HIV-1 superinfection in homosexual men
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Chapter 1

Introduction
HIV-1 superinfection is defined as acquisition of another HIV-1 strain after immune responses have been elicited against an initial HIV-1 strain. Dual infection comprises, next to HIV-1 superinfection, also HIV-1 co-infection, with co-infection defined as (nearly) simultaneous infection with multiple viral strains at or around seroconversion. Worldwide, close to fifty cases of HIV-1 superinfection have been documented in a broad variety of settings [1-23], starting in 2002 with two detected superinfections in an injecting drug user (IDU) cohort in Thailand [1], followed by superinfections in cohorts of female sex workers [7, 10, 12, 13, 18, 20], homosexual men [8, 16], and in 2009, in a longitudinal cohort of women [23]. HIV-1 superinfection incidence rates differ between reports, from no HIV-1 superinfection [24, 25] to equal rates of initial HIV-1 infection and HIV-1 superinfection [9, 12, 18, 20]. Reasons for these differences have remained unresolved as yet, but may be multifarious.

**HIV-1 superinfection and disease progression**

Although the notion that increased plasma viral load might be a potent indicator for the occurrence of HIV-1 superinfection has been discarded [26], HIV-1 superinfection was in some cases still associated with accelerated disease progression [1, 2, 4-6, 8-10, 14]. Yet, in other cases, viral load (VL) and CD4+T cell numbers remained stable upon superinfection, and for most cases neither VL nor CD4 counts were available for follow-up. Together with the unresolved cause(s) of incidence differences between studies this resulted in uncertainty regarding the impact of HIV-1 superinfection on HIV-1 disease outcome.

**HIV-1 superinfection, adaptive immunity and elite control**

Upon initial HIV-1 infection, HIV-1 specific cytotoxic CD8+T cells and neutralizing antibodies are generated by the immune system (reviewed in [27]); contradicting evidence exists whether adaptive immunity might influence rates of superinfection or not [3, 28-30]. Lack of neutralizing antibodies [28] as well as low cross-reactive T cell responses predisposed to HIV-1 superinfection [29]; on the other hand, HIV-1 superinfection occurred despite neutralizing antibody responses [30] and broad CD8+ T cell responses [3]. In addition, unknown mechanisms might be involved in resistance against superinfection raising the question whether those mechanisms might also account for other yet unexplained phenomena in HIV-1 disease, such as long-term non-progression or elite control.

**HIV-1 superinfection and vaccine research**

HIV-1 superinfection is invariably linked to HIV-1 vaccine research. Although the immune system of a healthy, vaccinated HIV-1 negative individual differs from that of an HIV-1 infected individual, both settings were considered comparable such that adaptive immune responses generated upon initial infection or vaccination might
confer resistance against incoming HIV-1. This was supported by SIV superinfection in an animal model, which could only be established during a brief time period after initial infection [31-33]. With the first HIV-1 superinfection cases published in 2002, which were followed by increasing numbers of superinfection cases due to more in-depth and systematic cohort screenings [18, 23], concerns for a realistic protective HIV-1 vaccine grew. Subsequently, trials on elicited T cell immunity vaccines failed to demonstrate a protective effect against initial HIV-1 infection, and left the research community uncertain of how to proceed.

**Differences in HIV-1 superinfection incidences between studies and putative causes for underreporting of HIV-1 superinfection**

Differences in HIV-1 superinfection incidence rates between cohorts in conjunction with absent superinfection or low HIV-1 superinfection incidences in some studies evoke the question whether the scientific community is facing to date only the tip of the HIV-1 superinfection iceberg. One possible reason for differences in HIV-1 superinfection incidences rates between studies might be variances in (sexual) risk settings, including differences in (sexual) risk behavior, in HIV-1 prevalences within (sexual) risk networks, presence of sexually transmitted infections (STI) other than HIV and of mucosal traumas, host or viral factors.

Furthermore, HIV-1 superinfection requires detailed phylogenetic analysis of HIV-1 sequences, including (optimally) the initial viral strain and the superinfecting strain, added to a set of reference sequences. However, HIV-1 superinfection strains might co-exist with initial HIV-1 strains, they might overgrow initial HIV-1 strains or they might be only intermittently present in blood, which makes timing and frequency of sampling crucial for detecting superinfection. In more recent publications, more frequent sampling clearly increased numbers of detected HIV-1 superinfections [18, 23]. With HIV-1 being a recombining retrovirus with high replicative capacity, superinfection might remain undetected if recombined strains differ in genome parts other than the investigated region. One study showed that analyzing an additional genome part increased the number of detected superinfections [20]. Optimally, whole-genome sequences would deliver the most complete picture; unfortunately, due to the highly laborious and costly nature of these methods, this remains unrealistic for standard investigations of cohorts.

Additionally, approaches and methods applied varied largely from pre-screening cohorts before detailed in-depth analysis to investigating single individuals by restriction fragment length polymorphism (RFLP), multiregion hybridization assay (MHA), heteroduplex tracking assay (HTA), heteroduplex mobility assay (HMA), sequencing of single copy PCR amplicons, population-based sequencing, clonal sequencing, followed by phylogenetic analysis. Study designs varied largely in number of participants, number of time points studied, length and numbers of
investigated genome fragments and sequences analyzed per time point, rendering reported HIV-1 superinfection incidences most likely biased. Finally, sensitivity of detection methods seems crucial. Methods failing to reveal superinfection strains present at lower percentages within viral quasispecies might have involuntarily assisted in underestimating superinfection incidences. For instance, during population-based sequencing viral strains present at less than 20-30% are not detectable [34-38], hence phylogenetic analysis deals with a viral quasispecies subset only, lowering chances of HIV-1 superinfection detection.

**Scope of this thesis**

In this thesis, research questions in relation to HIV-1 superinfection in homosexual men in the Netherlands will be addressed. The Amsterdam Cohort Studies on HIV Infection and AIDS (ACS) provides a unique collection of serum and peripheral blood mononuclear cell (PBMC) specimen of HIV-1 positive homosexual men who have been enrolled in the ACS since 1984 and who provide self-reported sexual behavioral data in 6-monthly questionnaires. In chapter 2, a case of HIV-1 superinfection of a long-term elite controller of HIV-1 infection is described which suggested that mechanisms involved in elite control of HIV-1 disease might not confer resistance to HIV-1 superinfection. In chapter 3, superinfection sexual risk settings, such as the number of sexual partners, condom use, and anal intercourse are addressed in relation to absence of HIV-1 superinfection in homosexual men in their first year after seroconversion during the early HIV epidemic in the Netherlands. Chapter 4 describes the investigation of superinfection in a group of homosexual men exhibiting higher-risk sexual behavior during long-term follow-up. In chapter 5, the absence of HIV-1 superinfection in a documented donor-recipient pair that continued to engage in unsafe sexual behavior after the initial transmission event will be delineated. In chapter 6, sensitivity of pre-screening methods for HIV-1 superinfection will be compared.
References