Two Low Doses of Tenofovir Protect Newborn Macaques against Oral Simian Immunodeficiency Virus Infection

Koen K. A. Van Rompay,1 Michael B. McChesney,1 Nancy L. Aguirre,1 Kimberly A. Schmidt,1 Norbert Bischofberger,2 and Marta L. Marthas1

1California Regional Primate Research Center, University of California, Davis, and 2Gilead Sciences, Foster City, California

Simple affordable interventions are needed to reduce vertical human immunodeficiency virus (HIV) transmission in developing countries. The efficacy of 2 low doses (4 mg/kg, subcutaneously) or 1 high dose (30 mg/kg, subcutaneously) of the reverse-transcriptase inhibitor 9-[2-(phosphonomethoxy)propyl]adenine (PMPA; tenofovir) to protect newborn macaques against simian immunodeficiency virus (SIV) infection was investigated. Thirteen newborn macaques were inoculated orally with virulent SIVmac251. The 4 placebo-treated animals (group A) became persistently infected. Groups B and C (n = 4 in each group) received 24-mg/kg doses of PMPA, either 4 h before and 20 h after (group B) or 1 and 25 h after SIV inoculation (group C). One animal (group D) received a single 30-mg/kg dose of PMPA 1 h after SIV inoculation. Despite evidence of an initial transient infection, 3 group B animals, 2 group C animals, and the group D animal were SIV negative and seronegative at ages 19–23 months. Immune activation with recall antigens or pharmacologic immunosuppression with corticosteroids failed to reactivate viral replication. These data suggest that 1 or 2 doses of PMPA may protect human newborns against intrapartum HIV infection.

It is estimated that every day ~1700 newborns become infected perinatally with human immunodeficiency virus (HIV) [1]. The majority of these infections occur in developing countries. Although transmission can occur in utero and postnatally (through breast-feeding), evidence suggests that a large fraction of infants become infected during birth, presumably by an oral route of infection [2, 3]. The recent discovery of simplified zidovudine and nevirapine regimens to reduce peripartum HIV-1 transmission is very promising [4–7]. The HIVNET012 regimen, which consists of 1 dose of nevirapine to the mother at the onset of labor and another dose to the baby within 3 days of birth, is currently the most simple and affordable strategy to reduce transmission of HIV-1 during delivery and for about the first week of breast-feeding [5].

A number of unanswered questions remain regarding the most effective and safe strategy to prevent vertical HIV transmission. With the HIVNET012 regimen, the resistance mutation K103N in reverse transcriptase (RT) was detected in 3 of 15 women 6 weeks after a single dose of nevirapine [8], which may limit the efficacy of subsequent nevirapine use to protect infants during breast-feeding. This finding of nevirapine resistance is especially worrisome, because studies in adults found that these mutants can persist for a long time and can be transmitted [9–11]. In addition, although the vertical transmission rate of HIV-2 is lower than that of HIV-1 [12], nevirapine is not active against HIV-2. Finally, it is unclear whether immediate administration of 1 or 2 doses of drug to only the neonate can still be effective in the absence of any maternal treatment. This is important to know, because the price of this postexposure regimen for the neonate would be even lower. Thus, even with the recent results of several clinical trials, there remains a need for additional effective, safe, and simple intervention strategies.

For many reasons, the reverse-transcriptase inhibitor 9-[2-(phosphonomethoxy)propyl]adenine (PMPA; tenofovir) is an attractive candidate for preventing vertical HIV transmission. Because it is an acyclic nucleotide phosphonate analogue, the rate-limiting initial phosphorylation step required for activation of nucleoside analogues (e.g., zidovudine and lamivudine) is bypassed, and phosphorylation to the active diphosphate form occurs in both activated and resting cells, including macrophages [13, 14]. This active form has a long intracellular half-life (~12–15 h and 33–50 h in activated and resting peripheral blood mononuclear cells [PBMC], respectively) [13]. In addition, results of ongoing trials in HIV-1–infected adults suggest that PMPA treatment does not select rapidly for resistant mutants and has a good
safety profile [15]. Most importantly, previous studies in juvenile and newborn macaques have shown that PMPA is highly effective in preventing simian immunodeficiency virus (SIV) infection; a 2–4-week dosage regimen of PMPA was very effective in protecting macaques against intravenous or oral SIV infection, even when the regimen was initiated after exposure [16, 17]. PMPA was still partially effective in protecting newborn macaques against oral infection with SIV mutants with reduced in vitro susceptibility to PMPA [18].

