Resistance of neonatal monkeys to live attenuated vaccine strains of simian immunodeficiency virus

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A major safety concern of using live-attenuated vaccine strategies against AIDS is the potential exposure of neonates or fetuses to vaccine virus from the mother. Here we report that high viral loads and disease were observed in only 2 of 18 neonatal monkeys infected with gene-deleted vaccine strains of simian immunodeficiency virus. Pathogenicity was restricted to neonates born to unvaccinated mothers, that is, lacking maternal immunity, and that received extremely high doses of vaccine virus orally. No *in utero* transmission of vaccine virus was observed in four neonates born to mothers vaccinated during the second trimester. Our results suggest that the live attenuated vaccine approach should remain a viable option for preventing HIV infection and disease in high-risk human populations.

A variety of vaccine strategies that are being considered for use against human immunodeficiency virus (HIV) have been evaluated in macaque monkeys using analogue vaccines and challenge with pathogenic simian immunodeficiency virus (SIV). Vaccines based on subunits or recombinant proteins[1,2], inactivated or pseudovirion particles[3-5], virus vectors[6,7], or prime and boost protocols[2,7] have not resulted in consistent high levels of protection following challenge with pathogenic SIVmac251 or SIVmac239. Live-attenuated vaccines prepared from SIV deletion mutants, however, have strongly protected monkeys against virulent SIV challenge[8,10].

Infection of juvenile and adult rhesus monkeys with the attenuated viruses SIVmac239Δnef (missing *nef*) and SIVmac239Δ3 (missing *nef*, *vpr* and upstream (US) sequences in U3) does not result in the immunodeficiency disease that is consistently observed following infection with the parental SIVmac239 (ref. 8, 11). Animals infected intravenously with the attenuated viruses remain persistently infected and exhibit cell-associated viral loads that are markedly lower than those seen after infection with wild-type virus during both primary and chronic stages (Fig. 1). Animals infected with *nef* deletion viruses develop rising neutralizing antibody titers for months to years after infection and have measurable cytotoxic T-cell responses to SIV gag and env proteins (R.P. Johnson and R.C.D., manuscript in preparation). When challenged after a long incubation period, animals vaccinated with SIVΔnef and SIVΔ3 can be completely protected against intravenous challenge[8,10].

Although live-attenuated vaccines for a variety of infectious agents are known to elicit broad immune responses and to be highly effective, the use of this strategy for HIV raises numerous safety issues. Prominent among these are the potential effects of such viruses on the unborn fetus and the neonate. Recently, pathogenicity has been reported in a neonate infected with an extremely high oral dose of SIVmac239Δ3 (ref. 12). This animal was infected with 1600 ng of SIVΔ3 equivalent to 5.3×10^5 tissue culture infectious doses ($TCID_{50}$). In order to investigate this phenomenon more fully, we designed several experiments to characterize neonatal infection with SIVΔ3.

Dose dependence

Nine pregnant rhesus monkeys were enrolled into three virus dosage groups. The stage of gestation and the expected delivery date for each fetus was determined by ultrasound. Approximately one week before the expected date of natural delivery, the neonates were delivered by cesarean section. Within 4 hours of birth, when each animal was breathing on its own and clinically stable, a virus inoculum of SIVmac239Δ3 was given orally. The highest virus dose was 5.7×10^5 $TCID_{50}$ of SIVΔ3, which contained 283 ng of p27. This dose was similar on a TCID basis to the dose reported previously to cause disease in a neonate[12]. Second and third groups of three neonates each were given lower doses of SIVΔ3 containing 50 ng and 5 ng of p27.

