

# Sexual transmission of HIV: infectiousness and prevention

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*AIDS* 1999, 13:155–166

**Keywords:** sexual transmission, infectiousness, HIV, susceptibility, prevention, antiretroviral treatment, sexually transmitted diseases, mathematical models, semen, genital secretions

## Introduction

HIV can be transmitted through contaminated blood and blood products; from a mother to her offspring during pregnancy, childbirth or breast feeding; or through sexual contact. Sexual transmission remains by far the predominant mode of transmission [1]. Vertical and blood borne transmission of HIV are highly predictable, and very efficient modes. The recipient of a unit of contaminated blood nearly always becomes infected, whereas only about 0.3% of people pierced with large bore needles seroconvert [2]. This difference in efficiency most probably reflects the dissimilar concentrations of viruses inoculated. Vertical transmission leads to infection in about 25% of newborns [3]. Sexual transmission of HIV, however, appears to be considerably less efficient and highly variable [4,5]. To develop effective prevention strategies, a better understanding of the factors affecting transmission of HIV is required.

The probability of transmission of HIV is a function of infectiousness of the index case, the mode of the sexual contact and the susceptibility of the person exposed to the virus. Whereas non-transmission of HIV in steady partnerships is found frequently, some individuals have transmitted the virus to the majority of their heterosexual partners [6]. Potential explanations for this variability in sexual transmission of HIV include biological differences in infectiousness, susceptibility or both, or differences in sexual behaviors. Susceptibility to HIV infection has recently been reviewed by Padian in this

Journal [7]. The purpose of this article is to review factors that contribute to HIV infectiousness.

## Epidemiological studies on infectiousness of HIV

### Disease stage and infectiousness

Epidemiological studies have documented the risks of HIV transmission by all routes [8]. Longitudinal partner studies have improved our understanding of the sexual transmission of HIV and factors influencing the infectiousness of HIV infected individuals [9–17]. In general, HIV transmission by any route is more likely when the index case is found to have a greater degree of immunosuppression (as manifested by reduced CD4 count or more advanced stage of disease, Table 1) [9,11,13,15,16,18,19]. When analyzed in partner studies, the transmission rate is approximately two to three times greater from infected males to females than from infected females to males [14,20]. In HIV epidemic areas in Africa, however, the rate of transmission is more evenly distributed between the sexes [21]. Hayes *et al.* [22] calculated the cofactor effect of genital ulcer diseases to be approximately five times higher for female-to-male than for male-to-female transmission. Thus, in countries with high sexually transmitted disease (STD) prevalence, the higher cofactor effect for female-to-male transmission could counterbalance the

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Date of receipt: 27 July 1998; revised: 9 September 1998; accepted: 17 September 1998.

**Table 1.** Summary of published partner studies evaluating factors associated with infectiousness.

Reference	Publication year	Type of cohort	n (male/female)	Factors associated with increased genital viral load or detection rate
[10]	1989	Homosexual contacts	11 (18/-)	high CD8 cell count
[11]	1991	HIV-positive men	368 (368/-)	CD4 depletion (< 400)
[12]	1993	Heterosexual contacts	343 (-/343)	CD4 depletion (< 400)
[13]	1993	Homosexual contacts	187 (187/-)	p24 positivity
[19]	1993	Heterosexual contacts	158 (11/147)	CD4 depletion (< 500)
[14]	1994	Heterosexual contacts	304 (108/196)	symptomatic disease
[15]	1994	HIV-positive women	224 (-/224)	CD4 depletion
[16]	1994	Partners of transfusion recipients	46 (14/32)	CD4 depletion (< 400)
[17]	1996	Discordant couple study	177 ( 34/143)	symptomatic disease
[66]	1996	Partners of transfusion recipients	45 (14/31)	CD4 depletion (< 200)
[67]	1998	Partners of HIV-positive hemophiliacs	39 (-/39)	symptomatic disease
				syphilis
				HIV-RNA viral load
				HIV-RNA viral load

higher risk of transmission of male-to-female sex in the absence of STDs.

