JOURNAL OF VIROLOGY, Oct. 1996, p. 6922–6928 Vol. 70, No. 10
0022-538X/96/$04.00+0

A Chimeric Simian/Human Immunodeficiency Virus Expressing a Primary Patient Human Immunodeficiency Virus Type 1 Isolate *env* Causes an AIDS-Like Disease after In Vivo Passage in Rhesus Monkeys

KEITH A. REIMANN,[1]* JOHN T. LI,[2] RONALD VEAZEY,[3] MATILDA HALLORAN,[1] IN-WOO PARK,[2] GUNILLA B. KARLSSON,[2] JOSEPH SODROSKI,[2] AND NORMAN L. LETVIN[1]

Division of Viral Pathogenesis, Beth Israel Hospital,[1] and Division of Human Retrovirology, Dana-Farber Cancer Institute,[2] Harvard Medical School, Boston, Massachusetts 02115, and New England Regional Primate Research Center, Harvard Medical School, Southborough, Massachusetts 01772[3]

Received 8 April 1996/Accepted 5 July 1996

The utility of the simian immunodeficiency virus of macaques (SIV_{mac}) model of AIDS has been limited by the genetic divergence of the envelope glycoproteins of human immunodeficiency virus type 1 (HIV-1) and the SIVs. To develop a better AIDS animal model, we have been exploring the infection of rhesus monkeys with chimeric simian/human immunodeficiency viruses (SHIVs) composed of SIV_{mac}239 expressing HIV-1 *env* and the associated auxiliary HIV-1 genes *tat*, *vpu*, and *rev*. SHIV-89.6, constructed with the HIV-1 *env* of a cytopathic, macrophage-tropic clone of a patient isolate of HIV-1 (89.6), was previously shown to replicate to a high degree in monkeys during primary infection. However, pathogenic consequences of chronic infection were not evident. We now show that after two serial in vivo passages by intravenous blood inoculation of naive rhesus monkeys, this SHIV (SHIV-89.6P) induced CD4 lymphopenia and an AIDS-like disease with wasting and opportunistic infections. Genetic and serologic evaluation indicated that the reisolated SHIV-89.6P expressed envelope glycoproteins that resembled those of HIV-1. When inoculated into naive rhesus monkeys, SHIV-89.6P caused persistent infection and CD4 lymphopenia. This chimeric virus expressing patient isolate HIV-1 envelope glycoproteins will be valuable as a challenge virus for evaluating HIV-1 envelope-based vaccines and for exploring the genetic determinants of HIV-1 pathogenicity.

While the simian immunodeficiency virus (SIV)-infected macaque has proven of enormous value in studying AIDS pathogenesis and evaluating vaccine strategies to prevent human immunodeficiency virus (HIV) infection, certain weaknesses remain inherent in this model. One of the most important limitations of this model arises from the divergence of the envelope glycoproteins of HIV type 1 (HIV-1) and SIV_{mac}. The envelope glycoproteins are so distinct that antibodies directed against the envelope of one virus show limited cross-reactivity with the other virus (6, 13) and do not cross neutralize (5). This feature has limited the utility of the SIV-macaque model for evaluating envelope-based vaccine strategies to prevent HIV infection. Furthermore, because the envelopes of HIV-1 and SIV are genetically divergent (12), molecular studies of envelope determinants of HIV pathogenicity have been difficult to pursue in macaque models.

Primary isolates of HIV-1 differ substantially from laboratory-adapted viruses in their cell tropism, replication kinetics in peripheral blood mononuclear cells (PBMC), and sensitivity to neutralization by antibodies and soluble CD4. These properties are determined primarily by differences in the envelope glycoproteins (1, 3, 20, 21). We have therefore sought to develop a chimeric SIV/HIV (SHIV) that expresses an HIV-1 envelope glycoprotein from a patient isolate for studies of AIDS pathogenesis and vaccine protection. Previous studies indicated that the envelope glycoproteins of a primary HIV-1 isolate, 89.6, conferred a high level of early replication on SHIV chimeras in vivo compared with envelope glycoproteins derived from the laboratory-adapted HIV-1 isolate, HXBc2 (17). Since a more detailed understanding of immune genetics exists for rhesus monkeys than for other macaque species, the present experiments were undertaken to generate a SHIV expressing a primary patient HIV-1 envelope capable of inducing AIDS-like pathology in rhesus monkeys.

* Corresponding author. Mailing address: Beth Israel Hospital - RE 113, 330 Brookline Ave., Boston, MA 02215. Phone: (617) 667-4583. Fax: (617) 667-8210. Electronic mail address: kreimann@bih.harvard.edu.

