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Immediate Zidovudine Treatment Protects Simian Immunodeficiency Virus-Infected Newborn Macaques against Rapid Onset of AIDS

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Simian immunodeficiency virus (SIV) infection of newborn rhesus macaques is a practical animal model of pediatric AIDS. Intravenous inoculation of rhesus newborns with uncloned SIV_{mac} resulted in a high virus load, no antiviral immune responses, severe immunodeficiency, and a high mortality rate within 3 months. In contrast, immediate oral zidovudine (AZT) treatment of SIV-inoculated rhesus newborns either prevented infection or resulted in reduced virus load, enhanced antiviral immune responses, a low frequency of AZT-resistant virus isolates, and delayed disease progression with negligible toxicity. These results suggest that early chronic AZT treatment of human immunodeficiency virus-exposed newborns may have benefits that outweigh its potential side effects.

About one-fourth of infants born to human immunodeficiency virus (HIV)-infected mothers will acquire the infection either in utero or during the perinatal period (1, 11). This rapidly increasing population of HIV-infected infants has prompted a number of intervention strategies.

An interim analysis of a clinical trial has recently shown that 3′-azido-3′-deoxythymidine (zidovudine [AZT]) administration to HIV-infected pregnant women beginning at 14 to 34 weeks of gestation and continuing to their newborns during the first 6 weeks of life reduces the rate of viral transmission by two-thirds (7a, 25). However, no data are yet available on the effect of such therapy on the subsequent course of HIV-related disease in infants who became infected.

Vertical HIV infection often results in a more rapid and severe disease than is seen for adults, as about one-third of these infants develop symptoms within 1 year of birth and die early (1, 10, 36). Previous studies have demonstrated benefits to perinatally infected infants of AZT administration started during the symptomatic stage of the disease, when these children were at least several months old (2, 24, 29). Little is known, however, about potential benefits of starting AZT treatment early in infection, before symptoms of AIDS develop. There are concerns that AZT treatment during the early stages of HIV infection might be harmful because of the risk of causing a less vigorous immune response (28, 39) or because it might induce early emergence of AZT-resistant HIV mutants (32), whose pathogenicity is unknown and which could reduce the efficacy of drug treatment at later disease stages. However, starting AZT administration early in infection may produce greater beneficial effects than waiting until symptomatic disease becomes apparent, especially for infants, who may show rapid disease progression. Because the rapid development of AIDS in these infants is associated with high levels of viremia and weak antiviral immune responses (4, 9, 30, 35), one would predict that AZT administration during the early stages of HIV infection may alter the natural history of infection by reducing the initial viremia which is responsible for early systemic dissemination.

Simian immunodeficiency virus (SIV) infection of rhesus macaques has been shown to be an excellent animal model of HIV infection in humans (reviewed in reference 8). We previously found that SIV_{mac} infection of newborn macaques resembled HIV infection of human infants in causing rapidly progressive disease with similar clinical symptoms (23). Therefore, we investigated whether early AZT therapy would alter the disease course in this animal model of pediatric AIDS.

MATERIALS AND METHODS

Animals, virus, and AZT administration. Eighteen newborn rhesus macaques (*Macaca mulatta*) from type D-retrovirus- and SIV-seronegative dams at the California Regional Primate Research Center, Davis, were removed from their mothers and hand reared in a primate nursery in accordance with American Association for Accreditation of Laboratory Animal Care standards. We strictly adhered to the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Resource Council. When necessary, animals were immobilized with 10 mg of ketamine HCl (Parke-Davis, Morris Plains, N.J.) per kg of body weight, injected intramuscularly. Samples were collected regularly for monitoring toxicity and viral and immunologic parameters (41). Heparinized blood was collected weekly for the first month, every 2 weeks for the next 5 months, and from then on, monthly; bone marrow aspirates were collected at 2 weeks, 4 weeks, and then monthly; and cerebrospinal fluid was collected at 2, 4, 8, and 12 weeks and at death.

To study toxicity of AZT, as well as its antiviral effects against SIV, the 18 newborn rhesus monkeys were divided into five groups (Table 1). Between 1 and 3 days after birth, 15 animals (Table 1, groups 1 through 4) were inoculated intravenously with 1 ml of a 10^{-3} dilution (equivalent to 10 to 100 100% animal infectious doses [AID_{100}]) of a cell-free uncloned SIV_{mac} virus stock propagated in human peripheral blood mononuclear cells (PBMC) (38). Six animals (Table 1, group 1) were used as untreated SIV-infected controls. Three groups of three animals each were SIV inoculated and treated with AZT; AZT treatment was started either 2 h prior to, simultaneously with, or 1 week after SIV inoculation (Table 1, groups 2 through 4). Three additional control animals were not SIV inoculated but only given AZT (Table 1, group 5).

