

# Evaluation of Oral Tenofovir Disoproxil Fumarate and Topical Tenofovir GS-7340 to Protect Infant Macaques Against Repeated Oral Challenges With Virulent Simian Immunodeficiency Virus

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**Summary:** Simian immunodeficiency virus (SIV) infection of infant macaques is a useful animal model of pediatric HIV infection to evaluate the potential of chemoprophylactic regimens to reduce mother-to-infant transmission of HIV. Previous studies have demonstrated that short-term subcutaneous administration of the reverse transcriptase inhibitor tenofovir was highly effective in protecting newborn macaques against infection after a single high-dose oral inoculation with virulent SIVmac251. In the current study, we mimicked HIV transmission through breast-feeding by repeatedly feeding infant macaques low doses of SIVmac251. Topical administration of a low dose of the second-generation tenofovir prodrug GS-7340 did not have detectable prophylactic efficacy. Oral administration of tenofovir disoproxil fumarate (DF; 10 mg/kg SID) lowered the infection rate at birth, but had lower efficacy against virus infection at 4 weeks of age, most likely because drug levels became suboptimal relative to those obtained with the current tenofovir DF regimen in humans. These prophylactic results further underscore the relevance of the current tenofovir DF prevention trials in pediatric and adult populations.

(*J Acquir Immune Defic Syndr* 2006;43:6–14)

Simple antiviral drug regimens, especially those based on zidovudine and nevirapine, have played an important role in reducing the rate of peripartum HIV transmission from mother to infant.<sup>1–4</sup> However, even when transmission during delivery is prevented, breast-feeding continues to be a considerable risk for HIV transmission in developing countries where replacement feeding is not always feasible.<sup>5–7</sup> Until an effective vaccine is available, the use of antiviral drugs may be an effective way to reduce the rate of HIV transmission via breast milk. Drug treatment of the mother results in lower viral RNA

levels in breast milk, which is expected to lower the risk for transmission.<sup>8,9</sup> Alternatively, prophylactic drug administration to the infant throughout the period of breast-feeding may reduce infection rates. Preliminary data suggest that lamivudine treatment of the breast-feeding infant may be effective.<sup>10</sup> Chronic nevirapine treatment of the nursing infant is also likely to be effective<sup>11</sup> but may not be ideal because of concerns for toxicity, the possibility of nevirapine-resistant virus in breast milk of women who previously received a dose of nevirapine,<sup>12</sup> and nevirapine's lack of activity against HIV-2. Thus, there is the need to explore additional simple regimens that may be able to protect infants against HIV infection both during delivery and throughout the period of breast-feeding.

Tenofovir (9-[2-(phosphonomethoxy)propyl]adenine) is a good candidate because of its favorable safety and resistance profile, and its high prophylactic efficacy in animal models such as simian immunodeficiency virus (SIV) infection of macaques.<sup>13–20</sup> Previous studies have demonstrated that short-term subcutaneous administration of tenofovir was effective in protecting newborn macaques against infection after an oral single high-dose SIVmac251 inoculation.<sup>16,21,22</sup> This included a 2-dose tenofovir regimen (4 mg/kg SC SID) expected to give newborn macaques a similar pharmacokinetic exposure (based on plasma drug levels) as the intravenous tenofovir regimen that was tested in the initial trial in human adults.<sup>22,23</sup>

Tenofovir, because of its charged anionic nature, has poor oral bioavailability. Accordingly, an orally bioavailable prodrug, tenofovir disoproxil fumarate (DF), has been developed and is currently used as a once-daily regimen to treat HIV infection.<sup>24</sup> The current study investigated whether oral dosing with tenofovir DF aimed at achieving similar plasma levels of tenofovir would protect infant macaques against oral SIV infection.

As described elsewhere, another approach to try to prevent SIV infection after oral exposure is the use of topical microbicides by administering compounds locally at the site of viral exposure in an amount too low to provide sufficient systemic drug levels.<sup>25</sup> In a previous study, topical administration of tenofovir DF did not have detectable prophylactic efficacy against oral SIV infection.<sup>25</sup> However, another prodrug of tenofovir, GS-7340, may be more attractive. In contrast to tenofovir DF, the GS-7340 prodrug is preferentially hydrolyzed by intracellular instead of extracellular esterases and, therefore,

Received for publication March 6, 2006; accepted April 13, 2006.  
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This work was supported by National Institutes of Health/National Institute of Allergy and Infectious Diseases grants AI58056 (K.V.R.) and AI46320-01 (M.L.M.), Gilead Sciences, and Public Health Science grant RR00169 from the National Center for Research Resources.  
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yields much higher intracellular levels of the active moiety tenofovir diphosphate.<sup>26</sup> Tenofovir GS-7340 was approximately 5-fold more potent than tenofovir DF, and 100- to 1000-fold more potent than tenofovir in inhibiting HIV-1 replication in cell lines, peripheral blood mononuclear cell (PBMC), and macrophages in vitro.<sup>26</sup> In dog studies, oral tenofovir GS-7340 administration had high oral bioavailability (>70%), targeted lymphoid tissues, and led to much higher intracellular levels of tenofovir in PBMC than equivalent oral doses of tenofovir DF.<sup>26</sup> Accordingly, we hypothesized that oral administration of tenofovir GS-7340 at a topical dose (ie, too low to give sufficient systemic drug levels) may be more efficient in inducing intracellular accumulation of tenofovir inside the cells at the mucosal site or in the draining lymphoid tissues of the oral cavity, with the potential for enhanced prophylactic efficacy.

