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Biological correlates of sexual transmission of HIV: practical consequences and potential targets for public health

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The probability of sexual transmission of HIV depends on the infectiousness of the index case and the susceptibility of the sexual contact. The risk of HIV transmission is heterogeneous and may be greatest during the initial sexual contacts in a steady partnership. Several factors, including systemic and mucosal acquired protective immune response, might be responsible for the apparent decrease of per-sex-act risk of transmission in a given partnership over time. Biological studies can be used to understand better the complex information obtained from epidemiological surveys. The infectiousness of HIV depends both on the inoculum and on virologic factors. The genital tract viral load of the index case is probably the most important determinant of transmission. At the population level, interventions that reduce the genital shedding of HIV by reducing systemic blood viral load and/or local inflammatory processes are likely to have a beneficial impact on HIV incidence. Antiretroviral drugs are likely to reduce sexual transmission of HIV; however, these drugs may not do so equally. Compartmentalised HIV replication in the male and female genital tract has been observed. Treatment with antiretroviral drugs that penetrate the genital tract poorly pose the risk of local production and spread of resistant viruses. In addition, increased risk-taking behaviour could offset the benefits of reduced probability of transmission at the population level. Biological data about HIV transmission must be used to inform public health policies and optimise HIV prevention strategies.

Keywords: HIV, antiretroviral therapy, sexual transmission, prevention.

INTRODUCTION

Worldwide, the predominant mode of HIV transmission is clearly heterosexual contact. However, the biological correlates for HIV transmission are less clear because of the heterogeneity of sexual transmission. This heterogeneity is best illustrated by individual case reports where transmission occurs in some partners after limited sexual contact, while other partners of the same individual remain uninfected [1]. The heterogeneity of sexual transmission makes the risk of a single contact much less predictable than for other modes of transmission.

The probability of sexual transmission of HIV is

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a function of the infectiousness of the index case, the mode of the sexual contact, and the susceptibility of the person exposed to the virus. We have recently reviewed factors contributing to the infectiousness of the infected individual [2] and a review of the susceptibility factors has been published by Buchacz [3]. Here we summarise recent findings about susceptibility and infectiousness, with a focus on infectiousness factors that can be influenced by public health prevention strategies.

ESTIMATING THE TRANSMISSION RISK: WHERE BIOLOGY MEETS EPIDEMIOLOGY

Estimates for the risk of sexual transmission of HIV have been generated from epidemiological surveys. In most instances, partner studies have been used to calculate the per-partnership transmission risk or the per-sexual-contact risk of transmission. The calculation of the per-sex-act risk is based on the assumption that the risk remains stable over a long period (stage) of the disease [4,5]. However, studies that report a high transmission risk after a single exposure to an HIV-infected prostitute question the validity of this assumption [6,7]. The data from partner studies is best predicted by mathematical models that account for a large heterogeneity in HIV infectiousness [8]. Increasing evidence is evolving that indicates a more complex temporal performance for both HIV infectiousness and susceptibility to HIV. Biological studies have therefore been used to complement the information obtained from epidemiological studies; the two combined approaches will undoubtedly improve our understanding of HIV transmission and facilitate interventions to reduce it.

Heterogeneity of the HIV transmission risk

Three major factors that determine HIV transmission risk contribute to the heterogeneity observed: differences in the susceptibility to HIV; variable infectiousness of infected individuals; and a wide spectrum of sexual behaviour in a given population. Heterogeneity of sexual behaviour has led to the concept of core groups in which rates of partner change and sexual activity are substantially higher than in the general population [9]. Behavioural issues that affect transmission have recently been reviewed [10].

