Frequency of HIV-1 dual subtype infections, including intersubtype superinfections, among injection drug users in Bangkok, Thailand

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Objectives: To estimate the frequency and incidence of dual HIV-1 subtype infections, including superinfections, among recent seroconvertors from a cohort of injection drug users (IDUs).

Methods: A total of 1209 HIV-negative IDUs were followed in a prospective cohort study at 15 methadone clinics in Bangkok, Thailand. After 2308 person-years (PY) of follow-up, 133 seroconverted to HIV-1, of which approximately 20% were subtype B and 80% were CRF01_AE (formerly called subtype E). Specimens from 126 individuals were available at time of first seropositive test and specimens from 80 of these 126 individuals were also available more than 12 months later. For each infected participant, we calculated the amount of time to superinfection, loss to follow-up, or to the closest visit more than 12 months after the time of initial seropositivity.

Results: Of all 126 seroconverters seen at the time of the first seropositive test result, there was no apparent case of concurrent dual subtype infection detected despite 2301 PY of observation. Overall, the incidence of superinfection was 2.2 per 100 PY [95% confidence interval (CI), 0.3–7.8]. The 1-year incidence of CRF01_AE superinfection following subtype B primary infection was 3.9 per 100 PY (95% CI, 0.1–21.9) and the incidence of subtype B superinfection following CRF01_AE primary infection was 1.5 per 100 PY (95% CI, 0.04–8.3).

Conclusions: Determination of the frequency and incidence of dual HIV-1 subtype infection demonstrates that HIV-1 superinfection is not uncommon in a population with high HIV-1 incidence with more than one circulating strain.

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Introduction

In 2002, our group and others published the first documented reports in the literature of persons who were initially infected with one strain of HIV-1 and subsequently were superinfected with a different strain [1–3]. Superinfection is defined as the re-infection of an individual, after a primary HIV 1 infection, with a

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heterologous strain belonging to the same or different subtype as the primary strain. Although the potential for HIV-1 superinfection was first demonstrated experimentally in chimpanzees [4] and dual infections and recombinants involving different strains or clades of HIV-1 have been reported over the past decade [5–8] it was not clear how frequently these events occurred.

The high genetic diversity of HIV-1 is well documented, and viruses have been classified into a number of clades or subtypes [9–11]. Dual infections may represent either: (1) concurrent infection of two or more viral strains occurring at or near the same time; or (2) sequential superinfection of a second viral strain after primary seroconversion to the initial strain. The first documented dual infection of two distinct HIV-1 subtypes B and E (later designated as CRF01_AE) was reported in Thailand [5]. Reported multiple subtype infections in individuals with known transmission histories are thought to have been the result of concurrent infections by different strains [12–15]. The routes of transmission for these concurrent infections included vertical transmission, sexual transmission, and blood transfusion. However, most of these reports have been identified from cross-sectional studies or from convenience samples from individuals for whom little or no information regarding the source and timing of transmission was available. Therefore, it is unknown if any of these multiple infections were the result of superinfection with a heterologous strain belonging to the same or different subtype as the primary strain. Moreover, currently available data are not clear as to whether the immune response to the first strain would be sufficient to prevent re-infection by a heterologous strain and also what the pathogenic consequences of superinfection might be.

Although reports to date show that recombinants are an integral part of many regional epidemics where more than one strain is prevalent [16] and that 10% or more of HIV-1 infections worldwide may represent intersubtype recombinants [8], determinations of the actual incidence of dual infection, especially intersubtype superinfection, from longitudinal follow-up of population-based cohorts are greatly needed. Two recent reports have failed to detect superinfections despite apparently long periods of follow-up [17,18]. A third report detected apparent dual infections with distinct subtype C viruses among a cohort of female sex workers during the first 3 months of infection but no evidence of superinfection over the course of 24 months of follow-up [19]. Finally, a recent research letter suggested a 5% incidence of subtype B superinfection within 6 to 12 months of primary infection [20].