AZT syrup (10 mg/ml; Retrovir) was given at an oral dose of 25 mg of AZT per kg of body weight (≈250 mg of AZT per m^2 of body surface area) every 8 h (7:00 a.m., 3:00 a.m., and 11:00 p.m.) to four groups of animals (Table 1, groups 2 through 5). For each dosing, animals were restrained physically and 2.5 ml of AZT syrup per kg of body weight was administered through nasogastric intubation. When animals were several weeks old, nasogastric intubation could be replaced by direct oral administration, often without the need for physical restraint. AZT dosages were adjusted weekly according to body weight.

Determination of AZT toxicity. Complete blood counts were performed on EDTA-anticoagulated blood samples from all animals. Samples were analyzed by using an automated electronic cell counter (Baker 9000; Serono Baker Diag-

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TABLE 1. Experimental design and disease outcome[a]

Group (size)	SIV inoculation[b]	AZT treatment[c]		Virus load[d]		Anti-SIV antibodies[e]	Disease outcome[f]
		Time started	Duration	Plasma	PBMC		
1 ($n = 6$)	+	NA	NA	High, 100 (6–285)	High, 100 (30–284)	−	5 of 6 died <3 mos p.i.; 1 died at 6 mos p.i.
2 ($n = 3$)	+	At inoculation	>15 mos ($n = 2$)	Reduced, 1.3 (0.04–2.5)	Reduced, 5.1 (4–6)	+	1 died at 10 mos p.i.; 1 healthy[g] at >15 mos p.i.
			6 wks ($n = 1$)	Absent, 0	Absent, 0	−	Healthy at >15 mos p.i.
3 ($n = 3$)	+	2 h preinoculation	>15 mos	Reduced, 0.7 (0.02–2.0)	Reduced, 8.9 (2–15)	+	1 died at 7 mos p.i.; 2 healthy[h] at >15 mos p.i.
4 ($n = 3$)	+	1 wk after inoculation	>12 mos	Reduced, 11.1 (0.17–31.8)	Reduced, 29.6 (5–76)	+	Healthy at >12 mos p.i.
5 ($n = 3$)	−	Day of birth	7 wks ($n = 1$)[i] 8 mos ($n = 2$)	NA	NA	NA	Healthy at >12 mos

[a] NA, not applicable; p.i., postinoculation.
[b] All animals (except group 5) were inoculated intravenously with 10 to 100 AID_{100} of SIV_{mac} <3 days after birth. +, SIV inoculated; −, not SIV inoculated.
[c] AZT was given at 25 mg/kg of body weight 3 times daily (groups 2 through 5).
[d] Comparisons of cell-free (plasma) and cell-associated (PBMC) virus titers were performed by calculation of the AUC, without logarithmic transformation, for each animal for the first 10 weeks after virus inoculation. The average AUC for group 1 was given a reference value of 100%. Values for each group represent the average percent and the range (in parentheses) of AUC values, in comparison with this reference value. Both plasma- and PBMC-associated virus levels in all AZT-treated SIV-infected animals of groups 2 through 4 ($n = 8$) were reduced in comparison with levels in the six untreated controls ($P = 0.002$).
[e] Anti-SIV antibodies were detected by whole-virus ELISA and immunoblotting.
[f] AZT-treated SIV-infected animals ($n = 8$) survived longer than the six untreated SIV-infected animals ($P < 0.001$).
[g] Animal had detectable CTLs, and AZT-resistant SIV was isolated after 8 months of AZT therapy.
[h] One of the two animals had detectable CTLs.
[i] AZT withdrawn due to neutropenia.

nostics); differential cell counts were determined manually. A standard serum chemistry profile (including liver enzymes) was done by the Dacos system (Coulter Electronics, Hialeah, Fla.). Bone marrow aspirates were obtained monthly, and smears were stained with Wright-Giemsa stain (with a Giemsa overlay) for morphologic evaluation.