Susceptibility to HIV

The susceptibility to HIV infection depends on host immunological and genetic factors. A homozygous CCR5 co-receptor deletion mutant has been shown to protect against HIV infection [11]. Genetic factors, such as CCR5 and SDF variants as well as certain HLA types, are also strongly associated with non-progression in HIV-infected individuals [12]. It is conceivable that these factors could also influence the susceptibility to HIV. At least one-third of individuals exposed to HIV develop a cell-mediated immune response without overt seroconversion [13,14]. Some animal models show a protective immune response after subinfectious exposure to HIV; by analogy, one might expect that some of the HIV-sensitized exposed partners also have a reduced susceptibility to HIV [15]. Some exposed individuals also develop an HIV-specific mucosal and systemic IgA response and these antibodies neutralise primary HIV strains [16]. These data are supported further by several reports of decreased susceptibility to HIV in multiply-exposed commercial sex workers (CSW) with continued high-risk behaviour[17,18]. Existence of a (partly) protective immune response after sexual exposure to HIV might explain the decrease of HIV transmission risk after a few sexual contacts in a given heterosexual partnership, as reported in the study that is discussed below.

Local genital factors are also likely to influence the susceptibility to HIV. Upregulation of the CCR5 co-receptor and increased activation of T lymphocytes present in the normal cervix have been documented [19]. This finding supports the vulnerability of the female genital tract for HIV and suggests a mechanism for the observed predominance of sexual transmission of HIV variants with CCR5 co-receptor usage [20].

Sexually transmitted diseases (STD) have long been shown to influence not only infectiousness (see below) but also susceptibility to infection. Local inflammatory reactions in response to STD are likely to attract CD4 lymphocytes and release of cytokines that enhance transmission of HIV. Changes in vaginal flora appear to affect susceptibility to HIV as well. Bacterial vaginosis (BV) is associated with a high concentration of vaginal anaerobes, reduced lactobacilli and an increase in vaginal pH [21]. Sturm-Ramirez and colleagues found *in vitro*

evidence for increased levels of pro-inflammatory cytokines (TNF-α and IL-1) in patients with BV, which could in part explain the association of BV with increased susceptibility to HIV [22].

Other local factors also potentially modulate HIV susceptibility: hormones that alter the vaginal epithelium have been investigated for their influence on vaginal HIV shedding. Recently, oestrogens were shown to inhibit HIV transmission in the macaque model; this effect was accompanied by thickening of the vaginal epithelial wall [23].

The effect of circumcision on HIV transmission is highly debated. The estimates for the odds against transmission in circumcised men are in the range of 1.5. Several mechanisms have been proposed to explain this effect, including the reduced surface area for mucosal contact [24]. However, most studies investigating the role of circumcision on HIV transmission are confounded by religion and are therefore difficult to interpret. In a mathematical analysis of per-sex-act male-to-female transmission probabilities, Duerr *et al.* found similar per-act transmission risk in otherwise comparable US (mostly circumcised) and Italian (mostly uncircumcised) HIV-discordant couples [25]. This argues against a marked effect of circumcision status on HIV transmission. The role of circumcision on susceptibility to HIV needs to be examined further before recommendations for large-scale circumcision interventions are issued as recently proposed [26].

In summary, a combination of local genital factors, immune response mechanism, and genetic predisposition all influence the risk of transmission by affecting susceptibility to HIV. However, based on the epidemiological partner studies discussed below, infectiousness of the infected partner is likely to influence transmission probability even more than susceptibility.

Infectiousness of the HIV-infected partner

Viral clade, quasispecies and transmission

Based on differences in the replication kinetics in dendritic cells between subtype E and B, Essex *et al.* suggested that HIV subtype might play a role in transmission efficacy [27]. However, these results have not been confirmed by others [28] and it has been very difficult to document any effect of subtype differences on transmission risk. Miller *et al.* could not find a difference in transmissibility based on HIV macrophage tropism but found *in vivo* replication capacity to be predictive for the outcome of intravaginal inoculation in the macaque model [29]. Another animal study found that some HIV quasispecies were better adapted for vaginal transmission than others, but the significance of this finding for the case of HIV transmission is not known [30]. Ping *et al.* have argued that clade C expresses biological characteristics which favour sexual transmission [31]. A careful review of the debate has been published by Hu *et al.* [32]. In summary, systemic and local host factors remain the most important determinants for HIV infectiousness.