Because HIV transmission through breast-feeding involves prolonged, daily exposure to virus in breast milk, we previously developed a model in which infant macaques are repeatedly fed low doses of SIVmac251.<sup>25,27</sup> Using this animal model, the present study demonstrates that the topical administration of tenofovir GS-7340 did not have any detectable prophylactic efficacy. In contrast, an oral tenofovir DF regimen lowered the infection rate, and the level of efficacy was associated with age-related changes in pharmacokinetics.

## MATERIALS AND METHODS

### Animals and Parameters to Monitor Infection

All rhesus macaques (*Macaca mulatta*) were from the type D retrovirus-free and SIV-free colony at the California National Primate Research Center. The newborn macaques were hand-reared in a primate nursery in accordance with American Association for Accreditation of Laboratory Animal Care standards. We adhered to the *Guide for Care and Use of Laboratory Animals*.<sup>28</sup> As described previously,<sup>25</sup> for the first weeks of life, the main diet consisted of Enfamil<sup>®</sup> with Iron (Mead Johnson Nutritionals, Evansville, IN) treated with Lacteeze<sup>®</sup> lactase enzyme drops (Gelda Scientific, Mississauga, Ontario, Canada), with gradual addition of other food items at 2 weeks of age. For blood collections, animals were immobilized with 10 mg/kg IM ketamine-HCl (Parke-Davis, Morris Plains, NJ). EDTA-anticoagulated blood samples were collected for monitoring immunologic and viral parameters and blood cell counts according to methods previously described.<sup>22,27</sup> As described below, the 49 infant macaques were divided into 3 main groups: group A, untreated control animals; group B, tenofovir GS-7340-treated infant macaques; and group C, tenofovir DF-treated animals.

### Preparation and Oral Administration of SIVmac251 Inoculum

The uncloned SIVmac251 stock (with internal reference no. 2/02) was propagated on rhesus macaque PBMCs and had a titer of  $10^5$  50% tissue culture infectious doses (TCID<sub>50</sub>) and  $0.86 \times 10^9$  RNA copies/mL (as measured by SIV bDNA). As described previously,<sup>25,27</sup> we developed a repeated low-dose exposure model in which infant macaques are handheld and bottle-fed SIVmac251 (diluted

in a 1:1 mixture of RPMI-1640 and isotonic sucrose) 15 times (3 times per day for 5 consecutive days at 1030, 1230, and 1630 hours). For each SIV administration, the infant macaques were handheld without chemical restraint and were fed the solution using pet nursing bottles (Four Paws, Hauppauge, NY). In the current study, 24 newborn macaques (ie, all groups except subgroup A2) were started on a series of 15 oral inoculations within the first week of life; each virus dose consisted of 2 mL of a 1:40 dilution of the SIVmac251 stock ( $\sim 5000$  TCID<sub>50</sub> or  $43 \times 10^6$  SIV RNA copies/dose). Animals that did not have detectable viremia were reinoculated 4 weeks later with another series of 15 inoculations (3 times per day for 5 consecutive days) using the same volume but double the concentration of virus (2 mL of a 1:20 dilution of SIVmac251 corresponding to  $\sim 10,000$  TCID<sub>50</sub> or  $86 \times 10^6$  SIV RNA copies/dose). Twenty-five untreated control animals (from concomitant other experiments using an identical inoculum) received only the second set of SIVmac251 inoculations at approximately 4 weeks of age (subgroup A2).

### Preparation and Administration of Tenofovir Prodrugs

The 6 animals of group B received oral administration of tenofovir GS-7340 3 times daily for 7 days in a row (from 1 day before to 1 day after the 5-day series of virus inoculations). A low dose of GS-7340 was selected as an investigational topical regimen (ie, unlikely to give sufficient systemic antiviral drug levels). GS-7340 powder was dissolved at 0.5 mg/mL in RPMI-1640, and aliquots were frozen at  $-70^\circ\text{C}$ . For each dose, a thawed aliquot was diluted by adding 200  $\mu\text{L}$  to 2 mL of each virus inoculum (during the 5 consecutive days of thrice-daily virus inoculations) or 2 mL of the 1:1 mixture of sucrose and RPMI-1640 (for drug administration 3 times daily (1030, 1230, and 1630 hours) on the day before and the day after the 5-day series of virus inoculations). Thus, each GS-7340 dose consisted of 0.1 mg GS-7340 (equivalent to 0.064 mg of tenofovir) at a concentration of approximately 0.05 mg/mL ( $\sim 100$   $\mu\text{mol/L}$ ). This concentration of GS-7340 (100  $\mu\text{mol/L}$ ) is approximately 1000-fold higher than the in vitro 50% inhibitory concentration against the SIVmac251 inoculum (data not shown).

