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vpr Deletion Mutant of Simian Immunodeficiency Virus Induces AIDS in Rhesus Monkeys

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In previous experiments, animals infected with SIVmac239 containing a point mutation in the *vpr* and *nef* genes developed AIDS-like symptoms after early reversion of the *vpr* and *nef* genes. Here we show that two animals in which the *nef* gene but not the *vpr* gene had reverted in the first few months did not develop disease during a 3-year observation period even after reversion to a functional *vpr* gene 70 weeks postinfection. To study the influence of a stable *vpr* mutation on virus load and pathogenesis, a 43-bp deletion was introduced into the *vpr* gene of SIVmac239on, a *nef*-open mutant of SIVmac239. Four rhesus monkeys were inoculated with the *vpr* deletion mutant (SIVΔvpr), and two control animals were infected with SIVmac239on. Both control animals had persistent antigenemia, high cell-associated virus loads, and elevated neopterin levels. They had to be euthanized 20 and 30 weeks postinfection because of AIDS-related symptoms. However, all four rhesus monkeys inoculated with SIVΔvpr showed only transiently detectable antigenemia. The cell-associated virus loads were high in three of the four animals. Two animals with AIDS-like symptoms had to be euthanized 71 and 73 weeks postinfection. The two remaining monkeys infected with SIVΔvpr were still alive 105 weeks postinfection. In contrast to the SIVmac239on-infected animals, SIVΔvpr-infected animals had strong humoral immune responses and intermittent cellular immune responses to SIV antigens. Our data show that a functional *vpr* gene is not necessary for pathogenesis. However, *vpr*-deficient SIVmac239 variants might be slightly attenuated, allowing some animals to resist progression to disease for an extended period of time.

In addition to the *gag*, *pol*, and *env* genes, which are present in the genomes of all retroviruses, human immunodeficiency viruses (HIV) and simian immunodeficiency viruses (SIV) express at least five regulatory proteins. The genes *tat* and *rev* are essential for virus replication, while *vif*, *nef*, *vpu*, *vpr*, and *vpx* can be dispensable in vitro. The fact that the reading frames of the auxiliary genes are conserved suggests that they are important in vivo, as recently demonstrated for the *nef* gene (16). The accessory protein Vpr is highly conserved among all primate immunodeficiency viruses (23). It is incorporated into the virus particle by association with the Gag protein (3, 21, 29, 30), suggesting a function of Vpr early in the viral replication cycle. An arginine-rich sequence in the carboxy-terminal part of Vpr functions as a nuclear localization signal in the absence of Gag (21). Vpr facilitated the nuclear localization of viral nucleic acids in growth-arrested cells, and, therefore, it was suggested that Vpr supports the translocation of the preintegration complex from the cytoplasm to the nucleus of nondividing cells (12). This might explain the impaired replication of *vpr*-deficient HIV-1 and HIV-2 strains in macrophages (1, 11, 12, 14), but not in T-cell lines and peripheral blood mononuclear cells (PBMCs) (10, 18). However, replication of a macrophage-tropic variant of SIVmac239 in macrophages was not significantly affected by *vpr* (10). In addition to the *vpr* gene, SIV and HIV-2 contain the closely related gene *vpx*. An overlapping function of these genes in SIV could explain the unimpaired replication of SIVmac239 *vpr* mutants in macrophages. The Vpr of HIV-1 was also reported to transactivate the HIV-1 long terminal repeat and a number of heterologous promoters (4) and to induce cell differentiation in a rhabdomyosarcoma cell line (19).

Little is known about the function of *vpr* for virus replication in vivo and for pathogenesis. In previous experiments, two of three animals infected with SIVmac239 containing a point mutation in the *vpr* gene developed disease after the mutated *vpr* gene had reverted to a functional *vpr* gene early after infection (18). The function of *vpr* in vivo was now investigated by analyzing the course of infection in animals in which the *vpr* point mutation had not reverted during the first year after inoculation and by infecting rhesus monkeys with a SIVmac239 *vpr* deletion mutant.

MATERIALS AND METHODS

Construction of the SIVmac239 mutants. The molecular clone SIVmac239 carries the stop signal TAA at position 93 of the *nef* reading frame (25). To obtain an open *nef* reading frame, we replaced a 469-bp *Nco*I-*Nde*I fragment (positions 9280 to 9749 according to the numbering system of reference 25) with a PCR-generated fragment spanning *nef* from a SIVmac239 isolate from an infected rhesus monkey. This change converted the TAA stop codon to CAA. The inserted *nef* gene also carries the silent point mutations AGT to AGC at codon 110 and CCA to CCG at codon 197. The resulting virus was called

