I-B

Acute HIV Infection: Implications for HIV Spread, Disease Progression, and Vaccine Development

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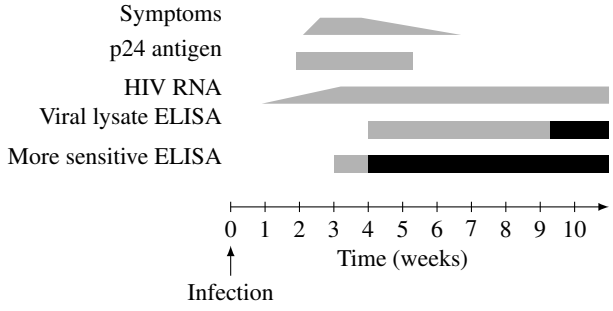
I-B-1 Acute HIV infection and its detection

The natural history of initial HIV infection can be arbitrarily divided into phases that include acute HIV infection, the first stage of disease (when HIV RNA can be detected in blood) before most HIV-specific antibodies form; recent or early infection, when antibodies in reduced concentration or reduced avidity can be detected; and established chronic HIV infection (Figure I-B-1 [Fiebig *et al.*, 2003; Pilcher *et al.*, 2004a]). Finding patients before seroconversion is critical to understand the HIV transmission event(s) effects and the effect(s) of host defenses on the viral population.

Figure I-B-1: Acute/early HIV infection timeline.



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In *HIV Molecular Immunology 2005.* Bette T. M. Korber, Christian Brander, Barton F. Haynes, Richard Koup, John P. Moore, Bruce D. Walker, and David I. Watkins, editors. Publisher: Los Alamos National Laboratory, Theoretical Biology and Biophysics, Los Alamos, New Mexico. LA-UR 06-0036. p. 21–31.

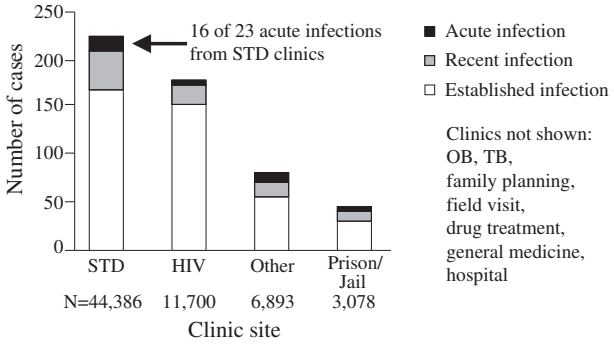
It is generally believed that half or more of patients with acute or early HIV infection may develop a "mono-like illness" [Schacker *et al.*, 1996; Lavreys *et al.*, 2000; Pilcher *et al.*, 2005]. Patients with acute HIV can present either for acute symptomatic care [Schacker *et al.*, 1996], for care of sexually transmitted disease (STD) symptoms [Pilcher *et al.*, 2005], or (realizing their elevated risk) for HIV testing. Although the diagnosis is rarely considered in acute patient care or testing settings, the potential for diagnosis is clearly present. For example, Rosenberg *et al.* [1999] reported that 1.0% of patients with negative tests for EBV (Epstein-Barr virus) infectious mononucleosis had serology consistent with acute HIV infection, and Pincus *et al.* [2003] found that 1.0% of patients with "any viral symptoms" in a Boston urgent care center had unsuspected acute HIV infection. However, searching for symptomatic subjects is a relatively inefficient strategy to detect subjects with acute or early HIV infection. For example, Rosenberg *et al.* found only about 150 subjects over 8 years [Kassutto *et al.*, 2005].

Alternative approaches involve "building a cohort" or longitudinal evaluation. The cohort approach requires enrollment of high risk, HIV seronegative subjects. For example, Lavreys *et al.* [2000] followed 883 female sex workers for up to five years at three month intervals. A total of 103 seroconversion events were observed. Fever, vomiting, diarrhea, headache, joint pain, body aches, skin rash, lymphadenopathy, fatigue and pharyngitis were more common among women who seroconverted. However, these patients were virtually all beyond the acute (antibody negative) phase of infection at the time of study.