MATERIALS AND METHODS

SHIV. The chimeric virus that served as the parental virus in this study, SHIV-89.6, was composed of SIV_{mac}239 expressing HIV-1 *env* and the associated auxiliary genes *tat*, *vpu*, and *rev* as described previously (10, 17). The *env* sequences were derived from a cytopathic, macrophage-tropic primary HIV-1 isolate (2). After transfection into CEMx174 cells, the virus was expanded on lectin-activated rhesus monkey peripheral blood lymphocytes (PBL) and its titer was determined; the virus was then used for animal inoculations.

Animal inoculations and passages. The infection of rhesus monkeys (*Macaca mulatta*) with SHIV-89.6 has been described elsewhere (17). One monkey infected in this previous study served as the initial donor for three serial passages into naive rhesus monkeys. For each virus passage, 10 ml of heparinized whole blood was obtained from the donor animal and inoculated intravenously into a naive recipient. Virus was reisolated from the PBL of monkey 345-91 7 days after inoculation, and 1 ml of supernatant with high levels of SIVmac p27 antigen was inoculated into four additional, naive rhesus monkeys.

The rhesus monkeys used in this study were maintained in accordance with the guidelines of the Committee on Animals for the Harvard Medical School and the Guide for the Care and Use of Laboratory Animals (14). Monkeys were anesthetized with ketamine-HCl for all blood sampling and inoculations.

PBL phenotyping. PBL were phenotyped for CD4 (OKT4-fluorescein isothiocyanate; Ortho Diagnostic Systems, Raritan, N.J.), total CD8 (T8-phycoerythrin; Dako, Inc., Carpenteria, Calif.), and CD20 (B1-fluorescein isothiocyanate; Coulter Corporation, Hialeah, Fla.) subsets, using a commercial whole blood lysis kit (Coulter) as previously described (18). Absolute lymphocyte counts in blood were determined on an automated hematology analyzer that provided a partial differential count (T540; Coulter).

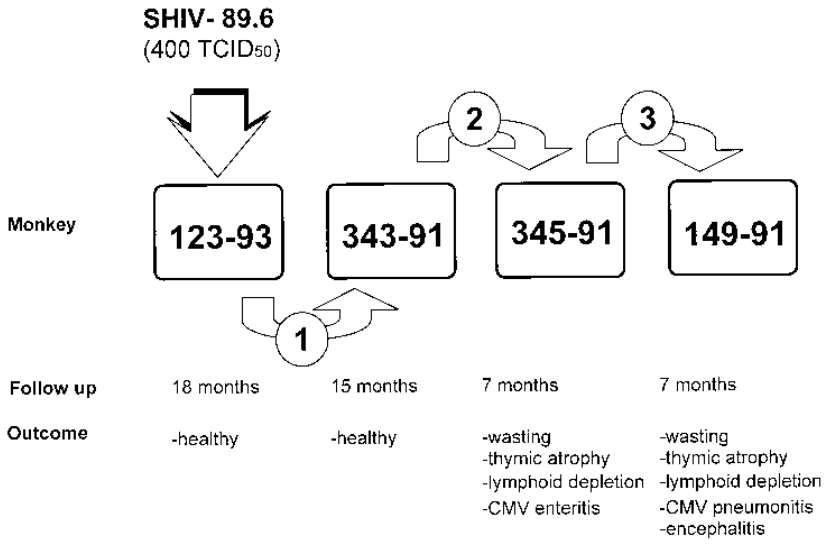

FIG. 1. Outline of serial passage of SHIV-89.6 in rhesus monkeys. Animal 123-93 was inoculated with 400 50% tissue culture infective doses ($TCID_{50}$) of SHIV-89.6 as previously described (17). Ten milliliters of whole blood was injected intravenously into a naive rhesus monkeys 14 weeks postinoculation (passage 1), 4 weeks postinoculation (passage 2), and 12 weeks postinoculation (passage 3). CMV, cytomegalovirus.

Plasma viral RNA quantitation. Quantitative assays for the measurement of SHIV RNA were performed by Chiron Corporation (Emeryville, Calif.), using a branched DNA signal amplification assay for SIV similar to the Quantiplex HIV-RNA branched DNA assay (15). In the SIV assay, target probes were designed to hybridize with the *pol* region of the SIV_{mac} group of strains, including $SIV_{mac}239$. The assay results were quantified by comparison with purified and quantitated in vitro-transcribed SIV *pol* RNA.