Systemic factors influencing HIV infectiousness

Epidemiological studies (mostly partner studies) have previously demonstrated an increased HIV transmission from patients with low CD4 cell count, advanced stages of disease, p24 antigenaemia and the presence of STD (reviewed in [2]). Subsequently, time variation of HIV infectiousness was proposed by Jacquez *et al.* based on mathematical models of monogamous partner studies [5]. The authors argue that HIV infectiousness is increased during primary infection (which is consistent with an increase of blood viral load during this phase). However, a key assumption of this model, a stable per-contact transmission rate, was challenged by Downs *et al.* [33] and discussed further by Shiboski and Padian [34]. Several factors that might explain the observed decline in transmission risk after primary infection need to be considered. Firstly, the large heterogeneity of transmission itself may explain the finding, also termed 'frailty selection'[35]. Secondly, the assumption of constant transmission rates during a given partnership might influence the results. Thirdly, key variables on infectiousness and susceptibility that might influence the curve are usually missing in these partner studies. Shiboski and Padian conclude that the nature of variation in infectiousness cannot be confirmed, but that the findings indicate a declining tendency of transmission risk in monogamous heterosexual couples with increasing length of exposure [34]. It is just as likely that the declining risk of transmission in a given partnership results from an acquired protective immune response in the non-infected partner. This would also explain the

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substantially higher risk estimates (3–8%) for single sexual exposures to commercial sex workers [6,7].

In general, transmission of a pathogen is concentration dependent. Vertical transmission of HIV and occupational transmission through needle-stick injuries increase in situations with high viral load in the index person [36,37]. Several partner studies have demonstrated the association of blood viral load with sexual transmission of HIV [38–40]. In these studies, neither CD4 cell count nor the number of sexual contacts affected the risk of transmission. In a recently published subanalysis of discordant couples included in a large STD-intervention study in Rakai, viral load was the most powerful predictor of HIV transmission [41]. Of note, none of the 53 HIV-infected individuals with a viral load < 1500 copies/ml transmitted the virus to their partner despite the negligible use of condoms.

In all of the epidemiological studies on transmission risk mentioned above, measurement of HIV-RNA concentration in blood was not available. It is likely that these studies were confounded by blood viral load. Low CD4 cell count, advanced stage of disease, p24 antigenemia and primary HIV infection are all associated with higher levels of HIV-RNA in the blood. While an independent role of all those factors on infectiousness cannot be excluded, it is apparent that blood viral load is by far the most important cofactor.

HIV load in the genital tract as a measure of infectiousness

The strong predictive power of blood viral load for HIV transmission risk is biologically plausible. In general, transmission of a pathogen is concentration dependent. Likewise, vertical transmission of HIV and occupational transmission through needle-stick injuries increase in situations with high viral load in the index person [37]. Semen can transmit HIV [42]. We and others have therefore studied the concentration of HIV in genital secretions and postulated that the measurement of HIV in semen can be used to estimate an individual's infectiousness (reviewed in [2,43]). Similar results have accumulated for female genital secretions, but the variability of the assays for the detection of HIV-RNA or DNA in female secretions is considerably higher than for semen [44–48]. The menstrual cycle further increases the biological variability of the virus shedding in the female genital tract [49].

The combined information on HIV viral load and tropism in semen and the CCR5 receptor density on target cells in the female genital tract has been used to predict the transmission probability of HIV in the genital tract [50]. In a mathematical model that we have developed, HIV transmission was unlikely (1 in 10 000 episodes of intercourse) when HIV RNA in semen was low (i.e. < 5000 copies/ml) but rose to 3 per 100 episodes of intercourse if HIV RNA in semen was high (10^6 copies/ml). Such high seminal RNA values have been found in semen from HIV-positive men with STD in Malawi. Thus, seminal viral load can serve as a surrogate marker to determine infectiousness.