In an attempt to identify the shortest and most affordable prophylactic regimen possible, we previously demonstrated that 2 doses of PMPA (30 mg/kg of body weight) administered subcutaneously 4 h before and 20 h after oral SIV inoculation are sufficient to protect newborn macaques [19]. Because this 30-mg/kg regimen is higher than the PMPA dose that has been used in human clinical trials, the goal of the present study was to investigate whether a much lower dose of PMPA (4 mg/kg) could still be effective. In addition, we investigated whether a 1- or 2-dose regimen of PMPA initiated 1 h after oral SIV inoculation could still protect newborn macaques against infection.

Materials and Methods

Animals. All newborn rhesus macaques (Macaca mulatta) were from the type D retrovirus- and SIV-free colony at the California Regional Primate Research Center and were hand reared in a primate nursery, according to the American Association for Accreditation of Laboratory Animal Care standards [20]. When necessary, animals were immobilized with 10 mg/kg of ketamine-HCL (Parke-Davis) injected intramuscularly. Heparin- and EDTA-anticoagulated blood samples from all animals. Samples were analyzed by complete blood counts performed on EDTA-anticoagu-

Virus inoculation. By age 3 days, newborn macaques were inoculated orally under ketamine anesthesia with 1 mL of an uncloned SIVmac251 virus stock administered intravenously by dispensing the inoculum slowly into the mouth. Animals were monitored to ensure that the inoculum was swallowed. The SIVmac251 stock used in this study was propagated on rhesus PBMC, had a titer of 10⁸ TCID₅₀/mL, and was shown to infect newborn and juvenile macaques by the oral route and to cause persistent viremia and simian AIDS, as described elsewhere [16, 19, 22]. In the current study, animals that developed persistent viremia were removed from the study at age 3 weeks to become part of a different antiviral drug therapy study.

Administration of drugs and immunizations. PMPA (Gilead Sciences) was suspended in distilled water, dissolved by the addition of NaOH to a final pH of 7.0 at 60 mg/mL, filter sterilized (0.2 μm; Nalgene), and stored at 4°C. For animals given a 4-mg/kg dose, PMPA was first diluted to 6 mg/mL in sterile 0.9% sodium chloride (Abbott Laboratories). PMPA was administered subcutaneously into the back of the animal at a regimen of either 4 or 30 mg/kg of body weight per dose. Placebo-treated animals received an equivalent volume of 0.9% sodium chloride per kilogram of body weight.

All infant rhesus macaques were immunized subcutaneously with 0.1 mg of cholera toxin B subunit (List Biological Laboratories) at age 2 weeks, and a booster immunization was given at age 10 weeks. The cholera toxin–specific IgG ELISA has been described elsewhere [23]. Tetanus toxoid in alum (Wyeth Laboratories) was administered intramuscularly at a dose of 0.5 mL at ages 8 and 16 weeks.

Methylprednisolone (Solu-Medrol; Upjohn) was administered intramuscularly at 30 mg/kg of body weight for 7 days in the midafternoon. A repository form of methylprednisolone (Depo-Medrol; Upjohn) was administered intramuscularly at a dose of 10 mg/kg/day twice weekly (Tuesdays and Fridays) during 2 consecutive weeks (i.e., total of 4 administrations).

Virus isolation (cell associated and cell free). Levels of infectious virus in cells and plasma of peripheral blood were determined by a limiting dilution assay of PBMC and plasma, respectively, in cultures with CEMx174 cells in 24-well plates and subsequent p27 core antigen measurement by methods described elsewhere [24, 25]. Levels of infectious virus are expressed as TCID₅₀ per 10⁸ PBMC or per mL of plasma. For animals with low or undetectable virus load, 1×10⁶ to 2×10⁸ PBMC were cocultivated for 8 weeks with CEMx174 cells in tissue culture flasks, as described elsewhere [25]. For animals with undetectable virus levels, an axillary lymph node was recovered by transcutaneous biopsy and was teased aseptically into single-cell suspensions of mononuclear cells (LNMC). LNMC were cocultured with CEMx174 cells similarly to the method described above for PBMC.