Knowledge of the frequency and timing of dual infections, especially in the case of superinfection, is important for our understanding of the relevance of dual infections within the context of correlates of immune protection and as a prerequisite for recombination. Since this information is directly applicable for current interventional research such as HIV vaccines, we sought to identify the appropriate conditions where population-based estimates of dual subtype infection incidence, and in particular superinfection, could be determined. The primary objectives of this study were to estimate the frequency and incidence of dual subtype infections among recent HIV-1 seroconvertors from a high-risk cohort of injection drug users (IDUs).

Methods

Description of the study population: prevalence and incidence of HIV-1

In 1991, it was estimated there were nearly 40 000 IDUs in Bangkok, Thailand [21]. The Bangkok Metropolitan Administration (BMA) manages a large municipal drug treatment program where approximately 8000–10 000 drug users are seen annually. As described previously, HIV-1 incidence is high and infections are caused by two distinct HIV-1 group M variants: subtype B and CRF01_AE (previously designated as subtype E) [22,23]. The prevalence of HIV-1 among IDU in Bangkok has been reported to be around 30–40% [22,23].

Between May 1995 and December 1996, 1209 HIV-negative IDUs were initially enrolled and followed in a prospective longitudinal cohort study at 15 BMA methadone clinics until December 1998 [23]. In addition to methadone treatment and HIV prevention counseling, these participants were seen at 4-month intervals where they were interviewed about their behavioral and medical history and sera were tested for antibodies to HIV-1 by enzyme immunoassay.

Following an HIV-1 seropositive test, study participants were offered, with voluntary informed consent, enrollment for subsequent prospective follow-up. The estimated date of seroconversion was assumed to have occurred as the midpoint of the time between the last seronegative (LN) test and the first HIV-1-seropositive (FP) test. At each visit, participants provided information on social and risk behavior as well as an update on any recent medical conditions. Most seroconverters had contributed specimens around four to five times within approximately 1 year following the estimated date of seroconversion.

Participants were evaluated and referred for treatment of any conditions as per BMA treatment guidelines. Although the treatment of HIV-infected persons has expanded in Thailand, no antiretroviral drug therapy was reported for any of the seroconvertors during the first year following seroconversion (1995–1998). As described previously, 126 seroconvertors were enrolled and additional blood specimens from the LN visit, the FP visit, and all subsequent visits (plasma) following seroconversion

were collected for viral load measurements, lymphocyte immunophenotyping by flow cytometry (FACScan; Becton-Dickinson,), and characterization of viral strains [23–25] Sequence analysis of HIV-1 strains in this population, close to the time of seroconversion, had revealed that approximately 80% were HIV-1 CRF01_AE and 20% were subtype B [25]. As also described previously, RNA derived from serum samples collected at FP and DNA from peripheral blood mononuclear cells collected more than 12 months later were screened by restriction fragment length polymorphism of the protease gene for evidence of dual subtype infection [1]. In addition, nested primers were used to amplify a 687 base-pair product, specific for subtypes B and CRF01_AE, within the envelope gene.

Prevalence and incidence of concurrent dual HIV-1 subtype infections

As of December 1998, after 2307.8 PY of observation, 133 IDUs seroconverted to HIV-1 yielding an incidence of 5.8 per 100 PY [95% confidence interval (CI), 4.8–6.8]. Since specimens from 126 (95%) of the original 133 seroconverters were available for characterization at the first seropositive visit, we calculated the number of person-years of observation up to that time as the total number of person-years of observation minus the contribution of those seven individuals whose specimens were not available. Adjustments for previously determined subtype-specific incidence rates [4.3 per 100 PY (95% CI, 3.5–5.2) for CRF01_AE and 1.2 per 100 PY (95% CI, 0.8–1.7) for subtype B] were also made [23].

