higher eukaryotic organisms (*21*). Nae I–L43K opens the possibility of engineering proteins that use similar combinations of sequence-specific endonuclease and ligase domains as valuable in vivo reagents for genomic manipulation at sequence-specific targets.

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Pathogenicity of Live, Attenuated SIV After Mucosal Infection of Neonatal Macaques

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Adult macaques do not develop disease after infection with a *nef* deletion mutant of the simian immunodeficiency virus (SIV) and are protected against challenge with pathogenic virus. This finding led to the proposal to use *nef*-deleted viruses as live, attenuated vaccines to prevent human acquired immunodeficiency syndrome (AIDS). In contrast, neonatal macaques developed persistently high levels of viremia after oral exposure to an SIV *nef*, *vpr*, and negative regulatory element (NRE) deletion mutant. Severe hemolytic anemia, thrombocytopenia, and CD4$^+$ T cell depletion were observed, indicating that neither *nef* nor *vpr* determine pathogenicity in neonates. Because such constructs have retained their pathogenic potential, they should not be used as candidate live, attenuated virus vaccines against human AIDS.

SIV infection of rhesus monkeys is considered to be the best model for human immunodeficiency virus–type 1 (HIV-1) infection of humans (*1*). The viral genomes of HIV-1 and SIV are closely related, and both viruses infect similar target cells. Moreover, SIV disease in rhesus macaques is comparable to human AIDS; SIV infection of adult macaques results in high levels of virus replication, CD4$^+$ T cell depletion, and immunosuppression (*2*). In contrast, a molecular construct of SIV with a deletion in the auxiliary *nef* gene replicated poorly in adult macaques after intravenous injection (*3*). Although these animals were persistently infected with low amounts of virus, they maintained normal CD4$^+$ T cell counts. During a follow-up of more than 3 years, no signs of immunodeficiency developed. When challenged with pathogenic SIV, these animals were protected from disease (*4*). On the basis of these data, *nef*-deleted mutants have been proposed as live, attenuated virus vaccines to protect humans against HIV-1 (*4*, *5*). Vaccine studies with *nef*-deleted HIV-1 viral constructs are ongoing in chimpanzees (*6*).

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The data generated from adult macaques infected with the *nef*-deleted SIV also led to the hypothesis that *nef* is a major determinant of pathogenicity in vivo for immunosuppressive lentiviruses (*3*). The *nef* gene product, a myristylated phosphoprotein of 34 kD apparent molecular size, down-regulates CD4 surface antigen expression but is not required for virus replication in vitro in T cell lines (*7*). Other Nef functions are more controversial; various effects on transcription from the HIV-1 long terminal repeat (LTR) have been described (*8*). According to more recent reports (*9*), Nef facilitates virus replication in unstimulated peripheral blood mononuclear cells (PBMCs).

Studying the pathogenicity and prevention of perinatal retrovirus transmission is important, given the increasing numbers of HIV-1–infected children (*10*). It appears that some newborns become infected after mucosal exposure to infectious maternal blood or secretions (*11*). We have developed a primate model of neonatal mucosal infection (*12*). Rhesus monkey (*Macaca mulatta*) neonates were exposed orally to cell-free SIV immediately after delivery. All exposed neonates became infected; high amounts of virus were seen in all animals, and one-half died of AIDS within 6 months.

As a potential live, attenuated vaccine virus, we evaluated a mutant of SIV deleted in *nef*, *vpr*, and NRE, termed SIVΔ3 (*13*). Like *nef*, *vpr* is an accessory gene that is believed to increase virus replication in cultured T cells and monocytes and macrophages (*14*). Vpr is virion-associated and has been implicated in achieving high amounts of virus replication and disease development in vivo (*15*). NRE, a negative regulatory element located in the LTR (*16*), had been removed to ensure adequate viral replication. The resulting triply deleted SIV mutant replicates well in cultured cells, including macaque PBMCs. In adult macaques injected intravenously with an amount of virus containing 5 ng of p27 [corresponding to 2000 50% tissue culture infectious doses ($TCID_{50}$)], virus could be isolated only transiently, but the animals remained seropositive and polymerase chain reaction (PCR)–positive. When challenged intravenously with 10 animal infectious doses of pathogenic SIV_{mac251}, protection was seen (*13*).