Polymerase chain reaction (PCR)–based detection of SIV proviral DNA sequences. Nested PCR was done in a GeneAmp 9600 thermocycler (Perkin-Elmer Cetus). Two rounds of 25 cycles of amplification were performed on aliquots of PBMC or LNMC lysates (5–10 replicates/sample with 10³ cellular DNA equivalents or 600 ng of genomic DNA [equivalent to 10⁶ cells]/reaction tube), by using SIVmac-specific gag primers and conditions, as described elsewhere [26] with the following modifications. After lysis and proteinase K digestion, lysates were incubated for 10 min at 95°C. A first round of amplification was performed for 5 min at 95°C and then 25 cycles (20 s at 95°C, 30 s at 65°C, 40 s at 72°C) by use of outer SIV gag primer pairs 5'-ACCTTCTGGTCTATC-GTGCG-3' and 5'-GTCCCAATCTGACGCCTCCTC-3'. The second PCR round was started with a 1-min incubation at 95°C and then 25 cycles (20 s at 95°C, 30 s at 64°C, and 40 s at 72°C) with the internal SIV gag primers 5'-CACGCGAAAGAGAACTGGA-AAC-3' and 5'-CTCTGATAACGTCAATCGGACC-3'. To detect potential inhibitors of Taq polymerase in cell lysates, β-actin DNA sequences were amplified with 1 round of PCR with the primer pairs 5'-ACCCAGCCATGCTAGTGCTATCC-3' and 5'-GCCGTCAGGGCGAGCCGCAAACGCTCA-3'. [26]. Positive controls included lysates of PBMC from known SIV-infected animals or SIV-infected CEMx174 cells. The sensitivity of this PCR assay is 1 copy of SIV gag sequence per reaction. Negative controls included reactions in which uninfected CEMx174 or PBMC lysates of uninfected animals were used and reactions in which the lysates were replaced by distilled water blanks.

Drug susceptibility assay. Phenotypic drug susceptibility of
SIVmac isolates was characterized by a previously described assay on the basis of a dose-dependent reduction of viral infectivity [24, 27]. This assay can detect SIV mutants with decreased susceptibility to PMPA and other antiretroviral drugs.

**Plasma viral RNA levels.** Plasma viral RNA levels were quantified with the SIV 3.0 RNA bDNA assay [28]. This assay is similar to the Quantiplex HIV RNA assay [29], except that target probes are designed to hybridize with the pol region of the SIVmac and SIVsm groups of virus strains, including SIVmac251. The lower quantification limit of this assay is normally 500 copies of SIV RNA per 0.5-mL specimen. However, because of the limited blood volume that can be collected from newborn macaques, plasma volumes of only 0.25 mL were available for testing during the early time points, which limited the sensitivity of this assay to >1000 copies/mL.

**Anti-SIV antibody determination.** The whole virus anti-SIV IgG ELISA has been described elsewhere [22, 27].

**Lymphocyte proliferative responses to SIV.** Antigen-specific proliferation was measured in PBMC from fresh blood samples, as described elsewhere [30]. The antigen was chemically inactivated, fusion-competent SIVmac239 (provided by J. Lifson, SAIC Frederick, National Cancer Institute–Frederick Cancer Research and Development Center, Frederick, MD) [31].

**Lymphocyte phenotyping by 3-color flow cytometry.** T lymphocyte antigens were detected by direct labeling of whole blood with PerCP-conjugated anti–human CD8 (clone SK1; Becton Dickinson Immunocyteometry), phycoerythrin-conjugated anti–human CD4 (clone M-T477; Pharmingen), and fluorescein-conjugated anti–human CD3 (clone SP34; Pharmingen). A separate aliquot of blood was labeled with fluorescein-conjugated anti–human CD3 and PerCP-conjugated anti–human CD20 (clone L27; Becton Dickinson). Red blood cells were lysed, and the samples were fixed in paraformaldehyde by use of the Q-prep system (Coulter). Lymphocytes were gated by forward and side light scatter and then were analyzed by FACSCalibur flow cytometer (Becton Dickinson). CD4 T lymphocytes and CD8 T lymphocytes were defined as CD3⁺CD4⁺ and CD3⁺CD8⁺ lymphocyte populations, respectively. B lymphocytes were defined as CD3⁻CD20⁺ lymphocytes.

**Statistical analysis.** Statistical analysis of protection against SIV infection was done by comparing findings of PMPA-treated with those of control animals, using 1-sided Fisher’s exact test and InStat version 2.031 software (GraphPad Software). Virus levels in peripheral blood during primary viremia were compared by calculating the area under the curve (AUC) for each animal for the first 3 weeks after SIV inoculation, followed by Wilcoxon rank sum test analysis [32]. We have shown elsewhere [24, 33–35] that these analyses can distinguish biologically relevant differences.