A minimum of eight of these nine neonates became infected with the vaccine virus on the basis of virus recovery from peripheral blood (Table 1). All nine had antibody responses to whole virus as tested by enzyme-linked immunosorbent assay (ELISA), but antibody levels in two of them (61-95 and 83-95) subsequently declined and did not persist above detectable levels (Fig. 2). Of these nine neonates, two that received the highest dose of virus maintained persistently elevated cell-associated viral loads after the primary phase of infection (Table 1). Animal 91-95 subsequently experienced a decrease in CD4/CDw29 cell percentages (Fig. 3), weight loss, and lethargy 7 months after infection and had to be killed. Marked lymphoid depletion and disseminated cryptosporidiosis characteristic of SIV-induced immunodeficiency disease were observed at necropsy. Axillary and mesenteric lymph nodes, thymus and ileum were examined by immunohistochemistry and *in situ* hybridization for the presence of viral antigen and nucleic acid[13-15]. Moderate to large numbers of cells containing viral antigen and nucleic acid were observed in the lymph nodes (Fig. 4). In contrast, few positive

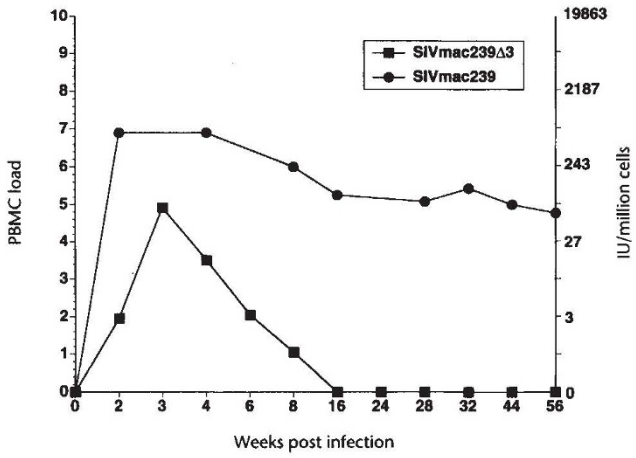

Fig. 1 Cell-associated viral loads in juvenile and adult rhesus monkeys. The numbers of PBMCs required to recover SIV at the indicated weeks after i.v. inoculation were determined. Juvenile and adult rhesus monkeys associated with different studies were used for this composite summary. Each data point represents an average of measurements from 12 animals for SIVmac239Δ3 and 8 for SIVmac239. Cell-associated viral loads are expressed on the *y*-axis on the left as in Table 1 and on the *y*-axis on the right as the number of cells per 10^6 PBMCs that yielded SIV.

cells were present in the lamina propria of the ileum and only a single, weakly positive cell was detected in the thymus and only by *in situ* hybridization. Although the numbers of infected cells in the lymph nodes were comparable to what we typically see in juvenile and adult animals in the terminal stages of simian AIDS with wild-type SIVmac239, the numbers of infected cells in the thymus and ileum were considerably less. The other animal, monkey 46-95, has maintained normal CD4 and CD4/CDw29 percentages for seven months post infection despite high viral loads (Fig. 3).

The one remaining animal that received the 283-ng dose (92-95) and the six animals that received the 50-ng and 5-ng oral doses of SIVΔ3 developed primary and chronic infection profiles similar to those described previously for SIVΔnef and SIVΔ3 in juveniles and adults[8,10,11]. These animals did not maintain persistent high viral loads after a peak approximately 2 weeks post infection (Table 1). Infection of two of these monkeys (61-95 and 83-95) may have been transient in nature, similar to what we have reported previously[10], in that antibodies declined to below detectable levels beyond month 4 (Fig. 2). Although virus was recovered from monkey 83-95 at week 2 after exposure, virus was never recovered from monkey 61-95. At 12 months post infection, these seven neonates are clinically normal, have registered normal weight gains and have maintained low or undetectable virus burdens and normal CD4 and CD4/CDw29 percentages.