For the 12 animals of group C, an investigational formulation of tenofovir DF powder for oral suspension was reconstituted with distilled water to a concentration of 20 mg/mL and stored at  $4^\circ\text{C}$  according to the manufacturer's instructions (Gilead Sciences, Foster City, CA). The 6 newborn macaques of group C1 received oral administration of tenofovir DF (10 mg/kg body weight) once daily in the morning ( $\sim 0700$  hours) for 7 consecutive days from 1 day before to 1 day after the first 5-day series of virus feedings. For the 6 animals of group C2, oral administration of tenofovir DF (10 mg/kg SID at  $\sim 0700$  hours in the morning) was started 2 days before the first set of virus inoculations and continued for all 6 animals until 2 weeks after the end of the second set of virus inoculations (ie, for a total of 7 weeks). While the animals were handheld, the small volume of tenofovir DF solution (0.5 mL/kg body weight) was administered directly in the mouth using a needle-less

syringe. To reduce the problem of poor palatability and to ensure swallowing of the drug dose, the animals were then immediately offered a bottle with formula.

### Virologic and Immunologic Assessment

Infectious virus was isolated from PBMC with CEM × 174 cells and subsequent p27 core antigen measurement via an enzyme-linked immunosorbent assay (ELISA), as described previously.<sup>29</sup> Plasma viral RNA was quantified using a bDNA signal amplification assay specific for SIV, version 4.0 (which has a lower quantitation limit of 125 copies/mL).<sup>30</sup> To detect and quantitate proviral DNA and RNA in PBMC, real-time polymerase chain reaction (PCR) assays with amplification of the SIV *gag* gene were performed according to methods described in detail previously.<sup>31,32</sup> The ELISA to detect SIV-specific IgG was performed as described previously.<sup>33</sup> Lymphocyte phenotypic analysis was performed using 4-color flow cytometry techniques as described previously.<sup>30</sup>

### Pharmacokinetic Analysis

At 6 to 8 days of age, 4 infant macaques were temporarily removed from their mother and administered a single dose of tenofovir DF suspension (10 mg/kg); the infants were then given the lactase-treated Enfamil formula described above ad libitum. EDTA-anticoagulated blood samples were collected over a 24-hour period (at 1, 2, 4, 8, and 24 hours after drug administration). The 24-hour pharmacokinetic study was repeated with the same dose of tenofovir DF when the same animals were 4 to 5 weeks of age.

Plasma samples were immediately stored at  $-70^{\circ}\text{C}$  and subsequently analyzed by MDS Pharma Services (Montreal, Quebec, Canada) using high-performance liquid chromatography methods with mass spectrometry detection (liquid chromatography–mass spectrometry–mass spectrometry), previously validated for monkey plasma, with a limit of quantitation of 1 ng/mL.<sup>34</sup> The values of the pharmacokinetic parameters were derived by noncompartmental analysis with WinNonlin software (version 3.1; Pharsight Corporation, Mountain View, CA). To account for the increased absolute dose (in milligrams) of tenofovir DF as the animals aged, week 4 pharmacokinetic parameters were dose-normalized to the dose administered on week 1.

### Phenotypic and Genotypic Drug Susceptibility Testing

Phenotypic drug susceptibilities of SIV isolates were characterized by a previously described assay based on a dose-dependent reduction of viral infectivity.<sup>35,36</sup> DNA sequence analyses of codons 0 to 320 of reverse transcriptase (RT) were performed on proviral DNA obtained from CEM × 174 cells infected with virus isolated from the SIV-infected animals and harvested as soon as culture supernatants were positive by p27 antigen-capture ELISA; genomic DNA was extracted and used for nested PCR according to methods and with primers described previously.<sup>30,37</sup> Amplicons were sequenced by Davis Sequencing (Davis, CA) with primers 239–2786 and SIV-RT3. This method, which can detect the

presence of a 20% subpopulation, has been used previously with success to detect the emergence of K65R viral mutants in tenofovir-treated animals.<sup>30,33</sup>