Virus isolation. PBMC were isolated from anticoagulated whole blood by density gradient centrifugation and activated overnight with 6.25 μg of concanavalin A per ml in RPMI 1640 supplemented with 10% fetal bovine serum, penicillin-streptomycin, and gentamicin. The cells were then washed, and $CD8^+$ lymphocytes were removed by using a CD8-specific monoclonal antibody and anti-mouse immunoglobulin magnetic beads (Dyna Beads; Dynal, Oslo, Norway) and a magnetic particle concentrator. The remaining cells were cultured at approximately 10^6/ml in medium additionally supplemented with 20 U of recombinant human interleukin-2 per ml. Cell number was adjusted, and culture supernatant was collected every 3 to 4 days for 2 weeks. Supernatants were assessed for SIV_{mac} p27 by using a commercial assay kit (SIVmac p27 core antigen assay; Coulter).

Immunoprecipitation of viral proteins. CEMx174 cells were infected with HIV-1 (HXBc2 strain), $SIV_{mac}239$ (*nef* open), and SHIV isolated from the PBMC of animals 149-91 and 236-84. The cultures were labeled overnight with [^{35}S]cysteine 1 to 2 days prior to the peak of syncytium formation, and cell lysates were precipitated either with a mixture of sera from HIV-1-infected individuals or with serum from an SIV_{mac}-infected rhesus macaque as described previously (22).

Sequence analysis of viruses isolated from SHIV-infected monkeys. Genomic DNA was purified from the PBMC used for isolation of in vivo-passaged SHIV-89.6 (SHIV-89.6P) from monkey 149-91 at 1 week postinoculation. Primer pairs specific for the HIV-1 *env* gene were used for PCR amplification of viral sequences in the infected cultures as described previously (9). PCR products were agarose-gel purified with Gene Clean II (Bio 101) and sequenced by using the *fmol* DNA sequencing system (Promega) and [γ-^{32}P]ATP as described previously (9).

Antibody responses in SHIV-89.6P-infected monkeys. Virion proteins were prepared by centrifugation through sucrose cushions of supernatants of [^{35}S]methionine-[^{35}S]cysteine-labeled CEMx174 cells infected with HIV-1 (HXBc2 strain) or with $SIV_{mac}239$ as described previously (16). Sera taken from animals 351-80, 236-84, and 145-84 at 23 weeks postinoculation were used to precipitate the labeled virion lysates as described previously (16).

TABLE 1. Viral load during serial passage of SHIV-89.6

Animal	Outcome	SIV RNA kEq/ml[a]			
		12–14 day[b]	41–43 day	97–113 day	152–169 day
343-91	Healthy	1,883	556	784	667
345-91	AIDS	442	<10	<10	<10
149-91	AIDS	<10	<10	<10	13

[a] Viral load in plasma was quantified by using a SIV branched DNA assay as described in the text.

[b] Specimens were obtained at the indicated times after blood inoculation as described in the legend to Fig. 1.

RESULTS

In vivo passage of SHIV-89.6 results in an AIDS-like disease in rhesus monkeys. A series of in vivo passages of SHIV-89.6 was performed in naive rhesus monkeys to generate a chimeric virus expressing a primary patient HIV-1 envelope that would be pathogenic in this nonhuman primate species (Fig. 1). Cell-free SHIV-89.6 was inoculated intravenously into a normal rhesus monkey (123-93). This inoculation has been described previously (17). Fourteen weeks following inoculation, 10 ml of heparinized blood from this animal was inoculated intravenously into a second naive rhesus monkey (343-91). Four

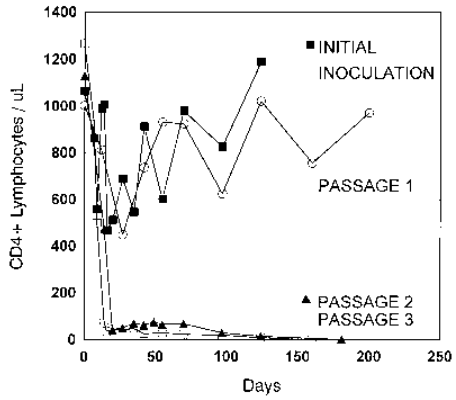

FIG. 2. Absolute CD4 counts in rhesus monkey recipients of serially passaged SHIV-89.6. CD4 counts are shown for animals 123-93 (initial inoculation with SHIV-89.6), 343-91 (passage 1), 345-91 (passage 2), and 149-91 (passage 3).