### Sexual behavior and infectiousness

Differences in sexual acts and other behavioral factors may also predict the efficiency of transmission of HIV. The risk of vaginal intercourse appears to be considerably less than insertive anal intercourse [11,19]. Whereas fellatio has been considered a much lower risk behavior [8,18] recent studies with macaques [23] as well as reports of homosexual men with primary infection [24] indicate that oral transmission may occur.

### Sexually transmitted diseases and infectiousness

The relationship between classical STDs and the transmission of HIV has been appreciated for a long time [25]. In a number of studies it has been shown that the prevalence and incidence of HIV is considerably greater in patients who present to STD clinics with genital ulcers and mucosal inflammatory diseases [22,26-32] and in patients with a history of STD [11,33,34]. HIV acquisition appears to be increased in women with inflammation of the cervix [35]. However, STDs might facilitate HIV transmission by increasing the genital shedding (infectivity) of HIV. In a study in Haiti, higher transmission risks were documented for patients with a diagnosis of syphilis [17]. In addition, concomitant transmission of HIV with STDs might play an important role. In a study of primary HIV infection, Kinloch de Loes *et al.* [36] reported genital ulcers in 13 out of 25 sexually infected primary infection cases examined. Regardless of whether STDs increase infectiousness of HIV or increase susceptibility, their importance in HIV transmission has been confirmed by Grosskurth and coworkers [37]. These investigators demonstrated a 42% reduction in incident cases of HIV in several African villages that implemented an aggressive STD treatment program.

### Use and pitfalls of mathematical models to describe HIV transmission

Several authors have used mathematical models to describe sexual transmission of STDs and HIV infection. In most partner studies the models do not address the question of whether the risk of transmission is a function of the number of sexual acts. Based on a number of pivotal studies by Anderson and May [38] several authors have previously suggested that the risk of transmission is a function of the number of partners rather than of the number of sexual contacts [13,18,34,39,40]. Recent work by Downs *et al.* [41] supports this concept, indicating that, in a given partnership, transmission of HIV is most likely to occur during the first few sexual contacts, after which the risk of transmission per contact becomes considerably smaller. In general, the risk of transmission per contact (in the range of 0.1–0.5%) is considerably smaller in studies of steady partnerships [14] than in studies of single sexual contacts with prostitutes [33,42]. Therefore, studies of steady partnerships are clearly subject to a selection bias favoring circumstances with low risk of transmission. The studies select for seronegative partners who may have natural as well as acquired resistance to HIV infection and may also select for seropositive cases with low infectiousness. It has been suggested that the uninfected partner may develop an HIV- or a partner-specific (e.g. anti-HLA) immune response during the first few sexual contacts with the infected partner which reduces the risk of transmission during subsequent sexual exposures [43]. Several lines of evidence suggest the possibility of acquired immunity [44-46] and additional longitudinal studies are needed to resolve these issues.

Data obtained from studies of artificial insemination illustrate further the possible risk of a single exposure to HIV contaminated semen. In a retrospective study of five fertility clinics, where 199 women were inseminated with unprocessed semen from their HIV infected

donor, seven (3.5%) were found to be HIV-positive [47]. This result further suggests that longitudinal partner studies may underestimate the per-contact transmission probability of HIV from men-to-women.

Jacquez and coworkers [48] have developed a model of the HIV epidemic which supports an increased HIV transmission during primary infection. This model assumes that recently infected patients continue to engage in high risk sexual behavior, and that biological factors (e.g. high viral load) render such patients particularly contagious. The model predicts per-contact infectivity during primary HIV infection to be 100 to 1000 times higher than during the asymptomatic period of the disease. However the model could be flawed, since it assumes a constant per-contact transmission probability which, as discussed above, may not be appropriate. In addition, the model does not consider changes in susceptibility of seronegative partners to infection nor is it based on empirical findings.

