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High Viral Load and CD4 Lymphopenia in Rhesus and Cynomolgus Macaques Infected by a

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Chimeric primate lentiviruses composed of SIV and HIV genes may allow the analysis of the role of these discrete HIV genes in viral pathogenesis in macaque monkeys. We have constructed a chimeric virus in which the *env, rev, tat,* and *vpu* genes of HIV-1 Lai replace the *env, rev,* and *tat* genes of the SIVmac239 genome. This virus, SHIVsbg, replicates efficiently in rhesus (Indian and Chinese subspecies) and cynomolgus monkeys with viral loads in PBMC and lymph nodes of up to one infected cell per 30 cells during the acute phase of the infection. Sera from all monkeys recognize specific HIV-1 glycoproteins. The onset of lymphadenopathy in all animals was concurrent with a depletion of CD4 lymphocytes in peripheral blood. The virulence of this SHIV for rhesus and cynomolgus monkeys therefore closely parallels that of HIV-1 for human in the acute phase of the infection. Changes in the *env* and *vpu* genes of a molecular clone of HIV-1 can now be analyzed after passage in nonhuman primate species as the SHIVsbg replicates efficiently. The SHIVsbg-macaque model is an important step in the development of a readily available animal model for HIV-1 vaccine studies.

INTRODUCTION

Since the description of the acquired immune deficiency syndrome (Gottlieb *et al.,* 1981) and the identification of human immunodeficiency virus (HIV) as the etiological agent (Barré-Sinoussi *et al.,* 1983), great progress has been made in understanding lentiviral pathogenesis (Fauci *et al.,* 1993). However, our knowledge about this immunodeficiency disease is still incomplete and the contribution of virus and host factors to HIV virulence is ill-defined. The ideal animal model of HIV-1 infection does not exist and this is a limitation on achieving the ultimate goals of successful prophylaxis and therapy. Due to the very narrow host range of the virus, small laboratory animals are poorly susceptible to HIV infection (Morrow *et al.,* 1987) including those transgenic animals which express the virus receptor (Lores *et al.,* 1992; Dunn *et al.,* 1995).

Despite the inherent drawbacks of using nonhuman primate models, the infection of chimpanzees (*Pan troglodytes*) with HIV (Fultz, 1993) and macaques (*Macaca* species) with simian immunodeficiency viruses (SIV) has greatly facilitated our understanding of HIV infection of human (Desrosiers, 1991). Chimpanzees are consistently susceptible to HIV-1 infection (Fultz *et al.,* 1986). However, viral loads are much reduced (ten Haaft *et al.,* 1995) relative to man and disease is rarely provoked although the DH12 isolate of HIV-1 may be more virulent than those tested up to now (Shibata *et al.,* 1995). Pig-tail macaques (*Macaca nemestrina*) are only poorly sensitive to HIV-1 infection (Agy *et al.,* 1992) and have not even been used as models for vaccine studies. Unfortunately the endangered nature of chimpanzees and the limited supply of the pig-tail macaque further restrict the utility of these primates as experimental models for HIV-1 infection.

A number of pathogenic and nonpathogenic clones of SIV have been characterized in the common species of macaque monkeys (Marthas *et al.,* 1989; Kestler *et al.,* 1990; Johnson *et al.,* 1991; Rud *et al.,* 1994). The attenuation of pathogenic SIVs has helped to define the role of specific primate lentivirus genes in viral pathogenesis (Kestler *et al.,* 1991, Lang *et al.,* 1993; Gibbs *et al.,* 1995) and the SIV-macaque model has also allowed the evaluation of a range of vaccine strategies (Stott, 1994). Although the SIVs are closely related to HIV-2 (Clavel *et al.,* 1986) and the disease induced in macaques is very similar to AIDS in man, it is the difference in the structure and biological properties between the envelope glycoproteins of SIVmac and HIV-1 which is most significant (Shioda *et al.,* 1991; Mori *et al.,* 1992; Robert-Guroff *et al.,* 1992; Hirsch *et al.,* 1994; Kirchhoff *et al.,* 1994). The variability and immunogenicity of the V3 loop of HIV-1 is much greater than the homologous region of SIVmac (Javaherian *et al.,* 1992; Almond *et al.,* 1993). This structure is an important target for HIV-1 neutralizing antibodies (Emini *et al.,* 1992), which has lead to interest in

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the envelope glycoprotein as a component of subunit vaccines.

Chimeric primate lentiviruses (SHIV) consisting of HIV-1 envelope and regulatory genes with the LTRs and *gag* and *pol* genes from SIVmac have been constructed in several laboratories (Shibata *et al.,* 1991; Li *et al.,* 1992; Luciw *et al.,* 1995; Mamounas *et al.,* 1995). This approach *in vivo* should allow the study of HIV-1 envelope subunit vaccines in readily available species of monkey as well as the study of discrete HIV-1 genes in lentiviral pathogenesis (Shibata and Adachi, 1992). Just as low virus load and lack of virulence must be considered deficiencies in the chimpanzee–HIV-1 model, similar problems have been encountered with the macaque–SHIV models (Igarashi *et al.,* 1994; Li *et al.,* 1995). Up to now, evidence for pathogenic effects such as immunodeficiency or even CD4 lymphopenia induced by a molecular clone of SHIV-*env* have not been reported, even for SHIV SF33, which appears to replicate very efficiently in rhesus monkeys (Luciw *et al.,* 1995).

We describe the construction of SHIVsbg from the SIVmac239 and HIV-1 Lai molecular clones and the infection of Indian and Chinese subspecies of rhesus macaque and cynomolgus monkeys. Cell-associated viral loads are similar to those observed in HIV-1 infection of human and the immune response to HIV-1 envelope glycoproteins is rapid and strong. Lymphadenopathy and a depletion of CD4 lymphocyte numbers in peripheral blood are additional indicators of a high level of replication of SHIVsbg in macaques. This model is of fundamental interest in studies of pathogenesis and an important advance for the testing of HIV-1 candidate vaccines for human.