Quantitative virus isolation (cell associated and cell free). Cell-associated and cell-free virus load in peripheral blood was determined regularly by limiting dilution culture assays (four replicates per dilution) of PBMC and plasma, respectively, with CEM×174 cells in 24-well plates and subsequent p27 core antigen measurement, according to methods previously described (41). In addition, for animals with a low or undetectable virus load, 1×10^6 to 10×10^6 PBMC were cocultivated for 8 weeks with CEM×174 cells in tissue culture flasks, as described previously (41). Virus load in fresh lymphoid tissues (spleen, thymus, and axillary lymph node) obtained at necropsy was determined by a limiting dilution culture assay of single-cell suspensions, by a method similar to that used for PBMC.

Anti-SIV isotype-specific antibody determination. The SIV_{mac} strain used for inoculations was concentrated by centrifugation, disrupted in 1% sodium dodecyl sulfate, and applied to microtiter enzyme-linked immunosorbent assay (ELISA) plates (Falcon 3912; Becton Dickinson) at 100 ng of total protein per well. The plates were incubated with test or control plasma samples (1:100 dilution) and washed, and then they were incubated with 1:1,000-diluted enzyme-conjugated goat anti-monkey immunoglobulin G (IgG) or IgA (Nordic), washed, and finally incubated with OPD (*o*-phenylenediamine) (Sigma) as a substrate and read spectrophotometrically. Immunoblotting was performed as described previously (37).

T-lymphocyte phenotyping. T-lymphocyte antigens were detected by direct labeling of whole blood with fluorescein-conjugated Leu-2a (anti-CD8; Becton Dickinson Immunocytometry Inc., San Jose, Calif.) and phycoerythrin-conjugated OKT4 (anti-CD4; Ortho Diagnostic Systems Inc., Raritan, N.J.). Erythrocytes were lysed, and the samples were fixed in paraformaldehyde by using the Coulter Q-prep (Coulter Corporation). Lymphocytes were gated by forward and side light scatter and were then analyzed with a FACSCAN flow cytometer (Becton Dickinson).

SIV-specific CTL responses. Cryopreserved PBMC were thawed, stimulated with concanavalin A (10 μg/ml), and cultured for 14 days in 5% human lymphocyte conditioned medium (Human IL-2; Schiapparelli Biosystems, Inc., Columbia, Md.) containing 20 U of recombinant human interleukin 2 (donated by Cetus Corp., Emeryville, Calif.) per ml, as previously described (19). For cytotoxic T-lymphocyte (CTL) assays, autologous B lymphocytes were transformed by herpesvirus papio (594S×1055 producer cell line; provided by M. Sharp, Southwest Foundation for Biomedical Research, San Antonio, Tex.), infected overnight with wild-type vaccinia virus or recombinant vaccinia viruses expressing $p55^{gag}$ or $gp160^{env}$ of SIV_{mac239} (provided by L. Giavedoni and T. Yilma, University of California, Davis), and then labeled with 50 μCi of chromium-51 (Na_2CrO_4; Amersham Holdings, Inc., Arlington Heights, Ill.). Effector and target cells were added together at multiple effector/target ratios in a 4-h chromium release assay, and percent specific lysis was calculated from supernatant chromium measured in a liquid scintillation counter (Microbeta 1450; Wallac Biosystems, Gaithersburg, Md.).

AZT sensitivity assay. AZT sensitivity of SIV_{mac} was characterized by an assay which is a modification of the AIDS Clinical Trials Group consensus HIV-1 drug susceptibility assay (13) and is based on a dose-dependent reduction of viral infectivity by AZT. Titration of viral infectivity of tissue culture supernatants (by making fivefold dilutions with eight replicates per dilution and using CEM×174 cells in 96-well plates, followed after 5 days by p27 antigen measurement [18]) was performed at various AZT concentrations. Infectious titers (50% tissue culture infective doses [$TCID_{50}$] per ml) were calculated by the method of Reed and Muench (31). Reduction of infectivity was determined for each AZT concentration by using the following formula:

$$\%\ \text{Reduction of infectivity at } x\ \mu\text{M AZT} = \frac{[\text{TCID}_{50}]_0 - [\text{TCID}_{50}]_x}{[\text{TCID}_{50}]_0} \times 100$$

where $[TCID_{50}]_0$ = infectious titer at 0 μM AZT (control) and $[TCID_{50}]_x$ = infectious titer at x μM AZT.

