

HIV Superinfection

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Worldwide, 16 cases of HIV-1 superinfection in humans have been reported since 2002. Superinfection is defined as the reinfection of an individual who already has an established infection with a heterologous HIV strain. Controversy exists surrounding superinfection, because it has implications concerning our understanding of worldwide HIV diversity, individual immunity and disease progression, and vaccine development. Here, we review the current understanding of HIV superinfection.

The first case of HIV superinfection was reported in 1987, in a chimpanzee model [1]. Fifteen years later, Ramos et al. described a case in a human [2]. Worldwide, 15 other cases of human superinfection have been reported since then. The initial published reports involved individuals who were reinfected with a virus that belonged to a clade different from that of the initial infecting virus (interclade superinfection) [2, 3]. Because different clades within HIV-1 are >30% different from each other in the *env* gene, interclade superinfections can be more readily detected than other superinfections. Moreover, immune responses to the initial infection might be less likely to be protective against such a divergent superinfecting virus. Subsequently, however, reinfection with a virus that belonged to the same clade as the initial infecting virus (in-

traclade superinfection) started to be reported [4, 5]. Here, we review the growing body of literature on HIV superinfection and discuss its potential implications.

WHAT IS SUPERINFECTION?

Similar to other chronic viral infections, such as those with cytomegalovirus and Epstein-Barr virus, infection of an individual with a second viral strain (dual infection) [6–22] occurs in HIV infection. The terminology related to dual infection is important. The differences between superinfection and coinfection may have significant implications. Dual infection occurs when an individual is infected with strains derived from 2 different individuals. Dual infections can be divided into coinfections and superinfections. Coinfection is defined as infection with 2 heterologous strains either simultaneously or within a brief period of time before infection with the first strain has been established and an immune response has developed. Arbitrarily, HIV coinfection would occur within the first month of infection. Superinfection is defined as infection with a second strain after the initial infection and the immune response to it has been established (figure 1) [23, 24]. “Reinfection” is a term that is often used in place of “superinfection,” because some feel that the “super” in “superinfection” may imply that the second infection is

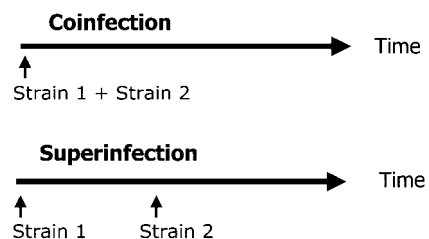


Figure 1. Types of dual infection

stronger, which may or may not be the case. We feel that the term “reinfection” may also be confusing, because it may imply that the first virus had been cleared before the second infection occurred, as happens with influenza virus and various paramyxoviruses. This is not the case with HIV superinfection, in which the initial virus is not cleared before the next virus is acquired.

Discrimination between coinfection and superinfection requires exclusion of a mixed viral population during acute infection [5]. Because it is impossible to prove the absence of a minor population of a second strain (i.e., the coinfecting strain), theoretically, all currently reported cases of superinfection could conceivably be cases of coinfection, with the “superinfecting” virus being present but undetectable early after the initial infection. This could theoretically happen if one virus initially remains localized to a cellular or anatomic compartment, such as the

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lymph nodes, genital tract, or central nervous system, and then appears in the blood later. Another possibility is that the coinfecting strain of virus persists at a low level until viral evolution, drug treatment, or host immunity results in a fitness advantage for the minority strain.

Overwhelmingly convincing epidemiologic linkage of the source partner of the superinfection to the recipient will be needed to prove more formally that HIV superinfection, not coinfection, has occurred [25, 26]. Furthermore, a significant amount of quality-control measures should be demonstrated when superinfection is reported. The potential of reporting a false case of superinfection is highlighted by the first reported case of superinfection in humans [27], which was reported in 2000 and was later proved to be the result of a contamination error [27, 28]. However, substantial circumstantial evidence remains that indicates that superinfection does occur, and this evidence will be discussed here.

WHAT DOES SUPERINFECTION MEAN TO THE GLOBAL EPIDEMIC?

The global epidemiologic profile of HIV clades represents a formidable pattern of evolution and geographic spread [29]. Nevertheless, the coexistence of almost all of the variants in west-central Africa (Cameroon and the Democratic Republic of the Congo) and the differential radiation of these variants throughout the world suggests that the introduction of HIV into humans occurred in this geographic area, which, not coincidentally, corresponds to the habitat of the chimpanzee, the likely zoonotic source of HIV-1, via simian immunodeficiency virus (SIV)_{cpz} [29–32].