Cross-sectional studies appear to offer the best strategy to find subjects before seroconversion. This approach was developed to make the blood supply safe, albeit at considerable cost [Ruiz *et al.*, 2001]. Recognizing that traditional antibody tests would inevitably miss some donors, blood banks in the United States and many other countries chose to eliminate infected (seronegative units) units with detection of HIV RNA [Engelfriet & Reesink, 2002; Roth & Seifried, 2002; Fang *et al.*, 2003; Mine *et al.*, 2003; Fiebig *et al.*, 2003; Koppelman *et al.*, 2005]. Bollinger *et al.* [1997] studied seronegative HIV in high risk sub-

jects in India. Among 6,495 consecutive attendees at two STD clinics in Pune, p24 antigen was detected in 58/3,874 (1.5%) of HIV seronegative samples. The majority of subjects with acute infection (51/58) were men. A group of 290 controls were matched on age and gender and compared for clinical signs of acute HIV infection. The most common symptoms associated with p24 antigenemia were fever, joint pain, inguinal lymphadenopathy, and night sweats; at least one of these symptoms was found in 47% of those with acute HIV infection.

Figure I-B-2: HIV-1 infections by testing site.

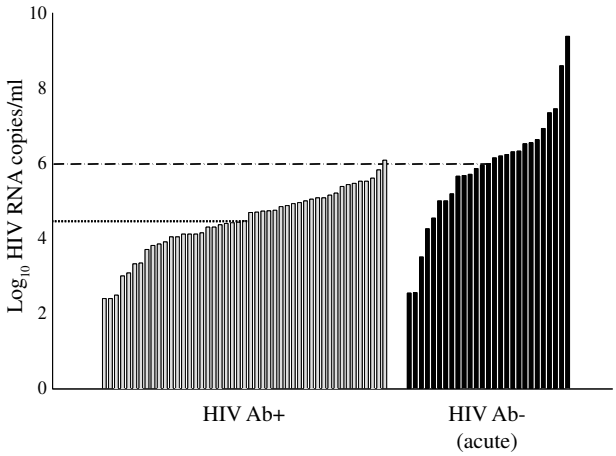


A series of studies have been completed that are designed to detect HIV RNA in blood in a large number of samples, using a serum pooling strategy adapted from the blood banking industry [Pilcher *et al.*, 2002, 2004b, 2005]. In North Carolina nearly 110,000 people seeking HIV testing were studied [Pilcher *et al.*, 2005]. A total of 563 people with established HIV infection were identified. A "detuned" ELISA assay designed to detect newly formed antibodies suggested 103 recent infections in the group of 563. Among HIV antibody negative specimens, 23 with HIV RNA (i.e., acute HIV infection) were detected. As shown in Figure I-B-2 most subjects with acute infection were detected in STD clinics. The median blood viral burden of subjects with acute HIV infection was 209,000 copies/ml, ten times greater than subjects with established infection.

These findings have led to increased focus on STD clinic subjects in developing countries [Pilcher *et al.*, 2004b]. As shown in Figure I-B-3, a group of 1360 men in an STD clinic in Lilongwe, Malawi were studied, and a total of 553 (40.6%) were HIV antibody positive. Analysis of HIV antibody negative specimens revealed that 24 men (1.8%) had unrecognized acute HIV infection, which represented 5.0% of all HIV infections detected. The factor most strongly associated with detection of acute HIV was attendance in the STD clinic [OR: 15.2 (95% CI: 2.04, 113.0)]. Patients with acute HIV infection had median blood viral burden > 10^6 copies/ml, significantly higher than the 10^4.5 copies/ml median observed in chronic infections (Figure I-B-3).

These results have recently been confirmed [Fiscus *et al.*,

Figure I-B-3: Viral load in Malawi: chronic and acute HIV infection



2005]. Among 1440 men and women studied in Malawi, 8% had established HIV infection and 1% (22 subjects) had acute infection. A heat-dissociated p24 antigen ELISA (see below) discerned 84% of the subjects with acute HIV infection. Of 1906 consecutive clinic attendees (47% female) recruited in the Hillbrow, Esselen Street STD Clinic in Johannesburg, SA, from April through October 2004 [Stevens *et al.*, 2005], a total of 672 individuals were HIV antibody positive (35.2%). In addition, 12 subjects (1% of antibody negative subjects) were HIV RNA positive (with acute HIV infection).

I-B-2 The importance of acute HIV infection

Detection of subjects with acute HIV infection raises three related concerns: i) implications for HIV surveillance; ii) opportunities to change the natural history of the disease; iii) opportunities for prevention of transmission.