MATERIALS AND METHODS

Animals

Three monkeys of each of the Indian and Chinese subspecies of rhesus macaque (*Macaca mulatta*) and three cynomolgus monkeys (*Macaca fascicularis*) were each infected with 440 $TCID_{50}$ of the SHIVsbg by the intravenous route using the saphenous vein. The rhesus monkeys were 2- to 3-year-old juveniles and the cynomolgus monkeys were young adults. All animals tested seronegative for type D simian retroviruses, simian T-cell leukemia virus type 1, SIVmac, and herpes B prior to the commencement of the experimental protocol. Blood sampling and lymph node biopsies of monkeys were performed under sedation with ketamine (10 mg/kg, Imalgene, Mérieux) and atropine (0.05 mg/kg). The right inguinal lymph node was chosen for biopsy in all cases and the left node was taken from the three animals biopsied a second time. Animals were bled prior to and on the day of infection to establish hematological values and subsequently bled on Days 4, 7, 11, 14, 21, 28, 42, 70, 112, 154, and 210.

Cell cultures

The continuous human cell line CEMx174 is a B/T cell hybrid and the MT-4 cell line is also of human lymphoid origin. Both lines are maintained in RPMI medium at 37° in 5% CO_2 and supplemented with 10% decomplemented fetal calf serum (Gibco, United Kingdom) and 2 m*M* Glutamax (Gibco, United Kingdom). Human and macaque peripheral blood mononuclear cells (PBMC) were isolated on a Ficoll–Hypaque gradient (Eurobio, France), spec. grav., 1.077. After washing in phosphate-buffered saline (PBS), the PBMC were cultivated in the above medium and stimulated with phytohemagglutinin (PHA; Murex Diagnostics Ltd., United Kingdom) at 2 μg/ml for 2 days, after which the PHA was removed by washing. Recombinant human interleukin-2 (Boehringer, Germany) at 20 IU per milliliter was added to all cultures of PBMC.

Construction of chimeric primate lentivirus, SHIVsbg

The genome of SHIVsbg was reconstituted *in vitro* by ligation of the 5′ and 3′ half genomes after *Sph*I digestion. The strategy used to obtain the two half-genomes is derived from that described by Li *et al.* (1992) so only the differences will be indicated here.

The parent plasmids were p239SpSp5′ and p239SpE3′ (Kestler *et al.,* 1990) for SIVmac239 and pJ19-6 and pJ19-13 (Alizon *et al.,* 1984) for HIV-1 Lai, formerly called LAV (Wain-Hobson *et al.,* 1985, 1991). The latter was originally replicated only on human PBMC prior to cloning and contains many point mutations compared to the HXBc2 molecular clone of HIV-1 used by Li *et al.* (1992) for the construction of SHIV-4. The 5′ half-genome was modified as described for SHIV-4 and was cloned into plasmid pBS (Stratagene, CA) and designated pTG4036. However, the 3′ half-genome of SHIVsbg differs from SHIV-4 in several respects other than the origin of the HIV-1 genes incorporated into SIVmac239.

The natural HIV-1 tat splice acceptor site was conserved upstream of the HIV-1 tat coding sequence. Therefore the *Eco*RI restriction site at position 5289 in the pJ19-13 fragment was mutated to a *Sph*I restriction site. These changes allow the recovery of the 3′ *vpr* SIV sequence and a stop codon for the *vpr* coding sequence was engineered (Fig. 1). The resulting *Sph*I-*Kpn*I (5289–5889) fragment was used in the SHIV construct. An additional *Sma*I site was introduced upstream of the gp120–gp41 cleavage site by site-directed mutagenesis of nucleotides 7277–7282 (Wain-Hobson *et al.,* 1985). This mutation results in a Lys to Arg change in the sequence VAPTKA of gp120 of HIV-1 Lai and was introduced to allow the exchange of different gp120 coding sequences by using the *Kpn*I–*Sma*I restriction fragment (positions 5889–7282).

The SIV nef gene and 3′ LTR were derived from p239SpE3′ in which the premature stop codon TAA in the SIVmac239 *nef* gene (Kestler *et al.,* 1990) was mutated to