A portion of the worldwide HIV genetic diversity is a result of the error-prone viral reverse transcriptase (RT), coupled with a high viral replication rate [29, 33]. Additional genetic diversity is introduced as a result of recombination that may take place during HIV replication [34]. HIV is

diploid; each virion contains 2 strands of RNA genome. If a single cell is infected with 2 different HIV strains, an RNA genome from each strain can be packaged into the same virion. Recombination can then occur when this virion infects the next cell and the viral RT enzyme switches from one viral template to the other, creating a mosaic of the parental viruses in the reverse transcript [35, 36]. This has been demonstrated in nonhuman primate models within 2 weeks of dual infection [37]. Recombination allows for a more rapid increase in viral diversity than does the accumulation of mutations through replication errors [38, 39]. Taken together, this genetic heterogeneity allows for rapid adaptation to host immune responses, target cell availability, and antiretroviral therapy, which can lead to increased viral pathogenicity and infectivity and decreased antiretroviral susceptibility [33, 40].

The best circumstantial evidence for dual infection is the presence of circulating recombinant forms (CRFs) and unique recombinant forms (URFs), both of which can be produced only by the above-described process of recombination. CRFs are mosaic viruses that are propagated from one person to another and spread geographically in one or more locations—for example, CRF02_AG in west-central Africa and South America [29, 41]. It is estimated that, worldwide, 10% of HIV infections involve these recombinant viruses [38]. Currently, there are 15 reported CRFs, which are represented on 4 continents [29, 42]. Additional CRFs are expected to arise in areas where the HIV epidemic is growing and multiple clades intersect, such as Africa, Southeast Asia, and South America [33, 38, 42, 43]. URFs are mosaic viruses that have not spread from their original location [44]. The high prevalence of URFs in certain locales suggests a high frequency of dual infection, but this has been difficult to document.

Presumably, intraclade recombination occurs at least as frequently as does recombination between clades; however, in

the context of the genetic diversity of HIV, only interclade recombinant forms are readily discernible by the techniques that are commonly used to identify mosaic viruses [45]. The URFs that have been described are also recognized in dually infected individuals in locations where multiple clades intersect [43]. Recently, a case of superinfection was described in which a URF was generated in a woman who was initially infected with a clade A virus and then was noted 9 years later to have acquired a clade C virus that had recombined with the initial infecting virus. This produced a clade A/C recombinant that fully replaced the initial clade A infection [46].

The magnitude of global HIV diversity, driven in large part by recombination, provides circumstantial but substantial evidence, with no alternative explanation, that dual infection—whether coinfection, superinfection, or both—is widespread. In mathematical models, superinfection during the window of primary infection could account for the proportion of recombinant forms observed in various populations throughout the world [26]. Therefore, given the global frequency of CRFs and URFs, the true incidence of interclade dual infection—and of superinfection, especially—is probably underestimated in current investigations. Moreover, the rate of intraclade superinfection is even more difficult to assess, given the difficulty of current techniques to distinguish between different viral strains of the same clade [47].

WHEN DOES SUPERINFECTION OCCUR?

Recently, a retrospective analysis of a convenience sample of chronically infected individuals receiving antiretroviral therapy was unable to detect HIV superinfection during >1072 person-years of observation [48]. A similar cohort of chronically infected injection drug users showed no evidence of superinfection during >215 person-years of exposure [25]. Currently, most reports of superinfection indicate that it occurred in the setting of primary infec-

tion [2, 5, 47, 49, 50]. This is similar to what has been observed in a macaque model of superinfection with HIV-2, in which all 4 macaques challenged 2 weeks after initial infection became superinfected with the alternate strain, whereas only 1 of 4 macaques challenged 4 weeks after initial infection became superinfected. No macaques were susceptible 8 weeks after initial infection [51]. Although the timing and character of the immunologic responses demonstrated in the acutely infected macaque may differ significantly from those in the acutely infected human, this animal model may demonstrate an important corollary with regard to a relatively limited window of susceptibility to superinfection. Compared with chronically infected individuals, individuals with early HIV infection—and, thus, an immature HIV-specific immune response—may be the most likely to acquire the second strain of virus. However, the type of exposure and the intrahost viral dynamics may also influence the risk of superinfection [25]. Superinfection has also been described in the setting of treatment interruption 3 years after initial infection, which may mimic the window of primary infection via a low HIV antigen load and a loss of HIV-specific immune response [4, 26]. However, ongoing antiretroviral therapy may protect an individual from superinfection [48].