### Mechanism of mucosal transmission

The exact source of the virus from the infected partner, the mucosal route of the virus during transmission, and the target cell in the mucosa of the recipient are still not known. From cases of HIV transmission by artificial insemination, it is clear that semen can transmit the virus [47,49]. This is also supported by the finding of a protective effect occurring with coitus interruptus [14]. The cellular fraction of semen contains spermatozoa, immature germ cells, leukocytes (lymphocyte, granulocytes and macrophages), and epithelial cells. HIV can be detected in lymphocytes/monocytes and cell free seminal plasma. Interestingly, vasectomy does not result in a reduction of shedding of HIV in semen as measured by HIV-RNA levels, indicating that most cell-free HIV in seminal plasma arises distal to the vas deferens [50]. HIV has also been found to be associated with sperm cells by electron microscopy and *in situ* polymerase chain reaction (PCR) [51,52], but these findings are highly controversial [53-55]. However, the fact that more than 1400 artificial insemination procedures with processed sperm from men with HIV failed to result in transmission of HIV indicates that the motile sperm fraction from semen is not likely to transmit the virus (A. Semprini, personal communication and [56]). The processing used by Semprini and others separates motile spermatozoa from contaminating leukocytes by density-gradient centrifugation and swim-up technique.

Whether HIV is predominantly transmitted as a cell-free virus or in a cell-associated form is not known. In the macaque model, vaginal infection with simian immunodeficiency virus (SIV) is established more efficiently using cell-free virus [57]. Information gained

from *in vitro* cell culture experiments with human cervical epithelia and HIV indicate a potential mechanism for cell-associated infection [58,59]. In this model, the HIV infected monocyte adheres to the monolayer and viral particles are internalized by pinocytosis into the epithelial cell. In addition, Furuta *et al.* [60] have demonstrated that vaginal epithelial cells can be infected by HIV via a CD4 independent mechanism similar to that described for neuroglial cells. This mechanism involves initial interaction of the HIV-1 envelope gp120 with a cell-surface glycosphingolipid which can be blocked by antibodies raised against gp120. Whether dendritic cells in the subepithelial tissue can serve as a direct target for HIV or are infected after a passage of the virus through the epithelial cell layer is not known. The relative contribution made by cell-free and cell-associated HIV in sexual transmission remains under investigation.

Information on the biology of female-to-male transmission is limited. HIV can be detected in endocervical swab specimens, cervicovaginal lavage samples, and CD4 positive cells [61,62] but little is known about potential target cells in the male genital tract [63]. Miller *et al.* [64] have been able to infect male macaque after placing cell-free virus on the penile urethra.

### The laboratory assessment of infectiousness of HIV

In general, infectious transmission of a pathogen is concentration and pathogen dependent. HIV infectiousness must reflect the effects of the level of HIV present in the inoculum and its phenotype. There are now several lines of evidence supporting the idea that HIV infectiousness is a function of virus concentration. Patients with high blood viral load were more likely to transmit the disease to recipients of blood [65], their sexual partners [16,66,67], and their offspring [3]. Conversely, antiviral therapy taken by an HIV infected mother late in pregnancy significantly reduces transmission to her offspring [68]. However, better understanding of sexual transmission of HIV requires evaluation of the effects of viral concentration in genital secretions. Given the transmissibility of HIV through semen and the high risk of male-to-female transmission, further analysis of the effects of semen is clearly essential. However, only recently have detailed large scale studies been undertaken. The lack of information probably reflects the relative difficulty of collecting semen specimens and also the technical limitations in quantifying HIV levels in semen samples, which have now been improved [69].

Several procedures for detecting and quantifying HIV have been employed and these include culture, and

amplification techniques for cell-associated HIV-DNA and cell-free HIV-RNA (Table 2). Seminal culture is labor and cost intensive, and potentially dangerous. Recovery of infectious HIV from seminal cells has been highly variable (i.e. 9–55%), and also variable when collected from individual subjects over time [53,69–75]. Recovery of infectious HIV from seminal plasma has proven difficult; this may be because seminal plasma is toxic to cells in culture in the presence of fetal calf serum[76]. Even under conditions that circumvent this toxic effect, however, recovery rates of HIV from seminal plasma are low [53]. These results suggest that seminal leucocytes may be primarily responsible for the infectiousness of HIV: a finding which contrasts with results gained from the macaque model where mucosal infection is achieved more easily by cell-free than cell-associated virus [57].