Local genital factors influencing HIV infectiousness

Semen studies have revealed a weak but significant correlation (r, 0.55–0.60) of blood viral load and seminal HIV RNA concentrations. The correlation was considerably higher (r, 0.80) in a recent study of patients with primary infection when a more sensitive HIV RNA detection assay was used [51]. Thus, in agreement with the epidemiological studies, the biological studies indicate that a major determinant of the viral inoculum size in semen is blood viral load; approximately 60% of the seminal viral load can be predicted by blood viral load alone.

Factors that explain the discordance between blood and semen viral load must be investigated. HIV-RNA concentration in semen is approximately one $\log_{10}$ lower than the respective level in blood. However, some patients have significantly higher levels of HIV in semen than in blood and have therefore been termed hypersecretors [52,53]. In one study, hypersecretor status has been associated with positive cell culture from the cellular semen compartment [54], supporting the potential effect of hypersecretor status on sexual transmission risk. Hypersecretor status has been associated with asymptomatic urethritis [55] and was an independent risk factor for the shedding of HIV-RNA in semen.

Symptomatic STD have long been shown to increase the risk of sexual transmission of HIV

[56,57]. Similar to the studies of blood viral load described above, biological studies have been performed to demonstrate the influence of STD on the viral load in genital secretions. Cohen *et al.* studied STD patients in Malawi and found an almost 10-fold increased level of HIV-RNA secretion in semen in patients with gonorrhoea as compared with control patients without urethritis [58]. This increase in genital viral load was not associated with an increased HIV RNA concentration in blood. In the same study, genital ulcer disease was also associated with an increased HIV shedding in semen, indicating the existence of an indirect mechanism by which genital inflammatory diseases stimulate the replication kinetics of HIV in semen [59]. Further analysis of the viral quasispecies in semen and blood samples from this study by heteroduplex mobility assay revealed a discordance of the two compartments in 40% of these individuals [31]. A number of other studies have documented similar associations between genital inflammatory diseases and HIV detection in both semen and cervical secretions [22,60–62]. In a recently published study conducted in Nairobi, treatment of women with cervicitis (*Neisseria gonorrhea, Chlamydia trachomatis* or non-specific cervicitis) was associated with a six-fold reduction in the shedding of HIV-1 RNA in cervical secretions [63].

Taken together, the biological findings support the significance of STD for HIV transmission and identify genital inflammatory diseases as another major determinant of genital shedding of HIV and HIV infectiousness.

REDUCING HIV INFECTIOUSNESS AS A PREVENTIVE STRATEGY

Efforts to reduce the spread of HIV can target sexual behaviour, or the susceptibility of uninfected individuals, or the infectiousness of the HIV-infected population. Reducing HIV susceptibility by the induction of a protective immune response is the central aim of HIV vaccine development. Until a protective vaccine is developed, other strategies – such as the application of vaginal microbicides or syndromic management of STD in high-incidence areas – are currently under development to help protect individuals from being infected.

HIV prevention efforts have only recently shifted to the infected person, who can now benefit from early detection of infection. Index cases can be encouraged to engage only in safe sex behaviours, with 100% condom usage. In addition, efforts to reduce HIV viral burden in the genital tract can be undertaken.

Treatment of STD and HIV infectiousness

STD increase the HIV load in the genital tract. The effects of treatment of STD on genital HIV shedding has been well-documented in men and women [58,60,63]. In addition, the benefits of STD treatment on the HIV epidemic have been translated to some, but not all populations. In a community-randomised trial in Mwanza, Tanzania, improved management of STD resulted in a 40% reduction of HIV incidence [64]. However, in a randomised study in the Rakai district of rural Uganda, mass STD treatment did not result in a significant reduction of HIV incidence [65]. In that study, absence of a detectable effect on HIV incidence was probably a result of the mature stage of the HIV epidemic in rural Uganda [66]. In such mature, generalised, epidemic settings, a saturating effect is observed in core groups with high-risk behaviour; therefore HIV incidence occurs at a lower level in the low-risk population and is less likely to be driven by STD. Mass treatment of STD cannot target individuals with the highest risk of transmitting HIV. On an individual level, however, treatment of STD is unquestionably an important intervention to reduce HIV infectiousness.