Three rhesus monkey neonates were given cell-free SIVΔ3 orally within 1 hour after cesarean section delivery (*17*). The oral SIVΔ3 dose given was 1.6 μg of p27, corresponding to 5.3×10^5 $TCID_{50}$. This dose was ~300 times the dose given intravenously to adult macaques as vaccine previously (*13*); it was chosen because mucosal infection in general requires a higher virus inoculum, as compared with intravenous inoculation (*18*). The selection of the dose

Table 1. Virus load in SIVΔ3-infected macaques. Preservative-free, heparinized blood was obtained serially from SIVΔ3-exposed macaques. Plasma and PBMCs were isolated from Ficoll-Hypaque density gradients and titrated by serial fourfold end-point dilution in quadruplicate cultures. Culture supernatants were tested for p27 antigen after 3 weeks of cocultivation with CEMx174 cells, as described. This assay does not cross-react with STLV-1 and simian type D retroviruses (*26*). Plus signs indicate that virus cultures were positive, but not titrated. For these assays, 10^6 PBMCs were used. Minus signs indicate that virus cultures were negative, but not titrated. For these assays, 10^6 PBMCs were also used. Plasma values are given as the $TCID_{50}$ per milliliter of plasma; PBMC values are given as the minimum number of PBMCs required for positive culture. Values given in parentheses were performed at the week indicated in parentheses.

Animal	Source	Weeks after virus exposure												
		1	2	4	6 (7)	10 (11)	15 (16)	20 (22)	24 (26)	31	35 (36)	40	41	46
93-7	Plasma		+	+	(−)	−	(+)	(32)	(8)	8	(1290)	256	8	0*
	PBMCs		+	+	(+)	+	(+)	(942)	(+)	1831	(610)	3900	2444	2444*
94-1	Plasma		32	8	0	(32)	1624	2048	4		128			
	PBMCs		244	1514	$>10^6$	(76)	38	153	610		610			
94-2	Plasma		4	0	4	(8)	0	0	0		0			
	PBMCs		3906	8789	1220	(9766)	9766	9766	9766		2444			
94-4†	Plasma	32	64	4	10	6		426‡						
	PBMCs	3.9×10^4	122	153	610	610		153‡						
E801§	Plasma	22	52	4	0	0		0‡						
	PBMCs	1.6×10^5	690	2444	1.6×10^5	$>10^6$‖		$>10^6$‡						
93-9¶	Plasma		110	−	(−)	26	(+)	(8)	(4092)					
	PBMCs	$>10^6$	+	+	(+)	+	(+)	(1.1×10^5)	(4900)					

*Animal 93-7 was euthanized 2 days after this blood sampling. †Infant 94-4 was exposed orally to 2.5 ml of whole blood from infant 93-7. ‡Tests carried out at 21 weeks after infection. §Mother E801 was exposed intravenously to 2.5 ml of whole blood from infant 93-7. ‖The negative result ($>10^6$ cells) was the same for week 8 and week 10. ¶Infant 93-9 was exposed orally to pathogenic SIV_{mac251} within 1 hour after cesarean section.

was based also on our other studies with SIV_{mac251} in fetal (*19*) and neonatal rhesus monkeys (*12*). All three neonates were viremic by 2 weeks of age, and all have maintained persistently high virus loads as determined by end-point dilution of plasma and PBMCs (Table 1). This result is in contrast to intravenous injection of SIVΔ3 into adult macaques, which cleared the virus rapidly (*13*); virus could not be recovered from PBMCs of adult macaques even when 10^6 cells were cocultivated, indicating at least a 1000-fold difference in virus load as compared with the wild-type virus. For comparison, we present the virus load in a neonate exposed orally to SIV_{mac251} within 1 hour after cesarean section delivery (Table 1, control animal 93-9).

Serial blood samples were analyzed for $CD4^+$ T cell counts. A persistent decrease in the number of $CD4^+$ T cells and inversion of the CD4/CD8 ratios were noted in animals 93-7 (Table 2) and 94-1 (Table 3). Thus far, infant 94-2 had one CD4 count that was low for its age (*20*). The CD29 subset of $CD4^+$ T cells, an indicator of immunosuppression (*21*), was also depleted in 93-7 and 94-1 (Tables 2 and 3). For comparison, values of an uninfected infant are shown in Table 4.