More recent studies have evaluated the actual concentration of HIV by measuring HIV-RNA in the seminal plasma [55,69,75,77,78]. The variability of the HIV-

RNA detected has also been evaluated with conflicting results obtained. Gilliam *et al.* [79] examined weekly semen samples from patients under stable conditions and found only limited inter-patient variability of less than 0.5 log<sub>10</sub> copies/ml. However, Coombs *et al.* [55] reported a much greater variability (1.0 log<sub>10</sub> copies/ml) in patients with stable blood viral load. In general, HIV-RNA levels in semen correlate with HIV-RNA levels in blood plasma. However, the degree of this association is weak (with r values in the range of 0.4–0.6), indicating that factors other than the viral load in blood influence the RNA level in semen [55,75,78,80,81]. HIV-RNA found in semen may also – in part – originate from local replication in the genital tract. An HIV-RNA concentration above 10,000 copies/ml in seminal plasma is highly associated with a positive HIV culture from seminal cells [55,75].

HIV-RNA measurements only assess the concentration of cell-free virus in semen. Recently, quantitative assessment of proviral HIV-DNA in seminal cells by

**Table 2.** Summary of published studies evaluating factors associated with increased shedding of HIV in the genital tract.

Reference	Year	N	Source*	Detection of HIV			Factors associated with increased genital viral load or detection rate
				Method	Quantitative	HIV-positives (%)	
<b>Semen samples</b>							
[71]	1992	95	SC	culture	no	9%	CD4 depletion (< 200) symptomatic disease seminal leucocytosis absence of zidovudine therapy
[72]	1994	33	SC	culture	no	54%	CD4 depletion (< 100) symptomatic disease
[74]	1995	215	SC	culture	yes	17%	CD8 count cytomegalovirus-shedding in semen
[83]	1997	74	SC	DNA	yes	65%	CD4 depletion seminal leucocytosis
[77]	1995	26	SP	RNA	yes	96%	No association with either blood CD4 count nor viral load
[75]	1997	101	SP	RNA	yes	62%	CD4 depletion absence of antiviral treatment
[78]	1997	29	SP	RNA	yes	66%	blood plasma RNA blood plasma RNA
[109]	1997	119	SP	RNA	yes	83%	urethritis, sexually transmitted diseases
[81]	1998	91	SP	RNA	yes	66%	blood plasma RNA
<b>Female vaginal secretions</b>							
[86]	1993	97	CS	DNA	no	33%	oral contraceptive pill use cervical mucopus cervical ectopy pregnancy
[111]	1994	92	CS	DNA	no	39%	cervical inflammation
[62]	1997	144	CVS	DNA	no	25%	HIV-1 subtype B absence of zidovudine therapy microbial cervicovaginal infection
[89]	1997	212	CS	DNA	no	32%	CD4 depletion cervical mucopus vaginal discharge
[97]	1997	318	CS/VS	DNA	no	51%	vitamin A deficiency CD4 depletion vaginal discharge & candidiasis
[61]	1997	609	CVL	RNA	no	24%	<i>Neisseria gonorrhoea</i> cervicitis CD4 depletion (< 14%) <i>N. gonorrhoea</i> infection <i>Chlamydia trachomatis</i> infection Genital ulcer disease
[95]	1998	28	CS/VS	RNA	yes	64%	blood plasma RNA
[93]	1998	49	CS	RNA	yes	49%	blood plasma RNA CD4 depletion
[140]	1998	17	CVL	RNA	yes	65%	blood plasma RNA CD4 depletion

\*SC, Seminal cells; SP, seminal plasma; CVL, cervico-vaginal lavage; CS, cervical swab; VS, vaginal swab.

quantitative competitive PCR (qcPCR) has been reported [82-84]. In a cross-sectional study of semen samples taken from 96 men, HIV-DNA was detected in semen from only 39 % of study subjects [84]. However, the level of HIV-DNA correlated with the recovery of infectious virus from seminal cells by culture ( $r = 0.5$ ,  $P < 0.001$ ). Whereas DNA-PCR cannot discern between infectious and defective provirus, the correlation between HIV-DNA and culture supports the use of DNA measurement as a surrogate for measuring infectious virus levels.

The concentration of HIV in the female genital tract can be expected to influence both sexual and vertical transmission of HIV. Whereas some investigators have grown HIV or detected HIV-DNA in cervicovaginal secretions [85,86], only recently have large scale studies been undertaken [62,87-89] and quantitative studies using RNA-PCR have also been performed [90-92]. These studies are complicated by the variability in collection techniques, since some investigators use endocervical or vaginal swab specimens and others cervicovaginal lavage.