### Statistical Analyses

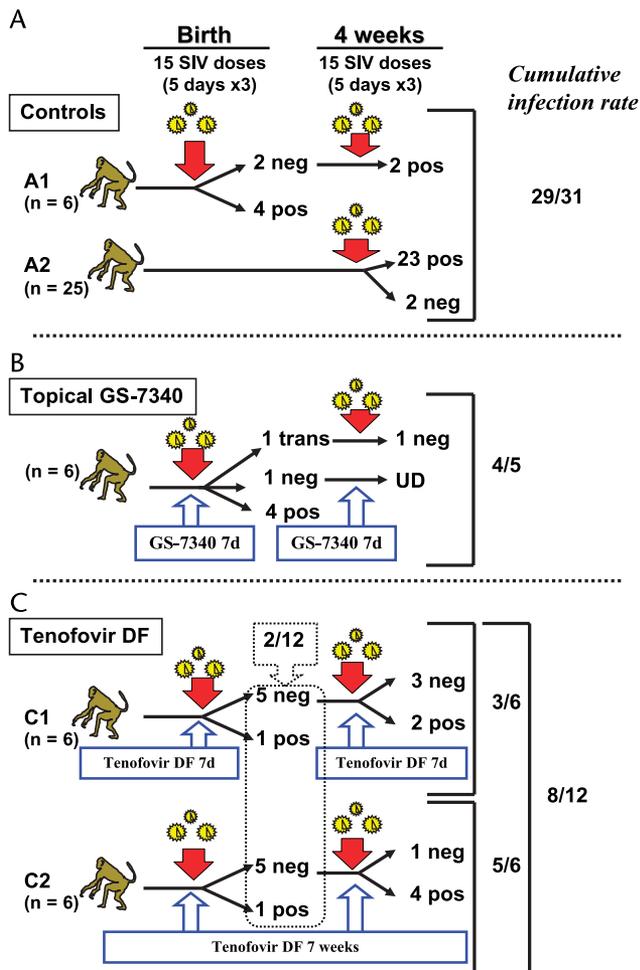
Statistical analyses were performed with Prism 4 for Mac and InStat 3 (GraphPad Software Inc, San Diego, CA). Differences between pharmacokinetics on weeks 1 and 4 were compared using a paired *t* test of the log<sub>10</sub> transform of tenofovir  $C_{\text{max}}$  and  $\text{AUC}_{0-\infty}$ .

## RESULTS

### Experimental Design and Summary of Infection Status and Viremia

As outlined in Figure 1, 3 main groups of infant macaques were handheld and bottle-fed diluted SIVmac251 3 times per day for 5 consecutive days, starting within the first week of birth. Animals that did not become persistently viremic were reinoculated with SIV again at 4 weeks of age. Group A consisted of 2 subgroups of untreated control animals: 4 of the 6 animals of subgroup A1 became persistently infected after the first set of inoculations, whereas the remaining 2 animals became infected after the second set of inoculations. Subgroup A2 had 25 untreated animals, which received only SIV inoculations at 4 weeks of age, and 23 of them became infected. Together, the cumulative infection rate for untreated animals after 2 series of inoculations was 29 (94%) of 31 animals, with the caveat that this infection rate may be underestimated (because the 2 animals that did not become infected had received only 1 set of virus inoculations at 4 weeks of age).

The 6 animals of group B received topical tenofovir GS-7340 3 times daily for 7 days (0.1 mg/dose, including mixed with the virus inoculum). The concentration of GS-7340 (100  $\mu\text{mol/L}$ ) is approximately 1000-fold higher than the *in vitro* 50% inhibitory concentration against the SIVmac251 inoculum (data not shown). Although GS-7340 was mixed with the virus inoculum, 4 of these 6 animals of group B became persistently viremic after the first set of virus inoculations. Although their infection rate (4/6 animals) was indistinguishable from that of the placebo-treated animals (group A), the viral RNA levels of these 4 animals at 2 weeks of age (ie, 1 week after the end of the 5-day SIV inoculation regimen) were lower than those of the 4 untreated infected animals of group A1 (Fig. 2B; 2-tailed *t* test on log-transformed values,  $P = 0.04$ ). However, starting at 3 weeks of age, there was no significant difference in viremia anymore. One animal of group B (number 35410) had evidence of transient viremia; although infectious virus was never isolated from PBMC and animal 35410 did not seroconvert, viral RNA levels in plasma at 2 weeks of age (ie, 1 week after this first set of virus inoculations) were low (1908 RNA copies/mL; Fig. 2). PBMC collected at this 2-week time point did not have detectable viral RNA but had detectable viral DNA (36 of 36 replicates were positive by quantitative TaqMan PCR technology; range, 456–1255 *gag* DNA copies/million PBMC). Starting at 3 weeks of age onwards, the animal was virus-negative by all criteria (including viral RNA in plasma,



