

Frequency and implications of HIV superinfection

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HIV superinfection occurs when an individual with HIV is infected with a new distinct HIV viral strain. Superinfection has been reported throughout the world, and studies have recorded incidence rates of 0–7.7% per year. Use of next-generation sequencing has improved detection of superinfection, which can be transmitted by injecting drug use and sexual intercourse. Superinfection might have incidence rates comparable to those of initial HIV infection. Clinicians should encourage safe sexual and injecting drug use practices for HIV-infected patients because superinfection has detrimental effects on clinical outcomes and could pose a concern for large-scale antiretroviral treatment plans. The occurrence of superinfection has implications for vaccine research, since it seems initial HIV infection is not fully protective against a subsequent infection. Additional collaborative research could benefit care of patients and inform future vaccine design.

Introduction

HIV superinfection occurs when an individual with HIV becomes infected with a new, phylogenetically distinct viral HIV strain. This differs from HIV dual infection, which is defined as being infected with two or more distinct viral strains at one point in time. Dual infection can be caused by superinfection as well as co-infection, which is initial infection with two or more strains. The possibility of superinfection was first discovered after the observation of co-infection with both HIV-1 and HIV-2, which are evolutionarily distinct viral species that share about 42% of nucleotide homology in their envelope genes.^{1–3}

Additional evidence for superinfection came from HIV-1 recombinant forms, which are HIV virions that contain separate genomic sections from distinct HIV-1 subtypes. HIV-1 is differentiated by genetic sequence into nine subtypes—A, B, C, D, F, G, H, J, and K, which have been associated with different rates of disease progression, viral load, detection method assay sensitivity, and distinct geographical regions.^{4,5} HIV-1 virions are diploid and viral strains are able to recombine when two distinct subtypes infect a single cell. If this new recombinant strain is transmitted it can become a circulating recombinant form. Roughly 10% of all HIV-1 infections involve recombinant viruses, providing further evidence of superinfection.⁶

Although HIV superinfection has been strongly suspected for many years, whether individuals were infected by two distinct HIV-1 viruses simultaneously (co-infection or dual infection), or whether a secondary infection occurred after the initial infection (superinfection), has been difficult to distinguish. Insufficient well documented longitudinal samples and absence of sensitive techniques for HIV superinfection detection prevented its documentation until 2002.^{7–9} Understanding of both intra-subtype and inter-subtype HIV superinfection is not only important for appropriate management, but could also provide insights into viral evolution and host immune responses to repeat HIV challenges before and after superinfection. Such information could have substantial implications for HIV vaccine development, global public health efforts, and care of patients.

Detection of HIV superinfection

The investigators in initial studies that identified individuals dually infected with HIV-1 and HIV-2 used serological assays that could easily distinguish between the two viral species;³ however, this approach cannot distinguish between different HIV-1 subtypes or strains. The initial cases of HIV superinfection were identified in injecting drug users in Thailand by restriction fragment analysis on amplified viral sequences from longitudinal samples followed by confirmatory viral sequencing.⁹ At the same time, two separate superinfection cases were identified in two men who have sex with men who were being monitored as part of larger clinical studies after they had unexplained spikes in their set-point viral loads.^{7,8} Samples from these individuals before and after the spike were subsequently analysed by subtype-specific PCR amplification⁷ or direct sequencing⁸ to confirm the presence of new viral populations. Other groups subsequently used these strategies of screening populations for spikes in viral load^{10–14} or subsequent restriction fragment analysis¹⁵ followed by direct sequencing to identify HIV superinfection.

After these initial studies, three diagnostic strategies were used to screen for HIV superinfection in populations: heteroduplex mobility assays,^{16–18} multiregion hybridisation assays,¹⁹ and bulk viral sequence analysis^{20–25} followed by selective cloning of those samples that suggested emergence of new viral variants. Multiregion hybridisation assays can identify only inter-subtype superinfection. Heteroduplex mobility assays, however, can detect samples with greater than 1.5% genetic difference but are susceptible to false positives caused by insertions or deletions.²⁶ Bulk sequencing can be used to examine for changes in the viral population by either searching for new phylogenetic species at a later timepoint or quantifying the amount of degenerate bases in a given sequence. The sensitivity of this strategy relies on the likelihood of amplification of the new viral population and not only the original strain. Not surprisingly, examination of degenerate bases poorly detected minor variants at levels of 20% or lower.²⁶ Additionally, all these methods need confirmation with cloning and sequencing.^{27–29} To avoid problems associated with less

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sensitive screening techniques, a range of populations with differing risk behaviours were screened for HIV superinfection by either single-viral genome amplification^{27,28} or cloning^{30,31} and then compared for evidence of phylogenetically distinct species. The capacity of these two approaches to accurately identify superinfection depends on the amount of sequences generated for each sample.^{28,29} The sensitivity of all sequence-based assays for identification of superinfection also depends on the number of viral genomic regions investigated.²⁸ The two approaches are therefore prohibitively expensive and too labour-intensive for large-scale studies.^{29,32}

Because of these limitations, we and other researchers have designed and verified next-generation-sequencing assays that can detect minor variants at 1% or less of the total viral population in a high-throughput way.^{29,33,34} We subsequently used this assay to identify HIV superinfection both in virally discordant couples and in a large population of African HIV seroconverters^{34,35} (figure 1). Next-generation-sequencing assays allow one to combine screening and verification, and can be done in a high-throughput manner, allowing for the accurate and timely assessment of large, at-risk populations or at an individual patient level.³⁵ Additionally, these assays can capture more

viral diversity than an approach based on amplification of single-viral genomes at 40% of the cost and with 20% less labour.²⁹ Consequently, next-generation-sequencing is becoming the assay of choice for detection of superinfection. Determination of the timing of the superinfection events relies on the sampling intervals available for the cohorts being examined. However, as knowledge improves of the rates of evolution and recombination after superinfection, better estimation of the timing of superinfection by use of phylogenetic and evolutionary modelling analyses is possible.