Table 1 Cell-associated viral loads in neonatal rhesus monkeys infected orally with SIV239Δ3

Neonate no.	SIV239Δ3 Dose (ng p27)	Viral load at months post exposure								
		0.5	1	2	3	5	7	9	10	11
46-95	283	3.5	2.0	5.5	6.0	4.5	6.0	6.0	4.5	4.5
91-95	283	7.5	3.0	7.5	8.5	8.0	dead			
92-95	283	3.5	2.5	1.5	2.0	1.0	1.0	1.0	0	
60-95	50	2.5	1.0	0	0	0	0	0	0	
61-95	50	0	0	0	0	0	0	0	0	
113-95	50	2.0	1.5	1.0	1.0	0	0			
81-95	5	6.5	2.5	4.0	0	2.0	1.0	1.5	1.0	
82-95	5	2.0	1.5	1.5	0	0	0	0	0	
83-95	5	6.5	0	0	0	0	0	0	0	

Viral load is expressed in code as the number of PBMCs required to recover SIV. 0 >10^6; 1, 10^6; 2, 333,333; 3, 111,111; 4, 37,037; 5, 12,345; 6, 4,115; 7, 1,371; 8, 457.

Maternal–fetal transmission

We next assessed whether infection of the dam could be transmitted to the fetus and whether immune responses in the dam might protect neonates from infection by the oral route or from disease. Pregnant rhesus monkeys were infected by intravenous (i.v.) inoculation of SIVΔ3 containing 11.3 ng p27 late in the second trimester of gestation between days 80 and 100. After initial infection, blood samples were collected for virus isolation and antibody determinations. On the day of delivery (by cesarean section), samples of amniotic fluid and umbilical cord blood as well as peripheral blood samples from mother and neonate were collected. The neonates were then administered orally SIVΔ3 containing 283 ng p27, the highest dose of the same stock of virus used in the titration study described above.

After vaccination, all four mothers became infected with the vaccine virus as evidenced by virus recovery from peripheral blood (Table 2). Two with quantitatively measurable cell-associated viral loads in peripheral blood mononuclear cells (PBMCs) showed 25 to 250 IU/10^6 cells on the weeks immediately following vaccination, consistent with previous viral load measurements in juvenile and adult animals. On the day of delivery, two of the four dams had SIV cultured from peripheral blood. No virus was recovered from amniotic fluid samples collected from the four dams at surgery.

No evidence of virus transmission from mother to fetus was observed in these experiments. All four neonates were negative both by virus isolation and polymerase chain reaction (PCR) analysis when cord blood and peripheral blood samples were tested on the day of delivery (Table 3). One neonate died within 24 hours of birth. Spleen, mesenteric and axillary lymph nodes, thymus and ileum of this animal (807-95) were examined by immunohistochemistry and *in situ* hybridization; no infected cells were detected in any tissue. Histologically, large portions of the lungs were filled with meconium and the tips of intestinal villi contained necrotic debris suggestive of a perinatal hypoxic episode. The thymus, peripheral lymph nodes and spleen of monkey 807-95 were normal, which further supports an acute cause of death.

Influence of maternal immunity

The three surviving neonates became persistently infected after oral administration of high-dose SIVΔ3 (Table 3). Thus vaccination of the dam did not result in passive transfer of sufficient protective immunity to prevent the infants from becoming infected when exposed to high doses of SIVΔ3 orally after birth. However, viral loads at months 5 through 8 for each infant were low and were not similar to the high viral loads observed in two of the three neonates that received high oral dose SIVΔ3 described above. Thus, although neonates born to

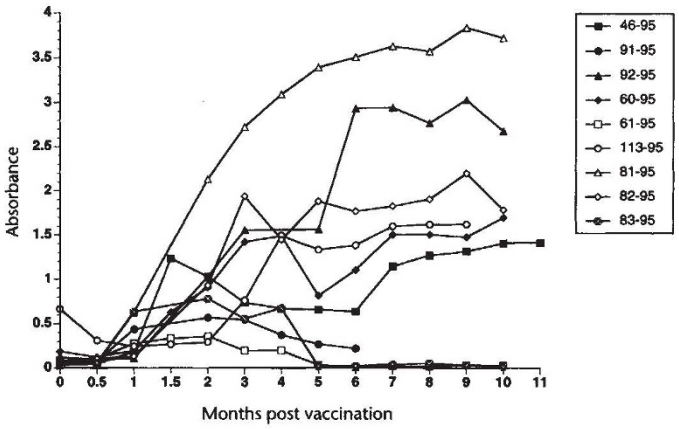

Fig. 2 Antibody responses. Anti-SIV antibodies to whole lysed virus were measured by ELISA in the neonates that received SIVΔ3 by the oral route.