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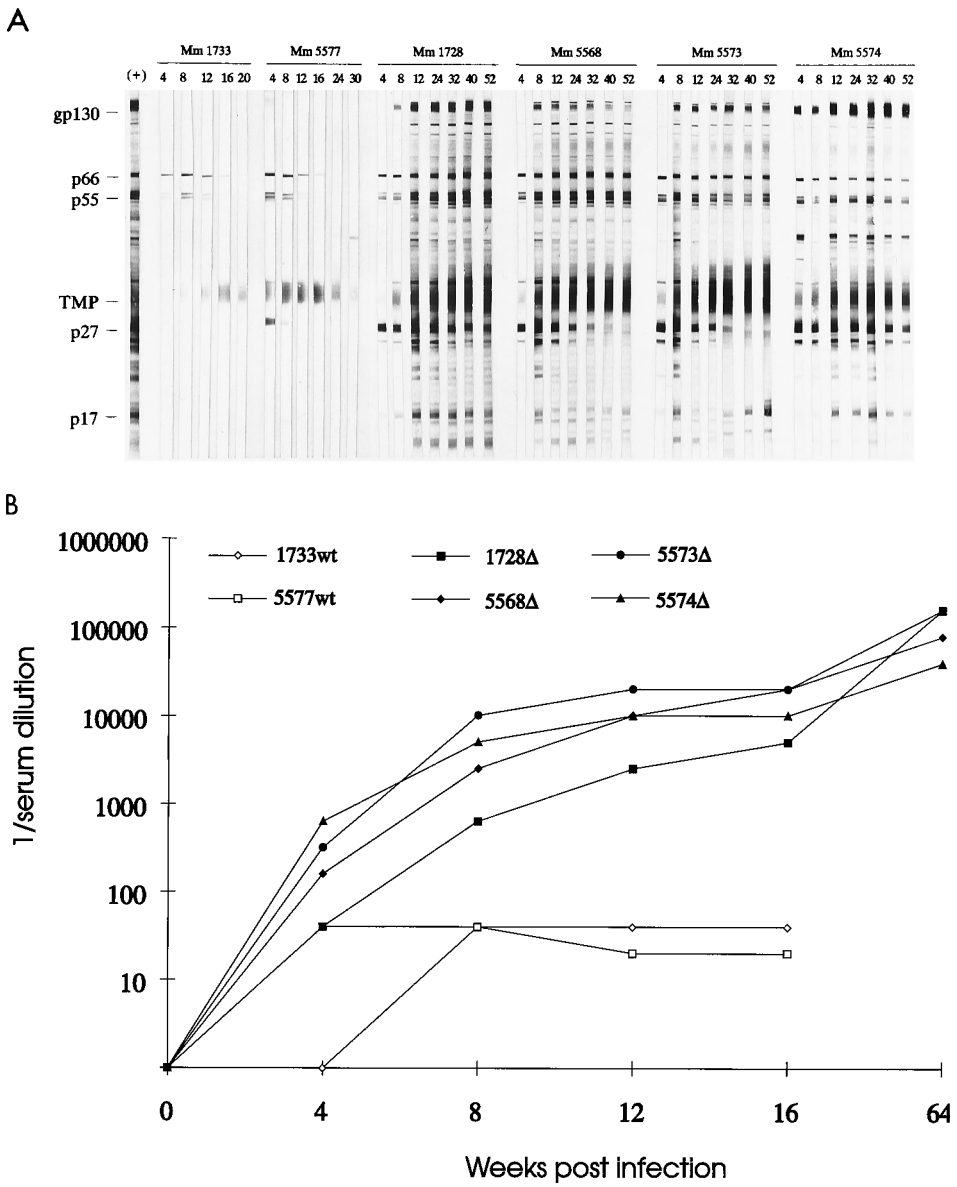

FIG. 1. Antibody response to SIV antigens during the course of infection. (A) A (1:10) dilution of plasma from animals infected with SIVmac239on (Mm 1733, Mm 5577) or a (1:50) dilution of plasma from SIVΔvpr-infected animals (Mm 1728, Mm 5568, Mm 5573, and Mm 5574) was used for Western blot analyses. As a positive control (+), plasma from a rhesus monkey infected with SIVmac251 was included. The numbers above the Western blot strips indicate the weeks after infection at which the plasma samples were collected. (B) Antibody titers in SIVmac239on (wt)- and SIVΔvpr (Δ)-infected animals. The reciprocal of the highest dilution giving absorption values twice above background is shown at different time points after infection.

SIVmac239 open-*nef* (SIVmac239on). To construct a stable *vpr*-deficient virus, a subcloned SIVmac239 fragment containing the *vpr* reading frame was cleaved with *Nco*I and *Hin*dIII, blunt ended with T4 DNA polymerase, and religated. One of the clones obtained (SIVΔvpr) had a 43-bp deletion in the *vpr* sequence from positions 6197 to 6239 (25), which also generated two stop signals at codons 16 and 18 of the *vpr* reading frame.