Reduction of viral load by HIV treatment

HIV-RNA concentration in maternal blood is associated with transmission risk [37,67–69] and antiretroviral treatment of mothers prepartum results in reduction in maternal viral loads and vertical transmission rates [70]. Both systemic and local genital effects of short-course zidovudine treatment have been shown to be independently associated with reduced vertical transmission rates [71]. Reduction of systemic and genital viral load is also likely to reduce the risk of sexual transmission.

A number of studies have demonstrated that highly active antiretroviral therapy (HAART) suppresses HIV replication, not only in the blood/lymphoid compartment, but also in the male and female genital tract [51,52,54,72–80]. While some of the earlier studies were con-

ducted among patients on suboptimal antiviral therapy, later studies have included patients with effective HAART. In our series of 114 patients with suppressed blood viral load < 400 copies/ml, only two patients had a seminal viral load slightly above the detection limit of the assay [81]. Although measurement of cell-free virus (HIV RNA) in semen is usually suppressed in HAART-treated patients, a significant number of treated individuals harbour HIV provirus (HIV DNA) in CD4-positive cells in their semen. In our recent study, the detection rate of HIV DNA in semen was 17% in treated individuals compared with 38% in a drug-naive control population. Zhang *et al.* have demonstrated that infectious virus can still be recovered *in vitro* from seminal lymphocytes after more than 6 months of suppressive HAART [75]. However, in a recent study on quadruple combination therapy in primary HIV infection, only two out of 22 patients had more than 50 HIV DNA copies-per-ejaculate after 1 year of HAART [51].

In an analysis of Swiss patients with primary infection, Yerly *et al.* demonstrated a significant reduction in the transmission of drug-resistant viruses after 1997 [82]. This reduction was paralleled by a gradual improvement of HAART effectiveness in the Swiss HIV Cohort Study. Currently, more than 60% of all treated patients in the Swiss HIV cohort study have HIV RNA levels below the detection limit of 50 copies/ml in blood.

It is not known how low the blood or genital viral load must be to completely suppress the HIV transmission risk (threshold level). In Quinn's study (cited above), a threshold level was defined at 1500 copies of HIV-RNA per ml of blood [41]. A similar observation was made for the case of vertical transmission [68]. However, any significant reduction in blood viral load is likely to result in reducing (but not eliminating) the risk of transmission.

Compartmentalisation and antiretroviral drug concentrations in the male genital tract

Not all drug combinations for HAART appear to be equally effective in the genital and systemic compartment. Compartmentalisation of HIV in semen has been clearly shown by the detection of different quasispecies in semen and blood in treated and untreated individuals [31,83–85]. Recent findings of a distinct mutation pattern in virus isolates from blood and cervical samples indicate a similar compartmentalisation in the case of the female genital tract [86,87]. By mathematical modelling, Kepler and Perelson have demonstrated that limited drug penetration into a small compartment is an important risk factor for the development of resistant HIV during treatment [88]. Thus, differential penetration of antiretroviral drugs into the genital tract is an important consideration if these drugs are intended to reduce the likelihood of sexual transmission of HIV-1 [89].

Theoretical factors that may influence antiretroviral drug distribution into the male genital tract, such as protein binding, drug transporters and drug ionization have been reviewed in detail elsewhere [90]. Seminal concentration data are available for all three classes of antiretroviral agents currently marketed and semen:blood ratio of antiviral drug concentrations (S:B ratio) vary from undetectable to > 1. In Fig. 1, median semen concentrations are shown in relation to the wild-type, protein corrected 50% inhibitory concentration of the drugs [91]. Of the nucleoside analogues, seminal concentration data are available for zidovudine, lamivudine and stavudine [92–96]. All three appear to achieve seminal plasma concentrations greater than that in blood plasma [97–99]. However, data on intracellular drug phosphorylation in seminal lymphocytes is lacking.