## Host factors associated with increased excretion of HIV in genital secretions

### Systemic factors

Whereas at least some of the HIV present in the genital tract is produced locally, HIV concentration in the genital tract is also a function of systemic factors. First, the concentration of HIV in blood is reflected in the genital secretions, this association arising either through blood and genital compartments being connected, or as a result of similar environmental pressures. As stated earlier, most studies that investigated HIV-RNA concentration in blood and genital secretions found a weak but significant correlation between the two measurements [55,69,75,80,93-95]. Conditions that increase the viral burden in blood (such as vaccines or systemic infections) could increase shedding of HIV in the genital tract. Second, systemic factors, including advanced stage of immunodeficiency, might also influence the functional or anatomical integrity of the genital mucosa. The rate of recovery of infectious HIV in seminal cells is increased in patients with more advanced stages of the disease [71,72]. Whereas smaller studies failed to demonstrate an association between HIV in seminal plasma and stage of disease or CD4 count [74,78], larger studies have shown a weak but significant association of HIV concentration in semen with both of these parameters [55,75,80]. HIV-DNA also correlates with CD4 lymphocyte counts in semen [83,84] and is inversely correlated with peripheral CD4 count.

HIV-DNA and HIV-RNA are also more readily detected in cervical secretions of women with a more advanced stage of disease and/or low peripheral CD4 count [89,92,96]. Hormone and vitamins appear to have local urogenital effects that have been particularly well studied in women. Increased detection of HIV-DNA in the genital tract has been documented in women with vitamin A deficiency [97], and in women receiving high dose oral contraceptive pills or depot contraception [86,97].

### Effect of primary infection

The concentration of HIV in semen in primary infection has only been studied to a limited extent. Dyer *et al.* examined the concentration of HIV-RNA in semen and blood in three subjects with primary HIV disease [98]. Whereas in subjects with acute disease the blood viral burden, as expected, was very high, HIV levels in seminal plasma were not outside the range of values found for a control group of subjects with chronic HIV infection. Celum *et al.* (personal communication) found similar results in a study of a larger group of men. These studies failed to provide a biological rationale to support the epidemiological models discussed earlier which postulate a 100- to 1000-fold increased risk of transmission during primary infection. If anti-HIV antibodies are absent from genital secretions early in primary infection, however, the infectious potential of these secretions may be greater for any given concentration of virus in semen.

### Local factors

The occurrence of HIV in the genital tract does not merely reflect the blood viral burden and several findings support the concept of compartmentalization within the genital tract. First, the correlation between the concentration of HIV in genital secretions and blood is weak in most studies with an  $r$  value between 0.4 and 0.6 [55,80,94]. Second, the variability of HIV-RNA content in semen over time may be higher than the variability of its presence in blood [55]. Third, sequence analysis has revealed some sequence evolution in HIV recovered from the genital tract as compared to HIV nucleic acid from blood [99-102].

It seems likely that local cytokines increase replication of HIV in the genital tract. *In vitro* work supports this hypothesis. Ho and coworkers demonstrated that chlamydial infection increases HIV replication *in vitro* [103]. Gonorrhoea caused increased concentration of tissue necrosis factor (TNF) and IL8 in men with urethritis [104]. Similar results have been obtained when HIV is exposed to spirochetal lipopolysaccharide [105]. Spear and coworkers found that cervicovaginal secretions from approximately 10% of women enhanced replication of HIV *in vitro* [106]. The as yet unidentified cervical factor was heat-stable and protease labile. Genital tract cytokines probably work through activation of the HIV-LTR [105].

Local inflammation can also be expected to increase HIV excretion by release of inflammatory cells into the genital secretions. The most common cause of inflammation in the genital tract is STDs, although other causes of inflammation and systemic factors maybe involved in women. STDs may increase the susceptibility to HIV by disrupting mucosal barriers, or by increasing the number of cells that are receptive to HIV infection. However, an alternative or additional hypothesis is that STDs render HIV-positive individuals more contagious.