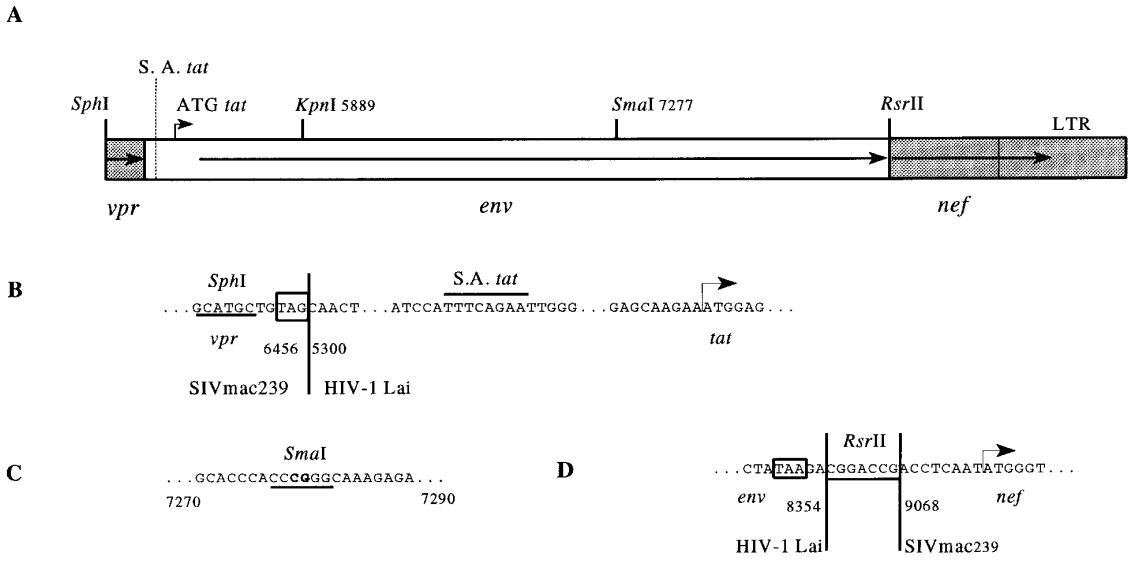

FIG. 1. General organization of the 3′ half-genome of the SHIVsbg. (A) Schematique representation of the 3′ half-genome of SHIVsbg. Gray boxes represent SIVmac239 sequences; the white box comprises HIV-1 Lai sequences coding for *tat, rev, vpu,* and *env.* The first splice acceptor site of *tat* (S. A. *tat*) is marked by a vertical dotted line. The major restriction sites are localized by vertical bars. The arrows represent open reading frames of *env, nef,* and the end of *vpr.* (B) Sequence of the 5′ junction between SIVmac239 and HIV-1 regions. (C) Sequences surrounding the *Sma*I site introducted by site-directed mutagenesis. The mutated nucleotides are in boldface. (D) Sequences of the 3′ junction between HIV-1 Lai and SIVmac239 regions. The A nucleotide located −5 from the start codon of the SIVmac239 *nef* gene is deleted in the SHIVsbg sequence. For (B), (C), and (D) restriction sites are underlined, start codons are marked by an arrow, and stop codons are boxed. The sequences of HIV-1 Lai and SIVmac239 are noted following the numeration of Wain-Hobson *et al.* (1985) and Regier and Desrosiers (1990), respectively.

GAA in order to ensure the presence of a functional Nef protein from the start of SHIVsbg infection. The plasmid containing the 3′ half of the SHIV genome was designated pTG5043.

Preparation of stock of SHIVsbg

The ligation products of the two half-genomes were transfected into the CEMx174 cell line by electroporation using the voltage and capacitance recommended for lymphoid cells (Easyject Plus, Eurogentec, Belgium). A total of 10 μg of viral cDNA was mixed with 10×10^6 cells suspended in 800 μl of RPMI culture medium and a potential difference of 280 V was applied across the chamber with a capacitance of 1500 μF. Cells were subsequently cultured at 10^6 cells/ml and supernatants were assayed for the presence of reverse transcriptase as previously described (Moog *et al.,* 1994).

The presence of infectious virus particles was demonstrated by the passage of this supernatant on PBMC isolated from a healthy rhesus macaque. Once these primary cell cultures became positive by the RT assay, supernatants constituting the virus stocks for animal infections were collected and stored daily. Viral infectivity was assayed by titration on CEMx174 cells using the end-point dilution method. Individual wells were determined to be positive for viral replication by the identification of RT activity 14 days postinfection. Titers were subsequently calculated by the method of Reed and Muench (1938).

Infection of human and macaque cells by SHIVsbg

Human and macaque PBMC, CEMx174 cells, and MT-4 cells were infected with SHIVsbg and its parental viruses. A dose of 300 $TCID_{50}$ was used per 10^6 primary cells or per 3×10^5 CEMx174 and MT4 cells. The infectivity of the stocks of the parental viruses was determined on CEMx174 cells as for the stock of SHIVsbg. Following a 1-hr adsorption at 37°, the cells were washed in culture medium and plated. Cultures were checked for the appearance of a cytopathic effect and supernatants were checked for RT activity twice weekly.

Radioimmunoprecipitation analysis

SHIVsbg and SIVmac251BK28, a clone having 98% sequence homology with SIVmac239 (Regier and Desrosiers, 1990), were grown in CEMx174 cells in the presence of ^{35}S-labeled cysteine and methionine as described previously (Liska *et al.,* 1994). Uninfected CEMx174 cells were used as a negative control and SHIVsbg02, which is similar to SHIVsbg except for the absence of the tat splice acceptor site, was also included. Viral proteins in cell-free supernatants and those associated with cell pellets were immunoprecipitated by sera from SIVmac-infected macaques and an HIV-1-infected human, chosen for its high reactivity with HIV-1 glycoproteins relative to other proteins. Analysis by electrophoresis on a 12.5% sodium dodecyl sulfate–polyacrylamide gel allowed the identification of SIVmac or HIV-1 glycoprotein and the full-length SIV Nef protein.

