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Viral Factors Determine Progression to AIDS in Simian Immunodeficiency Virus-Infected Newborn Rhesus Macaques

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To evaluate how viral variants may affect disease progression in human pediatric AIDS, we studied the potential of three simian immunodeficiency virus (SIV) isolates to induce simian AIDS in newborn rhesus macaques. The three virus isolates were previously shown to range from pathogenic (SIVmac251 and SIVmac239) to nonpathogenic (SIVmac1A11) when inoculated intravenously into juvenile and adult rhesus macaques. Six newborn macaques inoculated with pathogenic, uncloned SIVmac251 developed persistent, high levels of cell-associated and cell-free viremia, had no detectable antiviral antibodies, and had poor weight gain; these animals all exhibited severe clinical disease and pathologic lesions diagnostic for simian AIDS and were euthanatized 10 to 26 weeks after inoculation. Two newborns inoculated with pathogenic, molecularly cloned SIVmac239 developed persistent high virus load in peripheral blood, but both animals had normal weight gain and developed antiviral antibodies. One of the SIVmac239-infected neonates exhibited pathologic lesions diagnostic for SAIDS and was euthanatized at 34 weeks after inoculation; the other SIVmac239-infected neonate remained alive and exhibited no significant clinical disease for more than 1 year after inoculation. In contrast, three newborn rhesus macaques inoculated with the nonpathogenic molecular clone, SIVmac1A11, had transient, low-level viremia, seroconverted by 10 weeks after inoculation, had normal weight gain, and remained healthy for over 1 year. These results indicate that (i) newborn rhesus macaques infected with an uncloned, virulent SIVmac isolate have a more rapid, fulminant disease course than do adults inoculated with the same virus, (ii) the most rapid disease progression is associated with lack of a detectable humoral immune response in SIV-infected infant macaques, (iii) a molecularly cloned, attenuated SIV isolate is nonpathogenic in neonatal macaques, and (iv) SIV-infected neonatal macaques exhibit patterns of infection, virus load, and disease progression similar to those observed in human immunodeficiency virus-infected children. This SIV/neonatal rhesus model of pediatric AIDS provides a rapid, sensitive model with which to compare the virulence of SIV isolates and to study the mechanisms underlying the differences in disease progression in human immunodeficiency virus-infected infants.

Infants infected with human immunodeficiency virus (HIV) often develop immunodeficiency and die sooner after infection than do HIV-infected adults. About one third of HIV-infected infants have a rapidly fatal disease course and die within 1 year, while the majority develop clinical disease more slowly and survive for more than 5 years (reviewed in references 37 and 50). The levels or types of maternal antiviral antibodies, the timing of vertical transmission, and transmission of distinct maternal viral variants have been proposed to explain this bimodal pattern of disease progression observed in HIV-infected children (reviewed in references 37 and 50). Because the virus transmitted to infants represents one (35, 43, 51) or a few (20) minor variants in the blood of HIV-infected mothers, it has been proposed that rapid disease in HIV-infected infants may be due to transmission of highly pathogenic virus strains (reviewed in reference 33). However, the specific role of HIV variants in infant pathogenesis is difficult to assess because multiple factors affect the onset or the severity of disease.

The goal of this study was to evaluate the effects of viral variants on disease progression in newborn rhesus macaques infected with distinct simian immunodeficiency virus (SIV) isolates. SIV infection of adult and juvenile rhesus macaques is a well-established animal model for HIV infection of humans (reviewed in references 9 and 14). Variants of SIV have been isolated from rhesus macaques (SIVmac), and several have been molecularly cloned and sequenced; the biological properties of cloned and uncloned SIVmac isolates have been characterized in vitro as well as in juvenile and adult macaques (reviewed in reference 16).

In the experiments described here, three SIV variants whose virulence had been assessed previously in juvenile and adult animals were tested in newborn rhesus macaques. An uncloned isolate, SIVmac251 (21, 22, 36), and the molecularly cloned virus, SIVmac239 (17, 36), both produce a persistent infection and cause fatal immunodeficiency disease similar to AIDS in juvenile and adult rhesus macaques. In contrast, infection of rhesus macaques with the molecularly cloned virus SIVmac1A11 produces a transient cell-associated viremia and no immunodeficiency (26, 27). Although the nucleotide sequences of SIVmac1A11 and SIVmac239 differ by only about 2% (25, 40), the precise genetic differences that attenuate SIV virulence remain to be determined (26). We tested the hypothesis that the developing neonatal rhesus immune system would permit virus to persist and disease to progress more rapidly in newborn than in juvenile or adult rhesus macaques infected with any of the three SIV isolates. The results of this study demon-

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strate that the relative virulence of the three SIVmac isolates in neonatal macaques was similar to the relative virulence of these viruses observed previously in adults. In addition, only the uncloned pathogenic virus isolate, SIVmac251, produced a pattern of disease in infant macaques that was accelerated compared with disease in older macaques.