The inhibitory concentration which reduces infectivity by 50% (IC_{50}) for unselected SIV_{mac} isolates (uncloned SIV_{mac} and the molecular clones 1A11 and 239) all fall within a narrow range (0.05 to 0.2 μM AZT) (40), which is similar to the one for AZT-sensitive HIV-1 as measured by a plaque reduction assay (16), or as measured by the AIDS Clinical Trials Group consensus HIV-1 susceptibility assay (13) by using reduction of p24 core antigen as a read-out. To enrich for potential AZT-resistant mutants in a mixed population, virus was also isolated from PBMC and plasma of SIV-infected animals in the presence of 5 μM AZT and tested for AZT sensitivity. This single passage in the presence of AZT is not sufficient to induce AZT resistance (40).

Statistical analysis. Statistical analysis was used to compare AZT-treated versus untreated SIV-infected animals with regard to survival and virus load. Survival was compared by the generalized Wilcoxon test. Cell-associated and cell-free virus levels were compared between the two animal groups by calculation of the area under the curve (AUC) for each animal for the first 10 weeks after virus inoculation, followed by analysis according to the Wilcoxon rank-sum test.

RESULTS

Sustained levels of AZT in blood of newborn rhesus macaques following oral administration. Serum AZT concentrations after a single oral dose (25 mg of AZT per kg of body weight) to newborn rhesus macaques showed considerable individual variability (Fig. 1). High peak levels (37 to 76 μM) were obtained within 1 to 2 h, and after 8 h, levels were still about 3 μM. Our findings confirm that, similar to observations for human newborns (6, 27), AZT clearance in newborn ma-

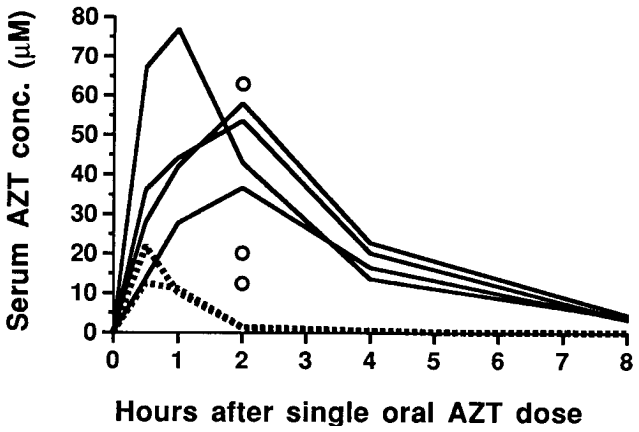

FIG. 1. Serum AZT levels in newborn rhesus macaques (<3 days old). Serum was collected by venipuncture without sedation at various time intervals after a single oral dose of AZT (25 mg/kg of body weight) to four newborn macaques (solid lines), and AZT concentration was measured by high-performance liquid chromatography (41). Open circles represent the AZT concentrations in the sera of three newborns (Table 1, group 3) at the time of virus inoculation (2 h after oral AZT). As a comparison, AZT levels following oral dosing of 50 mg of AZT per kg of body weight to two juvenile macaques (41) are given (dotted lines).

caques is substantially slower than for older animals, which results in higher and more prolonged blood AZT levels (20).

Chronic AZT administration to newborn rhesus macaques: tolerable toxicity and beneficial antiviral effects against SIV infection. (i) Chronic AZT toxicity. Outward signs of AZT toxicity were not apparent in any of the SIV-infected or uninfected animals that received chronic AZT administration (Table 1, groups 2 through 5). No significant changes were detected in any of the standard blood chemistry parameters (data not shown). After 4 weeks of AZT administration, one uninfected control animal (Table 1, group 5) developed persistent neutropenia (neutrophil count of $<500/\mu l$), which reversed rapidly when AZT was withdrawn at 7 weeks. Three other AZT-treated animals had short episodes of neutropenia, which resolved spontaneously (without AZT withdrawal).

The most obvious toxicity during prolonged oral AZT administration in both infected and uninfected animals was bone marrow erythroid hypoplasia, reflected in a mild macrocytic anemia. Eight of the 12 AZT-treated animals (Table 1, groups 2 through 5), but none of the 6 untreated animals (Table 1, group 1), developed a transient moderate anemia (hemoglobin level of <10 g/dl) during the first 8 weeks of AZT administration. This anemia, which was never severe enough to require reduction of the AZT dosage regimen, resolved spontaneously as animals got older, but hemoglobin levels still remained below values for age-matched control animals (data not shown). Transient or recurrent thrombocytosis (platelet counts of $>9 \times 10^5/\mu l$) was also observed in all 12 AZT-treated animals but in only 1 untreated animal.

