Rectal Transmission of Human Immunodeficiency Virus Type 1 to Chimpanzees

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Inoculation of chimpanzees with human immunodeficiency virus type 1 (HIV-1) has been used as a model system to define mechanisms of pathogenesis and to test protective efficacy of candidate HIV-1 vaccines. In most of these studies, the animals were inoculated intravenously. However, because HIV-1 is transmitted primarily across mucosal surfaces, future evaluations of vaccines should employ mucosal routes for administering infectious virus to immunized animals. To develop a model of rectal transmission of HIV-1, chimpanzees were exposed without trauma to 4 different HIV-1 strains at doses ranging from 200 to 10,000 TCID50. Infection, characterized by seroconversion and repeated isolation of virus from lymphocytes, was established in 1 of 5 animals. This animal was sequentially inoculated with a subtype B and then an E strain and was infected with both strains. The results show that rectal exposure of adult chimpanzees to cell-free HIV-1 was not an efficient mode of transmission in this cohort.

The major route of transmission of human immunodeficiency virus type 1 (HIV-1) among homosexual men is across mucosal surfaces of the rectum [1]. Therefore, any prophylactic interventions designed to prevent HIV transmission, such as a vaccine, should elicit responses that interfere with the ability of the virus to establish productive, pathogenic infections by this route. In addition, it is important to understand early events after rectal exposure to both cell-free and cell-associated virus (i.e., to identify mechanisms facilitating transit across the mucosa and to identify initial cells in which the virus replicates and secondary sites of replication). Animal models must be used to address these problems. Two model systems for evaluating various aspects of pathogenesis and therapeutic and prophylactic interventions for human HIV infections are infection of chimpanzees with HIV-1 and macaques with simian immunodeficiency virus (SIV) [2]. Several studies in which various SIV strains were used to infect macaques rectally demonstrated that the SIV-macaque model reproduces HIV-induced disease [3–7]. However, there have been no reports of attempts to establish a model of rectal infection with HIV-1 in chimpanzees.

The major use of the HIV-1 chimpanzee model has been to evaluate protective efficacy of candidate HIV-1 vaccines against intravenous transmission [2]. Since rectal transmission of HIV-1 continues to account for a significant proportion of new HIV-1 infections, it would be useful to include this route of infection in future HIV-1 vaccine studies. It has been shown that chimpanzees are the only species, other than humans, that can be infected reproducibly with multiple HIV-1 strains after intravenous or cervical exposure. Thus, to provide the potential to evaluate vaccine-mediated protection by the three major routes of transmission in the HIV-1 chimpanzee model, we exposed adult chimpanzees to various strains by the rectal route.

Methods

Animals. The chimpanzees (Pan troglodytes) were adult males housed at the Coulston Foundation (Alamagordo, NM). Before virus inoculation or collection of blood samples, the animals were anesthetized by intramuscular injection of ketamine hydrochloride (15 mg/kg body weight). Rectal inoculations were done by depositing 1 mL of diluted HIV-1 (~10 cm) into the rectum with a syringe to which flexible tubing was attached. This method was the same as that used successfully to establish infections in pig-tailed macaques with SIVsmmPBj14 [5, 7].

Viruses and virus isolation. Several HIV-1 strains were used in attempts to infect chimpanzees by the rectal route, including the 3 related subtype B strains (LAI/IIIB [8], LAI/LAV-1b [9], and IC499) [10] and a subtype E strain (90CR402) [11]. The dose of the inocula ranged from 200 to 10,000 TCID50. Chimpanzee peripheral blood mononuclear cells (PBMC) or single-cell suspensions of lymph node tissues were cocultured with mitogen-activated human PBMC as described [7]. All cultures were monitored for particulate reverse transcriptase activity in cell-free supernatants and were maintained at least 6 weeks before being designated as virus negative. In some cases, CD8+ T cells were removed from PBMC by use of immunomagnetic beads coated with anti-CD8 antibodies, and only enriched populations of CD4+ T cells were cocultured with human PBMC. To isolate HIV-1 from rectal biopsies, tissues (2–5 mm in diameter) were minced with sterile blades.
Table 1. Rectal inoculation of chimpanzees with HIV-1.