MATERIALS AND METHODS

Animals. Eleven newborn rhesus macaques (*Macaca mulatta*) from simian type D retrovirus and SIV-seronegative dams at the California Regional Primate Research Center were removed from their mothers and reared in a primate nursery in accordance with American Association for Accreditation of Laboratory Animal Care standards. We adhered to the *Guide for the Care and Use of Laboratory Animals* (7a). When necessary, animals were immobilized with ketamine HCl (Parke-Davis, Morris Plains, N.J.), 10 to 15 mg/kg of body weight injected intramuscularly. Each neonatal macaque was inoculated intravenously within 72 h of birth with one of the cell-free virus stocks described below. Samples were collected immediately before virus inoculation and regularly thereafter for monitoring viral and immunologic parameters: 0.5 to 1 ml of heparinized blood weekly for the first month, every 2 weeks for the next 4 months, and then monthly; and cerebrospinal fluid (CSF) at 2, 4, 8, and 12 weeks postinoculation (p.i.) SIV-infected infant macaques were euthanized as approved by the Animal Use and Care Committee, University of California, Davis. Euthanasia was indicated by three or more of the following clinical observations: (i) weight loss of greater than 10% in 2 weeks or 30% in 2 months, (ii) chronic diarrhea unresponsive to treatment, (iii) infections unresponsive to antibiotic treatment, (iv) inability to maintain body heat or fluids without supplementation, (v) persistent, marked hematologic abnormalities, including lymphopenia, anemia, thrombocytopenia, or neutropenia, and (vi) persistent, marked splenomegaly or hepatomegaly. A complete necropsy including gross and microscopic tissue examination was performed for each euthanized animal as described below.

Viruses. Three cell-free SIV stocks were used in these studies. The two molecularly cloned viruses, SIVmac1A11 and SIVmac239, have been previously described (3, 27, 36). SIVmac239 was grown on CEMX174 cells and contained 10^5 50% tissue culture infectious doses ($TCID_{50}$)/ml (26). SIVmac1A11 was propagated on rhesus peripheral blood mononuclear cells (PBMC) (24) and contained 10^6 $TCID_{50}$/ml. The uncloned SIVmac251 stock (obtained from Ronald C. Desrosiers, New England Regional Primate Research Center) was grown in human PBMC and contained 10^3 $TCID_{50}$/ml (31). The SIVmac251 stock and the SIVmac239 stock have been shown to cause persistent viremia and simian AIDS (SAIDS) in juvenile and adult rhesus macaques at the California Regional Primate Research Center (26, 32). The SIVmac1A11 stock causes transient cell-associated viremia and no disease in juvenile and adult rhesus macaques (24, 26, 28). All virus stocks were titered by endpoint dilution in CEMX174 cells as described previously (47) and stored frozen at −135°C without cryopreservatives. Aliquots of the SIV stocks were thawed at 37°C; appropriate dilutions were made by using sterile phosphate-buffered saline and then inoculated intravenously.

Quantitative virus isolation. Cell-associated and cell-free virus load in peripheral blood was determined by endpoint dilution culture (four replicates per dilution) of PBMC and plasma, respectively, with CEMX174 cells in 24-well plates (47). Aliquots of the culture medium were assayed regularly for the presence of SIV major core protein (p27) by antigen capture enzyme-linked immunosorbent assay (ELISA) (23). A detailed description of the technique and criteria to determine if an aliquot was antigen positive has been published elsewhere (26, 47). Cultures were considered positive if they were antigen positive at two consecutive time points. Endpoint dilution cultures were maintained and tested for 4 weeks before being scored as virus negative. In addition, for animals with low or undetectable virus load, 1×10^6 to 5×10^6 PBMC were cocultivated for 8 weeks with CEMX174 cells in tissue culture flasks as described previously (26). Virus levels were calculated by the method of Reed and Muench (39) and expressed as $TCID_{50}$ per 10^6 PBMC or per milliliter of plasma (47). Cell-free virus in CSF was detected by culture of 0.1 to 0.5 ml of filtered (0.45-μm-pore-size filter) CSF for 8 weeks with CEMX174 cells in tissue culture flasks as described previously (19).

PCR amplification. Nested PCR was carried out in a GeneAmp 9600 (Perkin-Elmer Cetus, Emeryville, Calif.). Two rounds of 30 cycles of amplification were performed on aliquots of plasmid DNA containing the complete genome of SIVmac1A11 (27) (positive control) or aliquots of PBMC lysates, using SIVmac-specific *gag* primers and conditions described elsewhere (26, 46). This nested PCR amplification procedure allows visual detection of a single copy of SIV *gag* sequences in as many as 200,000 PBMC (46).