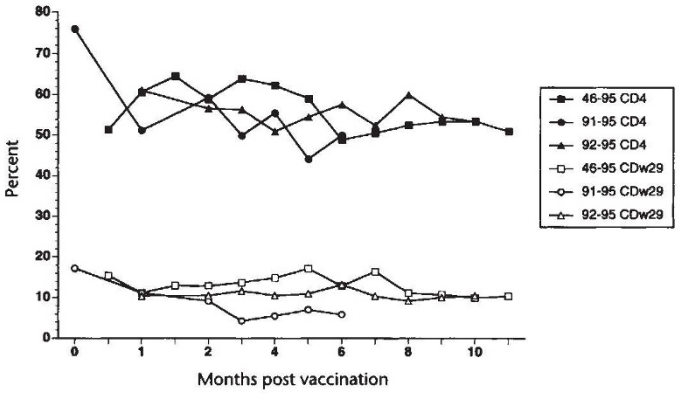

Fig. 3 CD4 and CDw29 subsets. CD4 cells and the CDw29 subset of CD4 cells in the group that received high-dose oral SIVΔ3 are expressed as the percent of cells in PBMCs.

previously vaccinated mothers were not protected from infection following high-dose oral administration at birth, their ability to maintain low viral loads contrasted with the high viral loads observed in two of three naive neonates that received the same high dose.

In two of the three mothers antibody levels to whole virus were well developed by the time of delivery (Table 2). One mother had a lower antibody response. The levels of antibodies directed to whole lysed virus in the dams were reflected in the relative levels in the neonates (Tables 2 and 3). Serum neutralizing activity to laboratory passaged SIVmac251 was also detected (Tables 2 and 3).

Other vaccine strains and routes

We have performed other experiments to test the effects of SIVΔ3 administered by the intravenous route to neonates and to test two other mutant viruses with alternate combinations of genetic deletions. One virus, SIVΔ3X, is missing *nef*, *vpx* and US sequences and the other, SIVΔ4, is missing *nef*, *vpr*, *vpx* and US sequences. SIVΔ4 is of interest because viral loads in juvenile and adult animals are very low and this strain is considerably more attenuated than SIVΔ3. SIVΔ3 administered intravenously at 50 ng resulted in low viral loads similar to those observed in neonates at the same dose administered orally. Both SIVΔ3X at 50 ng and SIVΔ4 at 300 ng were infectious orally without any evidence of persistent high viral loads for at least 12 months after infection (data not shown).

Discussion

In the course of these experiments, strains of SIV with deletions in multiple genetic elements were administered to 18 neonates (Table 4). The strains used have not caused disease in more than 40 juvenile and adult rhesus monkeys that were infected previously. Whereas a minimum of 17 of the 18 neonates became infected, only 2 of the 18 exhibited high viral loads and one of these has died from the infection. Both animals with high viral loads and evidence of disease progression were infected orally with an extremely high dose of SIVΔ3. Thus, there appears to be a dose-dependent pathogenic effect of SIVΔ3 in neonatal rhesus monkeys. This observation contrasts markedly with the lack of dose dependence of wild-type strains SIVmac239 and 251 and of the attenuated SIVmac239Δ3 in juvenile and adult rhesus monkeys. Numerous studies have shown that neither the severity of disease nor the rapidity of onset correlate with the dose of SIVmac239, SIVmac251 or other AIDS-inducing strains[16,17]. This independence of dose has been observed over dose ranges of more than 10,000-fold starting with high-titered undiluted stocks of virus. Similarly, we have infected 18 juvenile and adult rhesus monkeys with doses of SIVmac239Δ3 over a 100,000-fold range and saw no evidence of disease progression in any and no dependence of viral load on dose.