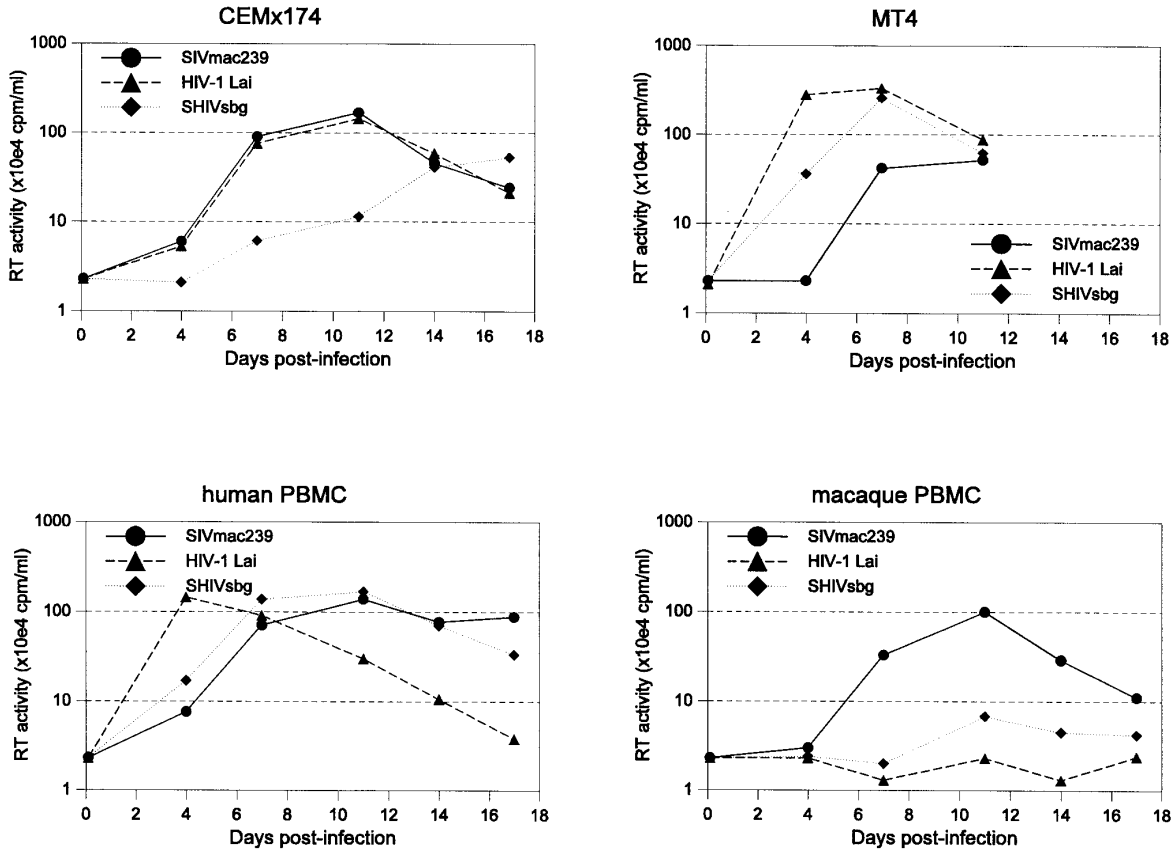

FIG. 2. Replication of SHIVsbg and its parental viruses on human lymphoid cell lines and human and macaque PBMC. 10^6 PBMC or 3×10^5 CEMx174 or MT4 cells were infected with $300TCID_{50}$ of SHIVsbg, HIV1 Lai, or SIVmac239. After a 1-hr adsorption, free virus was washed out, cells were resuspended at a density of 10^6 PBMC/ml or 1.5×10^5 CEMx174 or MT4/ml and distributed in culture plates. Virus production was monitored twice weekly by RT assays done on culture supernatants.

Hematology and immunophenotyping of lymphocytes

Standard hematological parameters of red and white blood cell populations were measured on a Coulter counter apparatus. The CD4 and CD8 phenotypes of lymphocytes from whole blood were determined by immunolabeling and flow cytometry. Whole blood collected in EDTA tubes was incubated with either the OKT4 monoclonal antibody (mAb; OrthoDiagnostic Systems, Raritan, NJ) coupled to phycoerythrin or the fluorescein-conjugated Leu2a mAb (Becton Dickinson, France). These mAbs recognize human CD4 and CD8 proteins, respectively, and cross-react with the homologous macaque antigens. After a 15-min incubation, the red cells were lysed (red cell lysis buffer, Becton Dickinson), washed in FacsFlow (Becton Dickinson), and resuspended in 2% paraformaldehyde in PBS. The fluorescence associated with the lymphocyte subpopulation using the respective mAbs was analyzed by a FACScan fluorescence-activated cell sorter using Lysis II software (Becton Dickinson).

Quantitation of virus load

The virus load associated with macaque PBMC isolated on a Ficoll–Hypaque gradient was determined by cocultivation of 2.5×10^6-, 10^6-, and 5-fold serial dilutions of 0.5×10^6 primary cells with 1.5×10^5 CEMx174 cells in 24-well plates. These cocultures were treated twice weekly, once to change medium and once to divide cells, and from Day 7 were checked for RT activity on each occasion. Rising levels of RT activity were taken as evidence of viral replication in a given coculture and then the number of infected cells was calculated per 10^6 PBMC. Similarly the free virus load in plasma was determined by the isolation of virus using CEMx174 cells incubated with 2-fold limiting dilutions of plasma. All these cultures were performed in duplicate.

Cocultures of CD8 lymphocyte-depleted macaque PBMC and CEMx174 cells were also performed. The depletion of macaque CD8-positive lymphocytes by immunomagnetic bead selection (Dynabeads M450, Dynal, Norway) was carried out following the protocol of the manufacturer. After a 30-min incubation at 4° with a bead-to-cell ratio of 10, the CD8 cells, rosetted by beads, were depleted using a magnet. The remaining cells were then cocultivated.

Biopsied lymph node tissue was dissociated by cutting with scissors and vigorous pipetting. After being filtered

through gauze to ensure a single-cell suspension, cells were pelleted and washed twice in PBS before being counted and cocultured in limiting dilutions as described above for PBMC.

Serology

Antibodies to HIV present in the serum of infected macaques were analyzed by a commercial ELISA test (Murex, Wellcome). This kit allows the detection of antibodies to HIV-1 and HIV-2 envelope and core peptides. Western blots using HIV-1 antigens (New Lav Blot I, Diagnostics Pasteur) determined the specificity of antibody against the relevant glycoproteins. Both these kits were used according to the protocols of the respective manufacturers.

RESULTS

Characterization of SHIVsbg

Following the transfection of the SHIVsbg viral cDNA into CEMx174 cells, RT activity in culture supernatants was detected after 12 days. These supernatants were stored and used to infect mitogen-stimulated macaque and human PBMC as well as the CEMx174 and MT-4 cell lines. A comparison of viral replication demonstrated that the SHIVsbg multiplies 10 times more efficiently on the human primary cells relative to the macaque cells but no cytopathic effect is observed (Fig. 2).

Both the parental viruses of SHIVsbg, SIVmac239 and HIV-1 Lai, replicate more efficiently on CEMx174 than the chimeric virus (Fig. 2), although SHIVsbg multiplies better on the MT-4 cell line. The replication of SHIVsbg relative to its parental viruses is dependent on cell type.

Sera from an HIV-1 seropositive individual, but not sera from SIVmac-infected macaques, were able to immunoprecipitate the envelope glycoprotein from the SHIVsbg (Fig. 3), demonstrating the presence of the HIV-1 envelope and the absence of the SIV envelope. Analysis of the immunoprecipitates from the cellular fractions demonstrates the presence of the Nef protein (34 kDa) of SIVmac239, which is larger than that of SIVmac251, in cells infected by the SHIVsbg.