Table 2. Hematological data on infant 93-7 exposed to SIVΔ3. WBC, white blood cell count; Hb, hemoglobin; Hct, hematocrit; Plts, platelet count; and Retic, reticulocyte count. Normal values for rhesus macaque infants at 12 through 122 weeks of age range between 1.1 and 2.3% (*36*). CD4, CD8, and CD29 are expressed as absolute counts; the percentages of positively staining lymphocytes are given in parentheses. Values for CD29 below 10% are abnormal, even in adults. $CD4^+$ T cells were measured by flow cytometry with antibodies Leu3a and OKT4. $CD8^+$ T cells were measured with antibody Leu2a, and the $CD29^+$ subset of $CD4^+$ T cells was measured with antibody 4B4.

Test	Weeks after virus exposure					
	33	33.5	39	41	43	46*
10^3 WBC	8.2	5.9†	19.9†	7.5	8.5†	9.1
Hb	6.5	6.0	6.0	4.7	5.5	4.2
Hct (%)	20.7	20.0	18.8	14.7	16.7	13.8
10^3 Plts	136	48	77	116	213	140
Retic (%)			11.6			9.8
CD4	380			195		311
	(5.9)			(4.6)		(4.8)
CD8	2187			588		1282
	(34.2)			(14)		(19.8)
CD29	151			50		173
	(2.4)			(1.2)		(2.7)

*The animal was euthanized 2 days after this blood sampling. †Corrected for nucleated blood cells.

In addition to the immunosuppression observed in HIV-1–infected individuals, several other manifestations of immune dysregulation have been reported, such as Coombs-positive hemolytic anemia and immune thrombocytopenia (*22*). Neonate 93-7 developed a life-threatening direct Coombs-positive hemolytic anemia. This animal had persistently elevated reticulocyte counts (Table 2). It also developed a transient severe thrombocytopenia that did not result in overt bleeding. A bone marrow biopsy confirmed the clinical diagnosis; severe erythroid hyperplasia was observed, as well as an abundance of megakaryocytes, indicative of peripheral platelet destruction. Platelet counts for animal 94-1 were also repeatedly low at weeks 24 and 35 (Table 3).

In human HIV-1 infection, an indicator of poor prognosis and imminent progression to full-blown AIDS is the loss of antibody to the major viral capsid antigen p24 (*23*). All three SIVΔ3-infected infant macaques exhibited a similar pattern of loss of antibody responsiveness to Gag (p26), predating the development of full-blown AIDS (Fig. 1, A and B).

We monitored all SIVΔ3-infected macaque neonates for the development of disease. Transient rash and lymphadenopathy were seen in 93-7. A lymph node biopsy revealed follicular hyperplasia. In situ hybridization to an SIV-specific riboprobe (*24*) showed substantial amounts of virus predominantly in the follicular B cell areas of the lymph node (*20*). By 46 weeks of age, the hematocrit of animal 93-7 had decreased to 13.8%. The animal became increasingly lethargic and was euthanized. A complete

necropsy revealed the stigmata of severe hemolytic disease (*25*). Surviving animals 94-1 and 94-2 continue to be observed.

The discovery that AIDS developed in animal 93-7 prompted a search for *nef* and *vpr* sequences by PCR analysis. DNA isolated directly from uncultured PBMCs obtained at week 22 yielded fragments of the size expected for SIVΔ3; we found no evidence of the wild-type *nef* or *vpr* alleles (Fig. 2). As a further control, product DNA obtained from the PCR analysis for *nef* and NRE deletions was subjected directly to DNA sequence analysis; the nucleotide sequence was identical to that obtained from the *nef*- and NRE-deleted control plasmid (*20*).

To test whether the virus in infant 93-7 had maintained its phenotype (attenuation after intravenous injection in adult macaques), we conducted an in vivo virus passage during week 39 after infection, after the disease had developed in infant 93-7. Immediately after cesarean section delivery, 2.5 ml each of infectious, whole blood from this infant were administered intravenously to recipient mother E801 and orally to her neonate 94-4. The infectious titer of 2.5 ml of blood was estimated at 3.4×10^3 $TCID_{50}$ total, as calculated from end-point titrations of plasma and PBMCs. The recipient animals were followed by virus isolation (Table 1) and protein immunoblots (Fig. 1C). Both mother and infant were virus-positive 1 week after virus exposure. The number of infectious PBMCs peaked at week 2 in the maternal circulation, and from week 8 onward, no virus could be isolated even when 10^6 cells were used in the cocultivation. This pattern of viremia was similar to that described for other adult macaques vaccinated intravenously with SIVΔ3 (*13*). In contrast, infant 94-4 has maintained a persistent, high level of viremia (Table 1), which is >1000-fold higher than that of its mother at week 10.