**Results**

**Selection of 4-mg/kg PMPA dosage regimen.** A 24-h pharmacokinetic study in 2 uninfected newborn macaques revealed that the AUC for a single 30-mg/kg subcutaneous dose of PMPA was 164 μg·h/mL (range, 153–174 μg·h/mL; data not shown). At the highest dose of PMPA tested to date in human adults (3 mg/kg/day, intravenously), the steady-state AUC was 22.5 μg·h/mL [36]. Accordingly, the required subcutaneous PMPA dose for newborn macaques to produce an AUC similar to this exposure in humans was estimated to be ~4 mg of PMPA/kg of body weight.

**Experimental design.** Within the first 3 days after birth, 13 animals were inoculated orally with highly virulent SIVmac251 (figure 1). Four of these infants (group A) were placebo-treated control animals given multiple subcutaneous administrations of 0.9% physiologic saline solution. Four animals (group B) received an initial low dose of PMPA (4 mg/kg of body weight, subcutaneously) 4 h before the oral SIV inoculation and were given a second and final dose of PMPA 24 h later (i.e., 20 h after SIV inoculation). Four infants (group C) received 2 low doses of PMPA (4 mg/kg of body weight, subcutaneously) at 1 and 25 h after the oral SIV inoculation. Finally, 1 animal (group D) received a single high dose of PMPA (30 mg/kg, subcutaneously) 1 h after the oral SIV inoculation.

To monitor immunocompetence to nonviral test antigens, all infant rhesus macaques were immunized with cholera toxin B subunit at age 2 weeks; a booster immunization was given at age 10 weeks. Immunizations with tetanus toxoid were given at ages 8 and 16 weeks. To reactivate viremia from either latent or occult systemic infection in virus-negative and -seronegative infant macaques in this study, pharmacologic doses of the corticosteroid methylprednisolone were used, as described in Materials and Methods.

**Prophylactic efficacy of short-term PMPA regimens.** After oral SIVmac251 inoculation, all 4 placebo-treated infant macaques became persistently infected, as determined by virus isolation, PCR, and SIV-specific antibody production (tables 1–3). Virus replicated rapidly to peak levels within 2–3 weeks after infection; peak levels were 316–4640 TCID₅₀/10⁶ PBMC, 46–5900 TCID₅₀/mL of plasma, and 4 × 10⁴ to 42 × 10⁴ copies of RNA/mL of plasma. A single oral inoculation with the same dose of the SIVmac251 stock that was used in the present study.
had previously infected 12 of 12 newborn macaques by the oral route [16, 19]. Accordingly, the course of infection of the 4 placebo-treated animals is representative of a larger number of historical SIV-infected control animals.

Of the 9 animals given 1 or 2 doses of PMPA, 3 animals (30847 [group B] and 30579 and 30844 [group C]), became persistently infected. The time course of the primary viremia and the peak levels in these 3 animals during the first 3 weeks of infection (316–3160 TCID50/106 PBMC, 46–17,780 TCID50/mL of plasma, and 17 × 106 to 152 × 106 copies of RNA/mL of plasma) were indistinguishable from those of the 4 placebo-treated infected animals and the historical control animals. All 7 animals maintained persistent viremia (viral RNA between 104 and 3 × 106 copies/mL of plasma; positive by virus isolation and PCR); 5 of the 7 animals developed simian AIDS and were killed by ages 2–5 months; the 2 remaining animals had a slower disease course.

In contrast to the 7 animals that became persistently viremic, 3 group B animals (30576, 30577, and 30842), 2 group C animals (30581 and 30845), and 1 group D animal (30478) were protected against persistent viremia and disease. Of these 6 “protected” animals, no infectious virus could be isolated from PBMC, plasma, or LNMC from 5 animals throughout the 1-year observation period (table 1). The exception was group C animal 30845; virus was isolated from 2 × 104 PBMC of this animal at 2 time points (ages 3 and 6 weeks). On the basis of a limiting dilution culture assay, infectious virus levels at these times were very low (<1 infected cell/400,000 PBMC). Viral RNA levels in plasma at these 2 time points also were below the limit of detection (<1000 copies/mL). Both virus isolates from 30845 had wild type in vitro susceptibility to PMPA. No virus was isolated from animal 30845 after age 6 weeks (table 1). In addition, for all 6 protected animals, no virus was isolated from plasma throughout the observation period or