The stock virus used to inoculate animals was grown on macaque PBMC. A titration of infectivity on CEMx174 cells demonstrated a titer of 4400 $TCID_{50}$/ml which represents 16.4 ng/ml of SIVmac p27 core protein as determined using the Coulter SIV Core Antigen Assay (Coulter Immunology, Hialeah, FL).

Clinical examination and immunophenotyping of lymphocytes

Clinical examinations revealed persistent lymphadenopathy of axillary and inguinal lymph nodes which was apparent from 2 weeks postinfection. Otherwise,

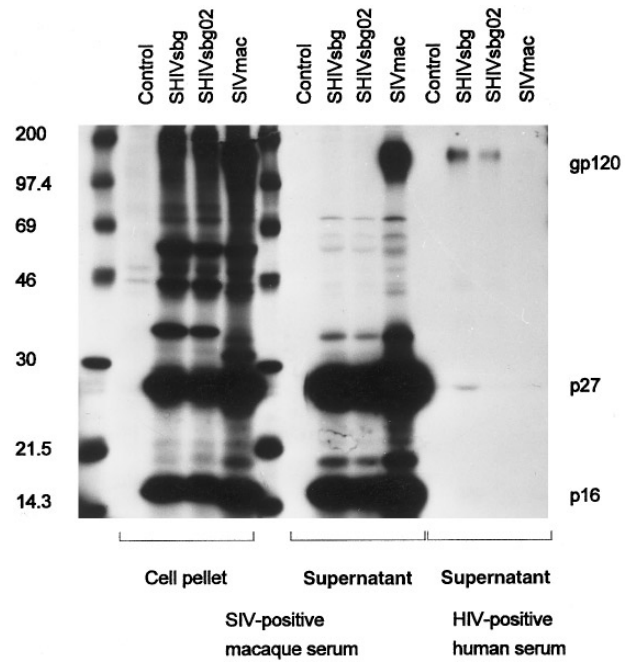

FIG. 3. Demonstration by radioimmunoprecipitation assay of the presence of HIV-1 envelope glycoprotein in chimeric stock virus grown on CEMx174 cells and the absence of SIV envelope glycoprotein. Nine days after infection with SHIVsbg, SHIVsbg02, or SIVmac (see Materials and Methods for characteristics) , cells were metabolically labeled for 18 hr with [^{35}S]cysteine–methionine. The cells and cell-free virus collected from culture supernatant by ultracentrifugation were lysed in dissociating buffer and subjected to immunoprecipitation with SIV-positive macaque serum. A fraction of virus lysate was immunoprecipitated with HIV1-positive human serum chosen as the HIV1 antibodies were mainly directed against the glycoproteins. Proteins of the immune complexes were resolved by SDS–polyacrylamide gel electrophoresis and autoradiography. Control, refers to uninfected cells labeled and immunoprecipitated in parallel. Unmarked lanes were loaded with molecular mass markers; masses are indicated in kilodaltons on the left side.

these macaques have not shown systemic signs of illness and no cutaneous exantem has been observed. Immunophenotyping of lymphocytes from peripheral whole blood to determine the CD4/CD8 ratio demonstrated inversion begining at 3 weeks postinfection which has persisted in certain animals (Fig. 4a). An analysis of the absolute numbers of CD4 lymphocytes shows that a CD4 lymphopenia is concurrent with the CD4/CD8 inversion (Fig. 4b). Although there may be some recovery of CD4 cell numbers, preinfection levels have not been fully attained except in the case of one cynomolgus monkey. This pattern of CD4 lymphopenia is similar in the three groups of animals and represents about a 50% depletion despite the variability in absolute numbers between individuals.

Serology

All animals seroconverted to HIV proteins, as detected by ELISA, 2–4 weeks postinfection and the titer of anti-