Epidemiology

HIV superinfection has been documented in observational studies and case reports in the USA^{8,23,31,36–38} and Canada,³⁹ Europe,^{7,10,13,22,40–42} Australia,¹¹ Asia,^{9,15} and Africa^{17,20,21,27,28,35,43} (figure 2); although a small study in Brazil did not find HIV superinfection when detection methods less sensitive than next-generation-sequencing were used.¹⁶ The widespread observation of superinfection suggests it is a substantial problem and has been under-reported.

Superinfection is transmitted in various ways. Two of the initial case reports and several observational studies^{23,37,40} were in men who have sex with men.^{7,8} Studies in Thailand and the USA have reported superinfection in intravenous drug users.^{9,15,31} Additionally, many cases of superinfection among female sex workers have been reported.^{17,20,27,28,43} Although first studied in high-risk populations, superinfection was recently found to occur among a heterosexual population in rural Africa.^{34,35}

HIV superinfection incidence rates of 0% to 7.7% per year have been recorded in several case reports and population-based studies.^{23,27,28,43–45} Incidence rates can vary substantially with population, the frequency of antiretroviral drug use, the length of follow-up, and the detection methods used. These differences in study design have led to several studies reporting no superinfection.^{45–47} However, a study using next-generation-sequencing assays in Uganda found superinfection incidence rates comparable to the rate of initial HIV-infection in the same area.³⁵ The study also drew attention to the difficulty of comparison between HIV superinfection and HIV primary incidence rates in a population because individuals with HIV are inherently at a higher-risk than the comparison group.³⁵ Results of this and other studies done in regions with multiple circulating subtypes have shown both inter-subtype and intra-subtype superinfection events, and a predisposition to either form is not apparent.^{27,28,34,35,48} Investigators doing future studies comparing incidences of superinfection and primary HIV should take into account individual risk practices, and ensure that the study is powered to identify a difference.

Acquisition of superinfection is suspected to have the same risk factors as primary infections, such as higher numbers of sexual partners,⁴⁹ non-marital relationships,^{49,50} limited condom use,^{51,52} no antiretroviral use,^{52,53}

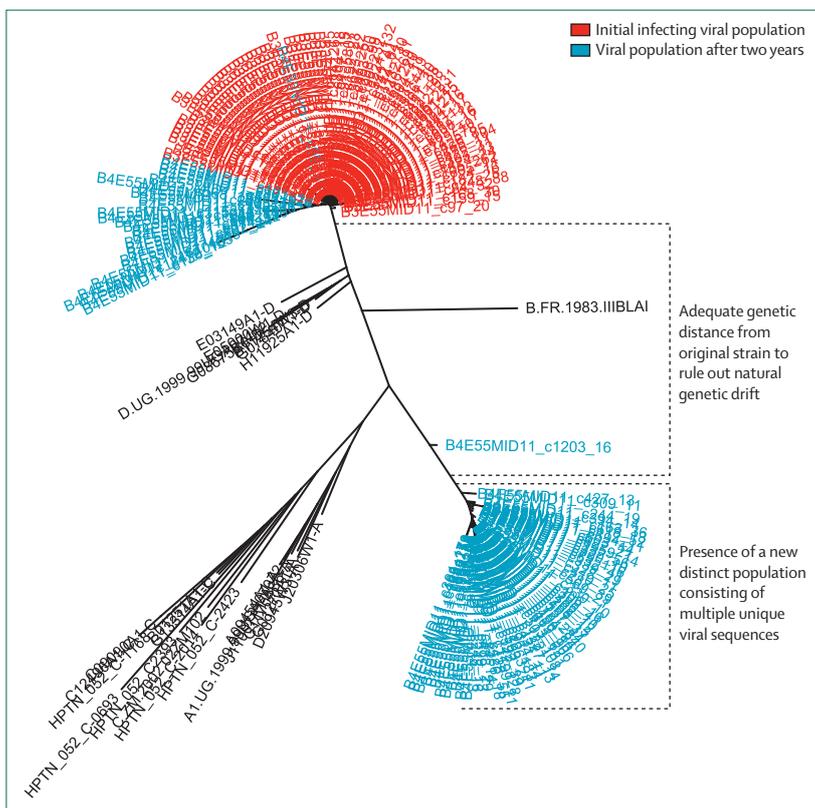


Figure 1: Representative phylogenetic tree for identification and confirmation of HIV superinfection
Phylogenetic tree of consensus viral sequences of a recent seroconversion sample (red) and a follow-up sample from 2 years later (blue) showing an inter-subtype superinfection event with a selection of subtype reference and random sequences from an individual in Uganda shown (black). The diagnostic requirements for superinfection are indicated.