Non-nucleoside reverse transcriptase inhibitor semen concentration data have been collected for efavirenz and nevirapine. Nevirapine concentration in semen reaches 60–100% of blood plasma levels [94], while seminal efavirenz exposure was 2.5–10% of that in blood [100].

The protease inhibitors generally achieve the lowest concentrations in the male genital tract, although S:B ratios vary for each compound (nelfinavir < saquinavir = ritonavir < amprenavir < indinavir). Detection of nelfinavir in semen was unsuccessful in one study [95] and both saquinavir and ritonavir reached < 5% of blood plasma levels in semen during co-adminstration of the two [101]. Amprenavir penetration into the seminal compartment was better than that of saquinavir (S:B ratio, 0.2; 95%CI 0.05–3.0) [102]. Two groups have demonstrated high levels of indinavir in semen (median S:B ratio 0.4 and 1.2) [103] and further improvement of

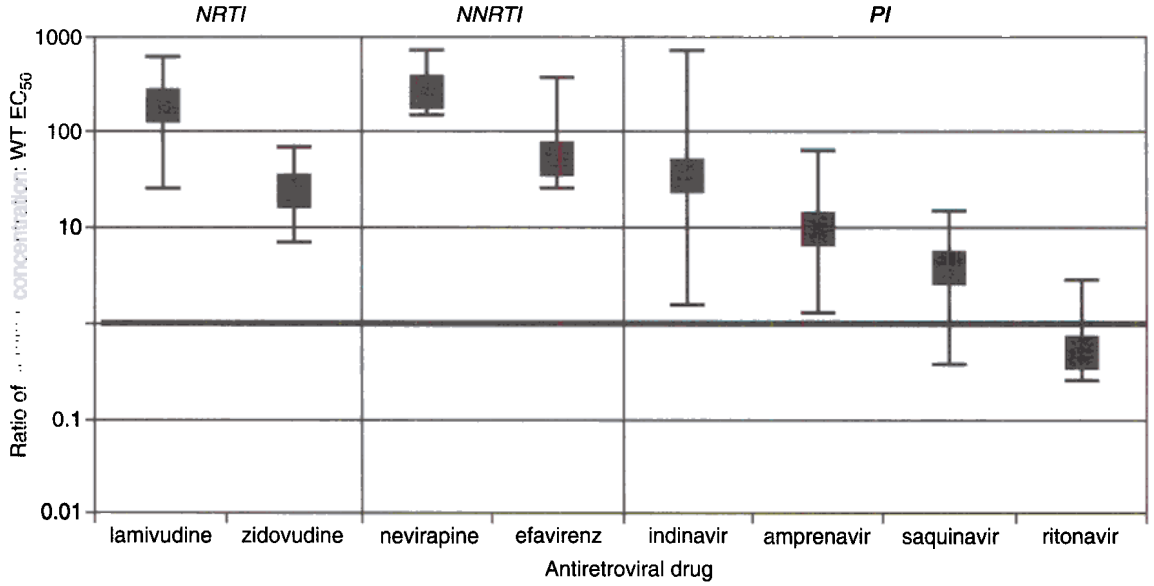

Fig. 1. Ratio of seminal plasma antiretroviral drug concentrations to HIV-1 wild-type 50% effective concentration (EC_{50}). Ratios were calculated based on median, minimum and maximum reported seminal drug concentrations. EC_{50} values were from Molla *et al.* [67].

indinavir penetration by co-administration with low-dose ritonavir [104,105].

Blood plasma protein binding, p-glycoprotein affinity and ionizing pH all influence drug penetration into and activity within the genital tract, but protein binding appears to be a major determinant of drug concentrations in semen (Fig. 2). Additionally, compounds with lower p-glycoprotein affinity (amprenavir and efavirenz) appear to achieve greater genital tract penetration than compounds with greater p-glycoprotein affinity (ritonavir and saquinavir) [106].