The argument might be raised that the neonates were carrying adventitious pathogens that could have augmented SIVΔ3 replication. During our review of serological testing, we discovered that mothers E968 and E801 were positive for antibodies to simian T lymphotropic virus–type I (STLV-I). Infants 93-7 and 94-4 tested negative for antibodies to STLV-I. Repeated serological testing for all mothers and serological testing of all infants for simian type D retroviruses was negative. Additionally, we subjected DNA isolated from all infants to PCR analysis to test for the presence of STLV-I and type D virus sequences. No sequences derived from these viruses were found in the infants. For STLV-I, PCR analysis allowed detection of one infected cell among 2000 PBMCs; for the detection of specific SIV *nef* alleles, the sensitivity was one infected cell among approximately 900 PBMCs; and for type D retrovirus sequences, the sensitivity was one infected cell among 8333 PBMCs, or ~10 times more sensitive than the PCR for SIVΔ3. According to our estimates for infant 93-7, SIVΔ3 would be >1000-fold more prevalent than type D sequences, if the latter were present. The high amounts of virus seen in all four infants were confirmed by SIV p27 antigen capture assay, which is specific for SIV core antigen and does not cross-react

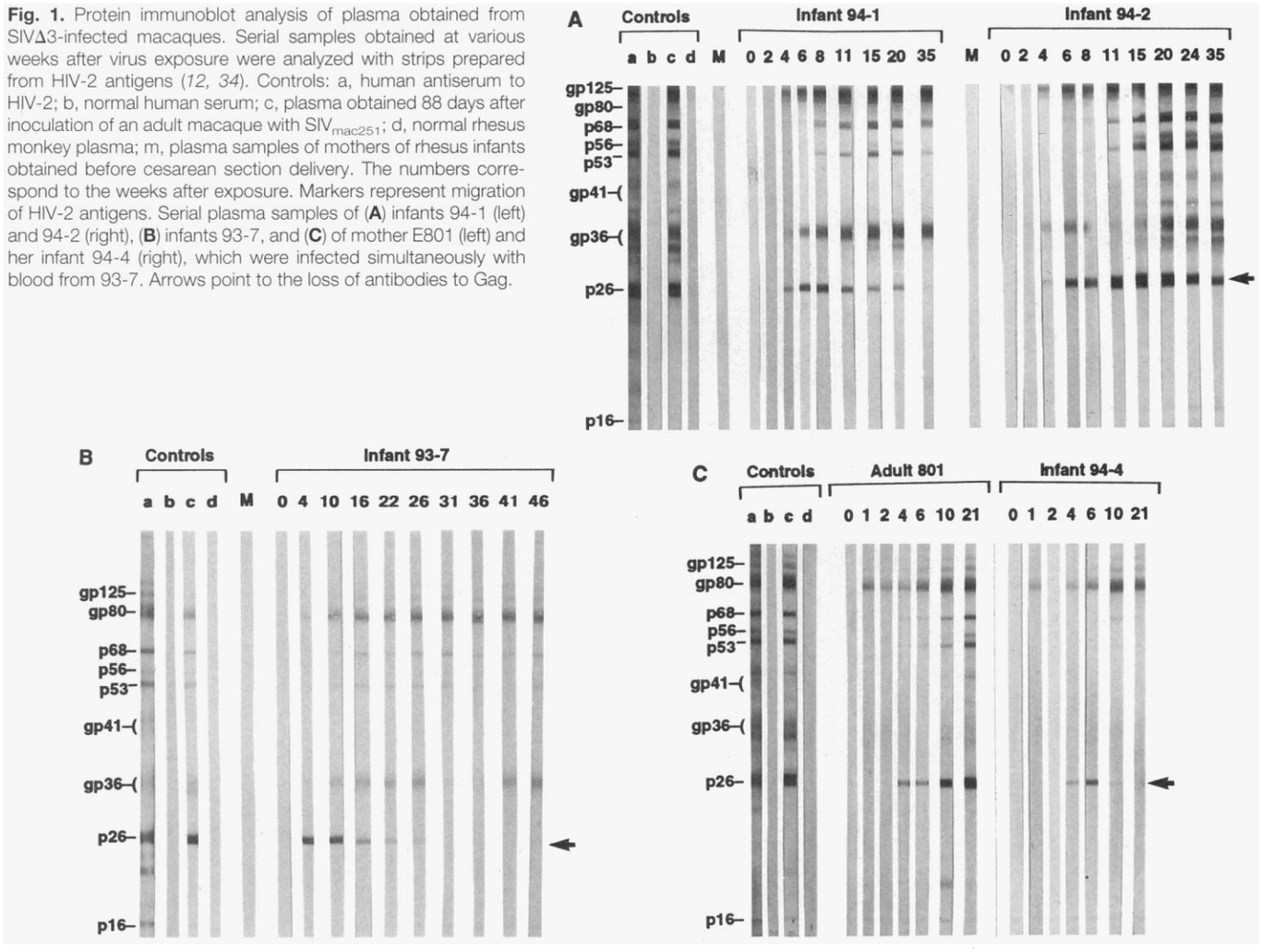

Fig. 1. Protein immunoblot analysis of plasma obtained from SIVΔ3-infected macaques. Serial samples obtained at various weeks after virus exposure were analyzed with strips prepared from HIV-2 antigens (*12, 34*). Controls: a, human antiserum to HIV-2; b, normal human serum; c, plasma obtained 88 days after inoculation of an adult macaque with SIV_{mac251}; d, normal rhesus monkey plasma; m, plasma samples of mothers of rhesus infants obtained before cesarean section delivery. The numbers correspond to the weeks after exposure. Markers represent migration of HIV-2 antigens. Serial plasma samples of (**A**) infants 94-1 (left) and 94-2 (right), (**B**) infants 93-7, and (**C**) of mother E801 (left) and her infant 94-4 (right), which were infected simultaneously with blood from 93-7. Arrows point to the loss of antibodies to Gag.

with either STLV-I or simian type D retroviruses (26).