Determination of immunological parameters. The humoral immune response of infected monkeys against viral antigens was determined by enzyme-linked immunosorbent assay (ELISA) and Western blotting (immunoblotting) with pelleted whole SIVmac251 as an antigen as described previously (27). Urinary neopterin levels were measured as described previously (6). The CD4/CD8 ratio in the PBMCs of infected animals was determined by fluorescence-activated cell sorter analyses with a fluorescein isothiocyanate-labelled anti-CD4 antibody (OKT4; Ortho Diagnostic Systems) and a fluorescein isothiocyanate-labelled anti-CD8 antibody (B9.11; Dianova, Hamburg, Germany). For the antigen-specific T-cell proliferation assays (28), PBMCs were obtained from citrate-treated blood by Ficoll-diatrizoate density gradient centrifugation. The cells were seeded in microtiter plates (10^5 per well) in 100 μl of CG medium (Vitromex, Vilshofen, Germany) supplemented with 1% human AB serum. Cells were stimulated with 0.25 μg of purified heat-inactivated SIVmac251 or 1 μg of keyhole limpet hemocyanin (KLH) for 7 days. During the last 6 h, 0.5 μCi of [^{3}H]thymidine was added per well. After the cells were harvested, the incorporated radioactivity was measured with a β-counter. The stimulation index was calculated from the mean counts of triplicate wells by dividing the mean counts of antigen-containing cultures by the mean counts of cultures without antigen. Stimulation indices over 2 were considered positive.

Virus cultures. To generate biologically active viruses, plasmid clones containing either the 5′ half of the SIVmac239 proviral DNA (p239SpSp5′ [15]) or the 5′ half of the SIVΔvpr DNA were ligated via *Sph*I sites to the 3′ half of SIVmac239on proviral DNA. The ligation reaction mixture was transfected into CEMx174 cells, and virus was harvested as described previously (18). The 50% tissue culture infective doses of the virus stocks obtained were determined on CEMx174 cells. For virus isolation, PBMCs were purified from citrate-treated blood by banding over a Ficoll gradient. Isolated PBMCs were stimulated with phytohemagglutinin (10 μg/ml) overnight. The next day, cocultures with CEMx174 cells were set up in RPMI 1640 medium (Gibco BRL), supplemented

TABLE 1. Follow-up of SIVvpr$^-$-infected animals that show late reversion of the *vpr* gene

Wk postinfection[a]	Minimal no. of PBMCs required for virus isolation[b]		CD4/CD8 ratio	
	HR69	HR70	HR69	HR70
0	ND	ND	2.1	1.8
66, 64	ND	ND	2.1	1.7
70, 68	ND	ND	2.3	1.6
90, 92	ND	ND	1.5	1.3
106, 104	75,000	Neg	1.1	1.2
118, 116	18,750	Neg	0.8	1.2
130, 128	4,688	Neg	0.7	1
134, 136	4,688	3×10^5	0.9	1
150, 152	1,172	Neg	0.8	1.5
154	586	Neg	ND	ND
166, 168	568	Neg	0.8	1.5
0, 172	ND	ND	0.4	1.5
174, 176	293	Neg	0.3	ND

[a] The first number indicates the time point at which the cell-associated virus load was determined, and the second gives the time point of the lymphocyte subset analysis. Note that results prior to week 66 have been published previously (18).

[b] ND, not done; neg, no virus could be isolated even in cocultures with more than 10^6 PBMCs.

TABLE 2. Cell-associated virus load in SIVmac239on (wt)- or SIVΔvpr (Δ)-infected animals

Wk post-infection	Minimal no. of PBMCs required for virus isolation[a]					
	1733wt	5577wt	1728Δ	5568Δ	5573Δ	5574Δ
2	1,172	18,750	4,688	586	147	1,172
4	293	1,172	37,500	293	147	4,688
6	293	293	9,375	1,172	293	75,000
8	4,688	293	4,688	18,750	1,172	5×10^6
10	1,172	1,172	4,688	2,344	1,172	10^6
12	1,172	147	586	2,344	9,375	4×10^6
16	586	293	18,750	2,344	18,750	4×10^6
20	2,344	293	9,375	2,344	4,688	10^6
24	D	293	4,688	1,172	1,172	Neg
32		D	18,750	293	1,172	Neg
36			9,375	4,688	4,688	Neg
40			9,375	293	2,344	8×10^6
44			4,688	2,344	1,172	Neg
48			9,375	2,344	1,172	Neg
52			4,688	1,172	2,344	Neg
56			2,344	2,344	2,344	Neg
64			2,344	2,344	2,344	Neg
68			1,172	586	2,344	2×10^6
72			ND	586	1,172	Neg
76			586	D	D	Neg

[a] The minimal number of PBMCs required for virus isolation in cocultures with Mm 5573/HVS488 cells (weeks 0 to 48) or CEMx174 cells (weeks 52 to 76) is shown. D, dead; ND, not determined; Neg, virus isolation not possible even in cocultures with more than 10^6 PBMCs.