### Table 1. Detection of infectious virus in infant macaques following oral SIVmac251 inoculation.

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**NOTE.** Animals were inoculated within 3 days of birth orally with uncloned SIVmac251. ND, not done or not available; PBMC, peripheral blood mononuclear cells; PMPA, reverse-transcriptase inhibitor 9-[2-(phosphonomethoxy)propyl]adenine; SIV, simian immunodeficiency virus.

a Group A consisted of 4 placebo-treated animals. Groups B and C (n = 4 in each group) received 2 4-mg/kg doses of PMPA, either 4 h before and 20 h after (group B) or 1 h and 25 h after SIV inoculation (group C). Group D consisted of 1 animal that received a single 30-mg/kg dose of PMPA 1 h after SIV inoculation.

b Virus isolation results shown as detectable (+) or undetectable (−) virus. No. of PBMC used for virus isolation from animals with low or undetectable virus levels depended on availability (range, 1 × 106 to 4.5 × 106 PBMC for the first 12 weeks and up to 20 × 106 PBMC afterward).

c Time of euthanasia because of simian AIDS.

### Table 2. Transient detection of proviral DNA in peripheral blood mononuclear cells of “protected” infant macaques after oral SIVmac251 inoculation.

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**NOTE.** Animals were inoculated orally within 3 days of birth with uncloned SIVmac251. NA, not available; PCR, polymerase chain reaction; PMPA, reverse-transcriptase inhibitor 9-[2-(phosphonomethoxy)propyl]adenine; SIV, simian immunodeficiency virus.

a Groups B and C (n = 4 in each group) received 2 4-mg/kg doses of PMPA, either 4 h before and 20 h after (group B) or 1 h and 25 h after SIV inoculation (group C). Group D consisted of 1 animal that received a single 30-mg/kg dose of PMPA 1 h after SIV inoculation.

b PCR to detect SIV gag sequences was done with 103 cellular DNA equivalents/reaction tube. Sensitivity of this assay is 1 copy/reaction.

c Lymph node (LN) mononuclear cells were recovered by biopsy of axillary LN. LNs were collected at age 44 or 48 weeks, except for animal 30478 (biopsy done at age 85 weeks).
from axillary lymph node–derived mononuclear cells obtained at ages 44–85 weeks (table 1).

PBMC and LNMC of the 6 protected animals also were tested for the presence of proviral DNA by nested PCR analysis. Proviral DNA was detected at varying frequencies in PBMC of all animals at ≥1 time point during the first 6 weeks after SIV inoculation. From week 8 onward, no proviral DNA was detected in either PBMC or LNMC (table 2).

Plasma samples of these animals also were tested for the development of anti-SIV IgG responses. As we have observed elsewhere [27, 33], the animals that developed persistent viremia had either weak and transient antibody responses (associated with high virus levels, rapid immunosuppression, and rapid disease progression) or persistent responses (associated with a slower disease course; table 3). Five of the 6 protected animals also had evidence of transient antibody responses. SIV-specific IgG was detected transiently at low titers (1:100) in animals 30576, 30577, and 30842 (group B) and 30845 (group C) within the first 5 months of life. Animal 30478 (group D), which was consistently negative by virus isolation (table 1), developed a moderate SIV IgG response (titer 1:1600) 3–6 weeks after SIV inoculation; the titer then declined and became undetectable from week 20 onward. This animal did not have any proliferative lymphocyte responses to SIV when tested at age 23 months (data not shown). The weak SIV-specific antibody responses in these protected infants could not be attributed to age-related immaturity of the immune system. Immunocompetence was demonstrated by the observation that all 6 protected animals mounted very rapid and strong antibody responses against cholera toxin subunit B after immunization at age 2 weeks (IgG titers >102,400 within 4 weeks after immunization; data not shown).

**Statistical analysis of protection.** Because some of the PMPA–treated infant macaques had evidence suggesting an initial transient or abortive infection (see above), prophylactic efficacy was defined as “the absence of persistent viremia, seroconversion, and disease.” According to this definition, animals 30576, 30577, and 30842 (group B), 30581 and 30845 (group C), and 30478 (group D) were considered to be protected.