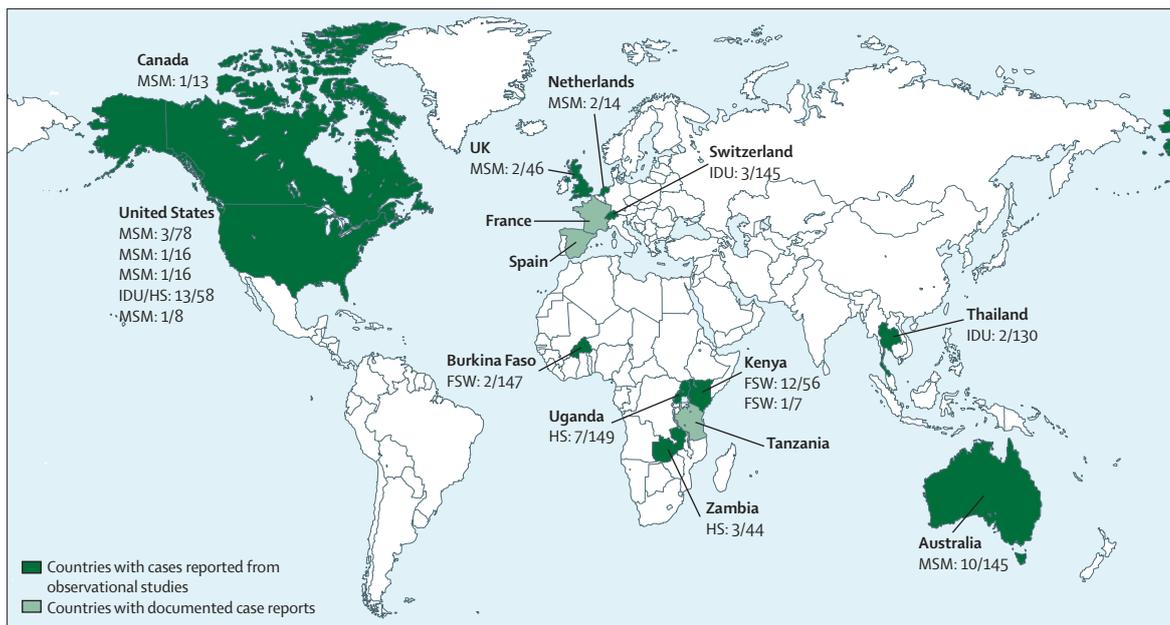


Figure 2: Worldwide cases of documented HIV-superinfection

Except for a small study in Brazil which did not find HIV superinfection when using less sensitive detection methods than next generation sequencing, all countries in white as reported in the medical literature have not been investigated for superinfection. Text indicates modes of transmission or risk groups and number of cases versus number screened by country for all observational studies in which HIV superinfection was detected. HS=heterosexual. FSW=female sex workers. MSM=men who have sex with men. IDU=injecting drug users.

and absence of male circumcision.^{54,55} However, risk factors have not been clearly defined because of the small number of cases studied. Future research is crucial to understand better the frequency and risk of superinfection on a population level.

A historical hypothesis that HIV superinfection most commonly occurs during the initial stage of primary infection because of reduced immunity was initially supported by many studies and case reports.^{9,12,23,56} The apparent close timing of the primary infection and superinfection, however, are probably due to the convenient sampling techniques. Time to superinfection has been reviewed,⁵⁶ and it can occur more than 2 years after primary infection.^{7,8,13} Determination of whether a predisposition in the timing of HIV superinfection exists is important to our understanding of the role an individual's immune system has in protecting them from a second HIV challenge.

Transmission and the global pandemic

Modelling studies have been undertaken to estimate the effect of HIV superinfection on recombination.^{57,58} These models suggest that superinfection was probably a pivotal component in creation and maintenance of recombination rates within a community; however, these models relied on many epidemiological assumptions that need to be clarified before the accurate effect of superinfection on the global pandemic can be ascertained.

One such assumption is how often HIV superinfection leads to transmission to partners who are not infected,

and whether the original, superinfecting, or a recombinant strain is transmitted (figure 3). Although no study has yet addressed this question directly, several investigators have attempted to find linked superinfection events by examining virally discordant HIV-infected partners.^{21,30,34,37} In two such couples, the HIV superinfection events were linked as verified by clonal sequencing²¹ or in-depth recombination analysis.³⁷ In view of the rarity of these linked cases, groups investigating superinfection-induced transmission events should combine their findings as much as possible.

Pathogenesis

Initial studies of HIV superinfection used distinct spikes in HIV viral load to identify new cases; the fact that in most cases superinfection will cause this type of response is generally accepted (figure 4).^{7,8,56,59} Whether superinfection results in a sustained rise in set-point viral load, however, is unclear. Results of several studies have shown an increased viral load in individuals with evidence of dual infection or superinfection,^{10,23,60,61} although others showed no such link.⁴⁰ In two studies in which confirmed superinfection events in Africa were examined, no consistent pattern of increased viral load set-point was found, even though set-point viral load increased by 0.5 log in seven of the 16 cases identified.^{27,28,35} Taken together, these data suggest that superinfection could lead to an increased viral load on a population level, but that it is not a necessary phenotype.

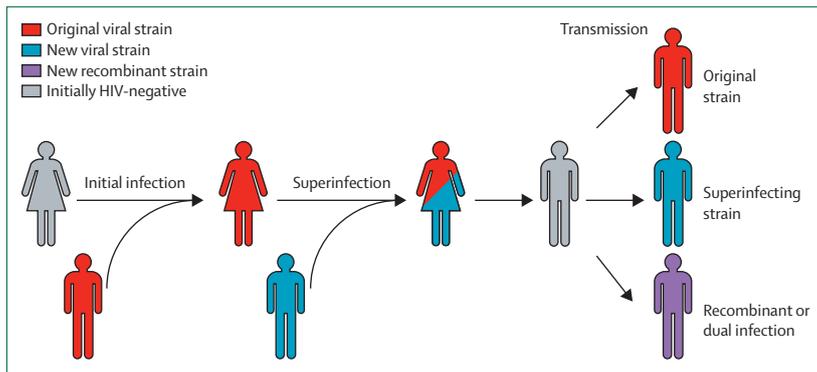


Figure 3: Hypothetical HIV superinfection transmission pattern

A sequence of a typical heterosexual sexual network involving initial transmission with an original viral strain followed by superinfection with a new viral strain. The sequence can result in the newly superinfected partner transmitting one or both of their two strains or a new recombinant strain to a subsequent partner. Although this demonstrates a heterosexual pattern, a similar pattern would be anticipated for men who have sex with men and intravenous drug users.