Our data revealed high levels of virus replication in all macaque neonates after mucosal SIVΔ3 infection. The virus maintained the deletions in *nef*, *vpr*, and NRE and its pattern of restricted replication in the macaque mother infected intravenously with blood from a diseased infant. Fatal disease, characterized by severe $CD4^+$ T cell depletion, anemia, thrombocytopenia, or renal disease developed in two infants. Early signs of disease were also present in the surviving infants. These findings are in contrast to those observed in adult macaques infected with *nef*-deleted SIV mutants. Our data demonstrate that neither Nef nor Vpr are required for high levels of virus replication or the pathogenicity of the NRE-deleted SIV mutant after mucosal infection of newborn macaques.

Neonates often develop more severe disease after infection with viruses such as herpes simplex virus, varicella zoster virus, and HIV-1, as compared with adults. In mice, the full pathogenic potential of Moloney murine leukemia virus and the neurotropic retrovirus Cas-Br-E is seen only after infection of neonates (27). The differences between our data and those derived from studies involving intravenous administration of SIVΔ3 to adults could be explained by age-dependent host factors. Perhaps neonatal animals in general allow virus to replicate vigorously in the absence of a functioning *nef* gene; unknown cellular factors found only in the neonatal period could compensate for *nef* function. It is also possible that unknown antigens encountered by neonates cause widespread T cell activation that could decrease or obviate the need for Nef. The transfer experiment in which equal amounts of blood from an infected, sick infant were given simultaneously to a recipient mother and her infant indicates that viral factors cannot account for the observed differences in virus load; the inoculum used led to persistent, high levels of virus replication and early signs of disease in the infant, whereas the mother was able to suppress virus replication within a few weeks after the intravenous injection.

One could postulate that the differential responses observed in adults as compared with neonates can be ascribed to the route of virus inoculation. We believe that SIVΔ3 initially encounters different target cells, depending on the portal of entry. Using cultured cells, we have found that acutely infected Langerhans cells, but not T cells, rapidly disseminate HIV-1 to activated T cells after a short cell-cell contact, resulting in a burst of virus production (28). Because mucosal tissues of the alimentary

Table 3. Hematological data on infant 94-1 exposed to SIVΔ3. Methods and abbreviations are as in Table 2.

