Oral Transmission of Primate Lentiviruses

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Oral transmission of human immunodeficiency virus type 1 (HIV-1) is well documented in children who become infected postnatally through breast milk. In contrast, epidemiologic surveys have yielded conflicting data regarding oral HIV-1 transmission among adults, even though case reports have described seroconversion and the development of AIDS in adults whose only risk was oral-genital contact. To study oral virus transmission in primate models, we exposed rhesus macaques of various ages to cell-free simian immunodeficiency virus (SIV), including uncloned and molecularly cloned viruses. In neonates, viremia and AIDS developed after nontraumatic oral exposure to several SIV strains. Furthermore, chimeric simian human immunodeficiency viruses containing the HIV-1 envelope can also cross intact upper gastrointestinal mucosal surfaces in neonates. In adult macaques, infection and AIDS have resulted from well-controlled, nontraumatic, experimental oral exposure to different strains of SIV. These findings have implications for the risks of HIV-1 transmission during oral-genital contact.

Postnatal oral transmission of human immunodeficiency virus type 1 (HIV-1) via infected breast milk is a well-recognized occurrence, and intrapartum oral-mucosal exposure might also be an important risk factor for HIV-1 transmission (reviewed in [1]), as suggested by the following three epidemiologic studies: (1) The higher rate of HIV-1 infection among first-born twins compared with rates among second-born twins is an indirect clue that infection occurs intrapartum via mucosal or skin exposure [2]. If infection resulted solely from virus crossing the placenta, this discrepancy would be difficult to explain. However, if virus entered via mucosal surfaces, the long time of exposure to infectious cervical secretions or maternal blood (or both) of the first-born twin, who typically spends many hours in the birth canal, could explain this difference. In contrast, the second-born twin is delivered usually within minutes. (2) The presence of serosanguinous blood in gastric aspirates of infants born to HIV-1–positive women was strongly associated with neonatal infection [3]. (3) Prolonged rupture of fetal membranes represented an increased risk for HIV-1 transmission [4].

Oral transmission of HIV-1 among adults has been a controversial subject. On the one hand, it was generally believed, on the basis of subset analyses of early epidemiologic studies [5], that no convincing epidemiologic evidence existed to support oral intercourse as a risk factor for HIV-1 transmission in adults. On the other hand, several case reports have described HIV-1 infection or AIDS over the years in individuals who claimed no risks other than oral-genital contact (reviewed in [6]).

We tested the permissiveness of the oral route in simian immunodeficiency virus (SIV)–rhesus macaque models by exposing both neonatal and adult animals to cell-free virus or infected blood.

Oral Inoculation of Neonatal Macaques with Pathogenic SIVmac251

Four neonatal monkeys were exposed orally to cell-free SIVmac251, an uncloned biologic isolate [7]. All infants became
viremic within 2 weeks after exposure and progressed to AIDS, with a median survival time of 43 weeks (range, 17–145 weeks).

**Oral Infection of Neonatal Macaques with Live Attenuated, Molecularly Cloned SIVmac239Δ3**

Next, we sought to develop immunoprophylaxis to protect neonates against oral challenge with SIV. Combining active and passive immunoprophylaxis is 98% effective in preventing maternal transmission of hepatitis B virus, an enveloped virus that is distantly related to HIV-1. We reasoned that combining active and passive immunization might also prevent maternal HIV-1 transmission. When we initiated the active immunoprophylaxis studies, live attenuated virus appeared to be the most promising vaccine [8]; however, it was not known whether the neonatal primate immune system could react appropriately to live attenuated virus vaccination. Live attenuated polio virus previously had been given to human newborns in studies designed to test the influence of maternal antibodies [9]. Vaccinated infants produced anti-poliovirus antibodies, and no infant was reported to develop poliomyelitis [9], indicating that exposure of neonates to live attenuated virus neither induced tolerance nor resulted in viral persistence. We concluded that, in general, the live attenuated vaccine approach was feasible in neonates.