The mechanisms responsible for the apparent dose-dependent pathogenicity of SIVΔ3 in neonates are open to speculation. A larger fraction of activated and/or dividing lymphocytes in gut tissue of the neonate may create a larger pool of susceptible cells able to support SIV replication in a Nef-independent manner. At higher doses of input virus in the neonate, virus replication may outstrip the ability of the immature immune system to respond in time. Alternatively, at the highest doses of input virus, large amounts of antigen may facilitate a state of immunologic tolerance, allowing virus replication to proceed unchecked. The dose-dependent effects of SIV in neonates appear to be analogous to dose-dependent effects of other agents in mice[18,20].

Vaccination of female monkeys during the second trimester of pregnancy did not appear to result in transmission of vaccine virus to any of the fetuses *in utero*. Although vaccination of the dam did not prevent infection of the neonates when high-dose SIVΔ3 was administered orally within 4 hours of delivery, none of these orally infected infants born to infected

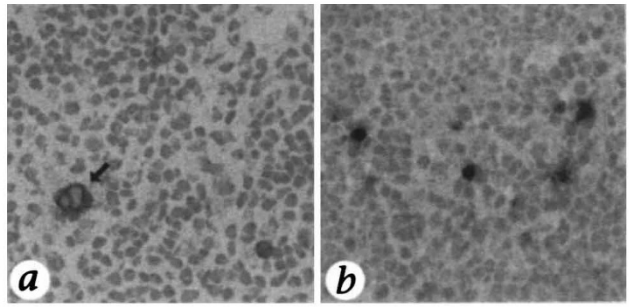

Fig. 4 Detection of virus-infected cells by immunohistochemistry for SIV gp-120 (*a*) and nonisotopic *in situ* hybridization for viral nucleic acid (*b*) on adjacent sections of lymph node from 91-95. Several cells positive for SIV gp-120, one of which appears to be binucleate (arrow) are visible (*a*). Many more positive cells were detected by *in situ* hybridization (*b*). Magnification, ×100.

Table 2 Response of pregnant females to SIV239Δ3 vaccination

	Pregnant female macaque			
	63-88	115-88	120-88	99-88
Post vaccination				
Day 15 PBMC load	6	4	+	+
Day 29 PBMC load	4.5	3	+	+
Day of delivery				
PBMC culture	–	+	+	–
Amniotic culture	–	–	–	–
ELISA antibody	1.348	0.352	1.786	ND
Neutralizing activity	1:672	1:94	ND	ND

*Cell-associated viral loads are presented as in Table 1. +, Cultures were positive with 10^7 PBMCs, but further dilutions were not tested; –,cultures were negative for SIV recovery using 10^7 PBMCs or 1 ml of amniotic fluid. ELISA antibodies are presented as absorbance at 405 nm for a 1:100 dilution of serum as previously described[10,16]. Procedures for measuring neutralizing antibody titers to laboratory-adapted SIVmac251 have also been described[16]. ND, not done.

Table 3 Infection by SIV239Δ3 of neonates born to vaccinated mothers

Neonate macaques	623-95	515-95	905-95	807-95
Day of birth				
PBMC culture	–	–	–	–
Cord culture	–	–	–	–
PBMC PCR	–	–	–	–
ELISA antibody	0.934	0.035	0.522	ND
Neutralizing activity	1:375	<1:30	1:139	ND
PBMC recovery/load				
Month 1	+	+	+	NS
Month 4	+	+	–	NS
Month 5	0	–	2.5	NS
Month 6	1.5	0	0	NS
Month 7	0	–	0	NS
Month 8	0	1.0	0	NS

Macaques nos. 623-95, 515-95, 905-95 and 807-95 were born to 63-88, 115-88, 120-88, and 99-88, respectively. SIV could not be recovered from 10^7 PBMCs or cord blood cells on the day of birth, and PBMCs were also negative for SIV sequences with a sensitive nested PCR procedure on the day of birth[10,27]. Cell-associated viral loads at the indicated months after SIVΔ3 was administered orally on the day of birth are presented as in Table 1, or as positive (+) or negative (–) with 10^7 PBMCs. ND, not done; NS, sample not available as animal died one day after birth.

mothers exhibited high viral loads or signs of disease progression. Passive transfer of immunity from the dams seems likely to have played a role in limiting virus replication in these neonates.