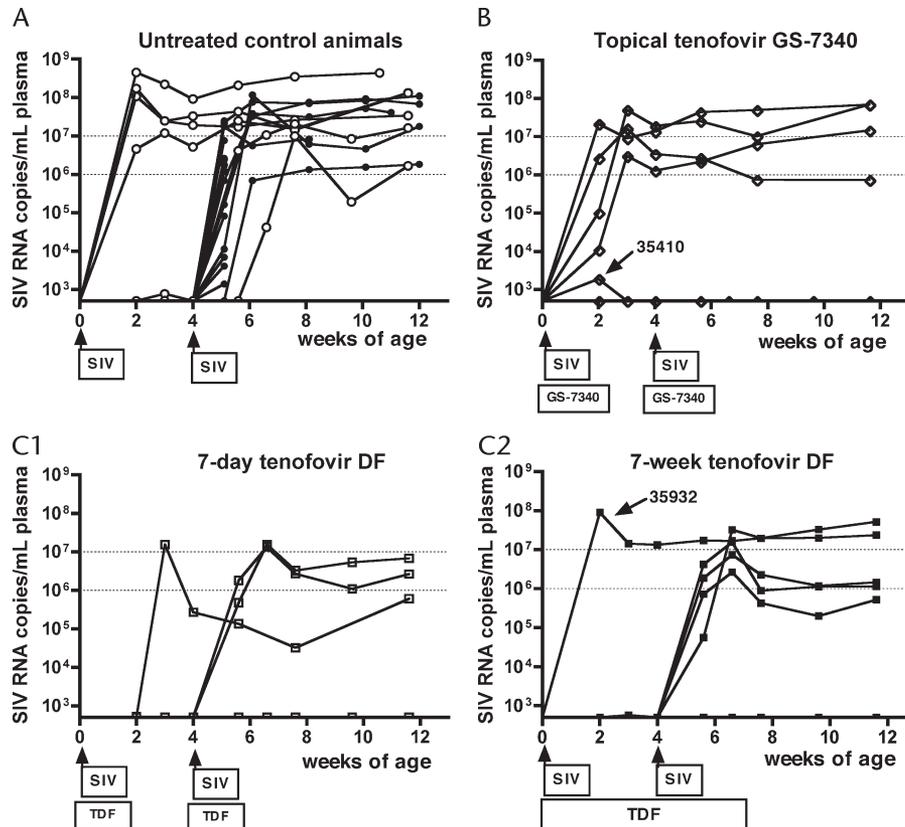
**FIGURE 1.** Summary of experimental design and outcome. Within 1 week of birth, groups of infant macaques were fed 2 mL of SIVmac251 (5000 TCID<sub>50</sub>/dose) 3 times per day (at 1030, 1230, and 1630 hours) for 5 consecutive days. Animals that did not become infected were reinoculated 4 weeks later with a similar series of 15 inoculations, but with a slightly higher virus dose (10,000 TCID<sub>50</sub>/dose). A, Untreated control animals, of which 1 subgroup (A2) received SIV inoculations only at 4 weeks of age. B, Six animals were given thrice-daily oral topical administration of tenofovir GS-7340 (0.1 mg/dose) starting 1 day before until 1 day after the 5 days of virus inoculations; on the 5 days of thrice-daily SIV inoculations, the tenofovir GS-7340 dose was added to the 2 mL of virus inoculum. C1 and C2, Starting 1 or 2 days before the first virus inoculation respectively, the groups received once-daily (at ~0700 hours) oral tenofovir DF administration (10 mg/kg body weight). C1, Tenofovir DF was given 7 days in a row (ie, the last dose given the day after the last virus inoculation) and then restarted at the same regimen only for the 5 animals that were reinoculated with SIV 4 weeks later. C2, Tenofovir treatment was continued for a period of 7 weeks (ie, including 2 weeks after the second set of virus inoculations) for all 6 animals. Pos indicates infected; neg, uninfected; trans, transient viremia (animal 35410); UD, animal loss because of unrelated death (aspiration pneumonia).

virus isolation from PBMC, and serology) and remained this way throughout the rest of the 1-year observation period. It is unclear whether the transient detection of virus at 2 weeks of age in animal 35410 may have had a protective effect on the outcome of the second series of virus inoculations. The sixth animal of group B was not viremic after the first set of virus inoculations but had to be euthanized because of an unrelated condition (aspiration pneumonia) at 5 weeks of age (shortly after the second set of virus inoculations); although the animal was aviremic at that time, it was too early to draw definite conclusions about the outcome of the second set of virus inoculations.

The 6 newborn macaques of group C1 received oral administration of tenofovir DF (10 mg/kg body weight) once daily in the morning (~0700 hours) for 7 consecutive days from 1 day before to 1 day after the first 5-day series of virus feedings. Although 1 animal became infected (Fig. 2, C1), the other 5 animals had no detectable viremia and were reinoculated with SIV at 4 weeks of age and given the same tenofovir DF regimen (10 mg/kg SID). This time, 2 animals became infected and had a viral RNA set point (6–7 log copies/mL) within the range of that of untreated animals (Figs. 1 and 2). The other 3 animals that received short-term tenofovir DF remained uninfected by all criteria throughout the observation period of 1 year. To see if a longer postexposure regimen would have higher prophylactic efficacy, another group of 6 animals (Fig. 1, C2) underwent the same series of oral SIV inoculations, except that oral administration of tenofovir DF (10 mg/kg SID) was started 2 days before the first set of virus inoculations and continued for all 6 animals until 2 weeks after the end of the second set of virus inoculations (ie, for a total of 7 weeks). Similarly to the results of group C1, 1 animal of group C2 became viremic after the first set of virus inoculations; this animal (35932; Fig. 2, C2) had high viremia (>7 log RNA copies/mL) despite continued tenofovir DF treatment for 7 weeks. Of the remaining 5 animals that were reinoculated with SIV at 1 month of age, 4 animals became infected and were already viremic while receiving daily tenofovir DF treatment. The sixth animal remained negative by all criteria. All uninfected animals of groups C1 and C2 had normal growth and normal clinical parameters (including serum chemistries, urinalysis, blood counts, CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocyte, and B lymphocyte counts) throughout the 1-year observation period, which is consistent with our previous observations of tenofovir’s safety profile in infant macaques.<sup>34</sup>