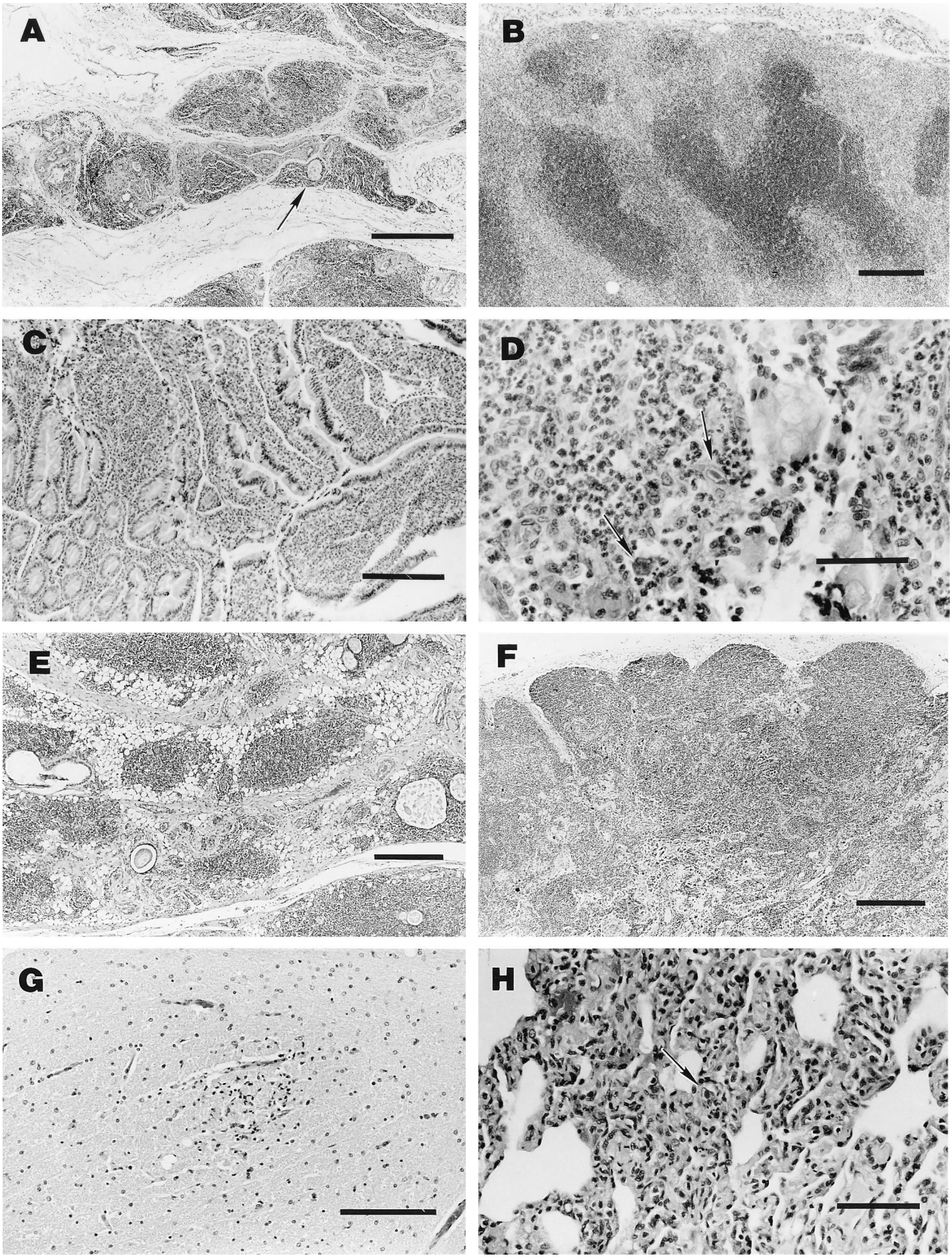
A
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FIG. 3. Histopathology of passage 2 and 3 monkeys. (A) Thymus of animal 345-91 (passage 2), showing marked atrophy and thymocyte depletion (dysinvolution) resulting in stromal collapse and loss of distinction between the cortex and medulla. Perivascular and interstitial fibrosis was present in the areas of stromal loss. Hassall's corpuscles were absent and replaced by cystic degenerate remnants containing amorphous to globular eosinophilic material (arrow). Bar = 500 μm. (B) Mesenteric lymph node of animal 345-91. Marked paracortical expansion, predominantly due to histiocytic infiltration, was noted. Follicles were irregularly shaped and often fused, with complete loss of germinal centers. Bar = 300 μm. (C) Jejunum of animal 345-91, showing severe enteritis characterized by expansion of the villus lamina propria as a result of marked infiltration of neutrophils. Bar = 200 μm. (D) Jejunum of animal 345-91. Higher magnification demonstrated intense suppurative inflammation and two cytomegalic cells containing large intranuclear inclusions typical of cytomegalovirus infection (arrows). Bar = 100 μm. (E) Thymus of animal 149-91 (passage 3), showing marked atrophy characterized by stromal collapse, thymocyte depletion, and loss of cortical and medullary distinction. Hassall's corpuscles are absent and frequently replaced by large cystic spaces filled with eosinophilic globular material. Perivascular and interstitial fibrosis is present. Bar = 300 μm. (F) Axillary lymph node of animal 149-91, showing mild lymphoid depletion with loss of primary and secondary follicle formation. Medullary vessels were prominent. Bar = 300 μm. (G) Cerebrum, frontal lobe, of animal 149-91, showing a glial nodule composed of perivascular accumulation of macrophages and glial cells. Bar = 200 μm. (H) Lung of animal 149-91, showing interstitial pneumonitis due to cytomegalovirus infection, characterized by mild alveolar septal thickening and infiltration of macrophages and neutrophils. Note the large cytomegalic cell containing a large intranuclear inclusion body (arrow). Bar = 100 μm.