We used the vaccine strain SIVmac239Δ3, a mutant containing large deletions in *vpr* and *nef* and in the negative regulatory element of the long terminal repeat [10]. SIVmac239Δ3 was attenuated when given intravenously (iv) to adult monkeys [10]. After the period of acute viremia, which lasted up to 12 weeks, it was difficult to isolate virus from peripheral blood, and the animals showed no signs of disease. When challenged with pathogenic SIVmac251, ~50% of the vaccinees were protected overall [10].

The clinical outcome was very different in neonatal macaques that were vaccinated orally; SIVmac239Δ3 itself induced persistent viremia and lethal AIDS [11]. Extensive polymerase chain reaction analysis of peripheral blood mononuclear cell DNA revealed no wild-type *nef* sequences in any infant at any time. In fact, with in vivo passage, the 3 long terminal repeat sequences in the negative regulatory region were replaced with further deletions [11].

Had the viral genome undergone deleterious mutations in segments that were not analyzed by polymerase chain reaction, or could differential host responses in adults versus neonates account for the drastic difference in clinical outcomes? To address these questions, we collected blood from a vaccinated macaque infant that had developed full-blown AIDS. We simultaneously administered one aliquot of blood orally to neonatal recipient 94-4 and another aliquot iv to this neonate’s mother, animal E801. The different routes of virus exposure were used to replicate our oral exposure protocol in neonates and to replicate the experimental conditions (i.e., iv administration) under which the adult macaques previously had received the vaccine virus [10]. Adult E801 has remained well for >3 years, and her virus appears attenuated. However, infant 94-4 died of AIDS and renal failure only 34 weeks after virus inoculation. Thus, virus from the same donor source caused rapid death in the infant but remained attenuated in the adult recipient. The differential pathogenicity of SIVmac239Δ3 thus cannot be ascribed solely to viral factors but rather to host influences.

Were the neonatally infected macaques rendered tolerant to the vaccine virus, thus precluding anti-SIVmac239Δ3 immune responses? This possibility can be ruled out because the infected infants produced anti-SIV antibodies, as determined by Western blot analysis [11]. Furthermore, neutralizing and infection-enhancing antibody responses in SIVmac239Δ3-infected infants and adults did not correlate with disease progression in the former or viral attenuation in the latter [12]. We now postulate that differences in cellular immune responsiveness in adult and infant macaques are responsible for the differential pathogenicity of SIVmac239Δ3. Perhaps, less mature cellular immune responsiveness in infants allows the virus to escape host control, reach high virus loads, and expose its underlying pathogenicity.

**Oral Exposure of Adult Macaques to Uncloned, Pathogenic SIVDeltaB670**

The difference in the route of virus inoculation (i.e., oral vs. iv) may also have influenced the clinical course. Would SIVmac239Δ3 remain attenuated if neonates received the virus iv? Conversely, would adult macaques develop progressive disease after oral inoculation? Because there was no information in the literature regarding SIV infection and AIDS in adult macaques exposed orally to wild-type, pathogenic, *nef*⁺ SIV, we inoculated adults with a stock of SIVDeltaB670, an uncloned, biologic isolate that had already been titrated in adults by iv and nontraumatic rectal inoculation [13].

When these experiments were designed, it was generally believed that oral transmission of HIV-1 was not a significant route of transmission in adults. While several case reports described individuals who attributed their HIV-1 infection solely to oral-genital contact (reviewed in [6]), early epidemiologic surveys did not identify oral sex as an independent risk factor for HIV-1 transmission [5, 14–16]. In contrast, transmission of HIV-1 infection to newborns via infected breast milk is well documented (reviewed in [1]). Our primate experiments demonstrated the permissiveness of the neonatal upper gastrointestinal mucosa to SIV; thus, we postulated that newborns may be susceptible to infection by oral exposure because they lack gastric acidity [7]. Did the few adults who reportedly became HIV-1-positive through oral-genital contact have defects in gastric acid secretion that allowed infection?