Twelve untreated historical control animals became persistently infected after oral inoculation with the same inoculum in the preceding 2 years. Inclusion of these control animals in the statistical analysis revealed that the 2-dose 4-mg/kg regimens of groups B and C offered statistically significant protection (P<.05; table 4). Combination of the results of the 3 PMPA–treated groups (B–D) strongly demonstrates the beneficial effects of early 1- or 2-dose PMPA regimens against oral SIV infection (P = .0005).

**Safety of the 1- or 2-dose PMPA regimen.** There was no detectable toxicity with 1 or 2 doses of PMPA. All the animals protected against persistent SIV viremia had normal CD4+ and CD8+ T lymphocyte counts and B lymphocyte counts throughout the observation period. In addition, these animals had rapid weight gain, which was similar to uninfected infants at the California Regional Primate Research Center, normal serum chemistry values, and normal urine parameters (absence of glucosuria

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**Table 3.** Anti-SIVmac251 antibody responses in reverse-transcriptase inhibitor 9-[2-[(phosphonomethoxy)propyl]adenine (PMPA)–treated infant rhesus macaques.

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**NOTE.** SIV, simian immunodeficiency virus.

a All animals were inoculated with SIVmac251 shortly after birth. Group A consisted of 4 placebo–treated animals. Groups B and C (n = 4 in each group) each received 2 4-mg/kg doses of PMPA, either 4 h before and 20 h after (group B) or 1 h and 25 h after SIV inoculation (group C). Group D consisted of 1 animal that received a single 30-mg/kg dose of PMPA 1 h after SIV inoculation. Underlined animal nos. indicate infants that were protected against persistent viremia (see tables 1 and 2).

b Anti-SIV IgG titers at indicated weeks after virus inoculation were determined by ELISA and are expressed as the reciprocal of the highest 4-fold dilutions (starting from 1:100, with 2 replicates per dilution) that gave a positive optical density above cutoff value. Titers >100 are indicated in bold type.

c Time of euthanasia because of simian AIDS.
and proteinuria). These animals were healthy at ages 19–23 months.

**Immune activation or pharmacologic immunosuppression fails to reactivate viral replication in animals with transient viremia.** Because it was unclear whether the protected animals had cleared virus or whether they still had an occult or latent infection [30], we used 2 strategies to reactivate viral replication. Selection of these regimens was based on their demonstrated ability to increase virus levels in HIV-1–infected chimpanzees [37, 38].

Immune activation provided by tetanus toxoid immunization (at ages 8 and 16 weeks) and cholera toxin subunit B immunization (at ages 2 and 10 weeks) failed to induce persistent viremia or seroconversion (tables 1–3). Next, to induce immunosuppression, the 5 protected group B and C animals (30576, 30577, 30581, and 30842, and 30845) were treated with corticosteroids at about age 1 year. Methylprednisolone (Solu-Medrol) was administered intramuscularly at 30 mg/kg of body weight during 7 days in the midafternoon. After 6 weeks without treatment, a repository form of methylprednisolone (Depo-Medrol) was administered intramuscularly at a dose of 10 mg/kg, twice weekly for 2 consecutive weeks.

Corticosteroid treatment induced transient 2–3-fold increases in neutrophil counts and 2–6-fold reductions in total lymphocyte counts in all animals. The reductions in total lymphocyte counts were reflected in both CD4 and CD8 T lymphocyte fractions (figure 2). Compared with the 1-week Solu-Medrol treatment, the 2-week repository Depo-Medrol treatment was more potent in suppressing lymphocyte populations, as nadir levels were 420–1550 total lymphocytes, 143–634 CD4 T lymphocytes, and 100–297/μL CD8 T lymphocytes.

Heparinized blood samples were collected from these 5 animals on a regular basis during an 8-month period after the start of the corticosteroid treatment (including all time points indicated in figure 2). No virus could be isolated from PBMC or plasma. For virus isolation at each time point, we used 1 × 10^6 to 10 × 10^6 PBMC and 0.2–0.5 mL of plasma. No proviral DNA was detected by PCR at these time points (with 5–10 replicates/sample; data not shown). Plasma samples collected at the 2 time points when lymphocyte counts were lowest (i.e., weeks 9 and 11; figure 2) also were analyzed for the presence of viral RNA by bDNA assay. All of these samples had undetectable RNA levels (<500 copies of RNA/mL of plasma). Thirteen weeks after the initiation of the Solu-Medrol treatment (i.e., 4 weeks after withdrawing Depo-Medrol treatment), an axillary lymph node biopsy was performed. No virus was isolated from 2.5 × 10^6 to 10.5 × 10^6 LNMC, and no proviral DNA was detected by PCR for any of the 5 animals. No SIV-specific IgG antibodies were detected in any of the plasma samples collected during and after the corticosteroid treatment (SIV-specific IgG ELISA titers <1:100). These animals remained negative, as determined by virus isolation, PCR, and serology throughout the rest of the observation period (i.e., until age 19–22 months).