SIV-specific antibody responses. SIV-specific antibodies in plasma were measured by ELISA, using SIVmac251 grown in HUT 78 cells as the antigen, by a modification of a method previously described (28). For these experiments, SIVmac251 was concentrated by centrifugation, disrupted in 1% sodium dodecyl sulfate, and coated onto microtiter ELISA plates (Falcon 3912; Becton Dickinson) at 100 ng of total protein per well. The SIV-coated plates were incubated with test or control plasma samples (diluted 1:100), washed, then incubated with 1:1,000-diluted enzyme-conjugated goat anti-monkey immunoglobulin G (Nordic, Capistrano Beach, Calif.), washed, and incubated with *o*-phenylenediamine (Sigma, St. Louis, Mo.) substrate, and the results were read spectrophotometrically. Each sample plasma and the positive control plasma (from an SIV-infected animal) were assayed in duplicate, and mean values of optical density (OD) were calculated. Samples were considered positive if the mean OD value was greater than 0.100 and exceeded three times the mean OD value of plasma obtained preinoculation. Immunoblots were performed to detect specific SIV proteins as described previously (45).

Necropsy collection and preparation of tissue samples. A complete necropsy examination was performed on all animals that died during the course of the study. Tissues collected at necropsy were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 6 μm, stained with hematoxylin and eosin, and examined by light microscopy.

Statistical analyses. Statistical analysis was used to compare the survival and virus load among neonatal rhesus macaques infected with each of the three SIVmac isolates and to compare the growth rates between uninfected and SIV-infected infant rhesus macaques. Survival was compared by the generalized Wilcoxon test (8). Cell-associated and cell-free virus levels during the first 10 weeks after virus inoculation were compared among the three groups of SIV-infected infant macaques by calculating the area under the curve without logarithmic transformation for each animal, and the area under the curve was then analyzed according to the Wilcoxon rank-sum test (8). Growth rates (weight gained in grams per day) during the first 10 weeks of life were calculated for more than 50 uninfected control infant rhesus macaques reared at the California Regional Primate Research Center and for SIV-infected infant rhesus macaques by performing regression analysis on daily body weight measured in kilograms during the first 10 weeks of age, using Microsoft Excel (version 5.0) software (Microsoft, Inc., Seattle, Wash.). The slopes (growth rates) of regression lines for daily weights of each group of SIV-infected neonates and of control neonates were compared by *Z* test for parallelism as described previously (18).

RESULTS

Outcome after intravenous inoculations. The pathogenicity of three SIV variants in neonatal rhesus macaques was evaluated by intravenous inoculation of 11 monkeys with cell-free virus. The design of the study and the dose of virus used for the inoculations are based on our experience with these virus stocks in juvenile and adult rhesus macaques (19, 26). In older rhesus macaques, uncloned SIVmac251 is uniformly pathogenic with a relatively short disease course (19, 21, 22, 36); infection with the molecular clone SIVmac239 is uniformly pathogenic but has a less rapid disease course than does SIVmac251 infection (17, 22, 26). Infection of older rhesus macaques with SIVmac1A11 does not result in disease (24, 26, 28). Thus, to compare the disease courses of neonatal and older macaques infected with the uncloned SIV and the molecular clones, the neonates were inoculated with doses of virus similar to those used in studies involving older animals (19, 26). Six neonates were inoculated with 10 $TCID_{50}$ of uncloned SIVmac251, two neonates were inoculated with 100 $TCID_{50}$ of molecularly cloned SIVmac239, and three neonates were each inoculated with 200,000 $TCID_{50}$ of molecularly cloned SIVmac1A11 (Table 1). By using the lowest dose of virus for the animals inoculated with the highly pathogenic SIVmac251 and the highest dose of virus for the animals inoculated with the attenuated clone SIVmac1A11, we increased the likelihood of observing high virus load or rapid disease in neonatal macaques infected with the two molecularly cloned SIV isolates.

Virus was recovered from the PBMC of each of the 11 animals during the first 2 weeks after inoculation, demonstrating that the viruses were all infectious in neonatal macaques (Fig. 1A). The patterns of subsequent virus isolation from PBMC, plasma, and CSF varied among the three groups, although some variation among animals within each group occurred, as is observed for older SIV-infected macaques (26).

Neonates inoculated with SIVmac251. SIVmac251 was the most rapidly fatal of the three SIV isolates tested in newborn macaques (Fig. 1; Table 2). Persistently high (10^3 to 10^5 $TCID_{50}$/10^6 PBMC) cell-associated viremia was observed in all animals inoculated with SIVmac251 (Fig. 1A). High levels (10 to 10^5 $TCID_{50}$/ml) of virus were also recovered from plasma of

TABLE 1. Experimental design for study of pathogenesis of disease for SIV variants in neonatal rhesus macaques

SIVmac stock (virulence)[a]	Virus dose ($TCID_{50}$)	Animal no.	Age at virus inoculation[b] (days)
251, uncloned, (pathogenic)	10	27159	0
		27161	0
		27164	1
		27318	3
		27328	3
		27370	1
239, molecular clone (pathogenic)	10^2	27971	3
		28214	3
1A11, molecular clone (attenuated)	2×10^5	27311	2
		27315	2
		27403	0