<table>
<thead>
<tr>
<th>Chimpanzee</th>
<th>Strain</th>
<th>TCID&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-1173</td>
<td>LAI/IIIB</td>
<td>200</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>LAV-1b</td>
<td>1000</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>E/CR402</td>
<td>10,000</td>
<td>No</td>
</tr>
<tr>
<td>C-1181</td>
<td>LAI/IIIB</td>
<td>10,000</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>LAV-1b</td>
<td>10,000</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>E/CR402</td>
<td>10,000</td>
<td>Yes</td>
</tr>
<tr>
<td>C-1196</td>
<td>LAI/IIIB</td>
<td>10,000</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>LAV-1b</td>
<td>10,000</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>E/CR402</td>
<td>10,000</td>
<td>Yes</td>
</tr>
<tr>
<td>C-1163</td>
<td>LAI/IIIB</td>
<td>10,000</td>
<td>No</td>
</tr>
<tr>
<td>C-1323</td>
<td>JC499</td>
<td>2000</td>
<td>No</td>
</tr>
</tbody>
</table>

Results

Most HIV-1-related vaccine studies in chimpanzees have used a specific stock of HIV-1<sub>LAI/IIIB</sub> as the challenge virus [8], and we have infected chimpanzees with this stock via the cervical os [13]; therefore, we used this stock during initial attempts to infect 2 chimpanzees by the rectal route. Chimpanzee C-1173 received 200 TCID<sub>50</sub> and C-1181 received 10,000 TCID<sub>50</sub> (table 1). The animals were evaluated for the presence of virus in PBMC, lymph nodes, and rectal biopsies by cocultivation with normal human PBMC. In addition, PBMC obtained 4 weeks after inoculation were enriched for CD<sup>4</sup> T cells before coculture. Universal primers were used to amplify either the p17<sup>env</sup> gene or the C2–V5 region of env in DNA from peripheral blood or lymph node cells. Serum samples were tested for antibodies to HIV-1 antigens by whole virus enzyme immunoassay (Sano; Seattle). All virologic and serologic assays were negative.

When there was no evidence of infection in either animal during 4 months of follow-up, a third animal (C-1196) was exposed to 10,000 TCID<sub>50</sub> of HIV-1<sub>LAI/LAV-1b</sub>, a strain of virus known to be more infectious for and to establish higher virus burdens in chimpanzees than HIV-1<sub>LAI/IIIB</sub> [9]. Six weeks after exposure, virus was isolated from C-1196’s PBMC, and at 8 weeks, HIV-1–specific antibodies were detected in serum (figure 1). Subsequently, virus was isolated from C-1196’s PBMC and lymph node biopsy tissue on every attempt and from rectal biopsies on four of six attempts over a period of 2 years. In addition, levels of HIV-1 virion RNA in plasma peaked at 1.1 × 10<sup>6</sup> copies/mL 8 weeks after inoculation and then gradually declined to ~10<sup>5</sup> copies/mL by 140 weeks of follow-up (figure 1).

Because HIV-1<sub>LAI/LAV-1b</sub> established infection after rectal inoculation, this strain was used to re-expose C-1173 and C-1181 (neither of which were infected by HIV-1<sub>LAI/IIIB</sub>) to inoculate a third animal (C-1163) not previously exposed to HIV-1. During 12 months of follow-up, HIV-1 was neither isolated nor detected by nested PCR, from PBMC, lymph nodes, or rectal biopsies from any of these animals. Using cells from different dates and HIV-1<sub>LAI</sub>–specific primers, multiple independent nested PCRs (20 for C-1173 and 12 each for C-1181 and C-1163) were also negative. Further, none of the animals developed detectable antibodies to HIV-1.