Implications for HIV surveillance

Established HIV infection is detected by antibody testing. In recent years a variety of assays have been developed to detect antibodies with reduced concentration or avidity that are believed to reflect early (incident) infection [Janssen *et al.*, 1998; Brust *et al.*, 2000; Stramer *et al.*, 2000; Glynn *et al.*, 2000; Chen *et al.*, 2002; Stramer *et al.*, 2004]. The precise relationship between the time of infection and the veracity of these assays is difficult to assure, but current evidence suggests that positive results are obtained for at least several months after infection. Obviously, acute (truly incident) infections are not detected with antibody testing so the results of the cross-sectional methods applied to the general population are complementary, and can be quite

important if a substantial number of subjects are identified [Pilcher *et al.*, 2005].

Implications for treatment

The earliest stages of HIV infection reflect a battle between viral replication and emerging host defenses. The magnitude of the viral burden when the "set point" is reached will help to determine the natural history of the diseases [Mellors *et al.*, 1996]. In addition, results from animals and humans demonstrate considerable destruction of gut lymphoid cells during the earliest weeks of HIV infection [Lifson *et al.*, 2003; Guadalupe *et al.*, 2003; Brenchley *et al.*, 2004]. Accordingly, there has been great interest in early treatment of HIV. However, to date no sustained benefit of such therapy has been observed [Klein, 2001; Gegeny, 2002; Clements *et al.*, 2003; Kaufmann *et al.*, 2004; Di Mascio *et al.*, 2004; Smith *et al.*, 2004; Hammer, 2005]. Newer trials are focused on patients with acute or very early infection, and the concomitant application of antiviral drugs and agents that affect the cell cycle and/or state of cellular activation.

Implications for transmission prevention

The efficiency of HIV transmission depends on the viral burden [Quinn *et al.*, 2000] and the viral phenotype. The great viral burden characteristic of acute HIV infection virtually assures increased transmission risk [Pilcher *et al.*, 2004c]. In addition, many people with acute HIV infection have untreated STDs [Pilcher *et al.*, 2004b] which further increase genital tract viral shedding [Cohen *et al.*, 1997]. Mathematical modeling exercises have predicted that subjects with acute infection could play a disproportionate role in the epidemic. Jacquez *et al.* [1994]; Koopman *et al.* [1997] and recent analysis of data from Uganda [Koopman *et al.*, 1997] provide compelling support for this idea. Wawer *et al.* [2005] estimated the probability of HIV transmission from a subject with "early infection" (measured an average of 2.5 months after seroconversion) as 8.2/1000 episodes of intercourse, with established infection as 7–15/10,000, and with advanced (unrestrained and untreated) infection as 2.8/1000. The authors estimated that 43% of new HIV infections observed in their study population could be ascribed to subjects with acute and early infections.

I-B-3 Clades and transmission

The viral burden represents only a part of the story. The genotype and phenotype of the viral population must also play a crucial, albeit poorly understood role.

A key feature of HIV-1 is its genetic heterogeneity represented by distinct subtypes (A-K) [von Briesen *et al.*, 1990; Leitner *et al.*, 2004]. The HIV-1 clades or subtypes are not equally distributed around the globe, with subtype C representing approximately one-half of all infections. The existence of these phylogenetic clades likely represents an early isolation or bottleneck to establish subepidemics out of a more diverse initial epidemic. In areas of cocirculating subtypes, recombinants are common, suggesting there is no genetic barrier that has isolated the subtypes. There has been widespread interest in the possibility that the subtypes have different biological properties. A study by Devito *et al.* [2002] suggests a differential response in neutralizing antibodies in seronegative individuals exposed to clades A and D vs. clade B. IgA purified from the blood and genital tract from sex workers from Nairobi and Kenya, wherein clades A and D predominate, demonstrated significant cross-clade HIV-1 neutralization. In contrast, a more clade-specific pattern of neutralization was found in noninfected sex partners of clade B individuals. In addition, one study that indicated subtype C isolates have reduced replicative capacity in cell culture relative to subtype B isolates [Ball *et al.*, 2003] runs counter to the world-wide success of the subtype C virus, suggesting simple correlations between *in vitro* measures of biology and subtypes may be problematic.