How frequently HIV superinfection occurs is not well characterized. In a cross-sectional study of 147 commercial sex workers in Burkina Faso, 2 cases of superinfection were identified, representing an annual incidence of 1.3%. This may be an underestimation of the true rate, because the investigators screened for superinfection on the basis of the HIV *env* gene and excluded individuals who were infected with HIV with *env* deletions. However, there was no other evidence of dual infection in the 2 cases identified with *env* deletions [52]. Our group evaluated the incidence of HIV-1 superinfection in a small cohort of newly infected individuals ($n = 78$) [47]. Superinfection was found at a rate of 5% per year (95% con-

fidence interval, 1.7%–13.3% per year), which was similar to the HIV-seroconversion rate observed in a comparably high-risk population participating in a VaxGen vaccine trial [53]. The finding of a superinfection rate that is similar to the HIV-seroconversion rate in a comparably high-risk cohort predominantly made up of men who have sex with men (MSM) suggests that the immunologic response after infection in our cohort offered negligible protection against superinfection with HIV during the first year after initial infection. The Swiss HIV Cohort Study found a similar annual incidence of HIV coinfection in 58 newly infected individuals (5% [3/58]). These individuals had injection drug use as their risk factor and were coinfecting with clades B and CRF-11. In this cohort, the investigators were also able to identify 1 individual who was superinfected during primary HIV infection and 2 individuals who were superinfected during chronic infection, >3 years after initial infection [23]. These data suggest that recent, and perhaps even chronic, infection with HIV does not offer distinguishable immunologic protection against reinfection. However, in a cohort of chronically infected sex partners (19 couples) who had distinguishable clade B HIV strains, no evidence of superinfection was detected during >38 person-years of follow-up, despite the self-report of >1500 unprotected vaginal or anal sex exposures [54]. All of these observations need to be confirmed and extended in larger populations, to better estimate the true risk of HIV superinfection after initial infection and to determine whether these rates change over time.

In many previous incidence studies, superinfection surveillance relied on population-based sequencing for initial screening, a method that may underestimate true incidence rates because it would not detect a superinfecting virus if it remained a minor variant below the level of detection, usually 30% of the circulating viral population [23, 25, 47, 48]. However, this was

not the case in 2 studies that screened for superinfection by use of other methods, heteroduplex mobility assays [52] and subtype-specific primers [23]. Superinfection may also have been missed if the superinfecting strain replicated transiently [23] or if it had replaced, through recombination, some of its genome with the initial infecting strain's genome by the time of the second sampling and that portion of the original genome was the portion being interrogated [25]. To better evaluate the true incidence of superinfection, large cohorts of newly infected individuals will need to be studied longitudinally. These investigations will need to include intensive sampling, epidemiologic partner tracking, and sensitive screening methods to detect minor viral populations and recombinant forms.

WHAT ARE THE INDIVIDUAL CONSEQUENCES OF SUPERINFECTION?

General assumptions that HIV-infected individuals cannot be reinfected may have contributed to the increase in risky sexual practices among HIV-infected individuals and, specifically, MSM [33]. Many urban centers have documented increases in rates of syphilis and other sexually transmitted diseases (STDs) among MSM [55] and the high prevalence of transmitted drug-resistant HIV [56]. These riskier sexual practices probably account for the seemingly high incidence of HIV superinfection in southern California [47].

Independent of typical STDs, risky sexual practices have other consequences, such as HIV superinfection. In most reported cases of superinfection, individuals have experienced a decrease in CD4⁺ cell count and an increase in HIV load (table 1). The magnitudes of the increase in HIV load and the decrease in CD4⁺ cell count are similar to those observed during primary infection [52, 60]. Both CD4⁺ cell count and HIV load are independent prognostic markers for HIV disease progression [61].

Even studies that could not distinguish

Table 1. Published cases of HIV-1 superinfection.