Test	Weeks after virus exposure					
	3	7	15	20	24	35
10^3 WBC	4.7	3.7	5.8	11.2	5.1	9.8
Hb	13.3	12.1	13.0	12.3	14.1	12.6
Hct (%)	40.7	37.7	42.1	39.1	41.3	40.1
10^3 Plts	630	664	418	314	174	215
CD4	1375 (40.6)	752 (29.5)	652 (27.4)	1493 (26.7)	742 (26.4)	844 (12.3)
CD8	1298 (38.3)	759 (29.8)	904 (38)	1987 (35.5)	724 (25.8)	1427 (20.8)
CD29	475 (14)	305 (12)	205 (8.6)	399 (7.1)	225 (8)	293 (4.26)

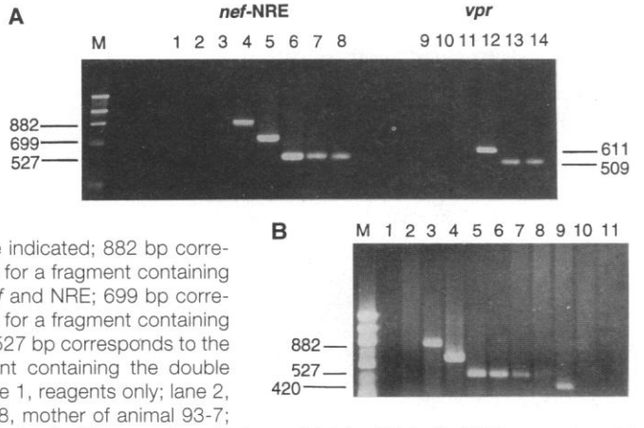

Fig. 2. PCR analysis of uncultured PBMC DNA of SIVΔ3-infected macaques. (**A**) PCR analysis of 93-7 DNA for deletions in *vpr*, *nef*, and NRE. Genomic DNA was prepared directly from PBMCs or CEMx174 cells. The sizes of the fragments (in base pairs) are indicated; 882 bp corresponds to the size expected for a fragment containing intact sequences of both *nef* and NRE; 699 bp corresponds to the size expected for a fragment containing the deletion only in *nef*; and 527 bp corresponds to the size expected for a fragment containing the double deletion in *nef* and NRE. Lane 1, reagents only; lane 2, CEMx174 DNA; lane 3, E968, mother of animal 93-7; lane 4, CEMx174 cells infected with SIV_{mac239}; lane 5, the *nef*-deleted 3′-half of SIV_{mac239} plasmid p239-3′Δ*nef*; lane 6, the doubly deleted 3′-half of SIV_{mac239} plasmid DNA p239-3′Δ*nef*ΔNRE; lane 7, CEMx174 DNA infected with SIVΔ3; lane 8, infant 93-7 infected with SIVΔ3; lane 9, reagents only; lane 10, CEMx174 DNA; lane 11, E968; lane 12, CEMx174 cells infected with SIV_{mac239}; lane 13, CEMx174 cells infected with SIVΔ3; and lane 14, infant 93-7. Lane M, ΦX174 Hae III restriction fragment molecular size marker. PCR was performed as described (35). (**B**) Absence of wild-type *nef* sequences in uncultured PBMC DNA of SIVΔ3-exposed macaques. A fragment of estimated size of 420 bp was produced as a result of an uncharacterized further deletion in the region of the primer pair used. One microgram of genomic DNA or 0.5 μg of plasmid DNA was used. Lane 1, reagents only; lane 2, CEMx174 DNA only; lane 3, CEMx174 infected with SIV_{mac239}; lane 4, plasmid p239-3′Δ*nef*; lane 5, CEMx174 cells infected with SIVΔ3; lane 6, infant 93-7; lane 7, infant 94-1; lane 8, infant 94-2; lane 9, infant 94-4 infected orally with blood from 93-7; lane 10, mother E968; and lane 11, mother E801 given blood from infant 93-7 intravenously.

Table 4. Hematological data on uninfected infant mm347-93 exposed to SIVΔ3. Methods and abbreviations are as in Table 2. As observed in normal human infants, CD4 counts are greater than those observed in normal adults (37).

Test	Weeks of age		
	38	51	56
10^3 WBC	7.0	4.4	8.6
Hb	13.9	13.9	12.8
Hct (%)	43.2	42	39.2
10^3 Plts	433	576	546
CD4	2134 (51.7)	1523 (51.6)	901 (45.5)
CD8	1286 (31.1)	932 (31.6)	710 (35.8)
CD29	631 (15.3)	683 (23.2)	415 (21)

Table 5. Hematological data on infant 94-4 exposed to SIVΔ3. Methods and abbreviations are as in Table 2. Animal 94-4 was severely immunosuppressed, became moribund 34 weeks after infection, and died with renal failure.