weeks later, a similar quantity of blood from monkey 343-91 was inoculated intravenously into a third naive animal (345-91). Finally, 12 weeks later, heparinized blood from monkey 345-91 was injected into another normal monkey (149-91).

These four rhesus monkeys were prospectively evaluated for evidence of clinical abnormalities, immunologic changes, and virus load. An initial decrease in circulating $CD4^+$ lymphocytes was documented during primary viremia in all animals (Fig. 2). However, this decrease was modest in the animal first inoculated (123-93) and in the passage 1 animal (343-91), in which nadirs of 400 to 500 $CD4^+$ lymphocytes per μl were observed. The CD4 counts in these two animals rebounded over the ensuing days, exceeding 1,000 cells per μl by 120 days following infection. However, in the passage 2 (345-91) and passage 3 (149-91) monkeys, the circulating $CD4^+$ lymphocyte counts decreased to less than 50 cells per μl by 14 days following infection and remained profoundly depressed thereafter.

Virus load was measured in these animals during the chronic phase of infection by quantifying plasma viral RNA. The passage 1 monkey (343-91) showed a stable level of viremia from 2 to 5 months after inoculation (Table 1). Paradoxically, virus load in the two monkeys that developed an AIDS-like disease (345-91 and 149-91) was low or below the limits of assay detection during the same postinoculation period. However, these two animals had fewer than 5 $CD4^+$ lymphocytes per μl of blood at these sampling points.

The passage 2 animal, 345-91, was euthanized 7 months after inoculation with a wasting syndrome, diarrhea, $CD4^+$ lymphopenia, and histopathologic evidence of thymic atrophy, lymphocyte depletion in secondary lymphoid organs, and an opportunistic viral enteritis. By the time of death, this animal had suffered a loss of 30% of its body weight. Histologic evaluation of the lymphoid organs revealed marked thymic atrophy and dysinvolution (Fig. 3A), and lymph nodes revealed mild lymphocyte depletion and marked follicular atrophy (Fig. 3B). Histologic evaluation of other organs demonstrated severe suppurative enteritis wherein the lamina propria was infiltrated with neutrophils and macrophages. Cytomegalic cells contain-

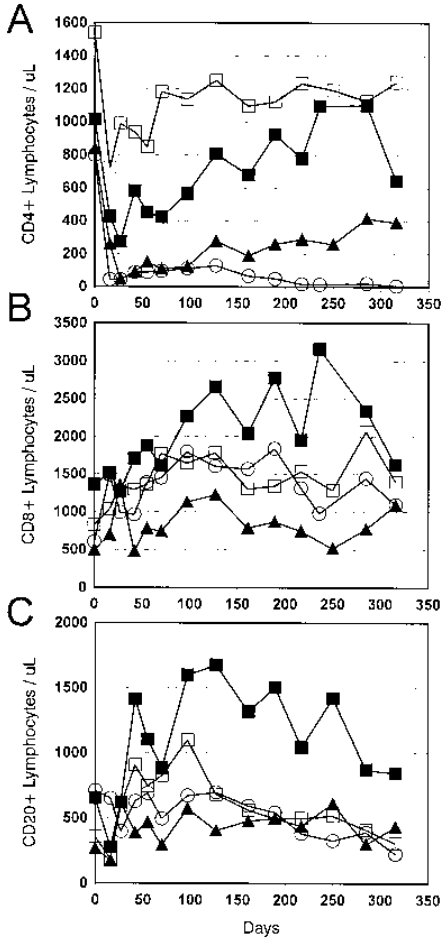

FIG. 4. Changes in PBL of rhesus monkeys 145-84 (□), 231-91 (■), 236-84 (○), and 351-80 (△) following inoculation with SHIV-89.6P. Absolute numbers of circulating $CD4^+$ lymphocytes (A), total $CD8^+$ lymphocytes (B), and $CD20^+$ lymphocytes (C) were determined.