### Statistical Analysis of Prophylactic Efficacy

The topical GS-7340 regimen, giving a similar infection rate as that of the untreated control animals after the first series of oral SIV inoculations, did not have any detectable prophylactic efficacy. The oral tenofovir DF regimen (10 mg/kg SID), aimed at giving systemic drug levels, had prophylactic efficacy during the first set of virus inoculations shortly after birth because the combined infection rate of groups C1 and C2 after the first set of virus inoculations (2/12 animals) was lower than that of the untreated animals (group A1, 4/6 animals; *P* = 0.057) and significantly lower than that of the combined untreated or



**FIGURE 2.** Viremia after repeated low-dose oral SIV inoculations. The 4 groups (A, B, C1, and C2) represent the groups of Figure 1. A, Open circles represent the untreated control animals of subgroup A1 (Fig. 1), which received oral SIV feedings at birth; 2 animals that did not become infected received another set of SIV inoculations a month later and became infected; the closed circles (A) are the infant macaques that received the repeated oral SIV feedings only at 4 weeks of age (Fig. 1, A2). B, Animal 35410 had transient viremia. Group C1 received 1 week of TDF per set of SIV inoculations (Fig. 1). Group C2 received 7 weeks of tenofovir DF administration, including animal 35932 that was already viremic after the first series of SIV feedings.

topically treated animal groups (groups A1 and B, 8/12 animals with persistently high viremia; 1-sided Fisher exact test,  $P = 0.018$ ; relative risk = 0.28). The same oral tenofovir regimen was less effective in protecting against the second set of oral SIV inoculations at approximately 1 month of age, as this time, 4 of the 10 tenofovir DF–treated and SIV-inoculated animals (subgroups C1 and C2) remained uninfected after the virus inoculations, compared with 2 of 27 untreated control animals (groups A1 and A2; 1-sided Fisher exact test;  $P = 0.03$ ). The cumulative infection rate (ie, after both series of virus inoculations) in the 12 tenofovir DF–treated animals of groups C1 and C2 (8/12 animals) was still significantly lower than that of the untreated animals (29/31 animals; 1-sided Fisher exact test,  $P = 0.04$ ; relative risk = 0.32).

### Evaluation of Phenotypic and Genotypic Susceptibility to Tenofovir

For the tenofovir DF–treated animals, virus isolated from PBMC was also tested for phenotypic susceptibility using assays previously able to detect mutant SIV isolates with approximately 5-fold reduced in vitro susceptibility to tenofovir and a K65R mutation in RT.<sup>30,36</sup> These virus

isolates included all those obtained at the first detection of viremia, and for group C2 (which had a longer postexposure treatment with tenofovir DF) also, virus isolated from all infected animals at the time when tenofovir DF administration was stopped at 7 weeks of age; this included the virus isolates of animal 35932, which had high viremia throughout the period of tenofovir DF treatment (Fig. 2, C2). All virus isolates tested had wild-type in vitro susceptibility to tenofovir, and all had wild-type amino acid (lysine) at codon 65 of RT, without evidence of any other mutations that are associated with reduced in vitro susceptibility to tenofovir.

### Pharmacokinetics of Tenofovir After Oral Administration of Tenofovir DF to Infant Macaques

To explore the pharmacokinetics associated with prophylactic efficacy of oral tenofovir DF administration, a pharmacokinetic study was subsequently performed to describe and assess possible age-related changes in tenofovir kinetics after oral administration during the first month of life. At approximately 1 week of age, 4 additional infant macaques (which were not exposed to SIV) were administered a single