Citation	Time to superinfection	HIV subtypes	Risk factor	Accelerated disease progression after superinfection ^a	Drug resistance
Ramos et al. (2002) [2]	<3 months	B after AE	IDU	Yes	Not reported
Ramos et al. (2002) [2]	<11 months	AE after B	IDU	Yes	Not reported
Jost et al. (2002) [3]	<28 months	B after AE	MSM	Yes ^b	Not reported
Yerly et al. (2004) [23]	18–24 months	B after CRF-11	IDU	No ^c	Not reported
Yerly et al. (2004) [23]	~36 months	CRF-11 after B	IDU	Yes	Not reported
Yerly et al. (2004) [23]	45–55 months	CRF-11 after B	IDU	Yes	Not reported
Fang et al. (2004) [46]	Unable to be determined	C after A	WSM	? ^d	Not reported
Manigart et al. (2004) [52]	Unable to be determined	G after AG	WSM	Yes	Not reported
Manigart et al. (2004) [52]	Unable to be determined	CRF-06 after AG	WSM	Yes	Not reported
Altfield et al. (2002) [4]	<32 months	B after B	MSM	Yes ^b	Not reported
Koelsch et al. (2003) [5]	<4 months	B after B	MSM	Yes	DS after DR
Gottlieb et al. (2004) [50]	<15 months	B after B	MSM	Yes	Not reported
Chakraborty et al. (2004) [58]	Not reported	B after B	Not reported	Yes ^b	DS after DR
Brenner et al. (2004) [59]	10 months	B after B	MSM	Yes	DR after DR
Yang et al. (2004) [49]	<5 months	B after B	MSM	Yes	DS after DR
Smith et al. (2004) [57]	<14 months	B after B	MSM	Yes	DR after DS

NOTE. CRF-06, mosaic of clades A, G, K, and J; CRF-11, mosaic of clades A, G, E, and J; DR, drug-resistant strain; DS, drug-sensitive strain; IDU, injection drug use; MSM, men who have sex with men; WSM, women who have sex with men.

^a Those individuals referenced as having disease progression were reported to have had increases in HIV load around the time of suspected superinfection.

^b Individual reported recent treatment interruption, which could explain the change in disease progression.

^c Superinfection was noted to be transient and was detected in only 1 sample.

^d Reported disease progression could have been the result of the natural history of advanced HIV disease.

between coinfection and superinfection have found that dual infection is associated with an elevated HIV load set point [50, 62]. Among a cohort of individuals with primary infection, one individual with dual infection had the fastest disease progression [50]. The pathogenic mechanism could be that 2 divergent viral strains replicating in a single host allows for rapid adaptation and immune escape; alternatively, individuals who are predisposed to dual infection could have an immune system that is incapable of slowing disease progression [62]. Another possibility is that only a more virulent or fit virus is capable of superinfecting in the face of an established infection. However, in an animal model, HIV-2-infected cynomolgus macaques superinfected with SIV experienced less disease progression than did macaques infected with SIV alone [63]. Because most cases of human superinfection have not been identified in systematic prospective studies, a significant bias may exist. In these studies, cases of superinfection were identified

only when the second virus emerged as the predominant strain (and, thus, was readily detectable). The virus became the predominant strain because it was more fit than the original strain and, thus, more pathogenic. Future studies that are able to identify cases of superinfection in which the superinfecting virus circulates only as a minority variant may help to clarify the impact that superinfection has on disease progression.

Superinfection has also been reported to complicate antiretroviral therapy [57] and, potentially, drug resistance testing [5, 49, 58]. Probably because of the relatively high prevalence of HIV drug resistance and drug-resistance testing in the United States, many reported cases of HIV superinfection have occurred in the setting of transmitted drug resistance [47, 59]. Another possibility is that drug-resistant strains have an impaired replicative fitness in the setting of no antiretroviral therapy, which may allow a drug-sensitive strain to superinfect [5, 49]. However, there is a report of an individual who was first in-

fecting with a drug-sensitive strain and then superinfected with a drug-resistant strain, which was unrelated to the replicative capacity of *pol* [57]. There is also a report of an individual who was infected and then superinfected with heterologous strains that both harbored multiple drug resistance-associated mutations [59]. Superinfection involving drug resistance can have serious clinical consequences. When the drug-sensitive strain is the superinfecting strain and becomes the predominant virus, the drug-sensitive strain may mask the underlying drug-resistant strain, making drug-resistance testing unreliable [5]. When the superinfecting virus harbors drug resistance, the effective antiretroviral options available to the individual are reduced [57].