Several clinical studies demonstrate increased excretion of HIV in the male genital tract of patients with STDs [107-109]. The largest study to examine this relationship was conducted in an STD clinic in Malawi, where 86 HIV-positive men with urethritis were compared to an HIV infected control group. In this study, urethritis caused an eight-fold increased excretion of HIV, which was reversed with appropriate antibiotic therapy. Although the therapy caused rapid cure and reduction in genital tract inflammation, the HIV concentration in semen decreased slowly [109]. Therapy for STDs had no effect on the blood viral burden. Results from this project also demonstrated that patients with genital ulcers also had a significantly higher concentration of HIV in semen, suggesting an unexpected mechanism (such as additional urethral lesions or inflammatory processes) by which ulcers might enhance transmission of HIV [110].

Similar studies have been conducted on HIV shedding in the female genital tract [61,111]. Working in the Ivory Coast, researchers from the Institute of Tropical Medicine, Antwerp, Belgium, demonstrated that cervical shedding of HIV-RNA was increased in women with *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, genital ulcers but not with cervical ectopy [61]. Treatment of STDs decreased the rate of recovery of HIV-DNA. A research group from the University of Washington has shown that detection of HIV-DNA from endocervical swab specimens from women attending an STD clinic in Mombassa is increased significantly with gonococcal cervicitis but not with cervical ectopy, *Chlamydia trachomatis* or *Trichomonas vaginalis* infection [97]. However, studies on women who have undergone hysterectomy indicate that the cervix is not required for the shedding of HIV in the female genital tract [112] and John *et al.* found increased cervical and vaginal shedding of HIV-DNA in women with purulent discharge [89]. Most recently, Levine and coworkers demonstrated increased recovery of CD4 positive cells in cervical swab specimens from patients with STDs [113]. Whereas these results are interpreted to suggest increased susceptibility to HIV, these cells could also be expected to increase infectiousness.

## Viral factors

A variety of viral factors have been suggested to play a role in the infectiousness of HIV; these include envelope proteins required for transmission, genetic factors that affect the replicative capacity and "fitness" of the virus, and resistance to antiviral drugs. Studies with macaques using SIV and SHIV have demonstrated that vaginal transmission of cell free virus requires particular envelope sequences [114]. Enose and colleagues compared vaginal and intravenous SIV infection of cynomolgous monkeys [115]. Intravenous inoculation resulted in infection with a viral population with heterogeneous envelope sequences, whereas vaginal inoculation led to infection with a minor envelope variant. Studies in humans with primary infection also demonstrate preference for sexual transmission of unique virus isolates. Zhu *et al.* observed selective transmission of a minor variant of HIV in a study of sexual transmission of HIV in five couples (four male, one female) [116]. In four of the five cases, the transmitted variants could be found in the semen of the index cases, and in three of these cases the variants transmitted were found in the semen but not in the blood. These findings suggest that some unique viral factors favor transmission, and/or that some selective mechanism(s) at the mucosal or systemic level restrict the transmission of some, but not all, viral isolates.

Envelope sequences that appear to favor sexual transmission of HIV have been characterized. Some HIV strains form syncytia in lymphocyte cultures, as a result of sequence variations in the V3 envelope region [117]. These strains are strongly associated with more advanced HIV disease [118]. Some authors suggested that the non-syncytia inducing (NSI) isolates of HIV are preferentially transmitted [119] whereas others have not been able to show a preferential transmission of NSI isolates [120]. In some patients differences in recovery of SI and NSI isolates in semen and blood have been reported [72]. However, detection of SI variants depends on a positive selection by the specific culture conditions and NSI isolates are almost always present as well [121]. Delwart and colleagues found both SI and NSI phenotypes in semen and no evidence for a semen specific signature amino acid sequence in the *env* gene [100]. Thus, if selective transmission of NSI isolates occurs, it is likely to occur in the recipient and the viral envelope is likely to play an important role in this selective event. In the animal model, Neildez *et al.* demonstrate a selective transmission of three specific envelope variants upon vaginal but not rectal or intravenous inoculation [122].