(ii) Prophylactic and therapeutic effects of AZT against SIV infection of rhesus newborns. No infectious virus was isolated from plasma or PBMC from one animal that was inoculated with SIV simultaneously with the start of AZT administration (Table 1, group 2), even after AZT administration was discontinued 6 weeks after virus inoculation, and the animal was monitored for 15 months. No proviral sequences could be detected in PBMC samples by PCR (41), and this animal never developed SIV-specific serum antibodies as measured by antibody ELISA and immunoblotting (data not shown). This inability to detect virus or antiviral responses suggests that SIV infection was prevented by the oral AZT regimen. That AZT administration starting prior to, or simultaneously with, virus inoculation (Table 1, groups 2 and 3) prevented infection in only one animal is likely due to the moderate virus dose (10 to 100 AID_{100}) used in this study, as previous studies have shown that using a low virus dose (<10 AID_{100}) greatly improves chemoprophylactic success (5, 41).

Although eight of nine newborn macaques treated with AZT became infected following intravenous SIV inoculation, comparison of AZT-treated versus untreated SIV-infected animals revealed significant therapeutic benefits of chronic AZT administration.

The six untreated, SIV-infected rhesus newborns (Table 1, group 1) developed a pronounced cell-free (plasma) and cell-associated (PBMC) viremia within 2 weeks, and this persisted until death (Fig. 2A and B). No SIV-specific IgG responses could be detected in any of these animals at any time point by immunoblotting (data not shown) or ELISA (Fig. 2C); only one animal had a weak yet detectable SIV-specific IgA response prior to death at 3 months of age (data not shown). While the absolute numbers of $CD4^+$ cells and $CD8^+$ cells for individual animals varied significantly over time (data not shown), the $CD4^+/CD8^+$ cell ratio for most infected untreated animals declined rapidly (ratio of <1 [Fig. 2D]). SIV could be isolated at least once from cerebrospinal fluid of four animals. Four of these six SIV-infected animals experienced poor weight gain (i.e., weight at 10 weeks of age was below the average minus 1 standard deviation for nursery-reared rhesus infants at the California Regional Primate Research Center [data not shown]). Five of the six animals died between 10 and 12 weeks with simian AIDS, while the sixth animal died at 6 months after virus inoculation. Their clinical abnormalities, plus gross and microscopic pathologic changes (including failure to thrive, enteritis or malabsorption, pneumonia, opportunistic bacterial infections, lymphadenopathy, and encephalopathy), were consistent with terminal stages of SIV infection. This rapid disease progression and mortality is also seen in newborn macaques inoculated with $SIV/Delta_{B670}$ (3), and the disease resembles that which is seen in a small percentage of SIV-inoculated adolescent and adult macaques that fail to mount a strong immune response (15). Persistent viremia and weak antiviral immune responses are also seen in perinatally infected children who show rapid disease progression (1, 4, 9, 10, 30, 35, 36).

In contrast, the SIV-infected animals that received chronic AZT (Table 1, groups 2 through 4) fared much better. Plasma viremia in these AZT-treated animals either was undetectable throughout the whole study period or was reduced, with occasional intermittent episodes; cell-associated virus replication was delayed and reduced in comparison with the levels in the untreated animals (Fig. 2A and B; Table 1). Of the eight SIV-infected AZT-treated animals, all but one produced a strong anti-SIV IgG response within 3 to 6 weeks; this animal, which was started on AZT 1 week after virus inoculation and which showed a delayed clearance of virus from the plasma, had a detectable anti-SIV IgG response after 5 months (Fig. 2C). In six of eight AZT-treated animals, the IgG response was followed by an anti-SIV IgA response (data not shown). No virus was isolated from the cerebrospinal fluid of any AZT-treated animal at any time point. The $CD4^+/CD8^+$ ratios in these AZT-treated animals generally remained higher than 1 (Fig. 2D) and were similar to values for uninfected control animals. Despite the initial transient anemia (due to AZT toxicity), these SIV-infected AZT-treated animals showed rapid weight gain during the first 6 months after virus inoculation (i.e., weight was within or above the normal range [av-