Although viral load is a strong predictor of HIV disease progression, the effect of superinfection is unclear.⁵⁶ Many studies have linked superinfection and dual infections to more rapid CD4 cell loss,^{19,23,56,62} although others have not shown an effect.⁶¹ One caveat is that the number of superinfection cases in these studies is small, so they are often grouped together with dual infections. Gottlieb and colleagues³⁶ described a case of a patient with superinfection who had a highly pathogenic dual-tropic HIV strain (using both CCR5 and CXCR4 as coreceptors for cellular entry), which led to rapid disease progression. In addition, others have documented cases of HIV superinfection causing either long-term non-progressors or elite controllers to progress to disease.^{41,63} However, another study documented superinfection in an elite controller who regained some control of the infection.⁶⁴ A mathematical model predicted that only superinfection by a more so-called fit strain would result in faster disease progression.⁶⁵ These data suggest that the full extent and potency of the detrimental effects of superinfection remain unclear and might depend on several viral and host factors.

Immunology

The immunological aspects of superinfection are inherently related to HIV vaccinology,⁵⁹ and the initial studies that described superinfection acknowledged this fact.⁷⁻⁹ These studies, and others in various populations, provide a sobering fact for HIV vaccine design—that initial HIV infection and the host's subsequent immune responses are not fully protective against a new HIV challenge. However, these findings also give investigators unique populations and novel research paths to identify which components of the natural HIV immune response could be protective against HIV superinfection, which components are not protective, and what happens to the immune response after a second successful HIV viral challenge.

Initially, Altfeld and colleagues⁸ showed in a case report that superinfection occurred even in the presence of a broad HIV-specific cytotoxic T-lymphocyte response.⁸ This absence of a protective effect for cytotoxic T-lymphocyte responses has since been confirmed in several studies.^{61,66,67} Neutralising antibodies (NAb) have long been thought to be an essential component of any successful protective HIV vaccine. Examination of NAb response before HIV superinfection in case-control studies has produced mixed results (figure 4). Smith and colleagues⁶⁸ observed that individuals before a superinfection event seemed not to have a NAb response. This observation was supported by another small study of heterosexual couples in Zambia that found individuals with HIV superinfection had a delayed NAb response to their autologous virus before superinfection;⁶⁹ however, results of a larger case-control study of Kenyan female bar workers showed no significant difference in NAb strength before superinfection.⁴⁸ This research group also reported that antibody-dependent cell-mediated viral inhibition was not associated with protection from superinfection.⁷⁰ Although the protective effect of NAb towards HIV superinfection remains unclear, results of several studies have shown superinfection boosted the NAb response after superinfection.^{48,68,69} The extent of this boost varies between cases, but seems to increase the potency of the NAb response, as measured by the plasma titre needed for neutralisation, as well as the breadth.⁴⁸ However, whether this level of NAb is protective against challenge from a third phylogenetically distinct strain of HIV is unknown.

Since HIV infection damages the host immune system, aspects of the anti-HIV immune response that are not fully protective against HIV superinfection might still protect against initial infection if recreated by a vaccine in the context of a healthy immune system. The underlying immunological health of patients examined for protection against superinfection should therefore be taken into account during study analysis.

One additional difficulty with study of the effects of HIV superinfection on the host immune response is that cases are difficult to identify in large enough numbers to make in-depth immunological analyses possible. In addition, longitudinal samples of a wide enough range of specimen types (peripheral blood mononuclear cells, mucosal excretions, serum, etc) to fully examine the host immune system are extremely rare. Therefore, for our understanding of the association between superinfection and host immunity to continue to expand, physicians and researchers examining this topic should work cooperatively with equivalent laboratory and clinical practices, so that studies can be easily compared and combined.

Implications for clinical care

Superinfection has implications for the clinical care of people with HIV. The risk of transmission of superinfection is greater among people with HIV who do not use safe sexual practices, and superinfection can lead to

increased viral load and disease progression.^{23,27,35} Thus, encouragement of safe sexual and injection practices is important, irrespective of HIV infection status. This includes counselling of patients with HIV about the risk of superinfection and encouragement of monogamous relationships, and use of condoms and clean needles. In a study of attitudes to HIV superinfection among men who had heard of the disease, 135 (82%) of 165 believed that superinfection could be damaging to their health and 122 (74%) of 165 practiced safer sexual practices because of concern about it.⁷¹ The most important aspect for men in this study in improvement of safer sexual practices was learning about the negative health consequences of superinfection; therefore, if counselling is done correctly, it could have a substantial positive effect on risk reduction. We therefore believe that clinicians and other health-care providers should counsel and give people with HIV information about the possible detrimental effects of superinfection as a component of their continuing care.