One difference that has been widely reported is that subtype C HIV-1 appears to be less likely to undergo a coreceptor switch from using CCR5 to using CXCR4 [Tscherning *et al.*, 1998; Abebe *et al.*, 1999; Bjorndal *et al.*, 1999; Ping *et al.*, 1999; Cecilia *et al.*, 2000; Morris *et al.*, 2001]. Since most transmission events involve an R5 virus, this might suggest subtype C HIV-1 has an advantage in the rate of transmission over subtypes that could have their R5 pool diluted with X4 viruses. However, the site of the block of transmission of X4 viruses is not known, if indeed there is one, so that the potential impact of this subtype difference is hard to evaluate.

Others postulate that HIV-1 clade C could theoretically be more genetically fit than other clades due to an increased number of NFκB sites (up to 3 in clade C) within the long terminal repeat which could enhance proviral DNA transcription [Montano *et al.*, 2000]. More direct indications of potential differences between subtypes are suggested by the detection of subtype C viral sequences, but not subtype A viral sequences, in vaginal secretions of a person coinfected with subtype C and subtype A viruses [Iversen *et al.*, 2005] and higher rates of vertical transmission of subtype C HIV-1 compared to subtypes A or D [Renjufi *et al.*, 2004], as well as higher rates of nevirapine resistance in women with subtype C vs. A or D after treatment with single-dose nevirapine [Eshelman *et al.*, 2005]. Taken together, these data suggest that different HIV clades may alter disease progression and pathogenesis.

I-B-4 The transmitted swarm

HIV-1 exists as a complex mixture of genotypic variants within an infected person. This complexity can be used to estimate the extent of the bottleneck that occurs during transmission. Early reports were conflicting as to whether the transmitted virus was homogeneous [Amedee *et al.*, 1995; Delwart *et al.*, 1994; Furuta *et al.*, 1994; Kliks *et al.*, 2000; McNearney *et al.*, 1993; Mulder-Kampinga *et al.*, 1993; Ou *et al.*, 1992; Pang *et al.*, 1992; Shankarappa *et al.*, 1999; Sodora *et al.*, 1998; Wolfs *et al.*, 1992; Zhu *et al.*, 1993], suggesting a strong bottleneck; or heterogeneous [Delwart *et al.*, 1997; Dickover *et al.*, 2001; Enose *et al.*, 1997; Kampinga *et al.*, 1997; Lamers *et al.*, 1993; Learn *et al.*, 2002; Liu *et al.*, 1997; Nowak *et al.*, 2002; Pilcher *et al.*, 2001; Scarlatti *et al.*, 1993; Sutthent *et al.*, 1998; Verhofstede *et al.*, 2003; Wolinsky *et al.*, 1996; Zhu *et al.*, 1996], suggesting the transmission of multiple variants [Rademeyer *et al.*, 2004; Ritola *et al.*, 2004].

Interest in this area was renewed with the report that with heterosexual transmission women are frequently infected with multiple variants while men are typically infected with a single variant [Long *et al.*, 2000]. This study was extended to detect transmission of multiple variants for several other subtypes, although in smaller studies [Sagar *et al.*, 2003]. Moreover, genital tract infections have been estimated to increase the risk of acquiring multiple vs. single variants nearly five fold in women [Sagar *et al.*, 2004], possibly secondary to transmission of cell-associated virus with multiple proviruses. The detection of single variants in men was reported but in a cohort where the risk factor was not known [Delwart *et al.*, 2002]. Conversely, multiple variants were detected early after transmission in a homosexual male cohort but these variants became more homogeneous in the subsequent weeks [Learn *et al.*, 2002].

Finally, we have detected multiple variants in half of the men infected through homosexual exposure, suggesting that this mode of transmission may be more similar to the risk of women in heterosexual exposure than of men in heterosexual exposure [Ritola *et al.*, 2004]. The question of the number of transmitted variants is important to resolve for two reasons. First, the transmission of multiple variants is inconsistent with transmission being infrequent and a virus particle being the minimal infectious unit, suggesting fundamental features of the mechanism of transmission remain unknown. Second, longitudinal follow-up of women infected with single versus multiple variants showed that infection with multiple variants was associated with more rapid progression [Sagar *et al.*, 2003]. Thus, features of virus transmission and the impact of this earliest event in infection on the entire disease course remain important areas of study.

I-B-5 Features of the transmitted virus

A long-standing interest in the vaccine field has been to understand the nature of the transmitted virus as this is ultimately the entity that a vaccine must protect against. The null hypothesis is that the transmitted variant is simply a randomly selected subset of the total virus population in the donor. However, compartmentalization of the virus population in the genital tract secretions that carry the transmitted virus or early selection for specific variants in the newly infected recipient could alter the composition of the virus population in ways that might impact vaccine design.