To test this hypothesis [13], we exposed 2 adult macaques to SIVDeltaB670 after pretreatment with omeprazole, a potent inhibitor of gastric acid secretion. The first animal was exposed orally to undiluted virus, and the second one was exposed to
a $1.2 \times 10^{-3}$ dilution of virus, a dose considered to be in the appropriate range for mucosal inoculation. Both animals became viremic. To test whether gastric acid neutralization was needed to achieve systemic infection, we gave the next animal the same virus dilution ($1.2 \times 10^{-3}$) without omeprazole pretreatment. It also became infected systemically, indicating that lack of gastric acidity is unlikely to play a major role in transmission of the virus. Next, we titrated the virus stock orally in the absence of omeprazole therapy. Of 7 adults tested, 6 developed systemic infection. After 2.5 years, 4 of the 6 animals appeared attenuated in adult macaques regardless of the route of initial virus exposure. In contrast, neonatally inoculated animals developed lethal AIDS. The minimal infectious dose for the oral route was 830 times greater than that required for iv inoculation. We were surprised to find that the minimal infectious dose needed to achieve nontraumatic mucosal infection after oral exposure was 6000 times lower than that required for nontraumatic rectal inoculation [13].

In humans, rectal exposure to HIV is significantly more risky than other mucosal routes, and unprotected oral-genital sex had been believed to be a low-risk activity [5, 14–16]. Thus, the outcome of our comparative titration experiment in macaques was unexpected because the minimal dose of cell-free SIV required for infection across intact rectal mucosa exceeded that required for the oral route. Most likely, rectal infection in humans is facilitated by mucosal tears that develop during sexual intercourse. However, in the controlled laboratory setting involving nontraumatic oral or intrarectal inoculation of cell-free SIV to young adult macaques, the uncloned biologic isolate SIVDeltaB670 could more readily cross the intact upper gastrointestinal mucosal barrier than the rectal mucosal cell. It will be of interest to evaluate cloned virus isolates with different tropism for patterns of infectiousness through various mucosal routes.

### Oral Exposure of Adult Macaques to Molecularly Cloned SIVmac239Δ3

After completing the control experiment in adult macaques that were exposed orally to nef<sup>+</sup> pathogenic SIV [13], we questioned whether the molecularly cloned, T cell–tropic SIVmac239Δ3 could induce disease in adult macaques after oral exposure. A titration experiment was conducted with cell-free virus: 5 virus-exposed animals became viremic after nontraumatic inoculation of doses ranging from $5.24 \times 10^2$ to $5.24 \times 10^3$ TCID<sub>50</sub> tissue culture infectious doses (TCID<sub>50</sub> [17]. The oral inoculations were administered as described [13]. A sixth animal that received 52.4 TCID<sub>50</sub> of SIVmac239Δ3 remained uninfected. The infected animals have not developed AIDS during 3 years of follow-up. Thus, SIVmac239Δ3 appeared attenuated in adult macaques regardless of the route of initial virus exposure. In contrast, neonatally inoculated animals developed disease after either oral or iv inoculation with cell-free SIVmac239Δ3 (unpublished data). These results led us to conclude that the initial route of virus inoculation does not determine the differential pathogenicity of SIVmac239Δ3 in adults and neonates.

### Neonatal Macaques Are Susceptible to SIVmac239Δ3 Infection and Disease after Oral Exposure: Confirmation

Wyand et al. [18] confirmed our finding that oral inoculation of neonatal rhesus monkeys with cell-free SIVmac239Δ3 not only leads to systemic infection but also to AIDS. Within only 7 months, 1 infant succumbed to disease, an event that the authors attributed to “extremely high doses of vaccine virus” [18]. It should be noted, however, that in a cohort given just a 5.7 times lower virus dose, systemic infection developed in only 2 of 3 inoculated animals, indicating that this lower dose is no longer fully infectious for the oral route. Furthermore, follow-up times significantly longer than the 10 months used [18] are needed to assess lentivirus—slow virus—pathogenicity or the lack thereof.