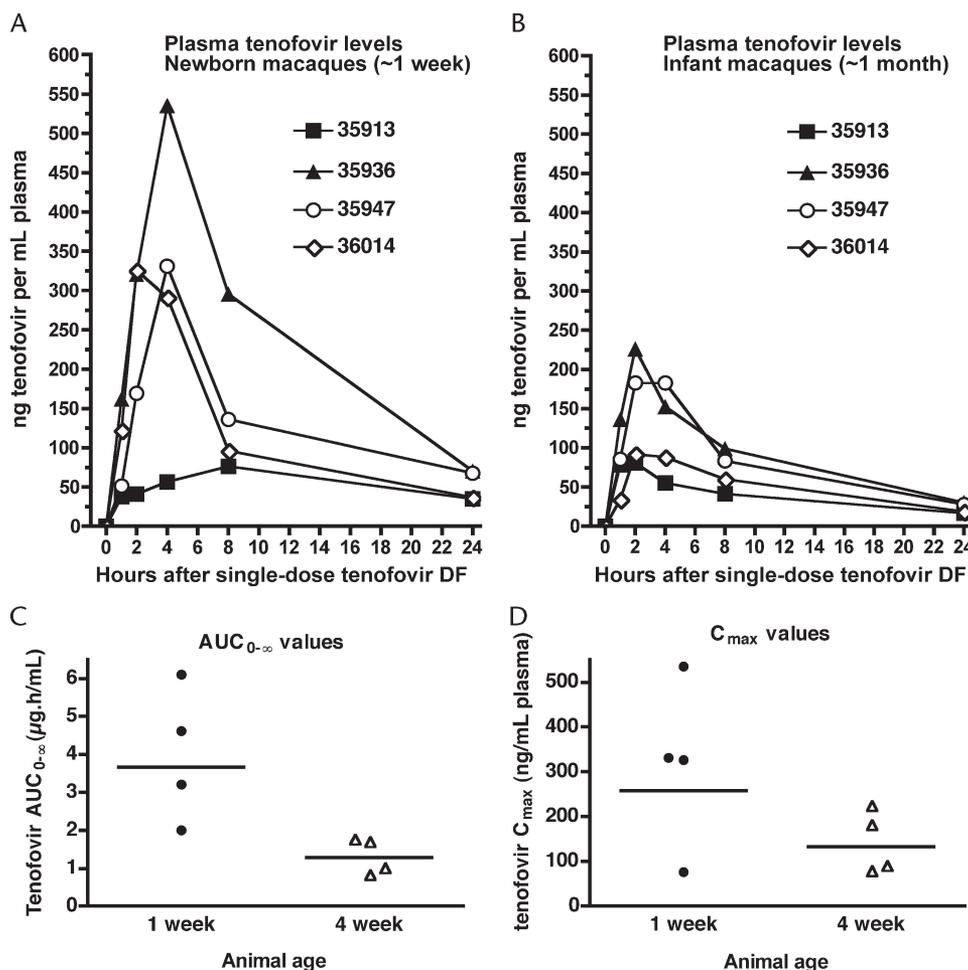
dose of tenofovir DF suspension (10 mg/kg) without fasting; a similar 24-hour pharmacokinetic study was repeated with the same dose of tenofovir DF when the same animals were between 4 and 5 weeks of age. As indicated in Fig. 3, systemic exposure to tenofovir (as measured by  $AUC_{0-\infty}$  values of tenofovir in plasma) was approximately 65% lower at 4 weeks of age than at 1 week of age ( $P = 0.001$ ). Geometric mean  $AUC$  values (95% confidence interval) were 3.67 (1.70–7.90) and 1.28 (0.712–2.31)  $\mu\text{g}\cdot\text{h}/\text{mL}$  at 1 and 4 weeks of age, respectively. Based on published drug levels obtained with subcutaneous injection of tenofovir in infant macaques,<sup>34</sup> the oral bioavailability of tenofovir DF in the current infant macaque study was estimated to be approximately 10% to 15%.

### DISCUSSION

To mimic better the daily exposure to HIV that occurs during breast-feeding, we had previously developed an

animal model in which infant macaques are fed repeatedly low doses of virulent SIVmac251.<sup>25,27</sup> In the present study, we used this repeated low-dose exposure model to evaluate the prophylactic efficacy of 2 prodrugs of tenofovir.

The novel GS-7340 prodrug of tenofovir was selected for topical administration because its rapid entry into cells and high intracellular accumulation in vitro are features expected to be advantageous for a topical virucide in vivo.<sup>26</sup> However, topical administration of a low amount of tenofovir GS-7340 was not effective in lowering infection rates, although tenofovir GS-7340 was given at a high concentration and was mixed with the relatively low virus inocula. The lower initial viremia in the GS-7340-treated animals (Fig. 2B) suggests that the “topical” administration of a low amount of tenofovir GS-7340 for 1 week may have led to some systemic intracellular levels of tenofovir, which were, as predicted, suboptimal and therefore only partially inhibited virus dissemination. In dog studies, oral tenofovir GS-7340



**FIGURE 3.** Pharmacokinetics of single-dose oral tenofovir DF administration to newborn and infant macaques. Four infant rhesus macaques were given a single oral dose of 10 mg tenofovir DF/kg body weight at approximately 1 week of age (A) and again at approximately 1 month of age (B), with collection of plasma to measure tenofovir levels over a 24-hour period. C and D, Values for area under the plasma concentration-versus-time curve ( $AUC_{0-\infty}$ ) and peak concentrations ( $C_{max}$ ), respectively; the horizontal lines represent the geometric mean values.

administration had high oral bioavailability (>70%) and led to very efficient intracellular accumulation of tenofovir (intracellular tenofovir AUC in PBMC was ~34-fold higher than that obtained with an equivalent oral dose of tenofovir DF).<sup>26</sup> For the infant macaque studies, we predict that a higher dose of tenofovir GS-7340 may have resulted in prophylactic efficacy because of systemic intracellular levels of tenofovir, rather than a direct topical effect. Thus, in the previous and present studies, we were not able to detect topical prophylactic efficacy with either the DF or the GS-7340 prodrugs of tenofovir against oral SIV infection.<sup>25</sup> This is in contrast to the demonstrated efficacy of a 1% tenofovir microbicide gel against intravaginal SIV infection (C. Miller, Z. Rosenberg, and N. Bischofberger, unpublished data); possible reasons for this discrepancy in efficacy include biologic differences in transmission events that occur across different mucosal surfaces and have been described in detail previously.<sup>25</sup>

In the current study, we also tested the efficacy of oral tenofovir DF to protect infant macaques against oral SIV infection. This study is highly relevant because the potential of a 2-dose oral tenofovir DF regimen (1 maternal dose and 1 infant dose) to reduce intrapartum transmission of HIV is currently being investigated.<sup>38</sup> We observed that a once-daily 10-mg/kg dosage regimen was quite effective in protecting infant macaques against infection during the first set of oral SIV feedings shortly after birth, but became less effective at 1 month of age. However, the cumulative results still demonstrated partial prophylactic efficacy of the oral tenofovir DF regimen against repeated oral SIV challenges ( $P = 0.04$ ).