Test	Weeks after virus exposure		
	6	10	21
10^3 WBC	6.6	5.3	8.3
Hb	12.9	12.2	9.5
Hct (%)	40.1	39.2	32.2
10^3 Plts	636	688	694
CD4	2100 (38.8)		1347 (28)
CD8	1665 (30.8)		1813 (37.7)
CD29	730 (13.5)		149 (3.1)

tract are known to be rich in Langerhans cells, such a mechanism could explain the pattern of virus replication we observed in the SIVΔ3-infected neonates.

This study also provides insight into the fundamental pathogenesis of immunodeficiency viruses. The course of retroviral infections can be divided into two phases: first, the early replication stage and then the disease stage. We hypothesize that the disease phase is critically dependent on a threshold level of virus replication that must be reached in order for disease to develop; in contrast, subthreshold levels of replicating virus induce protective cellular immune responses that eventually suppress or eliminate the infection. For example, when we suppressed Rauscher murine leukemia virus (R-MuLV) replication with antiviral drugs, we achieved a live virus vaccine effect without changing any genes in the virus genome, that is, without altering the molecular determinants of pathogenicity (*29*). Our live, attenuated R-MuLV vaccine, which was attenuated pharmacologically only, generated protective cellular immune responses (*29*).

On the basis of our data, we postulate that the lentiviral accessory genes *nef* and *vpr* do not determine viral pathogenesis per se but function only to modulate the degree of virus replication in certain cell types. Experimental systems used before now have not provided opportunities to differentiate virus replication from pathogenicity. We ascribe the lack of disease development in adult macaques infected intravenously with SIVΔ3 to subthreshold virus replication. In contrast, we have bypassed the need for these two accessory gene functions in our model; in the susceptible neonatal host, SIVΔ3, a virus deleted in *nef*, *vpr*, and NRE could replicate above threshold levels, thus allowing its full pathogenic potential to be unmasked. We conclude that the true virulence factor or factors must reside within the rest of the multiply truncated SIVΔ3 genome and remain to be defined.

The use of *nef*-deleted HIV-1 variants has been proposed as a live, attenuated vaccine strategy to protect humans against AIDS (*4*, *5*), on the basis of the belief that *nef* is required for full viral pathogenicity (*3*–*5*, *30*); however, this view has not been accepted universally because of the inherent risks associated with the use of replication-competent retroviruses (*31*). In contrast, our data in neonatal macaques support the hypothesis that *nef* is a determinant of virus replication rather than of pathogenicity. Thus, it follows that any factor capable of augmenting viral replication in adults could unlock the pathogenic potential of *nef*-deleted viruses. For instance, intercurrent illness with ensuing immunosuppression could enhance vaccine virus replication; the same could hold true for any agent capable of activating the viral LTR (*32*). If the threshold level of virus replication is reached, disease would then develop, even in adults. Of concern is the possibility of administering *nef*-deleted vaccine virus to individuals coinfected with other retroviruses, such as human T cell leukemia virus–types I and II, which have been shown to enhance HIV-1 replication (*33*). Long-term follow-up studies of adult macaques, including animals subjected to immunosuppression or coinfection with other pathogens, may support our hypothesis. The possibility of coinfection with other retroviruses remains in our study despite our efforts to rule it out because of limitations in sensitivity inherent in any tests, and thus our experiment mimics a real-life situation.

Because *nef*- and *vpr*-deleted viruses are pathogenic in neonatal primates, they should not be considered as candidate live, attenuated virus vaccines against human AIDS; however, they represent tools to help elucidate the nature of the protective immune responses in vaccinated adult macaques. We conclude that our macaque model of neonatal mucosal infection is an indicator of viral pathogenicity and propose that candidate live, attenuated lentivirus vaccines be evaluated in neonatal primates.

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Switch Transcripts in Immunoglobulin Class Switching

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B cells can exchange gene segments for the constant region of the immunoglobulin heavy chain, altering the class and effector function of the antibodies that they produce. Class switching is directed to distinct classes by cytokines, which induce transcription of the targeted DNA sequences. These transcripts are processed, resulting in spliced "switch" transcripts. Switch recombination can be directed to immunoglobulin G1 (IgG1) by the heterologous human metallothionein II_A promoter in mutant mice. Induction of the structurally conserved, spliced switch transcripts is sufficient to target switch recombination to IgG1, whereas transcription alone is not.