with 10% heat-inactivated fetal calf serum (Gibco BRL), penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (300 μg/ml), and interleukin-2 (100 U/ml; Eurocetus). The minimal numbers of PBMCs required for virus isolation in cocultures with Mm5573/HVS488 cells or CEMx174 cells were determined as a measure of cell-associated virus titers. The Mm5573/HVS488 cell line is a rhesus monkey T-cell line immortalized by herpesvirus saimiri as described previously (2). Virus replication was verified by measurement of the reverse transcriptase activity in culture supernatants (24) or by detection of viral antigens with a polyvalent antigen capture assay (Organon, Heidelberg, Germany). To isolate viral DNA from infected cells, Hirt extracts (13) were prepared shortly before peak syncytium formation. The amount of p27 capsid antigen in the plasma was determined with an antigen capture assay (Coulter Co.).

Infection of rhesus monkeys. Animals were housed at the German Primate Center in Göttingen, Germany. Handling of the monkeys and collection of specimens were performed according to institutional guidelines as described previously (26). Four rhesus monkeys of Indian origin (seronegative for SIV, D-type retroviruses, and STLV-1) were infected intravenously with 8×10^4 50% tissue culture infective doses of SIVΔvpr; two animals received 4×10^4 tissue culture infective doses of SIVmac239on as a control. Animals were euthanized when they developed cachexia, persistent anorexia, or severe respiratory symptoms. According to general in-house rules, all animals developing diarrhea were treated once for 10 days with the antiprotozoic agent paromomycine, which is effective in stress-induced diarrhea. Stress-induced diarrhea is sometimes observed in uninfected animals and is accompanied by massive excretion of protozoa. In SIV-infected animals, paromomycine therapy usually fails, indicating an opportunistic infection. Animals 5573Δ and 5568Δ developed therapy-resistant diarrhea with large amounts of *Giardia lamblia* cysts in their feces approximately 2 weeks prior to euthansia.

PCR amplifications and DNA sequencing. *vpr*- and *vpx*-spanning fragments were generated by PCR from Hirt DNA from infected cells and subcloned in the plasmid pBS(−) (Stratagene). The sequences of the specific oligonucleotides and the conditions of the PCR were described previously (18). The nucleotide sequence was determined with an ABI-373a automated sequencer by using dye-labelled dideoxynucleotides according to protocols provided by the manufacturer (Applied Biosystems).

RESULTS

Follow-up of animals showing late reversion of the SIV *vpr* gene. Previously, five rhesus monkeys were inoculated with SIVmac239 virus containing a point mutation of the *vpr* initiation codon (SIVvpr$^-$) and a premature termination codon in *nef* (18). Two animals were infected with SIVmac239, which also contains the premature termination codon in *nef*. The *nef* termination codon of the viral genome reverted within 4 weeks in all animals. In three animals infected with SIV vpr$^-$, the viral genome had reverted to a functional *vpr* 16 weeks postinfection, and two animals had to be euthanized 40 and 46 weeks postinfection because of AIDS-related symptoms (18). In two animals infected with SIVvpr$^-$ (HR69 and HR70), reversion to a functional *vpr* gene was not observed 58 (18) and 66 weeks postinfection, when six independent *vpr* clones from each virus reisolate were sequenced. However, 70 weeks postinfection, reversion was detected by PCR and partial sequence analysis in four of four clones from each animal. Reversion of the *vpr* genotype was confirmed for animal HR69 at 82, 86, 90, and 94 weeks postinfection. In the case of animal HR70, reversion was confirmed by sequencing the *vpr* gene of a virus reisolate recovered at week 90 and by sequencing the *vpr* genes amplified directly from PBMCs at weeks 78 and 94. In Western blot analyses, both animals had antibodies to Vpr 78 weeks postinfection (data not shown), which had not been detectable on week 58 (18). Therefore, the *vpr* gene must have reverted in both animals more than a year after infection.

One of these animals (HR70) had a low virus burden and normal CD4/CD8 ratios (Table 1). No clinical alterations were observed. In contrast, animal HR69 showed high cell-associated virus loads (Table 1) and developed a progressive lymphadenopathy. Histological analysis of a lymph node biopsy removed 146 weeks postinfection revealed an extreme follicular hyperplasia, signs of follicular fragmentation with single atrophic follicles, and a plasmacytosis. A slow decline in the CD4/CD8 ratio could also be observed in this animal (Table 1). Both animals were still alive 203 weeks postinfection and did not show any clinical signs of immunodeficiency.