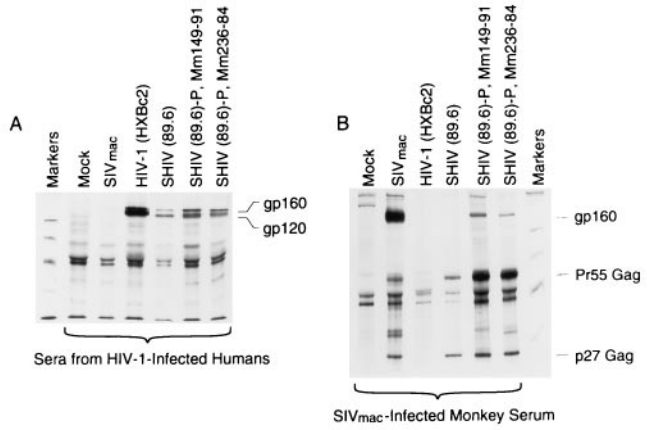

FIG. 5. Analysis of SHIV-89.6P proteins. Radiolabeled lysates from CEMx174 cells infected with SIV_{mac}, HIV-1, SHIV-89.6, or SHIV-89.6P isolated from monkeys 149-91 and 236-84 or from mock-infected cells were precipitated with a mixture of sera from HIV-1-infected individuals (A) or SIV_{mac}-infected monkey serum (B). Specific viral proteins are designated. Molecular mass markers shown are 200, 96, 69, and 46 kDa.

TABLE 2. SHIV isolations and viral loads in monkeys inoculated with SHIV-89.6P

Animal	Virus isolation from PBL (+, −), plasma SIV RNA (kEq/ml)[a]									
	7 day[b]	13 day	20 day	35 day	70 day	127 day	161 day	189 day	236 day	285 day
351-80	+	+, 20	+	+, <10	+	+, <10	+	+	+, <10	+
236-84	+	+, <10	+	+, 108	+	+, 20	+	+	+, 811	+
231-91	+	+, <10	+	+, 1,599	+	+, 741	−	+	+, 2,155	+
145-84	+	+, 63	+	+, 47	+	+, 122	+	+	+, 60	+

[a] Viral load in plasma was quantified by using an SIV branched DNA assay as described in the text.
[b] Specimens were obtained at the indicated times after blood inoculation as described in the legend to Fig. 1.

ing large, amphophilic intranuclear inclusions consistent with cytomegalovirus infection were frequently observed in affected areas (Fig. 3C and D).

The passage 3 monkey (149-91) was euthanized 7 months after inoculation following a similar clinical course. This animal also had a wasting syndrome, CD4 lymphopenia, thymic atrophy, lymphoid depletion, and opportunistic viral pneumonia. This animal had lost 25% of its body weight by the time of necropsy. Histologic evaluation of the lymphoid organs showed pathologic changes of thymic atrophy and lymphoid depletion very similar to those seen in the passage 2 animal (Fig. 3E and F). In addition, there were multifocal, rare glial nodules composed of accumulations of macrophages and glial cells in the frontal lobe (Fig. 3G) and several small foci of scattered mineralization throughout the basal ganglia and deep cortical white matter. Finally, this animal had interstitial pneumonia characterized by multifocal small areas of necrosis, perivascular edema, and infiltrations of macrophages in the alveoli. Occasionally, syncytial cells and macrophages containing large amphophilic intranuclear inclusion bodies typical of cytomegalovirus infection were observed (Fig. 3H). These findings were all consistent with the pathologic changes seen in macaques with the AIDS-like syndrome induced by pathogenic isolates of SIV_{mac} (7).

Cell-free SHIV-89.6P induces $CD4^+$ lymphopenia in rhesus monkeys. To study the consequences of infection with SHIV-89.6P in rhesus monkeys, cell-free virus isolated from the passage 2 monkey (345-91) was inoculated into four naive rhesus monkeys. All four inoculated animals showed an initial decline in circulating $CD4^+$ lymphocytes (Fig. 4A). Monkey 145-84 exhibited a modest decline in $CD4^+$ lymphocytes, with a return to normal counts of greater than 1,000/μl of blood by 70 days following infection. In a second animal (231-91), this decline was more striking, with a nadir of 230 cells per μl of blood. In this animal, circulating $CD4^+$ cells gradually returned to 800/μl of blood by 120 days following infection. However, in the final two monkeys (351-80 and 236-84), a profound drop in circulating $CD4^+$ cells was documented, with nadirs of less than 30 cells per μl of blood. A rebound in $CD4^+$ lymphocyte count to 400/μl of blood was seen in one of these monkeys in the ensuing 300 days. Interestingly, all infected animals showed a persistent $CD8^+$ lymphocytosis (Fig. 4B). Three of four animals showed increases of variable magnitudes in circulating B cells (Fig. 4C).