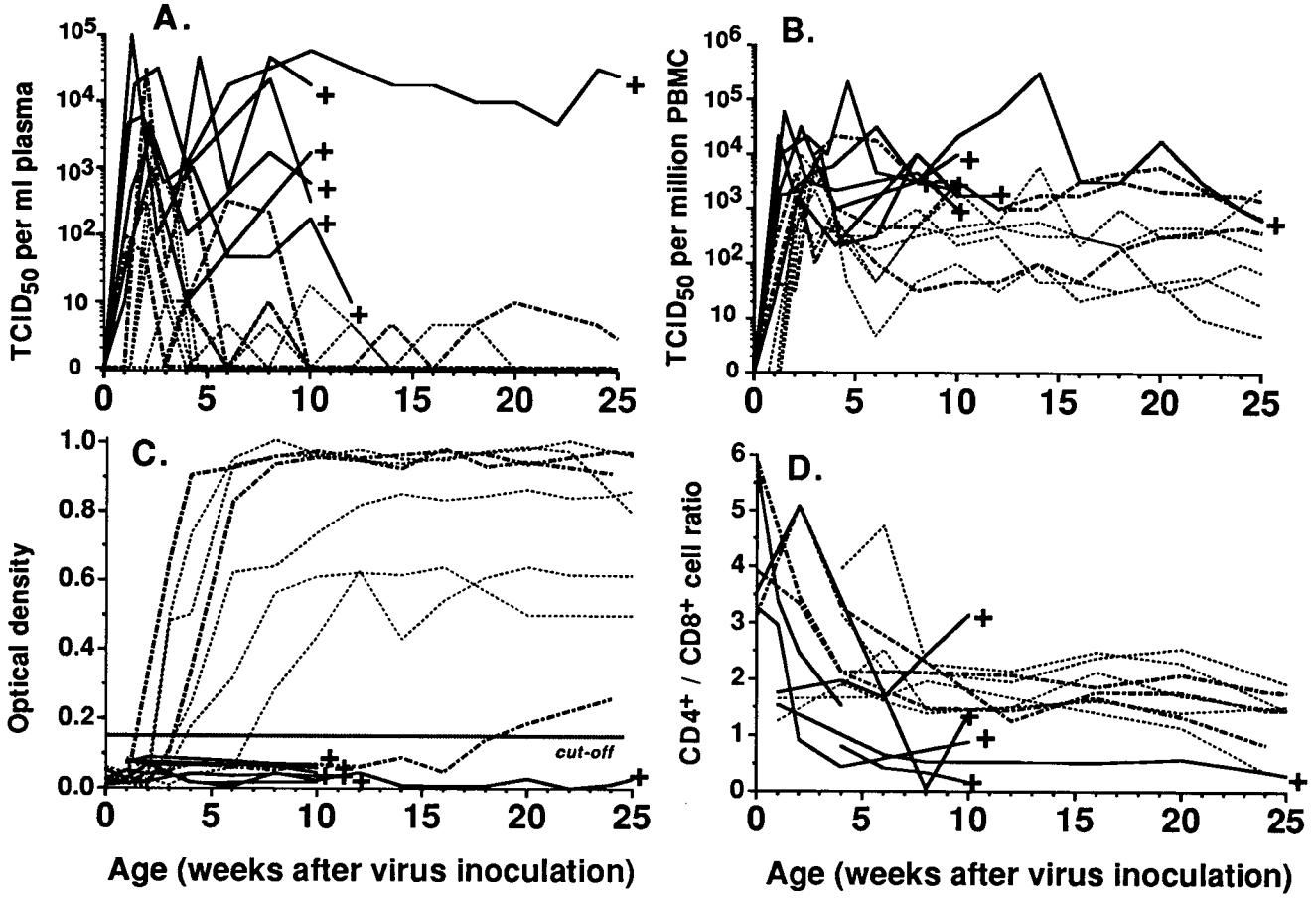

FIG. 2. Time course of SIV infection of newborn rhesus macaques and the therapeutic effects of AZT. AZT-treated animals, with AZT treatment (25 mg/kg 3 times daily) starting either prior to, simultaneously with (Table 1, groups 2 and 3) (thin dotted lines), or 1 week after (Table 1, group 4) (dashed and dotted lines) virus inoculation, are compared with the untreated (solid lines) SIV-infected rhesus neonates. Euthanasia because of simian AIDS is indicated (+). Cell-free (A) and cell-associated (B) virus loads were determined by limiting dilution culture of plasma and PBMC, respectively. SIV-specific IgG titers (C) were measured by ELISA; $CD4^+/CD8^+$ cell ratios (D) were determined by flow cytometry.

erage ± 1 standard deviation] for nursery-reared rhesus infants at the California Regional Primate Research Center [data not shown]).