Because the frequency of superinfection is poorly characterized—as are its clinical consequences—how to counsel individuals already infected with HIV has been debated. Many believe that clinicians must counsel patients already infected with HIV to continue vigilant personal protection,

through safe-sex practices or the use of clean needles for injection drugs, even if their risk exposures are with other HIV-infected people [4, 5, 47]. These prevention messages have been disseminated; up to 90% of HIV-infected MSM in San Francisco have heard of superinfection [54, 64], and 74% reported that they have engaged in “safer” sex practices because they were concerned about superinfection [64]. These conservative prevention messages to HIV-infected individuals may breed conflict and mistrust in the HIV-affected community, because the true rates and consequences of HIV superinfection have yet to be well delineated. Alternatively, some have proposed that HIV-infected individuals who do not wish to use barrier protection methods should be encouraged to have sexual encounters only with other HIV-infected individuals [54]. The same prevention message could be extrapolated to those who are exposed via needle sharing. This serosorting may be effective in preventing new HIV transmissions [54], but the risk and clinical consequences of HIV superinfection would not be addressed. Moreover, other consequences of unsafe sex, such as syphilis and other STDs, would remain.

HOW DOES SUPERINFECTION AFFECT VACCINE DEVELOPMENT?

Clinical cases of HIV superinfection may offer unique opportunities to investigate the level of immunity required to protect against HIV challenge [2]. Increasing evidence suggests that virus-specific CD8⁺ T cell responses may be an important component of an effective HIV-1 vaccine [2, 4], and animal models provide evidence that antigenic boosting may be important in training the cytotoxic T lymphocyte response [65]. However, in 3 cases of superinfection for which CD8⁺ T cell responses were evaluated, the superinfecting virus did not share apparently important epitopes with the initial strain [3, 4, 49]. This could explain the immune evasion that led not only to the superinfection but also to the increased replication of the su-

perinfecting virus. Ongoing efforts to develop candidate HIV vaccines must consider these “natural” immunizations to be failures in the hopes of better understanding the roles of virus-specific CD8⁺ T cell and other host immune responses. Vaccines designed to train the immune system to recognize specific viral epitopes could compromise the capacity of HLA to bind to viruses that do not share the vaccine epitope. Not only could this lead to an ineffective vaccine, but it could mean that individuals who were vaccinated and then infected would experience faster disease progression than would individuals who were not vaccinated. This hypothesis is supported by the cases of superinfection occurring in individuals who had good immune control of the initial infecting virus—with low viral burdens and high CD4⁺ cell counts—and then less immune control of the second infecting virus [3, 5, 47, 49]. Furthermore, superinfection seems to occur in individuals who have a mature immune response to the initial infecting virus, even when both strains belong to the same clade [4, 49]. This may not bode well for the development of a cross-protective vaccine [66].

On the other hand, it could be argued that superinfection is not reflective of the situation where an uninfected host is vaccinated. Unlike vaccination, infection highly activates the immune system, and activated CD4⁺ T cells are the primary host cells for HIV replication [67]. Two vaccine studies in animals have shown that vaccinated animals are hypersusceptible to lentivirus when challenged shortly after a vaccine boost, when the immune system may have been activated [68, 69]. Furthermore, the antibody responses to vaccination and superinfection also may not be the same—and although antibody responses to the superinfecting virus have been documented [2], neutralizing responses have not been investigated in detail in either animal models or humans. Future studies that are able to identify both incident cases of superinfection and unsuccessful superinfection challenges may be

necessary to better understand the immunologic correlates of protection.

FUTURE STUDIES

Lentiviral superinfection has been described with feline immunodeficiency virus in cats, SIV in macaques, and HIV-1 in chimpanzees [1, 51, 70], so it is not surprising that HIV superinfection occurs in humans. The mysteries of HIV superinfection are beginning to be unraveled, but continued collaboration between the HIV-research community and the HIV-affected community is required to make further progress. We propose that the following questions be addressed by future research.

1. What is the incidence of HIV superinfection and coinfection in various groups at risk for HIV acquisition?
2. What is the natural history of HIV dual infection with regard to disease progression and drug resistance?
3. How does dual infection and, specifically, superinfection influence global HIV diversity?
4. What is the window of susceptibility to HIV superinfection? Does it vary among HIV subtypes and routes of exposure?
5. Which immunologic correlates (i.e., cell-mediated and neutralizing antibody responses) are associated with HIV superinfection and unsuccessful superinfection challenges? How do these relate to vaccine development?
6. Does superinfection occur more readily with a virus that belongs to the same clade as that of the initial infecting virus (interclade superinfection) or with a virus that belongs to a different clade (intraclade superinfection)? How might this affect the selection of viral strains for vaccine development in given regions?
7. What are the psychological and behavioral consequences of HIV superinfection in HIV-affected communities, and how can HIV-prevention strategies be improved to incorporate this new paradigm?

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