Infection of rhesus monkeys with a SIVmac239 *vpr* deletion mutant. To generate a stable *vpr*-deficient virus, a 43-bp deletion was introduced into the *vpr* gene of SIVmac239on, a *nef*-open version of SIVmac239. When virus stocks of SIVΔvpr and SIVmac239on were used to infect CEMx174 cells, no obvious differences in either the rate or extent of viral replication were observed (data not shown). To analyze the effects

TABLE 3. p27 capsid antigen in plasma of SIVmac239on (wt)- or SIVΔvpr (Δ)-infected animals

Wk post-infection	p27 antigen levels in plasma (ng/ml)					
	1733wt	5577wt	1728Δ	5568Δ	5573Δ	5574Δ
2	1.9	0	0.5	0.3	0.1	0
4	0.3	0.2	0	0	0	0
6	0.5	0.4	0	0	0	0
8	0.8	1.1	0	0	0	0
10	1.2	0.9	0	0	0	0
12	1.8	0.8	0	0	0	0
16	3.2	0.7	0	0	0	0
20	1.8	0.8	0	0	0	0
24	D[a]	0.8	0	0	0	0
28		1.4	0	0	0	0
32		4.7[b]	0	0	0	0
36		D	0	0	0	0
40			0	0	0	0
44			0	0	0	0
48			0	0	0	0
52			0	0	0	0
56			0	0.2	0	0
64			0	0.2	0.4	0
68			0	0.2	0.3	0
72			0	0[c]	0	0
74				D	D	

[a] D, dead.
[b] Determined at 30 weeks postinfection.
[c] Determined at 71 weeks postinfection.

of *vpr* on virus load and pathogenesis in vivo, four rhesus monkeys were infected with SIVΔvpr (1728Δ, 5568Δ, 5573Δ, and 5574Δ), while two animals received SIVmac239on (1733wt and 5577wt) as a control. To quantitate the cell-associated virus load in the infected monkeys, the minimal number of PBMCs required for virus isolation was determined (Table 2). In three of the four monkeys infected with SIVΔvpr, cell-associated virus titers were similar to the titers observed in SIVmac239on-infected animals. In the fourth monkey infected with SIVΔvpr (5574Δ), virus could be isolated during the first 20 weeks and on only two occasions thereafter. Antigenemia was never detected in this animal, in contrast with the other monkeys infected with SIVΔvpr, which all had detectable p27 capsid antigen in the plasma at 2 weeks postinfection.

TABLE 4. CD4/CD8 ratio in PBMCs of SIVmac239on (wt)- or SIVΔvpr (Δ)-infected animals

Wk post-infection	CD4/CD8 ratio[a]					
	1733wt	5577wt	1728Δ	5568Δ	5573Δ	5574Δ
0	1.4	1.1	1.4	1.4	1.4	1.6
4	1.4	1.1	1.4	0.8	1.6	2.1
8	1.2	1.1	2.3	1.5	1.8	2.5
12	1.3	0.7	1.9	1.1	1.2	1
16	0.7	0.6	0.9	ND	0.8	0.8
20	D	0.8	0.9	1.3	1.9	2.6
28		1.0	0.9	0.5	0.7	1.7
32		0.6[b]	0.8	0.9	1.4	1.3
48		D	1.3	0.6	1.1	1.8
64			1.4	0.6	0.7	2.2
72			0.8	0.4	1.1	1.8
76			0.7	D	D	1.2
80			1.2			2.4

[a] D, dead; ND, not determined.
[b] Determined at 30 weeks of postinfection.

SIVmac239on-infected animals had persistent antigenemia throughout the observation period (Table 3). Two animals (5568Δ and 5573Δ) became antigenemic 56 and 64 weeks postinfection.

Clinical alterations and pathology. The two rhesus monkeys infected with SIVmac239on had to be euthanized at 20 and 30 weeks postinfection because of severe respiratory symptoms, anorexia, and poor health. The autopsies revealed a massive *Pneumocystis carinii* pneumonia, atrophy of T-cell areas of the lymphatic tissues, and a nearly complete loss of germinal centers of the lymph nodes in both animals. Three of the four animals infected with SIVΔvpr (5568Δ, 5573Δ, and 1728Δ) developed a transient, mild-to-moderate lymphadenopathy 10 to 28 weeks postinfection. Lymph node biopsies taken from the SIVΔvpr-infected animals 48 weeks postinfection revealed a mild follicular hyperplasia, with the exception of 5573Δ, which had a more prominent follicular hyperplasia. Animals 5568Δ and 5573Δ had to be sacrificed 71 and 73 weeks postinfection, respectively, because of therapy-resistant diarrhea and cachexia. Similar to SIVmac239on-infected animals, massive *P. carinii* pneumonia, atrophy of the T-cell areas of the lymphatic tissues, and nearly complete loss of the germinal centers of the lymph nodes were observed in both animals. Other pathological findings included pharyngeal and esophageal candidiasis, duodenal and cholangeal cryptosporidiosis, and microspirochetosis of the cecum and colon ascendens. Two of the animals infected with SIVΔvpr (1728Δ and 5574Δ) were still alive 105 weeks postinfection without showing any clinical signs of immunodeficiency. A decline in the CD4/CD8 ratio in SIVmac 239on-infected monkeys was observed 12 and 16 weeks postinfection (Table 4). As repeatedly observed in rapidly progressing SIV-infected macaques, the decline in the CD4/CD8 ratio was not severe (17, 20). In one of the SIVΔvpr-infected animals which progressed to disease (5568Δ), a drop in the CD4/CD8 ratio was observed 28 weeks postinfection. The other three animals infected with SIVΔvpr (1728Δ, 5573Δ, and 5574Δ) did not show a severe decline in the CD4/CD8 ratio, although CD4/CD8 ratios below 0.8 could be observed in animals with high virus loads (1728Δ and 5573Δ) at some time points. In 63 uninfected macaques, which were analyzed in the German Primate Center during these experiments, the CD4/CD8 ratios were always higher than 0.9, and in only two cases were ratios below 1 observed (data not shown).

