [20]. There are currently numerous interventions to prevent mother-to-child transmission, so such cohorts are dwindling. For the majority of cohorts established to examine mother-to-child transmission, excellent epidemiologic data are maintained, and the viral isolate and route of exposure can be defined through in-depth information on feeding practices and confounding issues (eg, mastitis).

For the sake of being complete, we must also mention healthcare workers who have suffered infected needlestick injuries. As HESN individuals, with an infection risk of ~1:250, the utility of this cohort as a model of HESN subjects is limited, compared with other cohorts, due to the single incidence of exposure. Although regular follow-up is easily carried out at their place of work and the precise time and isolate of exposure are known, investigations are further confounded by postexposure prophylaxis, which is the standard of care for healthcare workers in most countries and has been shown to reduce infection risk significantly [21]. There are also a number of Chinese community cohorts who were exposed to infected blood products, but they have not yet been well characterized in relation to a model of HIV resistance. In summary, these accidentally exposed groups also vary in their exposure to HIV and in definition of a resistance phenotype. The route of exposure may have limited relevance to development of a vaccine or treatment against a sexually transmitted virus. However, these groups probably share correlates with other HESN populations, and studies of them may be tremendously informative in identifying systemic correlates of protection (eg, *CCR5Δ32* polymorphism), rather than mucosal correlates.

CONCLUSIONS

Some similarities and differences of the main categories of HESN cohorts are summarized in Figure 2. Arguably, the 2 most common groups, CSWs and discordant couples, may provide the most useful data. CSWs are the most regularly ongoing highly exposed group, whereas discordant couples have much lower exposure rates but may be useful to study because the precise details of exposure are known, including the virus isolate. A key point to consider is that the differences between the different types of cohorts, including mode of exposure, lifestyles, and concurrent infections, make it important to keep separate the findings from different groups, because their resistance may well be derived from distinct mechanisms, even though there are probably commonalities in correlates of protection.

Given the number of cohorts being investigated and the discrepancies between groups concerning both follow-up and risk-taking information, it seems clear that we must define what constitutes sufficient exposure for a model of HIV resistance. Consensus is required to enable comparison of results between cohorts and accurate interpretation of the data. To achieve this goal, greater collaboration and interactions among researchers

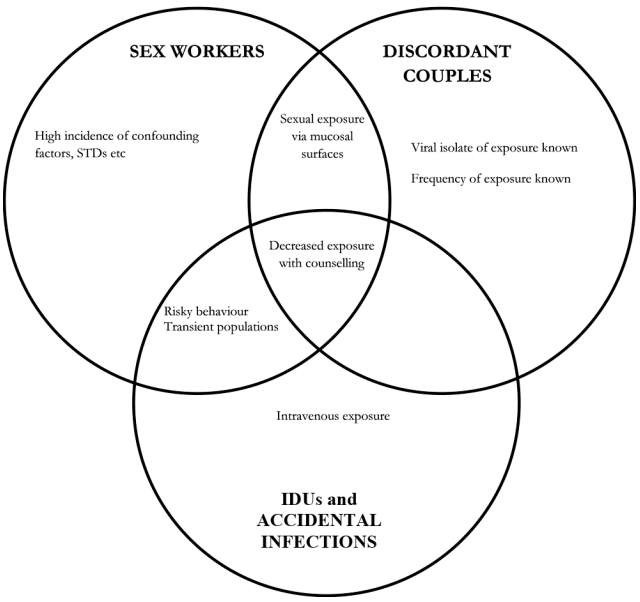

Figure 2. Similarities and differences between the main cohorts of human immunodeficiency virus–exposed but uninfected individuals. IDUs, injection drug users; STDs, sexually transmitted diseases.

studying HIV-resistant or HESN subjects should be encouraged and facilitated for the benefit of all.

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