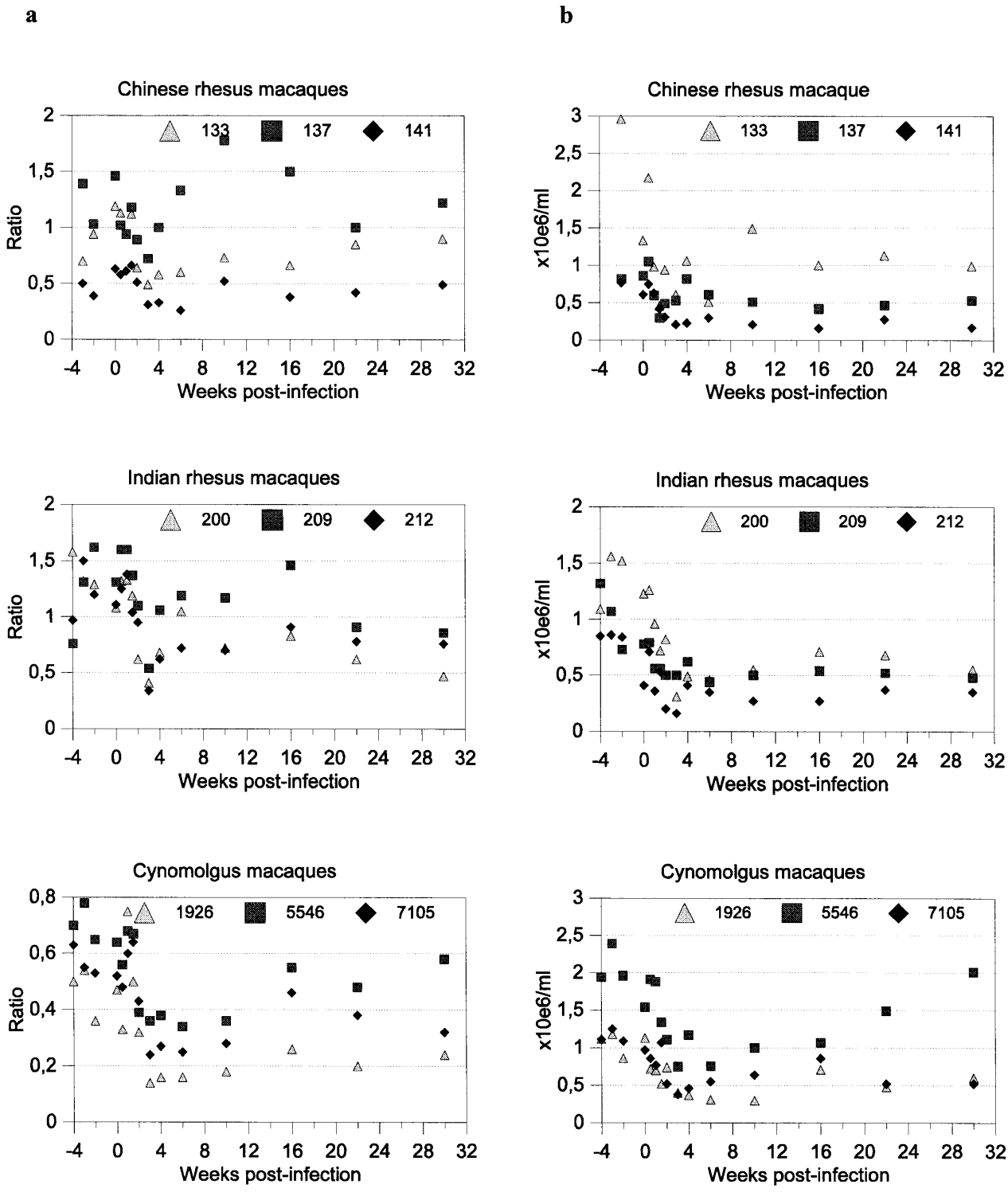

FIG. 4. Inversion of CD4/CD8 ratios (a) and absolute CD4 lymphocyte depletion (b) in peripheral whole blood of SHIVsbg-infected macaques. For more details, see Materials and Methods.

body continued to rise (Fig. 5). Antibody titers are approximately one log greater in the Indian rhesus macaques than in the Chinese rhesus and cynomolgus monkeys. Western blots performed 30 weeks postinfection show antibodies in the serum of all these macaques which recognize the glycoproteins of HIV-1 (Fig. 6). Reactivity is strongest against gp160 and gp120 but difficult to distinguish in certain animals for gp41. The band for p24 HIV-1 represents a cross reaction of antibodies normally recognizing p27 of SIVmac.

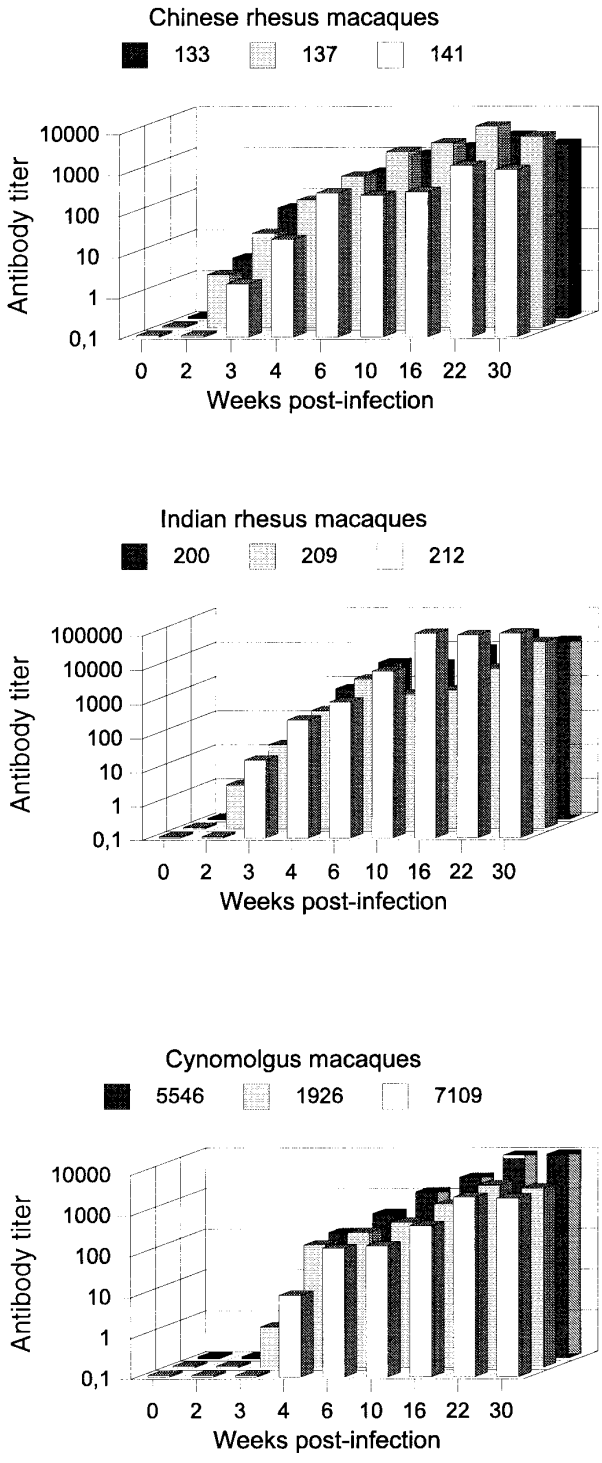

FIG. 5. Rising serum antibody titers in SHIVsbg-infected macaques. The temporal antibody response was determined from the day of infection and later, using a commercial HIV ELISA kit. Antibody titers are the reciprocals of the highest dilutions of sera giving an optical density of 0.1 above the cut-off values.

Virus load

Coculture of PBMC following limiting dilution with CEMx174 indicator cells has revealed high virus loads in all animals during the acute phase of the infection and virus has been isolated at all attempts during the chronic phase although only after CD8 depletion in certain cases (Table 1). The number of infected PBMC peaked at 2–3 weeks postinfection with up to one cell in 30 infected. Confirmation of elevated virus loads has been obtained from biopsied peripheral lymph nodes (Table 2). After the initial immune response, there is a sharp decline in the number of infected cells in both lymph node and peripheral blood. The dynamics of viral replication are closely paralled in the three groups of animals investigated.