Follow-up of immunological parameters. To analyze whether immunological alterations in SIVΔvpr-infected animals are similar to the alterations in SIVmac239on-infected animals, a number of parameters were determined at regular intervals. Western blot analyses revealed that rhesus monkeys infected with SIVΔvpr developed a strong humoral immune response against Gag, Pol, and Env, while antibody formation was very weak in SIVmac239on-infected animals (Fig. 1A). In animals 5568Δ and 5573Δ, a decline in the number of antibodies against the p27 capsid antigen could be observed at 32 weeks postinfection. To quantitate the antibody titers more precisely, a SIV whole-virus ELISA was performed (Fig. 1B). In animals infected with SIVΔvpr, antibody titers were approximately 100-fold higher than in animals infected with SIVmac239on.

Because of the marked differences in the humoral immune response to SIV antigens in SIVmac239on- and SIVΔvpr-infected animals, the cellular immune response was also analyzed in antigen-specific T-cell proliferation assays (Fig. 2). After KLH immunization, 20 weeks postinfection, KLH-specific T-cell proliferation could only be detected in animals infected with SIVΔvpr but not in the SIVmac239on-infected animal that was still alive at that time (Fig. 2A). The KLH response in animals infected with SIVΔvpr is similar to the

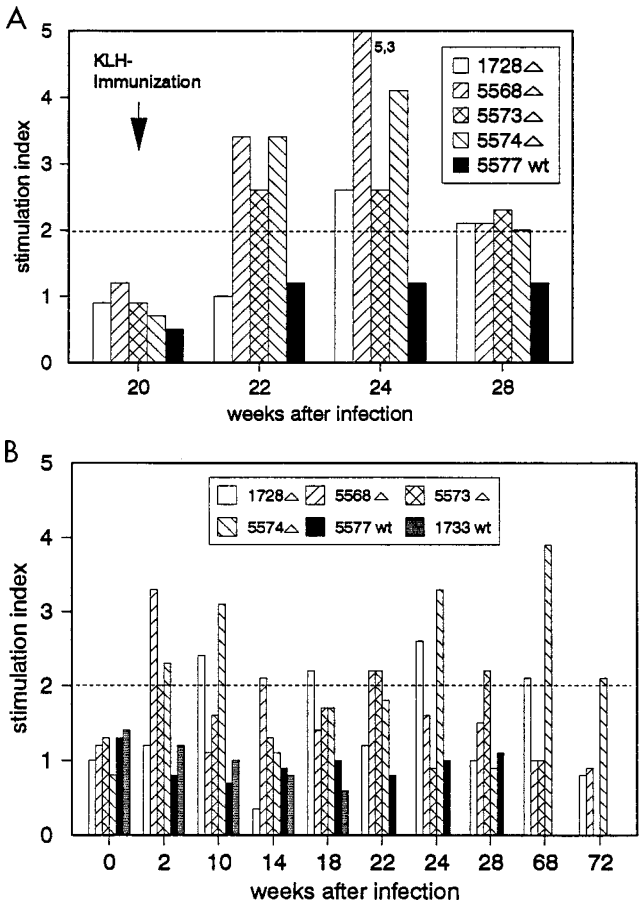

FIG. 2. Proliferation of T cells isolated from SIVmac239on (wt)- and SIVΔvpr (Δ)-infected animals. (A) KLH-specific T-cell proliferation. Twenty weeks postinfection, animals were immunized with KLH. Before, 2, 4, and 8 weeks later, [^{3}H]thymidine incorporation was determined in PBMCs cultured in the presence or absence of KLH. The stimulation index was calculated by dividing the counts of cultures containing KLH through the counts of cultures not containing KLH. (B) SIV antigen-specific T-cell proliferation at different time points after infection. [^{3}H]thymidine incorporation was determined in PBMCs cultured in the presence or absence of whole inactivated SIV. The stimulation index was calculated by dividing the counts of cultures containing SIV by the counts of cultures not containing SIV. Stimulation indices over 2 were considered positive.

response of uninfected animals (data not shown). The T-cell proliferation in response to SIV antigens was also analyzed at different time points after infection (Fig. 2B). SIVmac239on-infected animals never showed significant SIV antigen-specific proliferation of their T cells. However, an intermittent reactivity was observed for all animals infected with SIVΔvpr. The two animals progressing to disease (5568Δ and 5573Δ) had lost their SIV antigen-specific T-cell response 68 weeks postinfection.