Virus could be isolated from the blood of all inoculated animals through 285 days postinoculation (Table 2). Periodic viral load measurements showed persistently detectable viral RNA in the plasma of three of four animals (Table 2). However, viral load did not correlate with $CD4^+$ lymphocyte count. These findings indicate that cell-free SHIV-89.6P can persistently infect rhesus monkeys, with resulting immunologic abnormalities.

Characterization of reisolated SHIV-89.6P. The proteins encoded by SHIV-89.6P isolated at 1 week after inoculation from monkeys 149-91 (the passage 3 monkey) and 236-84 (the monkey receiving cell-free SHIV-89.6P with the most profound $CD4^+$ lymphocyte loss) were characterized by immunoprecipitation (Fig. 5). A mixture of sera from HIV-1-infected humans precipitated the gp160 and gp120 envelope glycoproteins of HIV-1, SHIV-89.6, and the viruses isolated from animals 149-91 and 236-84. As expected, this mixture of sera did not precipitate the SIV_{mac} envelope glycoproteins. Serum from an SIV_{mac}-infected monkey efficiently precipitated the $SIV_{mac}239$ envelope glycoproteins but demonstrated little reactivity with the envelope glycoproteins of HIV-1 or SHIV-89.6. Interestingly, the serum from the SIV_{mac}-infected monkey was able to precipitate the gp160 envelope glycoprotein of the viruses isolated from monkeys 149-91 and 236-84, albeit at efficiency lower than that seen for the $SIV_{mac}239$ envelope glycoproteins. The Gag p27 proteins of $SIV_{mac}239$, SHIV-89.6, and the isolated virus (SHIV-89.6P) were precipitated by the SIV_{mac}-infected monkey serum.

To confirm that the virus isolated 1 week postinoculation from monkey 149-91 encoded an HIV-1-like gp120 envelope glycoprotein, *env* gene segments were PCR amplified from infected PBMC and sequenced. Figure 6 shows the predicted amino acid sequence of the first conserved gp120 region of the isolated SHIV-89.6P compared with the analogous region of

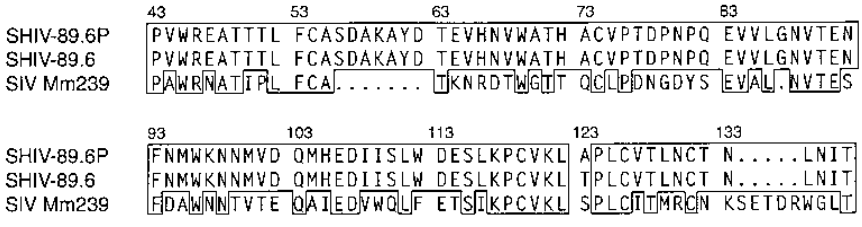

FIG. 6. Predicted partial gp120 sequence from SHIV-89.6 and SHIV-89.6P. The predicted amino acid sequence of the first conserved gp120 region of the SHIV-89.6P virus isolated from monkey 149-91 is compared with that of SHIV-89.6. The sequence of the analogous region of the $SIV_{mac}239$ gp120 glycoprotein is shown for comparison. Identical residues are enclosed by boxes.

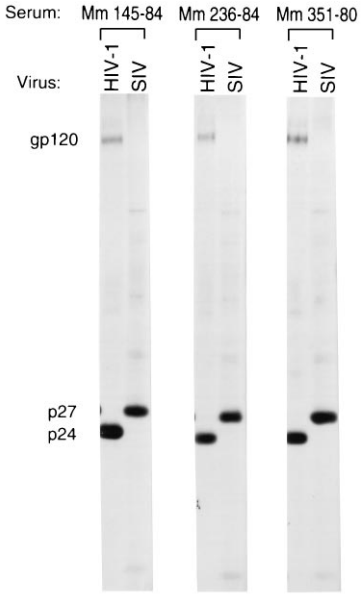

FIG. 7. Antiviral antibodies in SHIV-89.6P-infected monkeys. Sera from monkeys 145-84, 236-84, and 351-80 at 23 weeks postinoculation were used to precipitate HIV-1 and SIV_{mac} virion proteins. The positions of the gp120 envelope glycoprotein and the p24/p27 capsid proteins are indicated.

HIV-1-89.6 and SIV_{mac}239. Of the 95 residues comprising this region, only one amino acid change from the 89.6 envelope glycoprotein sequence was observed in the SHIV-89.6P gp120 glycoprotein. This result confirms that SHIV-89.6P encodes HIV-1-like exterior envelope glycoproteins.