For each participant who became infected with HIV-1, we then calculated the amount of time from the primary infection to the time of superinfection, until loss to follow-up or until the closest collection time more than 12 months after the time they first tested positive for HIV-1, whichever came first. As previously described, we detected two cases of intersubtype HIV-1 superinfection. Case 1 was initially infected with CRF01_AE and then became infected with subtype B and case 2 was initially infected with subtype B and then became infected with CRF01_AE. The one-year incidence of intersubtype superinfection with one subtype following primary infection with the other subtype was calculated by dividing the number of superinfections by the number of person-years of observed follow-up time following primary infection.

Results

Of all 126 seroconverters seen at the time of the first seropositive test result, there was no apparent case of concurrent dual subtype infection detected despite 2301.0 PY of observation. Among 80 seroconverters seen at both FP and more than 12 months later, two intersubtype superinfections were detected, yielding a prevalence of dual infections of 2.5% after more than 1 year following primary infection.

Overall, the incidence of superinfection as two cases observed divided by 92.5 PY of follow-up or 2.2 per 100 PY (95% CI, 0.3, 7.8). Specifically, the incidence of CRF01_AE superinfection following subtype B primary infection is given by:

I (AE/B) = 1 CRF01_AE infection observed per 25.5 PY of follow-up from 22 persons infected with subtype B = 3.9 per 100 PY (95% CI, 0.1–21.9)

This incidence was similar to the adjusted CRF01_AE-specific incidence cited in the Methods [23]. Similarly, the incidence of subtype B superinfection following CRF01_AE primary infection is given by:

I (B/AE) = 1 B infection observed per 67.0 PY of follow-up from 58 persons infected with CRF01_AE = 1.5 per 100 PY (95% CI, 0.04–8.3)

This is also comparable to the previously reported subtype B-specific incidence [23].

Discussion

Our population-based calculation of the incidence of intersubtype superinfections in high-risk IDUs is the one of the first reported in the literature. This accomplishment was possible due to the systematic and detailed prospective follow-up of at-risk individuals in a setting where more than one distinct viral strain was circulating. The calculated incidence of superinfection is very consistent with what is known about the overall and subtype-specific HIV-1 incidence rates in this population [23]. Nevertheless, there are a few caveats to consider. First of all, detection of intrasubtype dual or superinfection would have been more difficult to detect and confirm than the two cases of intersubtype superinfection we found in our cohort. For this reason, our estimate may be a minimal estimate. Secondly, transmission by injection drug use may be qualitatively different from sexual transmission where infecting viruses may encounter different immunologic hurdles such as mucosal immunity.

Dual infection, and especially superinfection, can be influenced by a number of factors. The most obvious factors, which are well known in this population, are the prevalence of the two primary HIV-1 strains and the respective incidence in the population. What is less clear is the timing of the superinfection with respect to the initial primary infection and the relationships with the level of host immunity. Despite the complex immune interactions which are not well understood, the likelihood of concurrent dual infection would be expected to

be quite low, based purely on probability. If we were to make the simple assumption that the risk of infection with one subtype was independent of infection with the other subtype, the probability of concurrent co-infection with both subtypes could be estimated as the product of the subtype-specific incidences or much less than one per 1000 years of follow-up. Our results confirm that concurrent dual HIV-1 subtype infections are indeed very rare and probably less than one per over 2300 person-years of follow-up.

On the other hand, superinfections were not uncommon, even among the relatively small number of IDUs followed. As the incidence of the two primary circulating strains was high in this population, the 1-year post-infection incidence of HIV-1 intersubtype superinfection could be determined with a measurable frequency. Since both detected dual infections were intersubtype superinfections, we were also able to calculate the subtype-specific incidence conditional on infection with the other subtype. Although two other studies attempted but failed to detect any superinfections among HIV-1 isolates from two different groups of patients, neither study controlled for time from infection despite relatively long periods of follow-up [17,18]. In contrast, three other recent reports suggest that infections with more than one viral strain are certainly not uncommon [19,20,26]. It is interesting to note that in one of these reports, apparent dual infections with distinct subtype C viruses were detected during the first 3 months of infection but no evidence of superinfection was found over the course of 24 months of follow-up [19].