Figure 1. HIV-1 viremia and serum antibodies in chimpanzee C-1196 after rectal inoculation of HIV-1 strains LAI/LAV-1b (time 0) and 90CR402 (week 40). The + and − signs identify times when rectal biopsy tissue was cultured in attempts to isolate HIV-1: +, virus was isolated; −, virus was not detected by reverse transcriptase assay or polymerase chain reaction. EIA = enzyme immunoassay.
Although there was no indication that these 3 chimpanzees were infected with HIV-1, it was possible that HIV-1 had initiated an infection that was rapidly contained by an immune response at the inoculation site. If there was a local immune response elicited after the first exposure of C-1173 and C-1181 to HIV-1, it might have hindered subsequent infection by the closely related LAI/LAV-1b strain. Therefore, we exposed C-1173 to 10,000 TCID₅₀ of a subtype E strain, 90CR402, that we had used previously to establish infection by the cervical route in a female chimpanzee [11]. C-1196, who became productively infected and seroconverted after rectal exposure to the LAI/LAV-1b strain, was also inoculated rectally with the subtype E strain. We obtained no serologic or virologic evidence of a productive infection in C-1173 with this unrelated strain. To enhance PCR sensitivity, LAI/IIIB- or 90CR402-specific primers were each used with 1.5 µg DNA per reaction in 10 independent PCRs. All of these assays for C-1173 were negative. However, using these same two sets of subtype B- and E-specific primers with DNA isolated from C-1196’s lymph node and blood cells, 11 of 11 and 12 of 12 independent PCRs with the subtype B- and E-specific primers, respectively, were positive. Thus, prior rectal infection of C-1196 did not prevent subsequent infection by a second HIV-1 strain via this same route.

Recently, we isolated an HIV-1, JC499, that established high virus burdens and induced rapid loss of CD4⁺ T cells in chimpanzees who were intravenously or cervically inoculated [14]. We inoculated a naive chimpanzee, C-1323, by the rectal route with 2000 TCID₅₀ of HIV-1JC499 because of its pathogenicity. Through 8 months of follow-up, virus was not isolated from PBMC or lymph node, and the animal did not seroconvert. All of 23 independent nested PCRs with JC499-specific primers (using a total of 62 µg DNA from PBMC and lymph node cells) were negative.

Discussion

On the basis of our experience and that of others relative to the outcome of rectal inoculation of macaques with various strains of SIV [3–7], it was assumed initially that it would be possible to infect chimpanzees by this route with similar amounts of cell-free virus. Therefore, different amounts of HIV-1 were used in hopes of identifying a minimal infectious dose. However, the fact that only 1 of 5 adult male chimpanzees was infected unequivocally after rectal exposure to HIV-1 indicates that rectal infection of macaques with SIV and of chimpanzees with HIV-1 might not be comparable. This difference might be explained by the age of the animals: Juvenile macaques were generally used in the SIV studies, whereas large adult chimpanzees were used in the HIV-1 studies. In these latter animals, the physical state of the rectal tissue may have provided more of a barrier than that in the young macaques. Since rectal transmission of HIV-1 among homosexual men is efficient, probably due to concomitant trauma to the rectal mucosa, lack of trauma upon rectal exposure of chimpanzees to HIV-1 could explain our failure to establish infection. Alternatively, the number of submucosal cells capable of supporting HIV-1 replication may differ between juvenile and adult animals (or between individual adult animals). All of the HIV-1 stocks used in the present study previously were shown to establish infections after exposure of the cervical mucosa of female chimpanzees [11, 13].

Although no evidence of infection was obtained in 4 of the 5 rectally exposed chimpanzees, it is possible that inapparent infections without seroconversion were established. Evidence for such infections by primate lentiviruses has been obtained in the SIV-macaque model after rectal or intravaginal inoculation of virus [3, 4, 15]. In addition, virus has been isolated transiently from PBMC from 1 chimpanzee that was inoculated via the cervical os but never seroconverted [13]. Moreover, that HIV-1 was isolated from small fragments of rectal tissue from C-1196 >1 year after its original exposure to HIV-1 suggests that HIV-1 might be retained in mucosal tissues at such low levels that the virus is not disseminated and the amount of viral antigen produced is insufficient to induce detectable humoral immunity. HIV-1-specific IgA and IgG antibodies have been documented in secretions from chimpanzees infected with HIV-1 by parenteral or mucosal routes [16]. It will be important to evaluate these animals for possible mucosal immunity and cellular immune responses against HIV-1.

Last, it is noteworthy that the only chimpanzee (C-1196) infected with HIV-1 by the rectal route was inoculated 9 months apart with 2 strains representing different subtypes, B and E. If one were to equate rectal infection by HIV-1 with mucosal immunization with an attenuated vaccine, then cross-clade protection at a mucosal surface was not achieved in this animal. This result has relevance for the planned Phase 3 vaccine trials in Thailand, where subtypes B and E are co-circulating.

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References