Antibody responses in SHIV-89.6P-infected monkeys. To examine the virus-specific antibody responses in monkeys infected with SHIV-89.6P, sera from animals 351-80, 236-84, and 145-84 were used to precipitate radiolabeled HIV-1 (HXBc2 strain) and SIV_{mac} virion proteins. These sera precipitated the HIV-1 gp120 envelope glycoprotein but not the SIV_{mac} envelope glycoprotein (Fig. 7). These sera also precipitated the capsid proteins of both HIV-1 and SIV_{mac}, consistent with the previously observed cross-reactivity of these Gag proteins (6, 13). These serological responses are similar to those previously seen in SHIV-infected monkeys and in HIV-1-infected humans.

DISCUSSION

The studies done to date in which macaque monkeys were infected with different SHIVs have failed to show an association between pathogenic effect and either persistence of infection or viral load during primary infection (9, 11). Even after 4 years of documented persistent infection with the original cloned SHIV-HXBc2, pathologic sequelae have not been seen in rhesus monkeys. Furthermore, in rhesus monkeys infected with the original cloned SHIV-89.6 (17), peak plasma p27 levels during primary infection were equivalent to those seen in pathogenic SIV_{mac} infections. Nevertheless, no pathologic consequences of these SHIV infections were observed, although provirus could be detected in PBL and virus could be readily isolated from blood.

Although SHIV-89.6P certainly induces a profound immunodeficient state in rhesus monkeys characterized by $CD4^+$ cell loss, the kinetics of this lymphocyte loss appears to differ from that observed in pathogenic SIV_{mac} infection of macaques or HIV-1 infection of humans. In these SIV_{mac} and HIV-1 infections, a transient fall in circulating $CD4^+$ lymphocytes during the period of primary infection is followed by a gradual loss of $CD4^+$ cells that eventually leads to opportunistic infections, tumors, and death (8). SHIV-89.6P infection appears to be capable of causing profound $CD4^+$ lymphocyte loss in some animals during primary infection, with no substantial increase in $CD4^+$ cell numbers thereafter. An explanation for the difference in the kinetics of $CD4^+$ lymphocyte loss induced by these various viruses is not readily apparent.

The profound loss of $CD4^+$ lymphocytes may have affected the virus load in infected animals, as measured by plasma viral RNA levels. In both the passage 2 and passage 3 monkeys that developed severe CD4 lymphopenia and an AIDS-like disease, viral loads were 1 to 2 log units lower than in the passage 1 monkeys, who maintained normal $CD4^+$ lymphocyte counts. In these animal, the $CD4^+$ lymphocyte loss may have been so extensive that infectable target cells capable of supporting SHIV replication no longer existed.

The pathogenicity of SHIV-89.6 changed dramatically between passage 1 and passage 2 in these monkeys. There is precedent in the SIV-macaque model for increasing lentiviral pathogenicity by serial in vivo virus passage. Even before the first isolation of SIV, there was a reported shortening in the time from inoculation of lymph node homogenates to morbidity in the serial transmission of an immunodeficiency syndrome in macaques that subsequently were shown to be infected with SIV_{mac} (19). The rapidly fatal PBj14 isolate of SIV_{smm} was derived from a monkey previously infected with a SIV_{smm} isolate that induced disease only after 1 to 2 years of infection (4).

It will be important to determine the precise molecular changes in the virus that occurred during in vivo passage of SHIV-89.6 resulting in its increased pathogenicity. Such changes may be localized to the *env* gene or may have occurred elsewhere in the viral genome. Sequencing of the first conserved gp120 region of isolated SHIV-89.6P showed few amino acid changes from the same region of the HIV-1 89.6 envelope. However, immunoprecipitation studies showed low but consistent reactivity of serum from an SIV_{mac}-infected monkey with the gp160 but not the gp120 envelope glycoprotein of the isolates of SHIV-89.6P and not with the envelope glycoprotein of HIV-1 or SHIV-89.6. These studies suggests that modifications of the SHIV-89.6P transmembrane envelope glycoprotein may have occurred to create epitopes shared with the SIV_{mac} envelope glycoproteins. Studies to define the molecular changes in SHIV-89.6P relevant to increased virulence await the generation of a pathogenic molecular clone of this virus.

ACKNOWLEDGMENTS

We thank Meredith A. Simon for assistance in histologic interpretation, Alison Hampson for photographic assistance, and Debra Ayles for preparation of the manuscript.

This work was supported by NIH grants AI-20729, CA-50139, AI-33832, AI-35166, RR-07000, and RR-00168 and Center for AIDS Research grant AI-28691; support from the G. Harold and Leila Mathers Foundation; and a gift from the late William F. McCarty-Cooper. John Li was supported by a Ryan Fellowship and a Harvard Merit Fellowship. Gunilla Karlsson was supported by a fellowship from Douglas and Judi Krupp.

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