Neopterin is released from activated T cells and macrophages. During the acute phase of infection with immunodeficiency viruses and with progression to disease, neopterin levels are elevated (6, 7). Elevated neopterin levels could be a consequence of a cytokine dysregulation leading to T-cell and macrophage activation as discussed previously (8). Therefore, urinary neopterin levels were determined as a marker for disease progression and immune stimulation (Fig. 3). Neopterin values increased in all animals between 5 and 15 days postinfection, reaching peak levels up to 16-fold above baseline. After this initial peak, neopterin concentrations declined at close to baseline values in the four animals that received SIVΔvpr, while the neopterin levels remained elevated in SIVmac239on-infected monkeys. In all animals that progressed to disease, increases in neopterin levels were observed approximately 3 months before death.

AIDS in the absence of *vpr*. To confirm the progression to AIDS in the absence of Vpr, the *vpr* genes of virus isolates recovered from the PBMCs of animals 5568Δ and 5573Δ on the day of death were cloned and sequenced. All five clones that were sequenced from each animal still contained the deletion followed by the two stop codons introduced. While there were a number of point mutations throughout the *vpr* gene, none of them led to the development of an ATG start codon (data not shown). Three of the clones sequenced from animal 5573Δ had a small additional deletion upstream of the *tat* initiation codon. Three bases (positions 6295 to 6297 in the numbering of reference 25) were deleted in one of the clones, and a 2-bp deletion (positions 6256 and 6257) was observed in two clones. An additional start codon was not introduced by these deletions. Virus reisolates recovered approximately 1 year after infection from the two animals that are still alive had also retained the deletion in the *vpr* gene.

DISCUSSION

In contrast with results obtained with a *nef* deletion variant of SIVmac239 (16), *vpr* is clearly not required for pathogenicity. Two out of four animals infected with SIVΔvpr developed an AIDS-like disease, as demonstrated by a decline in the CD4/CD8 ratio, a loss of SIV antigen-specific T-cell proliferation, an increase in urinary neopterin levels, the occurrence of opportunistic infections, and the finding of characteristic histopathological alterations of the lymphatic tissue. A high virus burden and decreasing SIV antigen-specific T-cell proliferation might indicate the progression to disease in the third animal infected with SIVΔvpr. One of the four animals infected with SIVΔvpr had a very low cell-associated virus load and showed no signs of progression to AIDS.

Both animals infected with SIVmac239on died with AIDS 20 and 30 weeks postinfection. Two of the four animals infected with SIVΔvpr died 71 and 73 weeks postinfection. The two remaining animals were still alive 105 weeks postinfection. Therefore, progression to AIDS might be delayed in the absence of *vpr*. In our previous study, in which two animals had been infected with SIVmac239 and five animals had been infected with SIVvpr$^-$, which contains a point mutation of the start codon of *vpr*, a similar tendency was observed (18). Both viruses used in this study had a premature stop codon, which quickly reverted in vivo. The two wild-type-infected animals died within the first year. Two of the five animals infected with the *vpr* deletion mutant were still alive 203 weeks postinfection and showed no signs of clinical immunodeficiency. In summary, four of nine animals infected with SIVmac239 *vpr* deletion mutants survived longer than 2 years, whereas four of four animals infected with viruses containing a functional *vpr* died within the first year postinfection. These observations may suggest an influence of *vpr* on the course of disease in SIV-infected rhesus monkeys. However, because of the limited number of animals available for these studies, it is not yet possible to firmly conclude that progression to AIDS is delayed in the absence of *vpr*.

In a recent study, J. S. Gibbs and coworkers also observed induction of AIDS in SIV-infected rhesus monkeys in the absence of *vpr* (9). In these experiments, the behavior of a SIVmac239 *vpr* deletion mutant was similar to that of the parental, wild-type virus, and none of four animals infected