Envelope sequences also can be used to define geographically distinct HIV subspecies, called clades. More than half of all HIV infections in the world result from the African variant, clade C. The HIV epidemic in the US and Western Europe has resulted from spread of

HIV clade B, whereas the rapidly growing epidemic in Thailand has resulted partly from clade E [123]. A segregation of subtype B in homosexual and subtype C in heterosexual contacts was found in a study conducted in South Africa [124]. Although behavioral and economic factors have been used to explain these epidemiological observations [125], it also seems possible that some clades are transmitted with greater efficiency than others, especially from men to female partners [126]. To explore this hypothesis, Soto Ramirez *et al.* compared the growth of primary isolates of different clades of HIV in dendritic cells; they reported more efficient growth of clade E than B [127]. However, other investigators have not been able to replicate these findings [128,129], and it seems clear that methodological details (such as source of dendritic cells and culture conditions) may greatly affect results.

Only recently has the biology of clade C been subjected to scrutiny. First, the concentrations of HIV in the semen and blood of study subjects in Malawi (mostly clade C) are significantly higher than those of patients in the US and/or Switzerland (clade B) matched for CD4 count [81]. This could relate to the growth rate of clade C *in vivo*, or it might reflect the effects of host and/or environmental factors (i.e. coinfection with tuberculosis, malaria, schistosomiasis) that are more common in developing countries. In addition, SI viruses of clade C are not readily recovered from blood or semen of patients with HIV [130] regardless of the stage of the disease. Availability of a predominant population of NSI isolates might favor transmission.

Miller and coworkers recently demonstrated that the replicative capacity of the viral isolate predicts the transmission efficacy of SIV for mucosal transmission in the animal model [131]. Finally, susceptibility to antiviral drugs might affect the infectiousness of HIV. Transmission of an HIV isolate that is resistant to nucleoside analogues has been reported [132-134]. Although a study on reduced *in vitro* infectivity of zidovudine-resistant virus has been presented [135], the fitness of these isolates for transmission has not been studied.

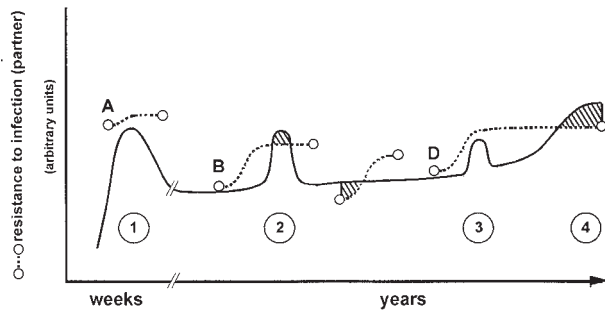
## **Reducing the infectiousness of HIV**

Prevention of HIV depends on interventions among both infected and susceptible people. Since only a fraction of HIV infected people are aware of their status, prevention programs focus primarily on those who are susceptible. However, the most effective way to prevent contagious diseases when vaccines are not available is to reduce infectiousness [136]. This approach requires the detection of as many HIV infected subjects

as possible – a daunting task, but one of benefit both to the patients and public. Behavioral and biological approaches can be used. Behavioral approaches to reduce the risk of transmission encourage responsible behavior in HIV infected people, including consistent usage of latex condoms. Biological approaches are designed to reduce shedding of HIV, with the hope that at some critical level patients with HIV will be less contagious. Behavioral and biological approaches must be complementary, and attempts to reduce viral shedding must not cause patients with HIV to conclude that they are not infectious.

Antiviral therapy can be expected to reduce the transmission of HIV. Reduced rates of vertical transmission have been documented, even when zidovudine is administered only to mothers late in pregnancy [137]. Patients receiving antiretroviral therapy have shown reduced transmission rates of HIV to their partners [138]. There has been an increase in the number of studies that demonstrate that antiretroviral treatment reduces detection of HIV in female genital secretions [139,140], and the concentration of HIV in semen [78,79,141-143]. In most patients the reduction of HIV in semen parallels that observed in blood, and in many patients no HIV-RNA can be detected in seminal plasma after potent antiretroviral therapy is initiated. Conversely, in some patients HIV persists in genital secretions during therapy, and genotypic resistance to nucleoside analogues has been demonstrated in HIV in semen [101] and female genital secretions [144]. Such resistance could correlate with selective pressure exerted by subinhibitory drug concentrations in genital secretions. To date, limited information is available on drug concentration in semen. Reports are available for zidovudine (six subjects) [145] and ritonavir/saquinavir (one patient) [146]. In these subjects, the concentration of zidovudine in seminal plasma was higher than in blood plasma and zidovudine-clearance from semen was delayed. Taylor *et al.* [146] reported that ritonavir and saquinavir levels were much lower in semen than in blood but still approximated *in vitro* ID90 levels. Studies to correlate zidovudine and 3TC in semen with the concentration of HIV in semen are in progress (Cohen M.S. *et al.*, unpublished data).