[a] The ability to cause fatal immunodeficiency in juvenile and adult rhesus macaques.
[b] Day of birth = day 0. All animals were inoculated intravenously within 72 h after birth.

each of the SIVmac251-infected infants at all times sampled. This differs from the transient plasma viremia seen in older macaques infected with SIVmac251. SIV was recovered at least once from the CSF of four (27164, 27318, 27328, and 27370) of six SIVmac251-infected neonates (Table 3). Detection of SIV in the CSF was not directly associated with specific clinical or morphological neurologic abnormalities; virus was recovered in multiple CSF samples from one (27328) of the two animals with encephalitis at the time of necropsy, but no SIV was detected in CSF samples from the other neonate (27161) with encephalitis (Table 3; see also Table 5). No SIV-specific antibody responses were detected in any of the six SIVmac251-infected animals at any time point by immunoblotting or ELISA (Table 4).

Table 5 summarizes the signs of clinical disease, time of death, and necropsy findings for all SIVmac251-infected neonates. By 4 to 8 weeks p.i., clinical disease signs were apparent in animals infected with SIVmac251. The most frequently observed clinical signs were recurrent bacterial infections, chronic diarrhea and wasting, loss of appetite, splenomegaly, and skin rash. Five (27159, 27161, 27164, 27328, and 27370) of the six SIVmac251-infected neonates experienced poor weight gain (significantly lower [$P < 0.01$] than the uninfected control infant weight gain of 6.2 g/day; Table 2) from 6 to 12 weeks p.i. (Fig. 2A).

Five of six SIVmac251-infected neonates had SAIDS and were euthanized at between 10 and 12 weeks p.i., and the remaining animal (27318) was euthanized at 26 weeks p.i. (Table 5). For all six SIVmac251-infected infants, the clinical abnormalities and pathologic changes (including failure to thrive, enteritis, pneumonia, opportunistic bacterial infections, lymphadenopathy, and encephalitis) observed at necropsy were consistent with terminal stages of SIV infection (Table 5). The pathologic findings at necropsy varied among the SIVmac251-infected infants, but colitis (six of six neonates) and interstitial pneumonia (five of six neonates) were routinely observed (Table 5). In one SIVmac251-infected animal (27328) with torticollis, cytomegalovirus meningoencephalitis and mineralization of cerebral blood vessels were observed.

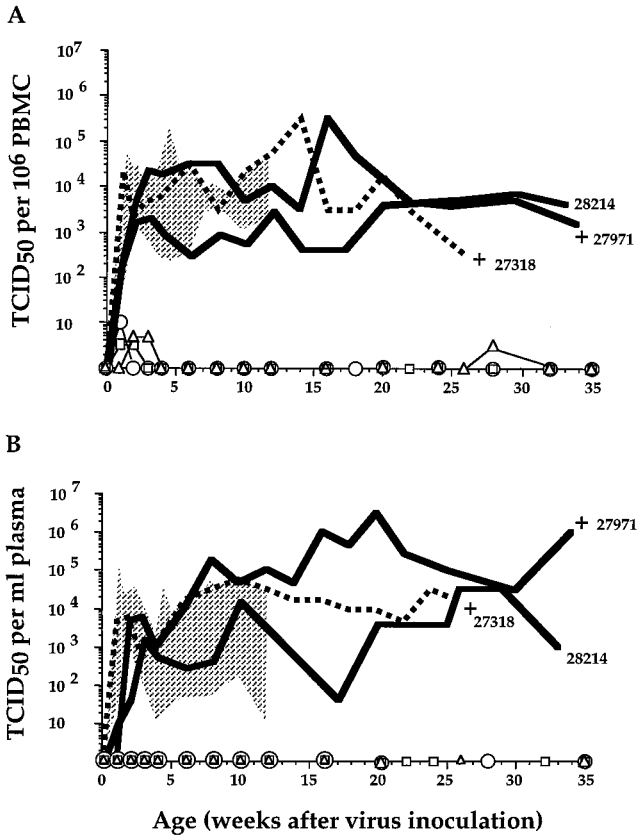

FIG. 1. Virus load in PBMC of newborn rhesus macaques over time after intravenous inoculation with SIV variants. Weeks after inoculation are shown along the *x* axis. The PBMC-associated (A) or plasma (B) viremia is shown on the *y* axis. The $TCID_{50}$ per 10^6 PBMC (A) or per milliliter of plasma (B) from each sample was determined as described in Materials and Methods. The shaded region (0 to 12 weeks) indicates the range of values observed for the six SIVmac251-infected animals; five of the six macaques had SAIDS and were killed by 12 weeks p.i. The dashed line represents the single SIVmac251-infected macaque (27318) that survived until 26 weeks p.i. Each heavy line represents one SIVmac239-infected macaque. Each line with open symbols represents one of the three SIVmac1A11-infected animals (triangles, 27311; squares, 27315; circles, 27403). Death with SAIDS is indicated (+).