The results of Baba *et al.* with SIVΔ3 (ref. 12) raised concerns over the safety of the live-attenuated vaccine approach for neonates. Our results suggest that the pathogenicity of SIVΔ3 in neonates is restricted to or heavily weighted toward the extreme conditions of very high input inocula and the absence of maternal antibodies. We feel that these extreme conditions do not reflect what would be expected naturally for fetal or infant exposure to such vaccine strains. Virus loads in monkeys vaccinated with SIVΔ3 and in chimpanzees vaccinated with the corresponding HIV-1Δ3 (ref. 21) are extremely low and are not likely to be adequate for fetal or neonatal exposure[22,23]. Indeed, we observed no maternal-fetal transmission even when four pregnant females were vaccinated during the second trimester. Furthermore, natural transmission of vaccine strain from mother to offspring would occur in the presence of maternal immunity, conditions in which disease progression did not occur in our studies. Although animal model studies have their limitations, including the limited numbers of animals used, it must also be remembered that most or all live-attenuated vaccines currently licensed are pathogenic in at least some immunocompromised individuals or some situations. This has not precluded their widespread use nor has it vitiated their efficacy[24]. The critical issue to be considered in using such a vaccine is the frequency of adverse events associated with the vaccine in the target population versus the frequency of wild-type virus infection and disease in that same target population.

Methods

Virus stocks. Derivatives of SIVmac239 with multiple gene deletions have been described previously[13,25]. SIVmac239Δ3 is missing *nef*, *vpr* and US sequences; SIVmac239Δ3x is missing *nef*, *vpx* and US sequences; SIVmac239Δ4 is missing *nef*, *vpr*, *vpx*, and US sequences. Cloned DNA was transfected into CEMx174 cells with a DEAE-dextran (diethylaminoethyl-dextran) protocol, and virus was harvested from the cell-free supernatant at or near the peak of virus production (9–13 days after transfection). Virus stocks were filtered through a 0.45-μm filter before freezing in aliquots at –165 °C. The concentration of p27 viral capsid antigen in virus stocks was measured with a commercial antigen capture kit (Coulter Immunology, Hialeah, FL). Tissue culture infectious doses ($TCID_{50}$) were measured using serial threefold dilutions in duplicate on CEMx174 cells. The stock of SIVmac239Δ3 that was used contained 113 ng/ml of p27 and 2.3×10^5 $TCID_{50}$ per ml.

Cell-associated viral loads. PBMCs were separated over Ficoll-Paque (Pharmacia, Piscataway, NJ) from heparinized whole blood. Twelve serial 1:3 dilutions of isolated PBMCs, beginning with 10^6 cells, were cocultured in duplicate with 10^5 CEMx174 cells per well in 24-well plates (total volume, 1 ml). After 3 to 4 days of culture, 1 ml of medium [RPMI 1640 containing 10% fetal bovine serum, HEPES, L-glutamine, penicillin-streptomycin, 2-mercaptoethanol, and amphotericin B (Fungizone; Gibco, Grand Island, NY)] was added to each well. The cultures were split every three to four days at a 1:1 dilution. Supernatant samples were collected after 21 days of culture and stored frozen at less than or equal to –70 °C until being assayed for p27 antigen with the Coulter p27 antigen assay kit.

CD4 counts. Whole blood collected in EDTA was analyzed for lymphocyte subsets CD4 [OKT4a (Ortho, Raritan, NJ) and Anti-Leu-3a (Becton Dickinson, Franklyn Lakes, NJ)] CD8 [Anti-Leu-2a (Becton Dickinson)], and CDw29 [4B4 (Coulter Immunology)] by a whole-blood lysis technique. Antibody (5 to 20 μl, depending on the antibody) was added to 100 μl of EDTA whole blood and incubated in the dark for 10 min. Lysing solution (2 ml; Becton Dickinson) was added, and samples were incubated for 10 min at room temperature. Stained cells were washed once in minimum essential medium with 5% fetal calf serum and then fixed in 0.5% paraformaldehyde. Samples were analyzed on a Becton Dickinson FACScan flow cytometer.