Switch recombination is induced in B cells upon activation. It occurs between highly repetitive DNA sequences, the switch (s) regions (located 5′ of the C_H genes), and is directed to distinct immunoglobulin (Ig) classes by cytokines (*1*). The mechanism of this control is not clear, but several molecular changes in the 5′ flanking regions of the switch regions correlate with targeting of switch recombination. These include specific cytokine-inducible protein binding, induction of deoxyribonuclease I–hypersensitive sites, and specific demethylation and transcription (*2*). Switch transcription starts upstream of the switch region, runs through the switch region, and terminates 3′ of the corresponding C_H gene. The transcripts are processed in a way that an exon located 5′ of the switch region (the I_H exon) is spliced to the C_H exons, generating a "switch transcript," also referred to as "germline transcript" or "sterile transcript" (*3*). Despite an almost perfect correlation of induction of switch transcription and switch recombination, the functional relation between the two events is still unclear.

Switch recombination to IgG1 or IgG2b is almost completely abolished in B cells in which the promoter and I exons of the IgG1 (*4*) or IgG2b (*5*) switch transcripts are deleted. Likewise, deletion of the Ig heavy chain gene (IgH) intron enhancer and part of the I exon of IgM disables recombination of the IgM switch region (*6*). However, switch transcription is apparently not sufficient to direct switch recombination. Replacement of the interleukin-4 (IL-4)–responsive promoter of IgE switch transcripts in the 18.81A20 pre-B cell line and in normal B cells by the promoter and enhancer of the Ig variable region genes (V_H promoter–IgH intron enhancer) results in only marginal switch recombination to IgE at about 1% of the frequency induced by IL-4 (*7*). Moreover, because they used V_H control elements, they could not exclude the possibility that even this marginal switch recombination is targeted to IgE by inherent V_H recombination control elements rather than by induction of transcription.

Here, we describe genomic replacement of the IL-4–responsive recombination control element for switch recombination to IgG1 by a heterologous promoter in normal B lymphocytes. The functional role of switch transcription and of structurally conserved, spliced switch transcripts in the targeting of switch recombination to IgG1 are assessed. We chose the human metallothionein II_A (hMT) promoter, which is not known to be involved in any recombination process and which responds to a variety of inductive stimuli like growth factors and differentiation factors [for example, lipopolysaccharide (LPS)] (*8*).

A 1.7-kb region of DNA directly upstream of the IgG1 switch region, containing all known IL-4–inducible molecular elements of the 5′s_γ1 region (*2*), was exchanged for an inversely oriented neomycin resistance gene and the hMT promoter by homologous recombination in murine embryonic stem (ES) cells. We used two different gene-targeting constructs (*9*) differing only in the presence of a 114-bp sequence (Fig. 1), which contains the splice donor site of the I_γ1 exon. Thus, IgG1 switch transcripts generated from the s-hMT–modified (s indicates the splice donor site), but not from the hMT-modified, allele contain the splice donor site of the I_γ1 exon (Fig. 1B). Mutant ES cells (*10*) were generated by homologous recombination of the hMT vector in E14-1 ES cells ($IgH^{a/a}$) and of the s-hMT vector in Δ5′s_γ1 ES cells ($IgH^{\Delta 5' s_\gamma 1 a/a}$); the latter are E14-1 cells with a deletion of the control region for IgG1 switch recombination on one allele, as described (*4*). Cloned heterozygous homologous recombinant cells were detected by polymerase chain reaction

Table 2. IgG1-positive splenic lymphoblasts of heterozygous mutant mice upon LPS stimulation.

Culture	IgG1-positive lymphoblasts (%) of mice		
	Control	hMT/+	s-hMT/s-hMT
1	0.07	0.00	6.12
2	0.03	0.02	7.77
3	0.04	0.00	5.55
Mean	0.05	0.01	6.5

Table 1. IgH loci and expected fragment lengths (in kilobases). The wild-type IgH^a and the mutated $IgH^{\Delta 5' s_\gamma 1}$ locus do not contain the neomycin gene.

Fragment	Length for allele				
	IgH^a	$IgH^{neo\Delta 5' s_\gamma 1}$	$IgH^{\Delta 5' s_\gamma 1}$	$IgH^{s\text{-}hMT}$	IgH^{hMT}
neo–Kpn I	–	2.9	–	3.6	3.5
s_γ1–Eco RI	16.5	10.0	10.0	10.7	10.6

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