Neonates inoculated with SIVmac239. Despite inoculation with a dose of infectious virus 10-fold higher than that used for animals inoculated with SIVmac251, neonatal monkeys infected with the molecularly cloned SIVmac239 had a longer clinical latency than those infected with SIVmac251. Both SIVmac239-infected neonates had persistent high viremia and developed moderate, persistent splenomegaly by 4 weeks p.i.; however, both animals had early weight gains exceeding those of control infants (Fig. 2A; Table 2) and survived with no other clinical signs consistent with SAIDS for more than 32 weeks p.i. One SIVmac239-infected animal (27971) euthanized at 34 weeks p.i. exhibited pathologic changes (including colitis, interstitial pneumonia, disseminated adenovirus infection, marked enlargement of the spleen, and meningoencephalitis) consistent with terminal stages of SIV infection (Table 5). The other SIVmac239-infected macaque remained alive at the end of the observation period (54 weeks p.i.).

As in the SIVmac251-infected infant macaques, persistently high (10^2 to 10^6 $TCID_{50}/10^6$ PBMC) cell-associated and plasma viremia was observed in both SIVmac239-infected neonates (Fig. 1A). Virus was also recovered from plasma of each the SIVmac239-infected infants at all times sampled. This re-

TABLE 2. Comparison of survival times, virus loads, and growth rates for neonatal rhesus macaques inoculated with SIV variants

SIVmac stock (virulence)[a]	Animal no.	Survival after inoculation[b] (wk)	Virus load[c]		Growth rate[d] (99% CI)
			Plasma	PBMC	
251, uncloned (pathogenic)	27159	10	8.0	53.6	3.7 (3.3–4.0)
	27161	10	12.4	30.4	2.0 (1.5–2.5)
	27164	10	125.8	87.3	3.3 (2.5–4.1)
	27318	26	163.4	112.2	6.3 (6.1–6.5)
	27328	10	284.9	284.4	4.1 (3.7–4.5)
	27370	12	5.6	32.0	3.9 (3.3–4.5)
	Avg		100	100	
239, molecular clone (pathogenic)	27971	34	420.0	169.9	7.0 (6.6–7.4)
	28214	>52	21.2	7.7	7.8 (7.2–8.4)
	Avg		220.6	88.8	
1A11, molecular clone (attenuated)	27311	>52	0	0.01	6.5 (6.2–6.8)
	27315	>52	0	<0.01	6.5 (6.0–6.9)
	27403	>52	0	0.01	6.4 (6.0–6.8)
	Avg		0	0.01	

[a] The ability to cause fatal immunodeficiency in juvenile and adult rhesus macaques.

[b] All three SIVmac1A11-infected animals survived longer than SIVmac251-infected animals ($P < 0.05$) or SIVmac239-infected animals ($P < 0.10$). SIVmac239-infected macaques survived longer than SIVmac251-infected animals ($P < 0.05$).

[c] Comparisons of cell-free (plasma) and cell-associated (PBMC) virus titers were performed by calculation and analysis of the area under the curve in Fig. 1 as described in Materials and Methods. The average area-under-the-curve value for SIVmac251-infected animals was assigned a reference value of 100%. Values for individual animals and each virus-infected group are shown. Both plasma and PBMC-associated virus levels in SIVmac1A11-infected animals were lower than in SIVmac251-infected ($P < 0.01$) or SIVmac239-infected ($P < 0.05$) animals. Virus titers in plasma or PBMC of SIVmac251-infected and SIVmac239-infected animals were not different ($P > 0.50$).

[d] Regression analysis of daily body weight (kilograms) during the first 10 weeks was performed as described in Materials and Methods. Growth rate (weight gained in grams per day) for each animal is shown with the 99% confidence interval (CI). Growth rates for five of the six SIVmac251-infected neonates that died within 12 weeks p.i. were significantly lower ($P < 0.01$) than those for uninfected control neonates (6.2 g/day, 99% CI = 6.0 to 6.4 g/day). Growth rates for SIVmac1A11-infected neonates were indistinguishable from those for control neonates. Growth rates for SIVmac239-infected neonates exceeded those for uninfected control rhesus neonates ($P < 0.01$).

sult differs from the transient plasma viremia seen in older macaques infected with SIVmac239. During the first 2 weeks, virus levels in the plasma samples of SIVmac239-infected neonates were 10- to 1,000-fold lower than levels in plasma samples of SIVmac251-infected neonates (Fig. 1B). From 4 to 12 weeks p.i., the levels of virus in plasma samples of the SIVmac251- and SIVmac239-infected animals were similar and ranged from 10 to 10^5 $TCID_{50}$/ml. However, the one SIVmac239-infected infant that survived longest (28214) exhibited from 10- to 10,000-fold lower virus load in plasma than did the other animal (27971) from 6 weeks p.i. through the remainder of the observation period (Fig. 1B). SIV was recovered at least once from the CSF of both of the SIVmac239-infected infant macaques; SIV was recovered multiple times from the CSF of the animal (27971) that had meningoencephalitis at the time of necropsy (Table 3).