Infectious free virus has been isolated from the plasma of all animals during the acute phase with the lowest titers being observed in the Chinese macaques and the most uniform load being identified in the cynomolgus monkeys (Table 3). Titers ranged between 8 and 266 $TCID_{50}$/ml of plasma. Due to the dilution of the plasma

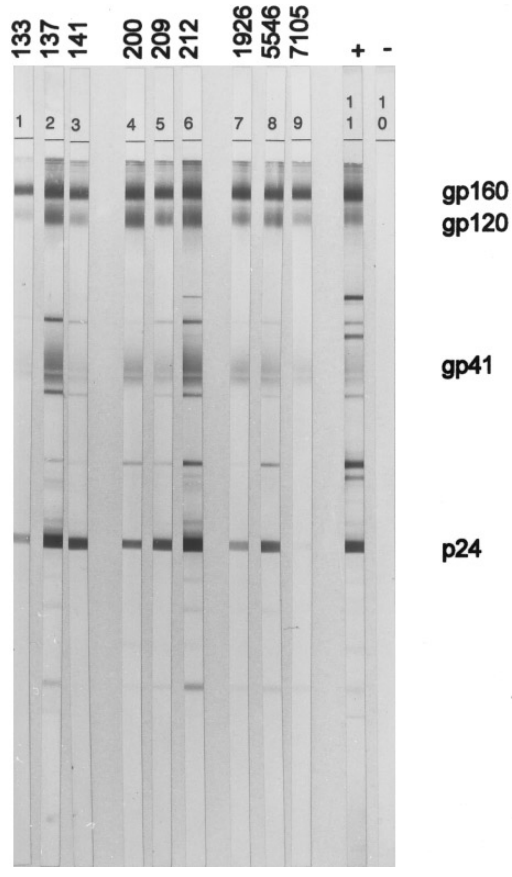

FIG. 6. Detection of HIV1 glycoprotein-specific antibodies in SHIVsbg-infected macaques. HIV1 commercial Western blots were probed with sera taken 30 weeks postinfection. The presence of antibodies against HIV-1 glycoproteins is confirmed; antibodies against SIVmac p27 protein cross-reacting with HIV1 p24 are also detected. The positions of the HIV1 envelope (gp160, gp120 and pg41) and capsid (p24) proteins are indicated on the right. Numbers above the lanes refer to the infected macaques: Chinese rhesus macaques, 133, 137, 141; Indian rhesus macaques, 200, 209, 212; cynomolgus macaques, 1926, 5546, 7105.

TABLE 1

Cell-Associated Virus Load Expressed as Number of Cells Infected per Million PBMC; Limiting Dilutions of PBMC Were Cocultured with CEM × 174 Cells and Virus Replication Was Detected by RT Activity

		Cells infected per 10^6 PBMC								
		Weeks postinfection								
Species	No.	1	2	3	4	6	10[a]	16[a]	22[b]	30[b]
Chinese rhesus macaque	133	250	6250	150	250	250	2/10	1/2	2	1
	137	50	3750	30	50	6	50/10	0/2	1	1
	141	30	3750	50	150	6	1/10	0/2	1	1
Indian rhesus macaque	200	150	1250	250	150	30	10/10	0/10	1	2
	209	50	18750	150	30	10	1/2	1/2	2	1
	212	750	6250	31250	50	30	10/50	10/10	10	2
Cynomolgus macaque	1926	30	31250	150	6	50	2/2	0/1	2	1
	5546	650	18750	750	150	150	2/10	10/50	2	10
	7105	50	6250	250	50	10	10/1250	0/2	1	0

[a] The second figure in each column is the coculture result following depletion of CD8 lymphocytes from macaque PBMC.
[b] Cocultures were performed only after CD8 lymphocyte depletion from macaque PBMC.

to avoid a toxic effect on the CEMx174 cells, the limit of detection of this technique is 4 $TCID_{50}$/ml.

DISCUSSION

The construction of an infectious recombinant virus consisting of genes from HIV-1 Lai and SIVmac239 should allow an analysis of the function of discrete genes of HIV-1 in nonhuman primates. Such a virus might also be used to challenge monkeys immunized by HIV-1 subunit vaccines. The SHIVsbg is a chimeric primate lentivirus which does not have *in vitro* replication characteristics predicted by those of the parental viruses. In addition, the high viral load and CD4 lymphopenia observed in infected *Macaca* species is not anticipated from the level of viral replication in macaque PBMC *in vitro*.

TABLE 2

Virus Load in Lymph Nodes Expressed as Number of Cells Infected per 10^6 Cells in a Single-Cell Suspension Prepared from Lymph Node Biopsies, Limiting Dilutions of Cells Were Cocultured with CEM × 174 Cells and Virus Replication Was Detected by RT Activity

		No. of lymph node cells infected/10^6			
		Weeks postinfection			
Species	No.	2	3	6	10
Chinese rhesus macaque	133	18750	ND	ND	10
	137	ND	3750	ND	ND
	141	ND	ND	250	ND
Indian rhesus macaque	200	31250	ND	ND	10
	209	ND	3250	ND	ND
	212	ND	ND	150	ND
Cynomolgus macaque	1926	ND	1250	ND	50
	5546	31250	ND	ND	ND
	7105	ND	ND	250	ND

Note. ND, not determined.

Previous studies have demonstrated that chimeric primate lentiviruses of SIV and HIV genes are infectious for macaque monkeys and viral replication can provoke an immune response (Li *et al.,* 1992, 1995; Sakuragi *et al.,* 1992; Igarashi *et al.,* 1994; Letvin *et al.,* 1995; Voss *et al.,* 1995). However, with the exception of SHIV SF33 (Luciw *et al.,* 1995), chimeric virus has not been consistently isolated from infected macaque PBMC and detailed quantifications of virus loads have not been established.