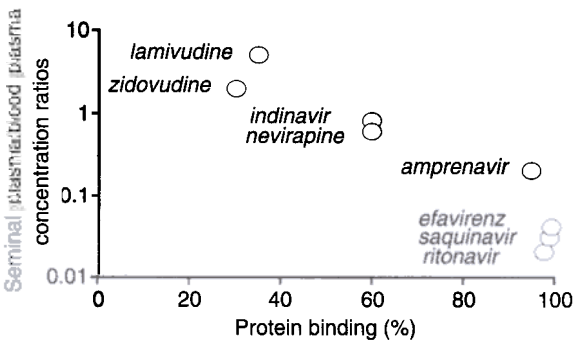

Fig. 2. Protein binding versus antiretroviral drug S:B concentration ratios. Protein binding predicts antiretroviral drug exposure in the male genital tract; r, 0.8 and $P = 0.017$ by non-linear regression analyses (see text for references).

To date, no investigation has fully evaluated and compared combination antiretroviral therapy in the context of genital tract pharmacology and virology to determine the most potent and durable regimen for suppressing viral replication in this compartment.

Sexual behaviour changes in response to HAART

The increased public awareness of the effects of HAART are likely to change concerns about HIV transmission in the population at risk. The first study examining changes in perceived transmission risk associated with HAART was presented by Kravcik *et al.* in 1998 [109]. The authors reported that 20% of HIV-infected individuals attending an HIV clinic thought that the risk of transmission was reduced during HAART and 19% believed that the need for safer sex practices was reduced under HAART. Increased-risk sexual behaviour was also documented in a survey of serodiscordant couples [108] from California. One-third of uninfected partners mentioned that they had already taken a chance with unprotected sex because of improved HIV treatment options and 40%

responded that HAART had changed their transmission concerns. HIV-positive gay men studied in London in 1998 also perceived a decreased infectiousness associated with HAART, but this perception was not associated with an increased risk behavior [109]. A marked increase in risk-taking behaviour (unprotected anal intercourse) – from 37% to 50% – was reported among gay men in the San Francisco Young Men's Health Study; this increase was also associated with increased rates of rectal gonorrhoea in STD clinics [110]. In a survey from the French SEROCO study group, gay or bisexual men were three times more likely to report unprotected sex with a seronegative partner after 6 months of HAART than they were prior to treatment initiation [111]. No increase in risk-taking behaviour was noted among heterosexual individuals; however, the sample size was small. A decrease in sexual risk-taking behaviour was documented in French injecting drug users who started HAART [112].

The increased sexual risk-taking behaviour in homosexual individuals is alarming. Reduced likelihood of HIV transmission due to the widespread use of HAART could be offset by an increase in risk-taking behaviuor.

What happens next?

HIV prevention activities were developed in the 1980s in the absence of reliable knowledge about HIV transmission. These prevention activities virtually excluded the infected index cases who would have been stigmatised with little benefit by learning their infection status. The situation in 2001 is completely different, and prevention strategies must be informed by ongoing scientific discoveries. First, vaccine and microbicide development directed at preventing infection must take advantage of our knowledge of mucosal exposure and mucosal immunity. Second, STD and behavioural interventions must embrace both HIV-infected and -uninfected people, and the optimal usage for STD interventions demand intensive and emergent study. The failure of mass STD therapy to prevent incident HIV infection in a population in no way reduces the importance of treatment of STD. Lastly, focus on HIV-infected subjects is a strategy whose time has come. Early recognition of infection allows improved care for the infected subject as well as critical prevention opportunities. Indeed, the US Centers for Disease Control and Prevention has dedicated substantial prevention resources to this goal (Project SAFE). In addition, antiretroviral therapy may help to prevent transmission, and this issue has the highest global research priority. In the absence of a successful vaccine, strategies to reduce infectiousness – including use of therapy – are likely to develop in the coming years.

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