Antiretroviral therapy is highly effective at disrupting and in many cases reversing the detrimental clinical effects of HIV infection. As use of antiretrovirals worldwide increases one of the biggest concerns about superinfection is the transmission of drug-resistant strains, or susceptible strains masking HIV-resistant strains. Individuals with resistant strains can acquire a susceptible strain,¹² and those with a susceptible strain can acquire a resistant virus.^{39,72} Because of this negative effect on treatment, clinicians should be aware of the risk of superinfection, and examine individuals with HIV who present with a substantial spike in their viral load or a drop in CD4 count for emergence of a new resistant strain. Standard HIV resistance testing soon after one of these events will probably detect the resistance profile of this secondary strain, since the strain is most likely rapidly replicating; however, ultra-deep sequencing technologies, which are becoming cheaper and easier, might soon become more clinically available for the routine detection of superinfection and acquired resistance.

HIV transmission is directly related to viral load and is rare among patients with loads less than 1500 copies per mL.⁷³ Randomised controlled trial data also confirmed that antiretrovirals substantially reduce HIV transmission.⁵³ The effect of treatment on superinfection reduction has not been recorded by a randomised trial; however, most cases of superinfection have occurred before drug initiation or during treatment interruption. In one study of 14 high-risk HIV-seroconcordant couples who were treated with antiretrovirals, no cases of superinfection were documented.⁴⁷ The advent of increased antiretrovirals use at earlier timepoints will also hopefully reduce incidence of superinfection, but further research is needed.

Future research questions and important issues

The observation and subsequent description of HIV superinfection can be viewed as either a setback or an

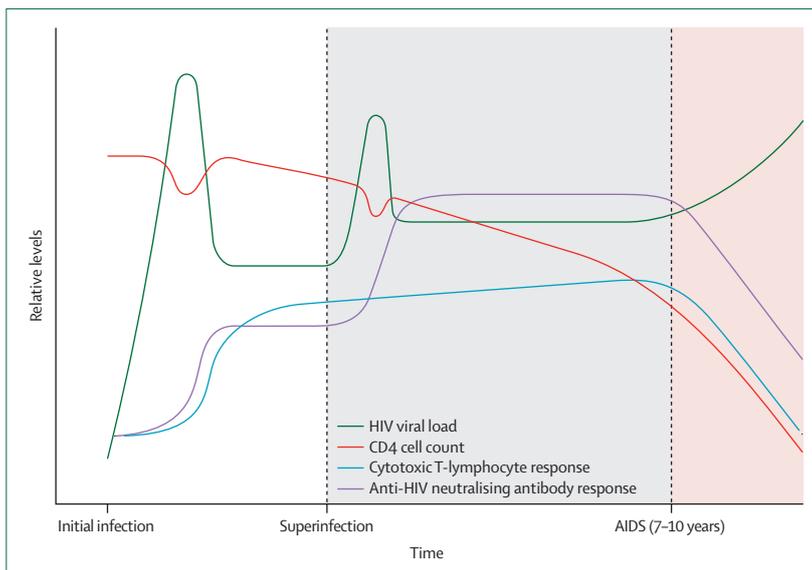


Figure 4: Representative clinical and virological aspects of HIV superinfection
Putative representative graph indicating the HIV viral load, CD4 cell count, CD8 cytotoxic T-lymphocyte response, and anti-HIV neutralising antibody (NAb) response of a typical case. The spike shown in viral load after superinfection is similar to what is found during acute infection, which might or might not result in a higher set-point viral load. An increase in NAb response is also shown after superinfection.

advancement of HIV research, depending on the point of view. The scale of the effect that superinfection will have on future HIV research is mostly unknown, and will rely on the ability of investigators to more fully answer some important fundamental biological questions. What is the effect of superinfection on disease progression, and what factors influence this (eg, subtype of superinfecting strain, timing of superinfection, magnitude of viral load change)? Can a robust NAb response protect individuals from superinfection, and what level of response is needed for this protection? What aspects of the immune response change after superinfection, and does this prevent a possible subsequent superinfection event? Are some individuals more susceptible to or protected from superinfection (eg, circumcised vs uncircumcised men, HLA-B57+ individuals)? What are the risk factors for superinfection, and do these differ from primary HIV-infection? Does superinfection increase the likelihood of transmitting to a partner, and what is the role of superinfection in transmission chains? Does antiretroviral therapy decrease superinfection and any subsequent transmission events?

The difficulty of identification of superinfection cases, and the labour and cost associated with screening large populations for these events, mean collaboration of researchers and clinicians will be essential to address these and other interesting questions. Therefore, when researchers use new and more technologically advanced diagnostic and experimental assays to examine superinfection, the strengths and limitations of every technique should be clearly stated and the sample population carefully described.

Search strategy and selection criteria

We searched PubMed for papers that were published in English between Jan 1, 1980 and Dec 1, 2012. The keyword search terms used included "HIV superinfection"[All Fields] OR "HIV dual infection"[All Fields] OR ("hiv"[MeSH Terms] OR "hiv"[All Fields]) AND double[All Fields] AND ("infection"[MeSH Terms] OR "infection"[All Fields] OR "communicable diseases"[MeSH Terms] OR ("communicable"[All Fields] AND "diseases"[All Fields]) OR "communicable diseases"[All Fields])) OR "HIV-1 superinfection"[All Fields] OR "HIV-1 dual infection"[All Fields] OR "HIV-1 double infection"[All Fields] OR "HIV reinfection"[All Fields] OR "HIV-1 re-infection"[All Fields]. Articles of relevance from reference lists were also incorporated. Only studies of original research or case reports of distinct HIV infection were included.