The SHIVsbg cell-associated loads described here are comparable with those determined by Luciw *et al.* (1995) for SHIV SF33 and for symptomatic HIV-1 infection of human (Daar *et al.*, 1991). Furthermore we have identified CD4 lymphocyte depletion and lymphadenopathy which is corroborative evidence for a high level of viral replication. These parameters represent markers of infection other than viral load which may be followed in the evaluation of vaccines in this model. Animals infected by our SHIVsbg virus stock do not produce antibodies to SIV glycoproteins and the phenotype of the virus isolated from animals on CEMx174 cells has remained noncytopathogenic. Glycoproteins of SIVmac have not been revealed by RIP assay using chimeric stock virus replicating on CEMx174 cells. Therefore our observations are unlikely to be due to a contamination of the virus stock with SIVmac. Routine serological screening of these animals has not identified evidence of other simian retroviruses which might complement SHIVsbg replication.

Several possibilities exist for the apparently higher viral load we describe for SHIVsbg relative to that of SHIV-

TABLE 3

Infectious Cell-Free Virus Load in Plasma of Monkeys Infected by SHIVsbg; Twofold Serial Dilutions of Plasma Derived from Heparinized Whole Blood Were Added to CEM × 174 Cells and Virus Replication Was Detected by RT Activity

		$TCID_{50}$/ml of plasma					
		Weeks postinfection					
Species	No.	1	2	3	4	6	10
Chinese rhesus macaque	133	0	266	0	0	0	0
	137	11	16	0	0	0	0
	141	0	16	0	0	0	0
Indian rhesus macaque	200	0	15	8	0	0	0
	209	0	0	22	0	0	0
	212	0	52	266	0	0	0
Cynomolgus macaque	1926	33	266	8	0	0	0
	5546	0	133	88	0	0	0
	7105	0	266	133	0	0	0

4 (Li *et al.,* 1992), which has a similar construction. The HXBc2 molecular clone of HIV-1 used for SHIV-4 differs from Lai as the former was adapted to lymphoid cell lines prior to cloning. SHIV-4 does not have an open reading frame for the *vpu* gene (Li *et al.,* 1992) and this has a definite effect on the ease of virus isolation and viral load (Li *et al.,* 1995). The presence of an intact tat splice acceptor site and mutations in the *env* gene to create a cassette to allow the insertion of other HIV-1 *env* genes are additional changes to SHIVsbg which might have an effect on *in vivo* replication. Unlike other chimeric viruses (Li *et al.,* 1992; Sakuragi *et al.,* 1992 Luciw *et al.,* 1995), the SHIVsbg replicated less well than the parental viruses on macaque PBMC and CEMx174 cells and is noncytopathogenic *in vitro.* This is a further example of the lack of correlation between *in vitro* and *in vivo* phenotypes of primate lentiviruses (Marthas *et al.,* 1989).

Comparisons can also be made between the infection of macaques with SIVmac239 and SHIVsbg. The viral loads in the acute phase of both infections are similar but once an immune response is established, cell-associated viral loads are up to 1000 times reduced for SHIVsbg compared to SIVmac239. Numerous studies have testified to the very high virus load maintained during SIVmac239 infection (Kestler *et al.,* 1991; Sharma *et al.,* 1992; Marthas *et al.,* 1993; Joag *et al.,* 1994; Lohman *et al.,* 1994; Lang *et al.,* 1993; Gibbs *et al.,* 1995; Hoch *et al.,* 1995; Luciw *et al.,* 1995). This indicates that the *env, rev,* and *tat* genes of SIVmac239 could be important viral determinants for such an elevated viral load relative to SHIVsbg in the chronic phase of the infection.

There has always been anecdotal evidence that Indian rhesus monkeys were more sensitive to pathogenic SIV infection than those of Chinese origin. Joag *et al.* (1994) have confirmed that Indian rhesus monkeys infected by SIVmac239 have viral loads which are higher than those of Chinese rhesus monkeys by a factor of 10–100. Even rhesus monkeys of different origins may vary in their sensitivity to infection by a stock of SIVmac251 (Lewis *et al.,* 1994) but despite the different species and subspecies we have studied, we have demonstrated uniform viral loads and CD4 cell depletions although there were higher antibody titers in the Indian rhesus macaques. That SHIVsbg can infect those species of monkey which are commercially available is an important practical consideration for an animal model for HIV-1 vaccine studies.

All the macaques inoculated with SHIVsbg have developed a humoral immune response to the proteins and glycoproteins of HIV-1. However, there is a 1-week delay in the time of seroconversion between the rhesus monkeys and the cynomolgus monkeys and this may explain why a greater quantity of infectious virus is found in the plasma of the latter. The rising titers of antibody imply sustained viral replication despite simultaneously falling viral loads in blood and tissue as determined by coculture. Thirty weeks after infection, an analysis by Western blot indicates that the animals have antibodies in serum which recognize the gp120 and gp160 of HIV-1 although the presence of antibodies to gp41 is more variable. For the macaque-SHIV model to be of use in studying the role of the immune response to HIV-1 glycoprotein in pathogenesis, this strong humoral response is indispensible. Further studies to exploit this model are underway to determine the existence and possible role of both neutralizing antibodies and viral variants in the pathogenesis of SHIVsbg infection.

To our knowledge this is the first time that a lentiviral molecular clone with an HIV-1 envelope has replicated with such efficiency and caused CD4 lymphopenia in nonhuman primates although recent reports have established that the passage of poorly replicating SHIV can considerably increase virulence in pig-tail macaques (Narayan *et al.,* 1995). Obviously the use of a chimeric virus with an envelope from HIV-1 maintained in PBMC

undermines the use of this virus for directly evaluating HIV-1 vaccines composed of envelope glycoprotein subunits from certain primary isolates. However, there is fundamental interest in the study of how the *env* and *vpu* genes of HIV-1 Lai might mutate in order to adapt to *in vivo* replication and how the insertion of other *env* genes from primary isolates of HIV-1 might affect the biological characteristics of the virus. A high viral load and CD4 lymphopenia have been demonstrated in these macaque monkeys infected by SHIVsbg and these are essential prerequisites for a pathogenic outcome.

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