Antibody responses. Antibody responses to whole lysed SIV were mea-

Table 4 Pathogenicity of SIV vaccine strains in neonates

No. of Neonates	Strain	Dose	Route	Status	High loads and disease
3	Δ3	high (283)	oral	naive	2/3
3	Δ3	med (50)	oral	naive	0/3
3	Δ3	low (5)	oral	naive	0/3
3	Δ3	high (283)	oral	maternal Ab	0/3
2	Δ3	med (50)	i.v.	naive	0/2
2	Δ3x	med (50)	i.v.	naive	0/2
2	Δ4	high (300)	oral	naive	0/2

Abbreviations: med, medium; Ab, antibody; i.v., intravenous.

sured by ELISA as described previously[10,16]. Briefly, SIV was concentrated by ultracentrifugation and purified by Sepharose chromatography (Pharmacia). Purified SIV was lysed in dilute Triton X-100 detergent and used to coat ELISA plates. Neutralizing activity was measured against laboratory adapted SIVmac as previously described[10].

Polymerase chain reaction. Viral DNA in PBMCs was detected with a sensitive nested PCR procedure as previously described[10,26].

***In situ* hybridization and immunohistochemical staining.** Tissues were collected immediately after animals were killed or died, fixed in neutral buffered formalin and snap-frozen in OCT compound (Miles Scientific, Elkhart, IN) by immersion in 2-methylbutane cooled on dry ice. Cryostat sections 6 μm thick were examined by immunohistochemistry and *in situ* hybridization. Immunohistochemistry and *in situ* hybridization for SIV were performed as described previously using a monoclonal antibody to SIV gp120 and a digoxigenin-labeled DNA probe[13,15]. The DNA probe used a combination of plasmids that spans essentially all of the SIVmac239 genome.