Derdeyn *et al.* [2004] have reported that subtype C HIV-1 isolated from newly infected subjects in a discordant couple cohort have env genes that are largely homogeneous, encode Env proteins that are more neutralization sensitive than the Env proteins of the donor virus, and have shorter variable loop lengths in Env compared to the circulating virus in the donor. The hypothesis to explain these observations is that the recipient initially represents an antibody-negative environment that rapidly selects for variants that can grow more rapidly but at the expense of having an Env protein that is more neutralization sensitive.

Similar reduced V1/V2 loop length and reduced number of glycosylation sites were observed in heterosexual transmission of subtype A HIV-1 [Chohan *et al.*, 2005]. However, no such differences in neutralization sensitivity, variable loop length, or glycosylation site number have been seen for (largely homosexual) transmission of subtype B HIV-1 [Chohan *et al.*, 2005; Frost *et al.*, 2005]. It is not obvious how different subtypes or different modes of transmission could have such a dramatic impact on determining the nature of the transmitted variant. At present these questions remain unresolved yet relevant to our understanding of HIV transmission and early infection.

I-B-6 HIV compartmentalization and implications for transmission

Substantial research has focused on HIV-1 variation within anatomical compartments in acute and chronic HIV-1 infection; compartmentalization can occur in a variety of areas including plasma, brain, genital tract secretions, or within different leukocyte compartments [Zhu *et al.*, 1996; Eron *et al.*, 1998; Staprans *et al.*, 1999; Kiessling *et al.*, 1998; Haddad *et al.*, 2000; Gupta *et al.*, 2000; Venturi *et al.*, 2000; Pilcher *et al.*, 2001; Poles *et al.*, 2001; Sutthent *et al.*, 2001; Zhang *et al.*, 2002; Potter *et al.*, 2003; Kemal *et al.*, 2003; Tirado *et al.*, 2004; Ghosn *et al.*, 2004; Potter *et al.*, 2004; Philpott *et al.*, 2005; Ritola *et al.*, 2005; Smit *et al.*, 2004; Sullivan *et al.*, 2005; Adal *et al.*, 2005]. One of the first examinations of viral compartmentalization was done by Zhu *et al.* [1996], and examined envelope gp120 se-

quences in plasma and genital secretions of patients acutely infected with HIV-1, and their chronically-infected sexual partners. Envelope sequences from the acutely infected group were largely homogeneous, and represented a minor variant of the population found in the semen of the transmitting partner. These data provided the first evidence that HIV-1 selection may occur during sexual transmission. Further analysis of envelope gp120 sequences during acute infection revealed that variants transmitted during acute infection form the genetic basis for subsequent viral diversification that leads to heterogeneity in chronic infection [Zhang *et al.*, 2002]. Kemal *et al.* [2003] found that in a majority of women, gp120 sequences from the genital tract and plasma are distinct. Envelope gp120 glycosylation sites, which are hypothesized to form a protective shield and facilitate neutralization escape, were significantly different between the two compartments.

These studies suggest that compartmentalization observed in chronic infection is likely secondary to gradual viral evolution from a dominant species, rather than differential migration of variants to various anatomical locations during acute infection. To analyze the dissemination of HIV-1 into various anatomical compartments during primary infection, including plasma, cerebrospinal fluid (CFS), semen, cervicovaginal lavage fluid, and/or saliva in 17 individuals, Pilcher *et al.* [2001] confirmed that viral dissemination is highly efficient and dynamic, and concluded that antiretroviral therapy is unlikely to limit initial virus spread to most tissue compartments, although it may reduce genital tract shedding and central nervous system (CNS) expansion. In those subjects that are infected with multiple variants, these variants appear to penetrate the seminal compartment and CNS (CSF) equivalently [Ritola *et al.*, 2004].

In addition, there is growing evidence that the genital tract of both males and females can serve as a reservoir for drug resistance mutations: paired analyses of plasma and genital secretions in nine of twelve women exhibited different drug-resistance mutations [Tirado *et al.*, 2004]. A similar result was obtained in men, wherein the rate and pattern of emergence of resistance varied between plasma and semen [Eron *et al.*, 1998]. Additional phylogenetic analyses of clones of the HIV pro gene have revealed variants in semen that originate not only from passive diffusion from blood, but also from local production within semen [Ghosn *et al.*, 2004]. Additional studies to analyze the interplay between cellular, humoral, and viral factors involved in drug resistance and compartmentalization are critical to HIV pathophysiology and controlling the infection.