Conclusions

HIV superinfection has a potentially deleterious pathogenic effect on individuals with the infection. Results of initial studies have suggested that clinicians could lower the incidence of risky behaviours by simply informing patients of the potential effects of superinfection. Information about the risks and prevention should therefore be included as part of any comprehensive counselling strategy. Over the past decade, HIV vaccine research has focused on the improvement of understanding how some people with HIV naturally control their initial infection, and investigators have attempted to replicate this state in healthy individuals to provide some level of protection against future virus challenge. The prevalence of superinfection reported around the world suggests that this approach will probably not succeed, and vaccine strategies attempting this approach have been largely unsuccessful. Conversely, the study of HIV superinfection allows examination of the aspects of the naturally occurring immune response that might, or might not, be important for protection against subsequent viral challenge in a natural environment. These insights will help the development of a more targeted HIV vaccine research agenda by suggesting promising aims for study, and, crucially, ruling out aspects of the immune response that do not seem to be protective.

Contributors

ADR and AART did the initial literature searches and wrote the first draft of the manuscript. All authors participated equally in the revision and final approval of this manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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References

- Guyader M, Emerman M, Sonigo P, Clavel F, Montagnier L, Alizon M. Genome organization and transactivation of the human immunodeficiency virus type 2. *Nature* 1987; **326**: 662–69.
- Georgoulas V, Fountouli D, Karvela-Agelakis A, et al. HIV-1 and HIV-2 double infection in Greece. *Ann Intern Med* 1988; **108**: 155.
- Rey F, Salaun D, Lesbordes JL, et al. HIV-1 and HIV-II double infection in Central African Republic. *Lancet* 1986; **328**: 1391–92.
- Kiwanuka N, Laeyendecker O, Robb M, et al. Effect of human immunodeficiency virus Type 1 (HIV-1) subtype on disease progression in persons from Rakai, Uganda, with incident HIV-1 infection. *J Infect Dis* 2008; **197**: 707–13.
- Novitsky V, Woldegabriel E, Kebaabetswe L, et al. Viral load and CD4+ T-cell dynamics in primary HIV-1 subtype C infection. *J Acquir Immune Defic Syndr* 2009; **50**: 65–76.
- Robertson DL, Sharp PM, McCutchan FE, Hahn BH. Recombination in HIV-1. *Nature* 1995; **374**: 124–26.
- Jost S, Bernard MC, Kaiser L, et al. A patient with HIV-1 superinfection. *N Engl J Med* 2002; **347**: 731–36.
- Altfeld M, Allen TM, Yu XG, et al. HIV-1 superinfection despite broad CD8+ T-cell responses containing replication of the primary virus. *Nature* 2002; **420**: 434–39.
- Ramos A, Hu DJ, Nguyen L, et al. Intersubtype human immunodeficiency virus type 1 superinfection following seroconversion to primary infection in two injection drug users. *J Virol* 2002; **76**: 7444–52.
- Doyle T, Garcia-Diaz AM, Ambrose J, et al. Detecting HIV-1 superinfection by pol gene population sequencing among untreated HIV-1-infected men who experience sudden rises in plasma HIV-1 RNA load. *AIDS* 2011; **25**: 542–44.
- Sidat MM, Mijch AM, Lewin SR, Hoy JF, Hocking J, Fairley CK. Incidence of putative HIV superinfection and sexual practices among HIV-infected men who have sex with men. *Sex Health* 2008; **5**: 61–67.
- Koelsch KK, Smith DM, Little SJ, et al. Clade B HIV-1 superinfection with wild-type virus after primary infection with drug-resistant clade B virus. *AIDS* 2003; **17**: F11–16.
- Yerly S, Jost S, Monnat M, et al. HIV-1 co/super-infection in intravenous drug users. *AIDS* 2004; **18**: 1413–21.
- Yang OO, Daar ES, Jamieson BD, et al. Human immunodeficiency virus type 1 clade B superinfection: evidence for differential immune containment of distinct clade B strains. *J Virol* 2005; **79**: 860–68.
- Hu DJ, Subbarao S, Vanichseni S, et al. Frequency of HIV-1 dual subtype infections, including intersubtype superinfections, among injection drug users in Bangkok, Thailand. *AIDS* 2005; **19**: 303–08.
- Diaz RS, Pardini R, Catroxo M, Operskalski EA, Mosley JW, Busch MP. HIV-1 superinfection is not a common event. *J Clin Virol* 2005; **33**: 328–30.
- Manigart O, Courgnaud V, Sanou O, et al. HIV-1 superinfections in a cohort of commercial sex workers in Burkina Faso as assessed by an autologous heteroduplex mobility procedure. *AIDS* 2004; **18**: 1645–51.
- Rachinger A, Stolte IG, van de Ven TD, et al. Absence of HIV-1 superinfection 1 year after infection between 1985 and 1997 coincides with a reduction in sexual risk behavior in the seroincident Amsterdam cohort of homosexual men. *Clin Infect Dis* 2010; **50**: 1309–15.
- McCutchan FE, Hoelscher M, Tovanabutra S, et al. In-depth analysis of a heterosexually acquired human immunodeficiency virus type 1 superinfection: evolution, temporal fluctuation, and intercompartment dynamics from the seronegative window period through 30 months postinfection. *J Virol* 2005; **79**: 11693–704.
- Fang G, Weiser B, Kuiken C, et al. Recombination following superinfection by HIV-1. *AIDS* 2004; **18**: 153–59.
- Kraft CS, Basu D, Hawkins PA, et al. Timing and source of subtype-C HIV-1 superinfection in the newly infected partner of Zambian couples with disparate viruses. *Retrovirology* 2012; **9**: 22.
- Pernas M, Casado C, Fuentes R, Perez-Elias MJ, Lopez-Galindez C. A dual superinfection and recombination within HIV-1 subtype B 12 years after primo-infection. *J Acquir Immune Defic Syndr* 2006; **42**: 12–18.
- Smith DM, Wong JK, Hightower GK, et al. Incidence of HIV superinfection following primary infection. *JAMA* 2004; **292**: 1177–78.
- Cornelissen M, Jurriaans S, Kozaczynska K, et al. Routine HIV-1 genotyping as a tool to identify dual infections. *AIDS* 2007; **21**: 807–11.

