JOURNAL OF VIROLOGY, Jan. 2003, p. 77–83 Vol. 77, No. 1
0022-538X/03/$08.00+0 DOI: 10.1128/JVI.77.1.77–83.2003
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Increased Virus Replication and Virulence after Serial Passage of Human Immunodeficiency Virus Type 2 in Baboons

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Received 18 March 2002/Accepted 27 September 2002

Similar to human immunodeficiency virus type 1 (HIV-1) infection of humans, the natural history of HIV-2 infection in baboons (*Papio cynocephalus*) is a slow and chronic disease that generally takes several years before an AIDS-like condition develops. To shorten the amount of time to the development of disease, we performed five serial passages of HIV-2$_{UC2}$ in baboons by using blood and bone marrow samples during the acute phase of infection when viral loads were at high levels. After these serial passages, virus levels in plasma, peripheral blood mononuclear cells (PBMC) and lymphatic tissues in the acutely infected baboons were increased. Within 1 year of the HIV-2 infection, all of the inoculated baboons showed specific signs of AIDS-related disease progression within the lymphatic tissues, such as vascular proliferation and lymphoid depletion. The HIV-2$_{UC2}$ recovered after four serial passages showed increased kinetics of viral replication in baboon PBMC and cytopathicity. This study suggests that the HIV-2 isolate recovered after several serial passages in baboons will be useful in future studies of AIDS pathogenesis and vaccine development by using this animal model.

In order to predict the clinical outcomes in humans, human immunodeficiency virus (HIV) vaccine candidates need to be evaluated in a nonhuman primate animal model that develops a chronic viral infection and has immune responses similar to those seen in human HIV infections (26; World Health Organization memorandum on animal models for HIV infection and AIDS, 1988). The most appropriate animal model for HIV type 1 (HIV-1) vaccine evaluation is the chimpanzee. However, due to its endangered species status, ethical considerations, and very slow rate of disease progression, most investigators have relied on the use of rhesus (*Macaca mulatta*) and pig-tailed (*Macaca nemestrina*) macaques infected with simian immunodeficiency virus (SIV) or chimeric simian-human immunodeficiency virus (SHIV) containing an HIV-1 envelope. The major drawback of these monkey models is usually a rapid disease course, with most monkeys dying within 1 year. This time period permits very little opportunity to study events leading to AIDS. Moreover, these monkey models do not use human-isolated retroviruses.

HIV-1 and HIV-2 isolates have been inoculated into several nonhuman primates (1, 11, 18, 33, 40), but with HIV-2, only baboons (*Papio cynocephalus*) and pig-tailed macaques develop an AIDS-like disease (3, 28, 31, 32). The infection course in baboons resembles the slow, lentiviral disease progression that is observed in chronic HIV-1 infection in humans. Moreover, the disease progression in the lymphatic tissues of HIV-2-infected baboons is similar to stages found during the development of AIDS in HIV-infected individuals (12, 28, 34).

Baboons are closely related to humans genetically (37), anatomically (44), and physiologically (20, 21) and are therefore widely used as animal models for human infections and vaccine development (31). Moreover, baboons, like humans, have four immunoglobulin G subclasses, whereas other monkey species only have three immunoglobulin G subclasses (10, 39). In addition, numerous monoclonal antibodies specific for human cytokines, chemokines, and immunophenotypic markers cross-react with baboon molecules (2, 46) (C. P. Locher and J. A. Levy, unpublished data). Furthermore, baboons are bigger than most monkey species and therefore can provide large blood draws with many peripheral blood mononuclear cells (PBMC) for the study of cellular immune responses. Finally, baboons are not naturally infected with herpes B virus, providing safer handling of the animals and blood products for animal care and laboratory workers (25).

We have found that baboons can be infected with four different HIV-2 isolates recovered from AIDS patients living in West Africa: HIV-2$_{UC2}$, HIV-2$_{UC3}$, HIV-2$_{UC12}$, and HIV-2$_{UC14}$ (3, 7, 30). Since HIV-2$_{UC2}$ can readily cause a persistent infection in baboons in vivo and over time induce an AIDS-like disease (3), we used this isolate in serial passage studies in attempts to shorten the pathogenic course.