I-B-7 Resistance characteristics of the transmitted virus

Although highly active antiretroviral therapy (HAART) has significantly decreased morbidity and mortality from HIV, treatment remains problematic due to the development of resistance to antivirals and evasion of the host immune system. Blower *et al.* [2001] have identified several factors that may enhance the transmission of resistant virus: an increasing proportion of HIV-infected patients who are on antiviral therapy (increased probability of acquiring infection from treated person), an increasing rate at which patients on antiviral treatment develop drug resistance (dependent on potency, adherence, genetic barriers), an increasing relative fitness of the resistant strains (dependent on replicative capacity of the resistant strain), a decreased transmissibility of drug-sensitive strains from treated patients, and individual risk factors.

Surveillance of the transmitted variants is crucial to understanding these factors, rational drug use and vaccine development. Indeed, transmission of drug-resistant viruses has been the subject of intense scrutiny in the recent past and remains a significant public health concern. A series of prevalence reports from North America and Europe indicate the transmission of HIV-1 resistance is steadfastly increasing [Salomon *et al.*, 2000; Grant *et al.*, 2002; de Mendoza *et al.*, 2005; Yerly *et al.*, 2001; Little *et al.*, 2002] with measurements ranging from 3% to 23% for one or more drugs. Differences are at least in part due to study design, number of patients, resistance testing, definitions of resistance, geography, variability in antiviral selection, timeline, and risk factors for transmission. Although multiple studies have confirmed the increasing frequency of transmission of drug-resistant virus, these estimates may be an underestimate as the sequencing techniques for genotypic analysis did not quantify minor populations below 25%. To determine the prevalence of these minor variants, Metzner *et al.* [2005] have devised a quantitative real-time PCR assay using allele-discriminating oligonucleotides for three key resistance mutations, L90M (protease), K103N and M184V (RT). In 49 acute seroconverters in Germany from 1999 to 2003, overall drug resistant variants were detected in 20.4%. The L90M, K103N, and M184V were found in 2%, 10%, and 12%, respectively. Thus, knowledge of the prevalence of major and minor resistance variants may impact antiretroviral therapy.

Transmission of drug resistance mutations has been shown to affect disease outcome and response to antiviral treatment. Little *et al.* [2002] found that among subjects infected with drug-resistant virus, there was a significant delay in viral suppression after initiation of antiretroviral therapy (median of 88 days vs. 56 days). Moreover, even though there was no significant difference in mean

baseline plasma viral loads between the susceptible and resistant cohorts, once suppression was achieved the time to virological failure was significantly shorter in the patients infected with resistant virus (approximately 80% vs. 40%, respectively, remaining suppressed 500 days after achieving viral suppression). These data indicate that antiretroviral therapy is twice as likely to fail in patients infected with drug-resistant virus. In addition, drug-resistant HIV-1 isolates have been shown to persist in the host after primary infection without reversion to wild-type, even in the absence of drug therapy [Barbour *et al.*, 2004; Brenner *et al.*, 2002; Chan *et al.*, 2003; Delaugerre *et al.*, 2004]. These data suggest that some drug-resistant mutants have altered genetic fitness. In addition, Turner *et al.* [2004] compared the incidence of HIV-1 variants containing resistance to thymidine analogues, RT inhibitors, and protease inhibitors in primary HIV and chronic HIV infection. M184V was found in early HIV infected patients significantly less than in the chronic (potential transmitter) HIV infected group, 10% vs. 70%, respectively. The reduced transmissibility of M184V in the potential transmitters may be secondary to reduced viremia (up to 0.8 log lower) or compartmentalization of variants lacking M184V.

I-B-8 Conclusions

Renewed interest has developed in acute and early infection in recent years. New strategies to identify subjects in acute infection in the course of standard HIV screening open the possibility of early intervention both to preserve immune function (early therapy) and to reduce transmission during this period of high infectiousness. Analysis of the transmitted virus has raised questions about the nature of the transmission event and about the earliest features of viral evolution with implications for vaccine design and disease progression. The study of acute infection will continue to extend our knowledge about the earliest response of the human host to the virus and its limitations that result in a failure to clear the virus. This knowledge will become the cornerstone of a new generation of vaccine strategies based on a deeper understanding of these earliest events of HIV-1 infection.

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