- 25 van der Kuyl AC, Zorgdrager F, Jurriaans S, et al. Incidence of human immunodeficiency virus type 1 dual infections in Amsterdam, The Netherlands, during 2003–2007. *Clin Infect Dis* 2009; **48**: 973–78.
- 26 Rachinger A, van de Ven TD, Burger JA, Schuitemaker H, van 't Wout AB. Evaluation of pre-screening methods for the identification of HIV-1 superinfection. *J Virol Methods* 2010; **165**: 311–17.
- 27 Piantadosi A, Chohan B, Chohan V, McClelland RS, Overbaugh J. Chronic HIV-1 infection frequently fails to protect against superinfection. *PLoS Pathog* 2007; **3**: e177.
- 28 Piantadosi A, Ngayo MO, Chohan B, Overbaugh J. Examination of a second region of the HIV type 1 genome reveals additional cases of superinfection. *AIDS Res Hum Retroviruses* 2008; **24**: 1221.
- 29 Pacold M, Smith D, Little S, et al. Comparison of Methods to Detect HIV Dual Infection. *AIDS Res Hum Retroviruses* 2010; **26**: 1291–96.
- 30 Rachinger A, Manyenga P, Burger JA, et al. Low incidence of HIV-1 superinfection even after episodes of unsafe sexual behavior of homosexual men in the Amsterdam Cohort Studies on HIV Infection and AIDS. *J Infect Dis* 2011; **203**: 1621–28.
- 31 Templeton AR, Kramer MG, Jarvis J, et al. Multiple-infection and recombination in HIV-1 within a longitudinal cohort of women. *Retrovirology* 2009; **6**: 54.
- 32 Redd AD, Collinson-Streng A, Chatziandreou N, et al. Previously transmitted HIV-1 viral strains are preferentially selected during subsequent sexual transmissions. *J Infect Dis* 2012; **206**: 1433–42.
- 33 Lehman DA, Wamalwa DC, McCoy CO, et al. Low-frequency nevirapine resistance at multiple sites may predict treatment failure in infants on nevirapine-based treatment. *J Acquir Immune Defic Syndr* 2012; **60**: 225–33.
- 34 Redd AD, Collinson-Streng A, Martens C, et al. Identification of HIV superinfection in seroconcordant couples in Rakai, Uganda using next generation deep sequencing. *J Clin Microbiol* 2011; **49**: 2859–67.
- 35 Redd AD, Mullis CE, Serwadda D, et al. The rates of HIV superinfection and primary HIV incidence in a general population in Rakai, Uganda. *J Infect Dis* 2012; **206**: 267–74.
- 36 Gottlieb GS, Nickle DC, Jensen MA, et al. HIV type 1 superinfection with a dual-tropic virus and rapid progression to AIDS: a case report. *Clin Infect Dis* 2007; **45**: 501–09.
- 37 Campbell MS, Gottlieb GS, Hawes SE, et al. HIV-1 superinfection in the antiretroviral therapy era: are seroconcordant sexual partners at risk? *PLoS One* 2009; **4**: e5690.
- 38 Chakraborty B, Kiser P, Rangel HR, et al. Can HIV-1 superinfection compromise antiretroviral therapy? *AIDS* 2004; **18**: 132–34.
- 39 Brenner B, Routy JP, Quan Y, et al. Persistence of multidrug-resistant HIV-1 in primary infection leading to superinfection. *AIDS* 2004; **18**: 1653–60.
- 40 Jurriaans S, Kozaczynska K, Zorgdrager F, et al. A sudden rise in viral load is infrequently associated with HIV-1 superinfection. *J Acquir Immune Defic Syndr* 2008; **47**: 69–73.
- 41 Casado C, Pernas M, Alvaro T, et al. Coinfection and superinfection in patients with long-term, nonprogressive HIV-1 disease. *J Infect Dis* 2007; **196**: 895–99.
- 42 Plantier JC, Leme V, Dorval I, et al. HIV-1 group M superinfection in an HIV-1 group O-infected patient. *AIDS* 2004; **18**: 2444–6.
- 43 Chohan B, Lavreys L, Rainwater SM, Overbaugh J. Evidence for frequent reinfection with human immunodeficiency virus type 1 of a different subtype. *J Virol* 2005; **79**: 10701–08.
- 44 Gonzales MJ, Delwart E, Rhee SY, et al. Lack of detectable human immunodeficiency virus type 1 superinfection during 1072 person-years of observation. *J Infect Dis* 2003; **188**: 397–405.
- 45 Tsui R, Herring BL, Barbour JD, et al. Human immunodeficiency virus type 1 superinfection was not detected following 215 years of injection drug user exposure. *J Virol* 2004; **78**: 94–103.
- 46 Angel JB, Hu YW, Kravcik S, et al. Virological evaluation of the 'Ottawa case' indicates no evidence for HIV-1 superinfection. *AIDS* 2004; **18**: 331–34.
- 47 Chakraborty B, Valer L, De Mendoza C, Soriano V, Quinones-Mateu ME. Failure to detect human immunodeficiency virus type 1 superinfection in 28 HIV-seroconcordant individuals with high risk of reexposure to the virus. *AIDS Res Hum Retroviruses* 2004; **20**: 1026–31.