Patients need to be carefully informed about the significance of treatment induced reduction of genital shedding of HIV. Reduced infectiousness does not equal lack of transmission and more importantly, not every antiviral treatment does result in reduced infectiousness. In order to be effective on a public health basis, reduced infectiousness must be coupled with continued safer sex practices. Kravcik and colleagues reported that 21 % of 147 HIV infected subjects felt that potent antiretroviral therapy would reduce the need for safer sex practices [147]. Whereas Kelly *et al.* [148] reported similar results, Lavoie and colleagues were unable to find a



**Fig. 1.** Hypothetical relationship between HIV infectiousness and susceptibility. The infectiousness of a hypothetical patient during the course of HIV infection is depicted by the solid line. In this diagram it is assumed that infectiousness increases during primary infection (1) and during later stages of disease (4). The arbitrary level of infectiousness during the asymptomatic phase of the infection (2) is interrupted by two episodes of a sexually transmitted disease (3). The inverse susceptibility (relative resistance) of four hypothetical steady partners (A–D) is depicted by dotted lines. Transmission to the HIV-negative partner can occur during time periods when infectiousness in the index case exceeds the relative degree of resistance in the partner (shaded area). Partner A appears to be relatively resistant to HIV infection and does not become infected. Partners B and D acquire a protective HIV specific immune response early in the relationship and do not become infected until the infectiousness of the index case increases either because of a genital inflammatory process (partner B) or as a result of progressing immunodeficiency (partner D). Partner C becomes infected during the first sexual contacts.

link between attitudes toward new therapies and a lessening of safer sex practices [148,149]. In the latter study, 98 % of participants continued to believe in the need for protecting themselves.

Antiviral therapy is currently too expensive and cumbersome to be used globally to reduce HIV transmission. Alternative methods to reduce HIV concentration include treatment of systemic and genital tract infections that increase shedding of HIV. Whereas the frequency of untreated STDs in developing countries has been widely recognized, recent studies show a considerable prevalence of STDs in patients with HIV in Europe [150] and the US. Vitamin deficiencies (e.g. vitamin A) that increase genital tract viral burden or local shedding should be corrected. Intensive research should be focused on: (i) antiviral therapy specifically designed to reduce shedding of HIV in the urogenital tract; (ii) topical microbicides that inactivate HIV, to be used to reduce infectiousness and susceptibility; and (iii) examination of immune factors that reduce shedding in the genital tract, so that vaccines that help to prevent HIV transmission can be developed.

## Conclusions

The risk of HIV transmission depends on several factors that influence infectiousness and susceptibility (Fig.1). Some of these factors are highly variable. Higher levels of infectiousness can be expected during later stages of disease, during conditions that increase the blood viral burden, as well as during episodes associated with local inflammation in the genital tract. Since susceptibility to infection appears to be higher during the first few sexual contacts, the risk of transmission obtained from discordant partner studies may underestimate the risk associated with a single sexual contact. Quantifying HIV levels in genital fluids appears to be a valuable tool to estimate an individual's degree of infectiousness.

HIV prevention programs have been heavily focused on protecting susceptible people. However, rapidly accumulating biological data suggest that reducing infectiousness of HIV-positive subjects may also be an efficient and effective strategy. An improved understanding of HIV infectiousness is essential for this strategy to be developed.

## Acknowledgment

Special thanks to Diane Feldman for editing assistance. This work was supported by NIH grants and the USAID IMPACT Program (Awards UO31496 and RO149381) and by a grant of the Swiss National Science Foundation (3233-048902.96/1).

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