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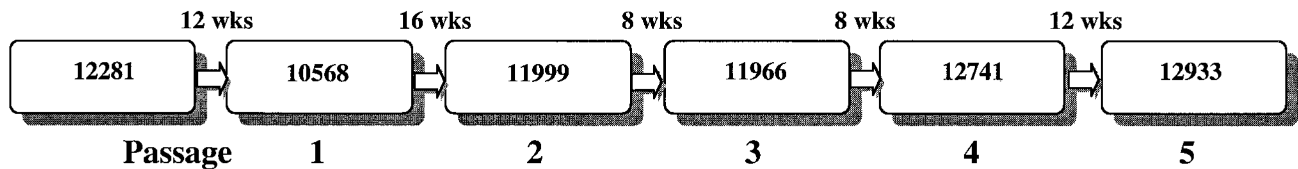

FIG. 1. Serial passage of HIV-2_{UC-2} isolates in baboons. Samples (10 ml of whole blood and 3 ml of bone marrow aspirates) were taken from baboons infected with HIV-2_{UC2} only. The first uninfected baboon (animal 12281) received 10,000 $TCID_{50}$ of HIV-$2_{UC2/9429}$ isolated from a baboon (animal 9429) with an AIDS-like disease (3). Serial passages were then made at shorter intervals of time as indicated.

MATERIALS AND METHODS

Virus. HIV-2_{UC2} was isolated from a woman who was originally from Burkina Faso but was living in the Ivory Coast. She had developed AIDS and was coinfected with HIV-1. By coculturing PBMC from this individual with the CEM T-cell line, the HIV-2_{UC2} isolate was separately recovered (6, 13). HIV-2_{UC2} is a dualtropic virus isolate (macrophage and T-cell line-tropic) and utilizes the CCR5 and CXCR4 chemokine coreceptors (22). A more virulent variant of the HIV-2_{UC2} isolate was recovered from an infected baboon with an AIDS-like disease (animal 9429) (3) and has been used as a challenge virus for studies of resistance to superinfection (26, 29).

Inoculation procedures in baboons. Juvenile baboons of the sacred or olive subspecies (*P. cynocephalus* subsp. *hamadryas* and *P. cynocephalus* subsp. *anubis*, respectively) received serial passages of HIV-2_{UC2}. They were housed under biosafety level 2 conditions at the Southwest Foundation for Biomedical Research in San Antonio, Tex., throughout the course of the present study. The protocol and procedures were approved by the institutional Animal Research Committee and were in accordance with the Animal Welfare Act. Each baboon was negative for antibodies by enzyme-linked immunosorbent assay (ELISA) to the simian retroviruses (SRV-1 and SRV-2) and SIV.

Virus isolation and viral load determination. For virus isolation from inoculated animals, 6×10^6 baboon PBMC were obtained from heparinized blood samples by Ficoll-Hypaque gradient centrifugation and were mixed with an equal number of human PBMC stimulated for 3 days with 3 μg of phytohemagglutinin (PHA; Sigma, St. Louis, Mo.)/ml (8). Virus replication was detected by the reverse transcriptase (RT) assay (23).

To determine the number of HIV-2-infected cells in baboon PBMC and lymphatic tissues, the infected cell titration assay was used as previously described (5). Briefly, 5- and 10-fold serial dilutions of 10^6 baboon PBMC were cocultured in a 24-well tissue culture plate with 10^6 human PBMC that had been stimulated with PHA. These infected cell titration cultures were then monitored over a 28-day period for virus production twice weekly by using the RT assay (23). All viral cultures were conducted in RPMI 1640 medium (BioWhittaker, Walkersville, Md.) supplemented with 10% heat-inactivated (56°C, 30 min) fetal bovine serum (Gibco, Grand Island, N.Y.), 10% natural interleukin-2 (T-stim; Roche, Indianapolis, Ind.), 2 mM L-glutamine, 100 U of penicillin/ml, and 100 μg of streptomycin/ml (Tissue Culture Facility, University of California at San Francisco).