- 48 Cortez V, Odem-Davis K, McClelland RS, Jaoko W, Overbaugh J. HIV-1 superinfection in women broadens and strengthens the neutralizing antibody response. *PLoS Pathog* 2012; **8**: e1002611.
- 49 Potts M, Anderson R, Boily MC. Slowing the spread of human immunodeficiency virus in developing countries. *Lancet* 1991; **338**: 608–13.
- 50 Tobian AA, Ssempiija V, Kigozi G, et al. Incident HIV and herpes simplex virus type 2 infection among men in Rakai, Uganda. *AIDS* 2009; **23**: 1589–94.
- 51 Beyrer C, Sullivan PS, Sanchez J, et al. A call to action for comprehensive HIV services for men who have sex with men. *Lancet* 2012; **380**: 424–38.
- 52 Curran K, Baeten JM, Coates TJ, Kurth A, Mugo NR, Celum C. HIV-1 prevention for HIV-1 serodiscordant couples. *Curr HIV/AIDS Rep* 2012; **9**: 160–70.
- 53 Cohen MS, Chen YQ, McCauley M, et al. Prevention of HIV-1 infection with early antiretroviral therapy. *N Engl J Med* 2011; **365**: 493–505.
- 54 Tobian AA, Gray RH, Quinn TC. Male circumcision for the prevention of acquisition and transmission of sexually transmitted infections: the case for neonatal circumcision. *Arch Pediatr Adolesc Med* 2010; **164**: 78–84.
- 55 Tobian AA, Gray RH. The medical benefits of male circumcision. *JAMA* 2011; **306**: 1479–80.
- 56 Smith DM, Richman DD, Little SJ. HIV superinfection. *J Infect Dis* 2005; **192**: 438–44.
- 57 Gross KL, Porco TC, Grant RM. HIV-1 superinfection and viral diversity. *AIDS* 2004; **18**: 1513–20.
- 58 Taylor JE, Korber BT. HIV-1 intra-subtype superinfection rates: estimates using a structured coalescent with recombination. *Infect Genet Evol* 2005; **5**: 85–95.
- 59 Chohan BH, Piantadosi A, Overbaugh J. HIV-1 superinfection and its implications for vaccine design. *Curr HIV Res* 2010; **8**: 596–601.
- 60 Grobler J, Gray CM, Rademeyer C, et al. Incidence of HIV-1 dual infection and its association with increased viral load set point in a cohort of HIV-1 subtype C-infected female sex workers. *J Infect Dis* 2004; **190**: 1355–59.
- 61 Pacold ME, Pond SL, Wagner GA, et al. Clinical, virologic, and immunologic correlates of HIV-1 intra-clade B dual infection among men who have sex with men. *AIDS* 2011; **26**: 157–65.
- 62 Gottlieb GS, Nickle DC, Jensen MA, et al. Dual HIV-1 infection associated with rapid disease progression. *Lancet* 2004; **363**: 619–22.
- 63 Clerc O, Colombo S, Yerly S, Telenti A, Cavassini M. HIV-1 elite controllers: beware of super-infections. *J Clin Virol* 2010; **47**: 376–78.
- 64 Rachinger A, Navis M, van Assen S, Groeneveld PH, Schuitemaker H. Recovery of viremic control after superinfection with pathogenic HIV type 1 in a long-term elite controller of HIV type 1 infection. *Clin Infect Dis* 2008; **47**: e86–9.
- 65 Fung IC, Gambhir M, van Sighem A, de Wolf F, Garnett GP. Superinfection with a heterologous HIV strain per se does not lead to faster progression. *Math Biosci* 2009; **224**: 1–9.
- 66 Blish CA, Dogan OC, Jaoko W, et al. Cellular immune responses and susceptibility to HIV-1 superinfection: a case-control study. *AIDS* 2012; **26**: 643–46.
- 67 Streeck H, Li B, Poon AF, et al. Immune-driven recombination and loss of control after HIV superinfection. *J Exp Med* 2008; **205**: 1789–96.
- 68 Smith DM, Strain MC, Frost SD, et al. Lack of neutralizing antibody response to HIV-1 predisposes to superinfection. *Virology* 2006; **355**: 1–5.
- 69 Basu D, Kraft CS, Murphy MK, et al. HIV-1 subtype C superinfected individuals mount low autologous neutralizing antibody responses prior to intrasubtype superinfection. *Retrovirology* 2012; **9**: 76.
- 70 Forthal DN, Landucci G, Chohan B, et al. Antibody-dependent cell-mediated virus inhibition (ADCVI) antibody activity does not correlate with risk of HIV-1 superinfection. *J Acquir Immune Defic Syndr*. Published online Jan 22, 2013. DOI:10.1097/QAI.0b013e3182874d41.
- 71 Colfax GN, Guzman R, Wheeler S, et al. Beliefs about HIV reinfection (superinfection) and sexual behavior among a diverse sample of HIV-positive men who have sex with men. *J Acquir Immune Defic Syndr* 2004; **36**: 990–92.
- 72 Smith DM, Wong JK, Hightower GK, et al. HIV drug resistance acquired through superinfection. *AIDS* 2005; **19**: 1251–56.
- 73 Quinn TC, Wawer MJ, Sewankambo N, et al. Viral load and heterosexual transmission of human immunodeficiency virus type 1. Rakai Project Study Group. *N Engl J Med* 2000; **342**: 921–29.