At 4 and 10 weeks after virus inoculation, three of the six untreated and all eight AZT-treated SIV-infected animals were tested for SIV-specific CTL responses by a chromium release assay that is able to detect these responses in most SIV-infected adolescent and adult rhesus macaques (19). SIV-specific CTLs were not observed in any of the untreated SIV-infected animals but were detected in two of eight AZT-treated SIV-infected animals (Fig. 3). This difficulty in detecting SIV-specific CTLs following neonatal SIV infection of rhesus macaques resembles observations for HIV-infected human infants: HIV-specific cytolysis is often undetectable in vertically infected infants by means that readily detect these responses for adults (7, 21, 22).

Only two AZT-treated animals developed fatal disease during the observation period, at 7 and 10 months, respectively (Table 1). Both animals had recurrent diarrhea and dehydration (which failed to respond to supportive fluid and standard antibiotic treatment) and progressive weight loss starting about a month prior to euthanasia. Neither of these animals had detectable SIV-specific CTL responses, while both had high anti-SIV IgG levels. Plasma viremia or p27 antigenemia was undetectable in both animals, and $CD4^+/CD8^+$ cell ratios at

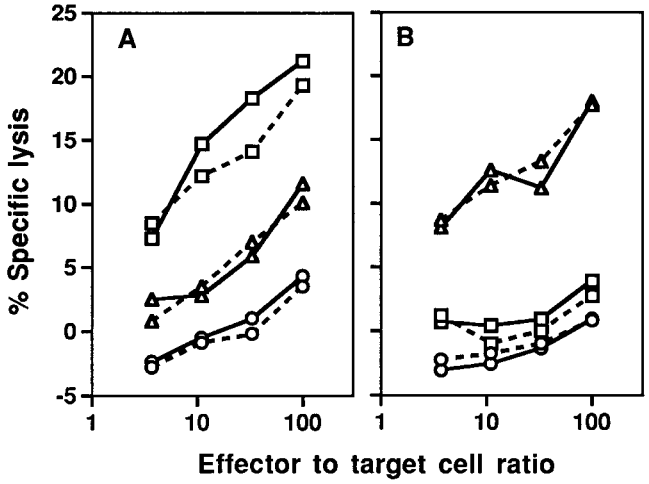

FIG. 3. SIV-specific CTL responses were detected in two of the eight AZT-treated SIV-infected animals. Specific lysis of autologous B cells infected with either wild-type vaccinia virus (circles) or SIV gag-expressing (squares) or SIV env-expressing (triangles) vaccinia virus recombinants was measured at 4 weeks (dashed lines) and 10 weeks (solid lines) after SIV inoculation. One animal (Table 1, group 3) has CTL against both gag and env, and the other one (Table 1, group 2) has CTL against env only (panels A and B, respectively).

the time of death were 0.18 and 0.97, respectively. The most important histopathologic finding for the first animal, which had persistent thrombocytopenia starting 4 months after virus inoculation, was an unexplained megakaryocytic myelosis. The second animal had lymphocytic enterocolitis and marked thymic atrophy. Its low cell-associated virus load in peripheral blood as well as in lymphoid tissues (<1 infected cell per 10^5 to 10^6 cells) and the absence of detectable plasma viremia suggest that virus replication in this second animal was limited. There was no indication of AZT toxicity. Thus, the lymphocytic enterocolitis in this animal may have been unrelated to SIV infection. All other AZT-treated animals remained healthy at least 12 to 15 months after virus inoculation (Table 1).