After lymphocyte counts had returned to baseline values, PBMC of these corticosteroid-treated animals were tested for SIV-specific T lymphocyte proliferative responses at 4 time points (11, 14, 17, and 23 weeks after withdrawal of Depo-Medrol). Three animals (30577, 30581, and 30845) had negative results (stimulation index [SI], <2) at all 4 time points. Weak proliferative responses (SI, 2–2.5) were detected for the other 2 animals (30576 and 30842) at a single time point; no responses (SI, <2) were detected at all other time points. Because uninfected macaques can occasionally have SI values between 2 and 2.5, the significance of this single detection of a weak response in animals 30576 and 30842 is not clear. In conclusion, neither immune activation nor immunosuppression resulted in detectable viral replication.

**Discussion**

Our results extend a number of observations previously made in our animal model, which suggests the strong potential of short-term PMPA administration to reduce intrapartum transmission of HIV-1 and HIV-2 from mother to infant. In particular, our present studies demonstrate that very short regimens (1 or 2 doses) of PMPA are still effective, including a low dose (4 mg/kg), and when PMPA was administered only after exposure to virus.

We previously demonstrated that 2 relatively high doses of PMPA (30 mg/kg) given 4 h before and 20 h after oral SIV inoculation protected all 4 newborn macaques; these animals had no evidence of an initial transient or abortive infection [19]. In the present study, we found that 2 lower doses of PMPA (4 mg/kg) given at the same times (−4 and +20 h relative to virus inoculation) were still partially protective, since 3 of the 4 animals were protected against persistent viremia and disease. For
practical reasons, we did not administer a first dose of PMPA to pregnant female macaques at the onset of delivery. Instead, the first dose of PMPA was given directly to the newborns before virus inoculation. However, this approach should be equivalent to giving the first dose to a pregnant woman at the onset of delivery, because PMPA crosses the primate placenta quite efficiently [16, 39].

The present study is the first to demonstrate that a 1- or 2-dose postexposure regimen can be protective. The prophylactic success for such short regimens is probably because drug administration was started very soon (1 h) after virus exposure. Currently, only 2 compounds have been very successful in preventing infection of macaques when treatment was started after virus inoculation: PMPA and 2',3'-dideoxy-3'-hydroxymethyl cytidine (BEA-005). Studies with both of these compounds demonstrated that the timing (i.e., the interval between exposure and the first dose of drug) and the duration of treatment greatly affect the success of postexposure prophylaxis; the sooner the first dose of drug is administered, the shorter the minimum duration required for protection [17, 40–42]. A 1-day treatment regimen of BEA-005 (3 10-mg/kg/day doses) protected cynomolgus macaques against intravenous low-dose SIVsm challenge, if started 1 h after virus inoculation; if treatment was delayed until 3 h after exposure, 3 days of BEA-005 treatment were required to achieve protection [42]. In a similar way, postexposure prophylaxis regimens of PMPA (30 mg/kg) were effective when started 24 h after intravenous SIV mne inoculation, but only with a longer duration of treatment (i.e., 28 days) [41]. In contrast, a 28-day combination regimen of zidovudine, lamivudine, and indinavir failed to protect cynomolgus macaques, even when started 4 h after intravenous SHIV89.6P inoculation [43].

Evidence suggests that postexposure HIV prophylaxis can be effective in humans. An observational study in New York state found that, in the absence of zidovudine administration to the HIV-infected mother before or during delivery, initiation of a 6-week zidovudine treatment course to the infant within the first 3 days of life still lowered the rate of infection in the infants [44]. Zidovudine prophylaxis to health care workers after occupational exposure to HIV is estimated to reduce the risk of infection by 80% [45]. Current recommendations for postexposure prophylaxis of health care workers include administration of a combination of antiviral drugs for 4 weeks [45, 46]. Although a postexposure drug regimen for 4–6 weeks probably is more effective than very short regimens, our data suggest that, in situations where issues such as costs, compliance, or fear of toxicity prohibit the ideal duration of treatment, a 1- or 2-dose regimen with potent drugs, such as PMPA, can still be beneficial if administered as soon as possible after exposure.