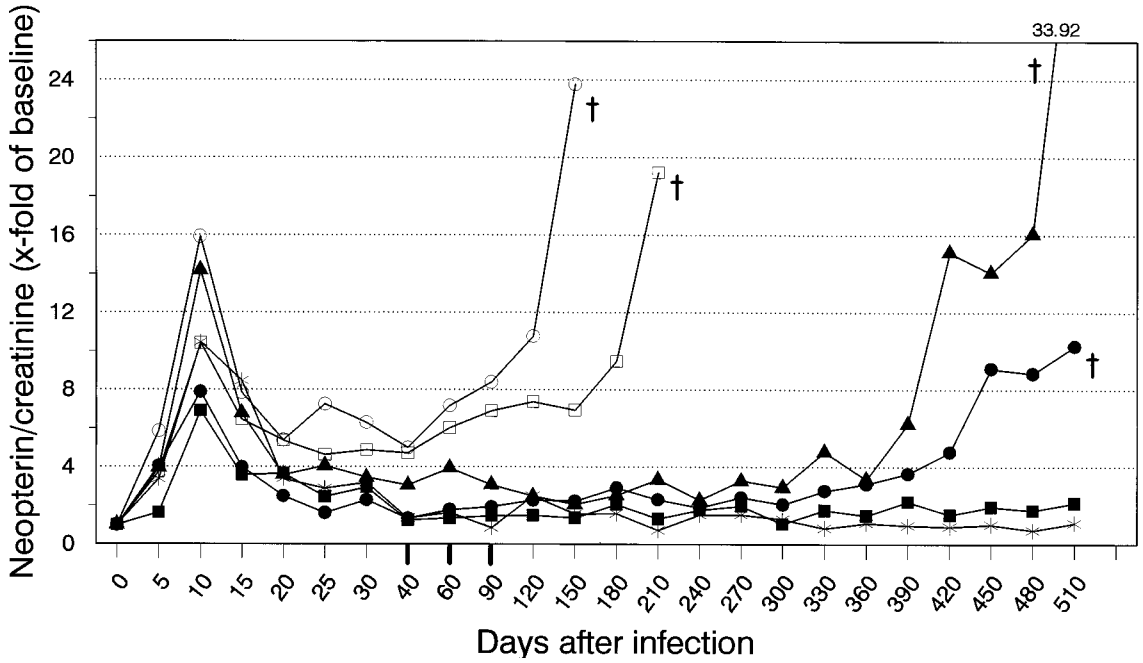

FIG. 3. Urinary neopterin levels in SIVmac239on (wt)- and SIVΔvpr (Δ)-infected animals. The neopterin/creatinine ratio at different time points after infection is expressed for each animal as the *x*-fold of the mean of six to eight independent neopterin/creatinine ratios determined before infection. †, time of death. ○, 1733wt; □, 5577wt; ■, 1728Δ; ▲, 5568Δ; ●, 5573Δ; ∗, 5574Δ.

with SIVmac239 died within the first year. In previously published studies, approximately 50% of the animals infected with SIVmac239 are rapid progressors, which do not develop a strong antibody response and which die within the first year (15, 16, 20, 22). In our experiments, all animals infected with SIVmac239 variants containing a functional *vpr* gene were rapid progressors, suggesting that the monkeys used in our study could have been particularly sensitive to rapid progression to disease. As noted before (20), the source of the rhesus monkeys can have a strong influence on the course of disease after infection with the same SIVmac stocks. Therefore, the differences between our studies and the study of Gibbs et al. could be explained if *vpr* had an influence only on rapid progression to disease. In animals in which the course of the disease is slower for other reasons, a supportive effect of *vpr* would then be less prominent. In addition to the *vpr* gene, SIV contains the closely related gene *vpx*. An overlapping function of these genes might be an explanation for the progression to disease in the absence of *vpr*. The observation that SIVmac239 *vpr* and *vpx* deletion mutants are more severely attenuated than single deletion mutants (9) is consistent with this hypothesis.

The course of the disease in animals infected with SIVΔvpr differed from the one observed in SIVmac239on-infected animals in a number of parameters. SIVmac239on-infected animals had persistent antigenemia, hardly any antibody response, and no antigen-specific T-cell proliferation. In contrast, in the first year, antigen could only be detected 2 weeks postinfection in three of the four animals infected with SIVΔvpr, although the cell-associated virus load in these three animals was similar to that in SIVmac239on-infected animals. The strong antibody response to the p27 capsid antigen in SIVΔvpr-infected animals probably prevents the detection of the p27 antigen. In addition to a strong humoral immune response, antigen-specific T-cell proliferation could be intermittently detected in SIVΔvpr-infected animals but not in SIVmac239on-infected animals. In previous experiments, macaques infected with nonpathogenic HIV-2 had acquired a SIV antigen-specific T-cell proliferation, which was not observed in animals infected with pathogenic SIVmac251, suggesting a partially attenuated phenotype of the *vpr* deletion mutant (5).

The causes of the observed differences in the immune responses are not understood. An immunosuppressive effect of *vpr* could explain the strong immune responses against SIV observed in the absence of *vpr*. Since high-level antibody responses were also observed in animals that progress slowly after infection with SIVmac239 in the presence of *vpr* (15), we suggest that a less-efficient replication of *vpr*-deficient SIVmac239 in certain cell types in vivo could result in a more vigorous immune response. The immune responses of some animals might then be able to control virus replication for an extended period of time, even after reversion to a functional *vpr* gene.

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