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1. Giavedoni, L.D. *et al.* Immune response of rhesus macaques to recombinant simian immunodeficiency virus gp130 does not protect from challenge infection. *J. Virol.* **67,** 577–583 (1993).
2. Cranage, M. *et al.* Immune responses to both cellular and viral proteins can mediate protection against infection with SIVmac. in *Vaccines 94: Modern Approaches to New Vaccines Including Prevention of AIDS.* (eds. Norrby, E., Brown, F., Chanock, R. & Ginsberg, H.S.) 203 –209 (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1994).
3. Cranage, M.P. *et al.* Intrarectal challenge of macaques vaccinated with formalin-inactivated simian immunodeficiency virus. *Lancet* **339,** 273–274 (1992).
4. Putkonen, P. *et al.* Whole inactivated SIV vaccine grown on human cells fails to protect against homologous SIV grown on simian cells. *J. Med. Primatol.* **22,** 100–103 (1993).
5. Stahl-Hennig, C. *et al.* Protection of monkeys by a split vaccine against SIVmac depends upon biological properties of the challenge virus. *AIDS* **7,** 787–795 (1993).
6. Mills, K.H.G. *et al.* Immunization of macaques with SIV env recombinants: Specificity of T cell and antibody responses and evaluation of protective efficacy. *J. Med. Primatol.* **22,** 104–109 (1993).
7. Daniel, M.D. *et al.* High titer immune responses elicited by recombinant vaccinia virus priming and particle boosting are ineffective in preventing virulent SIV infection. *AIDS Res. Hum. Retroviruses* **10,** 839–851 (1994).
8. Daniel, M.D., Kirchhoff, F., Czajak, S.C., Sehgal, P.K. & Desrosiers, R.C. Protective effects of a live-attenuated SIV vaccine with a deletion in the *nef* gene. *Science* **258,** 1938–1941 (1992).
9. Almond, N. *et al.* Protection by attenuated simian immunodeficiency virus in macaques against challenge with virus-infected cells. *Lancet* **345,** 1342–1344 (1995).
10. Wyand, M.S., Manson, K.H., Garcia-Moll, M., Montefiori, D. & Desrosiers, R.C. Vaccine protection by a triple deletion mutant of simian immunodeficiency virus. *J. Virol.* **70,** 3724 (1996).
11. Kestler, H.W., III *et al.* Importance of the *nef* gene for maintenance of high virus loads and for the development of AIDS. *Cell* **65,** 651–662 (1991).
12. Baba, T.W. *et al.* Pathogenicity of live, attenuated SIV after mucosal infection of neonatal macaques. *Science* **267,** 1820–1825 (1995).
13. Gibbs, J.S. *et al.* Progression to AIDS in the absence of genes for *vpr* or *vpx*. *J. Virol.* **69,** 2378–2383 (1995).
14. Horvath, C.J., Hunt, R.D., Simon, M.A., Sehgal, P.K. & Ringler, D.J. An immunohistologic study of granulomatous inflammation in SIV-infected rhesus monkeys. *J. Leukocyte Biol.* **53,** 532–540 (1993).
15. Ilyinskii, P.O., Daniel, M.D., Simon, M.A., Lackner, A.A. & Desrosiers, R.C. The role of upstream U3 sequences in the pathogenesis of simian immunodeficiency virus-induced AIDS in rhesus monkeys. *J. Virol.* **68,** 5933–5944 (1994).
16. Daniel, M.D. *et al.* Long-term persistent infection of macaque monkeys with the simian immunodeficiency virus. *J. Gen. Virol.* **68,** 3183–3189 (1987).
17. Lewis, M.G. *et al.* Titration and characterization of two rhesus derived SIVmac challenge stocks. *AIDS Res. Hum. Retroviruses* **10,** 213–220 (1994).
18. Ridge, J.P., Fuchs, E.J. & Matzinger, P. Neonatal tolerance revisited: Turning on newborn T cells with dendritic cells. *Science* **271,** 1723–1726 (1996).
19. Sarzotti, M., Robbins, D.S. & Hoffman, P.M. Induction of protective CTL responses in newborn mice by a murine retrovirus. *Science* **261,** 1726–1728 (1996).
20. Forsthuber, T., Yip, H.C. & Lehmann, P.V. Induction of T_H1 and T_H2 immunity in neonatal mice. *Science* **271,** 1728–1730 (1996).
21. Desrosiers, R.C., Daniel, M., Lifson, J. & Arthur, L.O. Infection of chimpanzees with attenuated HIV-1 deletion mutants. in *Dixième Colloque des Cent Gardes: Retroviruses of Human AIDS and Related Animal Diseases,* 23–25 October 1995, Marnes-la-Coquette, Paris, France (eds. Girard, M. & Dodet, B.) 197– (Elsevier, Amsterdam,1995).
22. Dickover, R.E. *et al.* Identification of levels of maternal HIV-1 RNA associated with risk of perinatal transmission: effect of maternal zidovudine treatment on viral load. *JAMA* **275,** 599–605 (1996).
23. Weiser, B. *et al.* Quantitation of human immunodeficiency virus type 1 during pregnancy: relationship of viral titer to mother-to-child transmission and stability of viral load. *Proc. Natl. Acad. Sci. USA* **91,** 8037–8041 (1994).
24. Deacon, N.J., McPhee, D.A., Crowe, S., Learmont, J. & Mills, J. Attenuated HIV vaccine: caveats: Response. *Science* **271,** 1790–1792 (1996).
25. Gibbs, J.S., Regier, D.A. & Desrosiers, R.C. Construction and *in vitro* properties of SIVmac mutants with deletions in the "nonessential" genes. *AIDS Res. Hum. Retroviruses* **10,** 607–616 (1994).
26. Kirchhoff, F., Kestler, H.W., III & Desrosiers, R.C. Upstream U3 sequences in simian immunodeficiency virus are selectively deleted in vivo in the absence of an intact *nef* gene. *J. Virol.* **68,** 2031–2037 (1994).