In contrast to the SIVmac251-infected infants, SIV-specific antibodies were detected in the plasma samples of all SIVmac239-infected neonates by 10 weeks p.i. (Table 4). Unlike the strong antiviral antibody response in juvenile or adult macaques infected with SIVmac239 (26, 27, 36), weak antibodies to viral proteins were observed in the SIVmac239-infected newborns. The two SIVmac239-infected infant macaques had stronger antibody responses to Gag (p17, p27, and p55) than to envelope (gp120) protein (Table 4).

Neonates inoculated with SIVmac1A11. SIVmac1A11 infection induced no disease in neonatal macaques even though the neonates were inoculated with a viral dose (200,000 $TCID_{50}$) that was 10 to 100 times greater than that which produces infection in adults (19, 26, 27, 29). None of the three infant macaques infected with SIVmac1A11 had clinical signs of immunodeficiency during the observation period (>15 months p.i.), and all three animals had normal weight gain (Fig. 2B; Table 2). This result is identical to the outcome seen in older rhesus macaques infected with SIVmac1A11 (19, 26, 27, 29).

The transient, low levels of virus (a maximum of 10 $TCID_{50}$/10^6 PBMC) detected from 1 to 4 weeks p.i. in PBMC from newborn monkeys inoculated with SIVmac1A11 were similar to virus levels found in PBMC from SIVmac1A11-infected juvenile macaques (26). After the initial period (1 to 4 weeks p.i.) of viremia, virus was recovered from PBMC of only one (27311) of the three SIVmac1A11-infected infants in a single blood sample (28 weeks p.i.) (Fig. 1A), and SIV DNA was detected by PCR only in the PBMC from the same SIVmac1A11-infected animal (27311) sampled at 26 weeks p.i. (data not shown). Consistent with results for SIVmac1A11-infected adult and juvenile macaques, virus was not recovered from any plasma (Fig. 1B) or any CSF (Table 3) sample from the three neonates infected with SIVmac1A11 (19). All three of the SIVmac1A11-infected neonates were ELISA and immunoblot seropositive by 10 weeks p.i. (Table 4); each of the three animals had antibodies to viral Gag proteins, but only two of the three (27311 and 27315) had antibodies to viral envelope protein detected by immunoblot (Table 4).

DISCUSSION

Although this study used relatively few animals, neonatal rhesus macaques inoculated with one of three SIVmac isolates showed distinct patterns of virus load in peripheral blood,

TABLE 3. Recovery of virus from the CSF of SIV-infected neonatal rhesus macaques

SIVmac stock (virulence[a])	Animal no.	Virus recovered from CSF[b]			
		2 wk p.i.	4 wk p.i.	8 wk p.i.	12 wk p.i.
251, uncloned (pathogenic)	27159	−	−		NA[c]
	27161	−	−		NA[c]
	27164	−	+		NA[c]
	27318	+	−	−	−
	27328	−	+	+	NA[c]
	27370	+	+		−
239, molecular clone (pathogenic)	27971	+	+	−	+
	28214	−	+	−	−
1A11, molecular clone (attenuated)	27311	−	−	−	−
	27315	−	−	−	−
	27403	−	−	−	

[a] The ability to cause fatal immunodeficiency in juvenile and adult rhesus macaques.

[b] Results are presented as virus positive (+) or negative (−) as described in Materials and Methods; no symbol indicates that virus isolation was not performed. NA, not applicable.

[c] As noted in the text, this animal died by 10 weeks p.i.