Although the incidence of intersubtype superinfection measured in our study was similar to the background subtype-specific incidences previously reported, it is difficult to assess how close our observed incidence is to the true incidence of intersubtype superinfection. On one hand, it is possible that some level of immune protection or interference would result in a lower incidence of superinfection [27]. On the other hand, any protective effect may be offset by the higher frequencies of risk behaviors among infected persons, on average, compared to uninfected individuals and thus it would be reasonable to expect that persons infected with either subtype are more likely to have subsequent viral exposure. Based on previously reported HIV-1 incidence rates from an earlier prospective study in this population, HIV-1 seroconversion was primarily associated with the frequency of heroin injection, the sharing of injection equipment, and incarceration, especially with drug injection [23]. For example, persons who reported daily injection and sharing of drugs or injection equipment had an incidence of 17.1 per 100 PY compared to the overall HIV-1 incidence of less than 6 per 100 PY [23]. It is of interest to note that both superinfections occurred during a documented period of high incidence where the prevalence of risk behaviors and sharing networks was high [1,28]. Both individuals with superinfections reported very frequent injection drug use during this period when a number of needle-sharing networks may have coincided with other factors that contributed to a period of high transmission and hence, higher probabilities of acquiring co-infection with distinct viruses.

As we previously reported, the vulnerability of the two individuals to superinfection raises concerns about the ability of future HIV-1 vaccine candidates to protect against heterologous infection [1]. A major issue hindering the current development of preventive vaccines for HIV is a lack of understanding of the quantity and quality of host immune responses over time required to protect against viral challenge. Although studies in non-human primates may provide clues on the correlates of protection against superinfection, [4,27,29,30] only systematic evaluations of HIV-1 superinfection in prospective cohorts offer the unique opportunity to evaluate the correlates of immune protection against HIV-1 in humans.

While documentation of measurable HIV-1 superinfection incidence may raise new challenges for HIV vaccine development, there are several caveats in interpreting these results with respect to vaccines. First, the superinfections documented here occurred in IDUs where direct intravenous inoculation of a relatively high inoculum of virus is unlike the setting in which most HIV infections are transmitted worldwide, where generally a lower dose mucosal challenge occurs from sexual exposure. Second, the nature of the immune response to primary HIV-1 infection will differ from the immune response to a vaccine. During primary infection, the loss of specific lymphocyte subsets creates a weakened immune system that may permit establishment of superinfection, while vaccine-induced immune responses will not have the same negative effects. Further analyses of the humoral and cellular responses may provide clues as to why these superinfections occurred.

In the absence of an effective HIV vaccine in the foreseeable future, continued prevention efforts for both HIV-infected and uninfected persons are crucial [31]. In settings where HIV-1 superinfection may not be an uncommon occurrence, such as in this and other populations with high HIV-1 incidence, infected individuals who continue to put themselves and others at risk through unprotected sexual or parenteral exposures could also be superinfected with a more virulent or drug-resistant strain [32–34]. In particular, there is evidence that infection with more than one HIV strain may be associated with higher viral loads or faster disease progression [19,33]. Since HIV-1 viral loads also tend to be significantly higher among recently infected persons, especially during periods of high incidence when superinfection may be most likely to occur, this may potentially contribute to not only faster disease progression but increased infectiousness or transmissibility to subsequent contacts [28]. Furthermore, subsequent recombination between distinct viral genomes

may accelerate the evolution of these types of viruses, leading to the emergence of a wider variety of new HIV variants with altered patterns of immunogenicity, transmissibility, pathogenesis, and fitness advantages such as drug resistance [33–35]. All of this evidence suggests that prevention efforts to reduce the effective size and turnover within sexual and drug using networks may have a significant impact on the epidemic by disrupting the rapid transmission of HIV-1 from recently infected, highly infectious individuals and by preventing the potential generation of drug resistant or more virulent viruses through superinfection and subsequent recombination.

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