As subcutaneous tenofovir regimens have been highly effective in protecting macaques against infection,<sup>15,16,18–22</sup> the partial efficacy observed with the oral tenofovir DF regimen in the present study was at first surprising, but made more sense when the pharmacokinetics were taken into account. Our decision of using a 10-mg/kg tenofovir DF dose was based on our prediction that this dose, if oral bioavailability would be similar to that in humans, would give plasma levels of tenofovir in infant macaques pharmacokinetically similar to those of the tenofovir DF regimen used in HIV-infected human adults and children (steady-state AUC ~3  $\mu\text{g}\cdot\text{h}/\text{mL}$ ,  $C_{\text{max}}$  ~300 ng/mL).<sup>24,39–41</sup> This hypothesis proved to be true when animals were 1 week of age; however, systemic exposures were quite variable and were significantly lower 4 weeks later (Fig. 3), which may explain the reduced prophylactic efficacy of this 10-mg/kg dosage regimen at this later time point (Fig. 1). Although our infant macaque study did not evaluate intracellular levels of tenofovir diphosphate (the active form of tenofovir), a pharmacokinetic study in juvenile macaques found that an oral tenofovir DF regimen that gave similar AUC values for tenofovir levels in plasma also gave intracellular levels of tenofovir diphosphate in PBMC within the range of those seen in adults who take the once-daily 300-mg tablet (A. Ray, K. Van Rompay, and B. Lee, unpublished data); thus, it is reasonable to consider that the variable and lower plasma tenofovir exposure levels in the 4-week-old infant macaques would yield lower intracellular levels of tenofovir diphosphate.

The virologic data of the infant macaques that became infected despite oral tenofovir DF administration further suggest that the drug regimen gave suboptimal systemic drug levels. In previous studies, subcutaneous injection of tenofovir to SIV-infected monkeys early in infection always resulted in a strong reduction and delay of primary viremia and/or selection for the emergence of virus with reduced *in vitro* susceptibility and a K65R mutation in RT.<sup>16–19,30,36,42</sup> Similarly, tenofovir DF monotherapy of HIV-infected adults induces rapid reduction of viremia.<sup>24</sup> In contrast, in the current study, for those animals that became infected in group C2 (which received 7 weeks of daily tenofovir DF treatment), the oral tenofovir DF regimen did not delay or dampen the initial viremia, although virus had wild-type susceptibility to tenofovir. This further suggests that drug levels were suboptimal.

Our present observations are consistent with those of other investigators who tested the potential of oral tenofovir DF to protect adult macaques that received weekly rectal inoculations with SHIV<sub>SF162P3</sub>; tenofovir DF-treated animals required more virus inoculations than untreated animals to eventually become infected, but drug levels in plasma were variable, and once animals became infected, viremia was not reduced, and there was no detectable emergence of K65R mutants despite the continued treatment with tenofovir DF for several months.<sup>43</sup> Together, these data suggest that the oral tenofovir DF regimens that were used in both these studies, although partially effective, were likely suboptimal to prevent infection. *In vitro*, tenofovir is active at lower concentrations in antigen-presenting cells (monocytes/macrophages, dendritic cells, and Langerhans cells) than in lymphocytes because of more efficient phosphorylation.<sup>44–47</sup> Considering that antigen-presenting cells may be the first cells that become infected during transmission, this may explain why a suboptimal tenofovir DF regimen may have partial prophylactic efficacy but may have no detectable therapeutic effect on reducing viremia once most virus replication occurs in lymphocytes.

With these considerations in mind, the partial prophylactic efficacy of an oral tenofovir DF regimen in the current macaque study is promising. Our data suggest that for human infants a tenofovir DF regimen that can consistently provide systemic drug levels similar to or higher than those obtained by a therapeutic tenofovir regimen has the potential to reduce the HIV transmission rate through breast-feeding. These same considerations apply also to the prophylaxis trials with tenofovir DF in adults. Because an efficacious HIV vaccine has so far not been identified, several ongoing clinical trials are investigating whether uninfected adult persons who engage in high-risk behavior will have a lower infection rate by taking a 300-mg tenofovir DF tablet once daily. These trials are held at several international sites and target different high-risk populations. Regardless of the ethical issues surrounding these trials (see review of the AIDS Vaccine Advocacy Coalition<sup>48</sup>), the combined data of the tenofovir studies in macaques provide further support to continue these pediatric and adult prevention trials because drug prophylaxis could be an additional strategy to reduce the risk for infection especially when other effective methods (such as abstinence,

mutual monogamy, and condoms) are not an option or are not consistently followed.

### ACKNOWLEDGMENTS

We thank D. Bennett, T. Dearman, L. Fritts, L. Hirst, A. Spinner, W. von Morgenland, the Veterinary Staff, Colony Services and Clinical Laboratory of the California Regional Primate Research Center, Davis Sequencing (Davis, CA), and A. Ray and T. Cihlar (Gilead Sciences) for technical assistance and useful discussions.

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