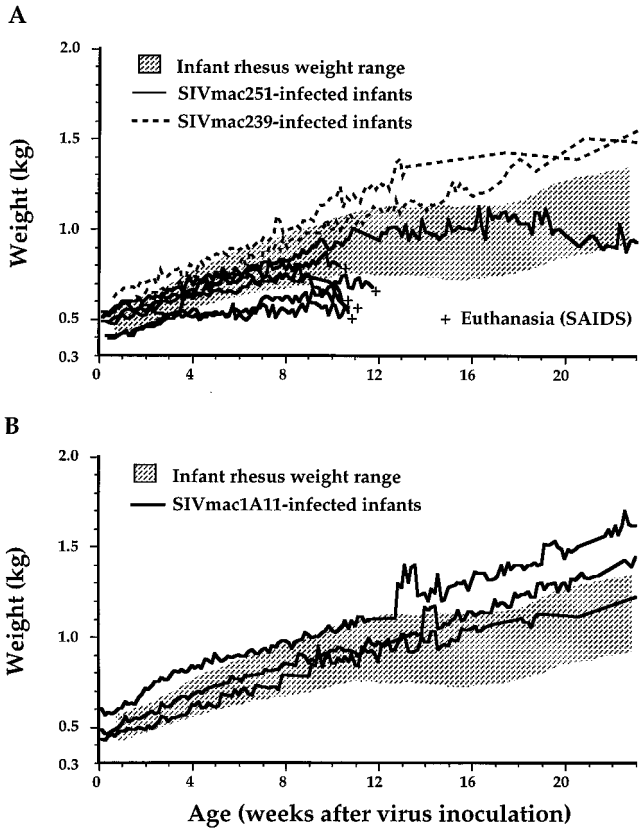

FIG. 2. Weight gain for newborn rhesus macaques inoculated with SIV variants. Weeks after inoculation are shown along the *x* axis. The *y* axis indicates weight in kilograms for macaques inoculated with pathogenic virus (uncloned SIVmac251 or molecularly cloned SIVmac239) (A) nonpathogenic virus (molecularly cloned SIVmac1A11) (B). Weights for SIV-infected animals are shown relative to the mean weights ± 1 standard deviation for more than 50 uninfected, age-matched controls (hatched area) that were reared at the California Regional Primate Research Center.

antiviral immune response, and clinical outcome. Uncloned SIVmac251 was the most rapidly fatal of the three viruses in neonatal rhesus macaques despite inoculation with the lowest virus dose. All neonates inoculated with SIVmac251 showed persistently high levels of cell-free and cell-associated viremia, no detectable antiviral antibodies, poor weight gain, and rapidly fatal immunodeficiency (five of six macaques died within 12 weeks p.i.). Although inoculated with a 10-fold-higher virus dose than SIVmac251-infected neonates, SIVmac239-infected neonates fared better. SIVmac239-infected neonates had persistently high levels of virus in plasma and PBMC, developed anti-SIV antibodies, and had a slower disease course (both animals showed good early weight gain and lived with few clinical signs for more than 32 weeks p.i.; one animal died by 34 weeks p.i.). Virus was isolated only from the CSF of neonates infected with SIVmac251 or SIVmac239; thus, detection of SIV in CSF samples was associated with high levels of cell-free and cell-associated viremia and viral virulence. In contrast, SIVmac1A11 was nonpathogenic in neonates even though animals were inoculated with a dose higher than that used in adults. SIVmac1A11-infected neonates had a transient, low-level cell-associated viremia, weak antiviral antibodies, normal weight gain, and no clinical disease during more than a year after infection.

The same spectrum of virulence was previously observed in older rhesus macaques inoculated with these three SIVmac isolates. However, in contrast to infant macaques, the majority of juvenile and adult animals infected with SIVmac251 or SIVmac239 have transient plasma viremia and strong antiviral antibodies (26, 32, 47). The SIVmac251-infected infants were the only animals that developed SAIDS more rapidly than adults. This accelerated disease progression and mortality has been reported for newborn macaques inoculated orally with SIVmac251 (2, 13) and macaque neonates inoculated intravenously with a different uncloned, pathogenic SIV isolate, SIVsm (5). Rapid onset of SAIDS is also observed in a small percentage of SIV-inoculated adolescent and adult macaques that fail to mount a strong immune response (17, 32).

A recent study (1) reported that a multiple-deletion variant (SIVΔ3) of the molecular clone SIVmac239 that does not cause disease in adult rhesus macaques (52) induced disease in orally inoculated neonatal macaques. In this paper, we report no difference in the virus load or the clinical outcome of infection for adults or neonates infected with the attenuated clone, SIVmac1A11. At least two factors may account for the difference in outcome for these studies. The routes of inoculation (oral versus intravenous) were different, and there were genetic differences between the viruses used. Comparison of the virus loads for neonates infected with each of the three cloned SIV isolates, SIVmac239, SIVΔ3, and SIVmac1A11, demonstrates a pattern of virus load that is similar to that observed for adult macaques infected with these viruses; i.e., virus load is highest for SIVmac239 infection, intermediate for SIVΔ3 infection, and lowest for SIVmac1A11 infection.

The only measured parameter that was associated with the most rapid disease course of the SIVmac251-infected neonatal macaques is the lack of antiviral antibody response. The inability of neonatal monkeys to mount an antiviral immune response to SIVmac251 infection may result from direct viral effects on the immune system that prevent development of antiviral antibody responses. The idea that this immunosuppression is determined not only by the level to which SIVmac251 replicates in neonates but also by additional factors

TABLE 4. Antiviral antibodies in plasma samples of SIV-infected neonatal rhesus macaques

SIVmac stock (virulence[a])	Animal no.	ELISA[b]	Immunoblot[c]				
			Gag			Envelope	
			p17	p27	p55	gp120	gp32
251, uncloned (pathogenic)	27159	−	−	−	−	−	−
	27161	−	−	−	−	−	−
	27164	−	−	−	−	−	−
	27318	−	−	−	−	−	−
	27328	−	−	−	−	−	−
	27370	−	−	−	−	−	−
239, molecular clone (pathogenic)	27971	+	−	+	+	w	−
	28214	+	−	+	+	+	−
1A11, molecular clone (attenuated)	27311	+	+	−	w	+	−
	27315	+	−	w	+	w	−
	27403	+	−	−	w	−	−

[a] The ability to cause fatal immunodeficiency in juvenile and adult rhesus macaques.