Drug-resistant mutants of HIV are often isolated from symptomatic children and adults after prolonged antiviral drug treatment and are found sooner in late stages than in early stages of HIV infection (12, 32, 34). To detect AZT-resistant SIV mutants, virus isolates from the eight AZT-treated SIV-infected animals after 6 to 10 months of AZT therapy were tested for AZT sensitivity. Virus isolates from seven AZT-treated animals (including the two AZT-treated animals that died) were AZT sensitive (IC_{50} between 0.05 and 0.2 μM). Emerging AZT resistance was, however, detected in one animal (Table 1, group 2): while virus isolated from this animal after 3 months of AZT therapy was still AZT sensitive ($IC_{50} \leq$ 0.2 μM AZT), virus isolated after 8 months of AZT therapy showed a high level of resistance (>100-fold increase in IC_{50}). This is the first time an AZT-resistant mutant has been isolated from an animal after prolonged AZT treatment. This animal had detectable anti-env CTL activity (Fig. 3B), a moderate cell-associated virus load (1 infected cell per 10^3 PBMC), but no plasma viremia; it had episodes of thrombocytopenia and lymphopenia starting 5 months after inoculation but was clinically healthy at 15 months. Because wild-type (i.e., AZT-sensitive) and AZT-resistant viruses coexist in this animal, the clinical significance of the AZT-resistant virus mutant in this animal is uncertain. Further characterization of this AZT-resistant mutant should allow identification of genetic changes and comparison with the mutations in HIV-1 reverse transcriptase that have been reported to confer AZT resistance (14, 17).

DISCUSSION

Results from this study with SIV-infected newborn rhesus macaques complement a recent interim analysis of a human trial showing that AZT reduces, but does not eliminate, vertical transmission of HIV (7a, 25). The present study of SIV-infected newborn macaques extends this demonstration of the prophylactic potential of AZT (41) and strongly suggests that prolonged AZT administration starting early after infection has tolerable side effects and may result in long-term clinical benefits for those infants that become HIV infected.

The most obvious side effect of prolonged oral AZT administration to newborn macaques was a moderate anemia, which resolved spontaneously and did not affect the growth of these animals. The spontaneous reduction of bone marrow toxicity in our rhesus newborns as they aged is most likely due to increased maturation of the hepatic and renal clearance pathways for AZT (20). Similar bone marrow toxicity has also been observed during AZT administration to infant macaques (41) and human adults and newborns (6, 33).

In this study, AZT had strong antiviral effects. Without AZT treatment, SIV-inoculated rhesus newborns had rapid and persistently high-level viremia, rapid immunosuppression, poor antiviral immune responses, and rapid disease progression. In contrast, early oral AZT treatment of newborns either prevented infection or delayed and reduced the initial viremia, presumably allowing the host to mount an anti-SIV immune response and resulting in delayed disease progression. Although infection can probably only be prevented if AZT is given prior to or immediately after exposure, our study showed that AZT administration starting 1 week after infection still had a favorable effect on the disease course, which suggests that AZT treatment starting at birth would still benefit newborns who became infected late in utero. Data for this newborn animal model do not support concerns that AZT, by reducing the initial viremia, might decrease or delay the antiviral immune response (28, 39), because most AZT-treated, SIV-infected animals mounted a rapid vigorous antiviral antibody response during AZT treatment.

We inoculated animals at birth because the rate of vertical transmission in macaques, as for humans, is low (26). The absence of maternal antibodies in these newborn macaques probably resulted in a more uniformly rapid disease development than in HIV-infected human infants, where levels of maternal antibodies vary and are likely to influence disease progression. The absence of maternal antibodies, however, made it easier to detect beneficial effects of chemotherapy. In addition, as untreated newborn macaques were not able to mount a detectable anti-SIV response following SIV_{mac} inoculation, the therapeutic effect of the drug was even more pronounced. This may explain why this is the first study to show that AZT clearly delayed disease progression in SIV-infected macaques. In previous antiviral drug studies done with older animals, it was often difficult to determine whether small differences in disease outcome were due to host factors or to the drug, because animals aged 3 months or older generally mount a strong immune response; in addition, the time between virus inoculation and progression to clinical immunodeficiency or fatal disease varies significantly among older animals and may occur from months to years after infection (42).

In contrast, because of the predictably rapid and fulminant disease progression of SIV_{mac} infection in newborns, effects of drug prophylaxis and therapy can be evaluated more reliably and quickly than with older animals. Therefore, this animal model of pediatric AIDS is very useful for testing chemo- and immunotherapeutic strategies, as efficacy can be assessed by monitoring clinical disease progression as well as viral and immune parameters, by using a limited number of animals and in a relatively short period of time (42).

This SIV-newborn rhesus model, which also allows rapid evaluation of the virulence of viral variants (23), will be useful to determine the clinical implications of antiviral drug resistance. While in humans, the causality between emergence of drug-resistant mutants of HIV and clinical disease progression is still unclear (32), inoculation of newborn rhesus macaques with drug-resistant SIV mutants provides a system to determine if drug resistance alters viral virulence and how this affects the efficacy of antiviral drug treatment.

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