### Oral Lentivirus Infection Studies in Primates: Summary

Virus strains that have been inoculated orally into neonatal and adult macaques by us and others are summarized in table 1. Among infant macaques, we did not find a statistically significant difference in disease progression rates between animals infected orally as neonates with cell-free SIVmac251 and those given cell-free SIVmac239Δ3. The former had survival times of...
17, 26, 60, or 145 weeks, whereas the 3 infants infected orally with cell-free SIVmac239Δ3 survived for 46, 162, or 233 weeks [17]. In both cohorts, a wide spread of disease progression rates was seen. The distribution of survival times after inoculation was compared with a log-rank test. Because of the small sample size, an exact test was performed using StatExact (Cytel Software, Cambridge, MA; $P = .33$). Other investigators reported that infection of neonatal macaques with uncloned SIVmac251 led to a more aggressive disease course [25] than we observed in our orally inoculated rhesus macaque infants. It is not known whether differences in the virulence of the particular virus stocks used, the route of inoculation (oral vs. iv), or host susceptibility factors account for this difference. We have also infected a neonatal macaque orally with SIV1A11 [19], an attenuated strain (unpublished data). Thus far, this virus has remained attenuated in the infected offspring (table 1), in contrast to all other viruses. No disease has been reported in SIV1A11-infected infant macaques regardless of the route of inoculation [25].

More recently, we also achieved systemic infection after oral inoculation of neonatal rhesus macaques with SHIV-vpu [20], a chimeric virus containing the env gene of HIV$_{in}$, a laboratory-adapted, T cell–tropic strain, and with SHIV89.6P, which encodes the env gene of the dual-tropic primary HIV-1 isolate 89.6 [21] (unpublished data).

Oral HIV-1 Transmission in Adults

Our results demonstrating the permissiveness of the oral route to lentivirus infection in adult primates should be viewed in the context of more recent studies that address oral HIV-1 transmission in humans. Schacker et al. [6] prospectively evaluated high-risk individuals who were initially HIV seronegative. Through frequent serologic surveys, they diagnosed 46 patients with an acute retroviral syndrome. Of these newly infected individuals, 12 recalled the day, partner, and sexual activity that resulted in their seroconversion. Among these 12 individuals, 4 (a surprisingly high 33%) reported having had oral-genital contact only [6].

Does oral HIV transmission occur more frequently than previously thought? The complexity of human sexual behavior renders identification of individuals who engage only in oral-genital contact difficult. Because the number of study subjects with no risk factors other than oral-genital contact was very small in the original surveys, the subset analyses have low statistical power. For example, a review of an early subset of the San Francisco Men’s Health Study [5] revealed only sufficient power to detect a relative risk of $\geq 4$ for oral transmission of HIV-1, with 49 seropositive cases among the 215 participants who reported no anal-genital contact. Risk ratios of 4 are far larger than those associated with other health hazards for which intervention is recommended. For example, aspirin is recommended as prophylaxis against myocardial infarction on the basis of a risk ratio 1.82 for placebo vs. aspirin. In another example, the risk ratio of fatal injury was 7.96 for individuals not wearing seat belts compared with those who did. The failure of the early epidemiologic studies to detect a significant association between HIV-1 infection and oral-genital contact should not be viewed as unequivocal evidence of a lack of an association given the problems of finding a sufficiently large group of individuals with only oral-genital exposure. A later study with a substantial number of subjects found odds ratios of 2.6–6.7 ($P$ values of .02–.15) for receptive oral intercourse, depending on the statistical model used for analysis [26]. The authors concluded that the data showed “some evidence indicating receptive oral intercourse to be unsafe” [26].

Summary

Oral transmission of lentiviruses has been demonstrated in neonatal and adult macaques. While the evidence is mounting that HIV-1 can be transmitted during oral-genital contact, the relative risks of various forms of such sex acts are not known. Many questions regarding the mechanisms involved in oral lentivirus transmission remain unanswered, chief among them are the site(s) of virus entry, initial target cells, the tropism of the transmitted virus, and the influence of local inflammation in the oral cavity or the upper gastrointestinal tract mucosa (or both) on virus transmission.

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References


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