[b] Samples from 10 weeks p.i. tested at a dilution of 1:100. Results are presented as OD values greater than (+) or less than or equal to (−) those for negative control plasma as described in Materials and Methods.

[c] Samples from 10 weeks p.i. tested at a dilution of 1:100. The symbols indicate strong (+), weak (w), or no (−) detectable reactivity of plasma with SIV antigens.

specific to SIVmac251 is supported by the observation that SIVmac239 reaches similar levels in plasma and PBMC of neonates, but the SIVmac239-infected neonates have some antiviral immune response and survive longer. It is possible that individual or multiple viral phenotypes in the uncloned SIVmac251 stock used for these studies contribute to accelerated disease progression in neonatal rhesus macaques.

These results demonstrate that virus-specific factors account for the differential virulence of these three SIV variants in adult and neonatal rhesus macaques, which supports the hypothesis that differences in the virulence of HIV variants may be responsible for the bimodal distribution of mortality observed in human pediatric AIDS patients. The virologic and clinical parameters (e.g., persistent viremia, weak or undetectable antiviral immune responses, clinical abnormalities, and pathologic changes [4, 6, 11, 12, 15, 30, 38, 42, 44]) of HIV-infected human infants who rapidly develop immunodeficiency and SIVmac251-infected infant macaques are similar. HIV-infected infants with persistent viremia but slower disease progression resemble SIVmac239-infected infant macaques. Rhesus infants infected with the nonpathogenic virus isolate SIVmac1A11 have a transient cell-associated viremia and weak antiviral antibodies that wane over time. It is possible that some HIV-exposed infants who have no detectable viremia, no antiviral antibody responses (after disappearance of maternal antibodies), and no disease may have been infected with an attenuated HIV (7).

The consistent clinical course in the neonatal macaques infected with each of the three viruses makes this SIVmac/newborn rhesus model of pediatric HIV infection useful for determining the relative importance of host and viral factors in disease pathogenesis. Although the precise SIV genotypes that determine high versus low virulence are unknown, the in vitro growth characteristics of SIVmac isolates used in these experiments do not correlate with their disease potential in macaques. All of the SIV isolates used in this experiment cause cytopathology in cultures of rhesus PBMC and transformed T lymphocytes (3). Both uncloned, pathogenic SIVmac251 and attenuated SIVmac1A11 grow in monocytes/macrophages (3). Thus, as reported for other SIVmac isolates (10, 34, 41), in vitro monocyte/macrophage tropism alone was not associated with virulence in macaques in this study. These results indicate that the ability to induce cytopathology and the monocyte/macrophage tropism of HIV variants are biological properties that may be necessary but not sufficient to cause disease. In addition, persistent, high viremia may not be sufficient to induce rapid immunodeficiency in HIV-infected individuals. Thus, observations from experiments with these SIVmac variants in neonatal macaques emphasize the need to evaluate potential correlates of HIV virulence in an animal model. The pediatric SIV model is particularly valuable because infection with pathogenic SIVmac reliably produces disease in a reasonable experimental time frame (i.e., 3 to 6 months). SIV-infected rhesus infants have some of the same clinical and sur-

TABLE 5. Most prominent clinical disease signs and pathologic findings in neonatal rhesus macaques infected with pathogenic virus isolate SIVmac251 or SIVmac239

SIVmac isolate	Animal no.	Most prominent clinical signs of SAIDS	Time (wk p.i.) when killed	Most prominent pathologic findings at necropsy
251	27159	Recurrent diarrhea,[a] failure to thrive	10	Interstitial pneumonia, enterocolitis, cholecystitis
	27161	Persistent diarrhea,[a] failure to thrive	10	Encephalitis, interstitial pneumonia, enterocolitis
	27164	Recurrent diarrhea,[a] weight loss	10	Enterocolitis
	27318	Recurrent dermatitis, conjunctivitis, recurrent diarrhea,[a] failure to thrive	26	Interstitial pneumonia, embolic nephritis (*Citrobacter freundii*), gastroenterocolitis
	27328	Recurrent diarrhea,[a] failure to thrive, torticollis	10	Meningoencephalitis (cytomegalovirus), interstitial pneumonia, colitis
	27370	Recurrent diarrhea[a] and vomiting (*Campylobacter*), weight loss	12	Interstitial pneumonia, colitis
239	27971	Recurrent diarrhea[a]	34	Meningoencephalitis (cytomegalovirus), disseminated adenovirus infection, interstitial pneumonia, colitis

[a] Recurrent diarrhea that failed to respond to standard antibiotic therapy and that was not associated with recognized pathogens.

rogate markers of infection (e.g., weight gain, antiviral antibodies, and virus load in peripheral blood) as HIV-infected children, and fatal immunodeficiency occurs within weeks after infection with highly pathogenic virus (i.e., uncloned SIVmac251), making the neonatal rhesus macaque model of pediatric HIV infection useful for evaluating the efficacy of new therapies or vaccines for AIDS very rapidly (48, 49).

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