Our data suggest that the protected PMPA-treated animals in the present study experienced an initial transient viremia or abortive infection. This finding was not completely unexpected. In a previous study with newborn macaques, a 2-week PMPA regimen (30 mg/kg) starting immediately after virus inoculation was effective in protecting 3 of the 4 animals against persistent viremia.
and disease, but these 3 animals also had evidence of an initial transient viremia with transient detection of antiviral immune responses and viral RNA [16]. Other investigators demonstrated that postexposure PMPA prophylaxis initiated 24 h after intravenous SIVsmE660 inoculation could protect juvenile macaques against persistent viremia, but the detection of SIV-specific lymphocyte proliferative responses suggests that these animals experienced a transient or low-level infection [31]. In HIV-1–inoculated chimpanzees receiving pre-exposure nevirapine prophylaxis, proviral DNA also was detected at some time points in the absence of infectious virus and seroconversion [47].

We hypothesize that the transient detection of virus and antiviral immune responses that we observed in our protected animals was due to infection with replication-defective or replication-impaired viral variants that were present in the virus inoculum, as explained elsewhere [16]. Alternatively, it is possible that infection with a pathogenic variant at a very low level can be contained by early innate and/or acquired immune responses. The limited blood volume that can be collected from newborn macaques did not allow for the assessment of cell-mediated immune responses during these early stages. Detection of antiviral immune responses or transient low levels of virus without persistent seroconversion also has been described in HIV-exposed adults and perinatally exposed infants [48–56]. Further studies of this phenomenon of transient/abortive infection are useful, as the ability to clear viremia provides hope for developing an HIV vaccine to prevent or abort HIV infection [31].

It is not clear whether infection in these protected animals was completely cleared or whether infection persisted at very low levels. We tried 2 approaches to reactivate virus, immune activation (by immunization with tetanus toxoid and cholera toxin B subunit) and immunosuppression (through administration of corticosteroids). Both of these approaches were expected to increase viral replication. Immune reconstitution antigens induced transient increases in plasma viremia in HIV-1–infected patients and HIV-1–infected chimpanzees [37, 57, 58]. In addition, the corticosteroid regimens that we used previously increased virus levels in HIV-1–infected chimpanzees [38]. Neither of these approaches led to detectable virus levels or seroconversion in our protected infant macaques. Although low levels of SIV-specific lymphocyte proliferative responses were detected at a single time point in 2 animals, these responses were not persistently detectable; thus, their significance is unclear. The animals were negative by all other criteria throughout the observation period to age 19–22 months. The finding of this transient or abortive infection in the protected animals, whereas other animals in the experimental groups demonstrated persistent infection (similar to that of the untreated animals), suggests that the reduced PMPA dosage regimens that were used in this study were at the borderline to achieve protection.

In prophylaxis studies in animal models, the virus inoculum dose required to induce persistent infection of 100% of untreated control animals is probably higher than the amount of virus that would be encountered during a normal sexual or perinatal exposure. This may explain why administration of zidovudine and other nucleoside analogs during several weeks usually was not effective in preventing SIV infection (reviewed in [59]). Our studies with newborn macaques used the highly virulent uncloned SIVmac251 isolate, which induces persistently high viremia and rapid disease in the majority of untreated newborn macaques [33, 34]. All available evidence suggests that prophylaxis is more difficult to achieve against highly virulent uncloned isolates than against molecularly cloned or attenuated virus isolates. In addition, protection is more frequently seen in nonpathogenic or attenuated models (e.g., HIV-1 infection of chimpanzees) than in highly virulent models (e.g., SIVmac251 infection of macaques). In this context, uncloned SIVmac251 has been one of the most difficult SIV isolates to protect against [60]. Altogether, these findings suggest that when an intervention strategy gives even partial protection in a highly virulent animal model, its efficacy in reducing intrapartum infection may be even more pronounced for human newborns. Thus, the prophylactic success of short and low PMPA dosage regimens in newborn macaques against the highly virulent SIVmac251 virus is impressive and promising.

In conclusion, our data further emphasize the potential of short PMPA regimens to reduce vertical transmission of HIV, especially in developing countries. The data suggest that administration of PMPA to HIV-infected pregnant women at the onset of labor and/or immediate administration to their newborns could reduce the rate of intrapartum transmission of HIV-1 and HIV-2.

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