Quantitative detection of HIV-2 RNA. HIV-2 RNA and DNA were detected in PCR-based assays as previously described (19) Briefly, primers RAR1000 (5′-GCTGGCAGATTGAGCCCTGGGAGGTTCTCT-3′) and RAR04 (5′-GAATGACCAGGCGGCGACTAGGAGAGAT-3′) were used to amplify a 201-bp fragment of the HIV-2 long terminal repeat. This region is highly conserved among known HIV-2 and SIV isolates because the upstream primer has no more than a two-base mismatch, whereas the downstream primer has no mismatches with known sequences. These primers were shown not to amplify HIV-1 sequences.

Flow cytometry and antibody detection. $CD4^+$- and $CD8^+$-T-lymphocyte numbers were measured by flow cytometry by using Leu3- and Leu2-specific monoclonal antibodies (Becton Dickinson, San Jose, Calif.) as described previously (27). Seroconversion and reciprocal antibody titers were determined by using a commercial HIV-2-specific ELISA kit according to the manufacturer's instructions (BioChem ImmunoSystems, Montreal, Quebec, Canada). The plasma dilution that had an optical density value of $\geq$0.2 was used to define the antibody titer.

In situ hybridization. Viral RNA was detected in paraffin sections of lymphoid tissue by using a ^{35}S-labeled antisense probe (Lofstrand Laboratories, Gaithersburg, Md.) (36, 42, 43). The antisense HIV-2 probe was synthesized from DNA templates that represent 90% of the viral genome as previously described for HIV-1 (45) and was provided by Cecil H. Fox. Evaluation of the tissues was performed in an Axiophot Zeiss microscope equipped with a Plan Neofluar, a three-dimensional color camera, and a PC-based image analyzer system (KS 400; Kontron).

Characterization of HIV-2 isolated from baboons after serial passage. To ascertain the biological tropism of the HIV-2_{UC2} isolates recovered after serial passage, 1,000 50% tissue culture infectious dosages ($TCID_{50}$) were used to infect 10^6 PHA-stimulated baboon PBMC taken from uninfected control baboons. The cultures were maintained in the presence of 100 U of recombinant interleukin-2 for 14 days, and virus replication was monitored by using the RT assay (23).

Human macrophages (2×10^6), isolated as previously described (17), were also inoculated with 2,000 $TCID_{50}$ of the HIV-2 isolates recovered from baboons or with positive control R5 macrophage-tropic HIV-1_{SF128A} and HIV-1_{SF162} viruses. In addition, duplicate cultures of 10^6 MT-2 cells were infected overnight with 1,000 $TCID_{50}$ of the recovered HIV-2 isolates or the positive control X4 HIV-1_{SF33} and the negative control R5 HIV-1_{SF128A} viruses. All virus cultures were monitored for syncytia induction for 8 days and virus replication was assayed every 2 days by using RT determination (23).

RESULTS

Serial passage of HIV-2_{UC2} using adoptive transfer of whole blood and bone marrow aspirates. Adoptive transfer of bone marrow cells during the acute phase of infection has been shown to enhance the virulence of SIV and SHIV isolates (24, 38). We therefore first inoculated 10,000 $TCID_{50}$ of HIV-$2_{UC2/9429}$ into baboon 12281 by the intravenous route. After 3 months, 10 ml of blood and 3 ml of bone marrow aspirate were transferred into a naive baboon (animal 10568). Four additional serial passages were then performed in naive juvenile baboons over the course of ca. 1 year (Fig. 1).

In five of the six HIV-2-inoculated baboons, persistent virus isolation from PBMC was achieved. Moreover, all of these baboons developed lymphadenopathy during the first few weeks of infection. In addition, numerous clinical signs of disease were observed, including circulating reactive lymphocytes, thrombocytopenia, alopecia, diarrhea, and gingivitis. A summary of the clinical findings and virus isolation is presented in Table 1. One of the baboons that developed diarrhea (animal 11966) was also infected with whipworm (*Trichuris trichiura*) and *Balantidium coli*. Another baboon that developed diarrhea was infected with the intestinal parasites *Entamoeba coli* and *Iodoamoeba butschlii*. These are not opportunistic infections, but these parasites were only observed in association with lymphadenopathy during the acute stage of infection.

After the fourth and fifth serial passages of virus in blood and bone marrow aspirates, the two baboons (animals 12741 and 12933) had a $CD4^+$-cell decline (321 and 269 $CD4^+$ cells/μl of whole blood) between weeks 2 and 8 postinoculation

TABLE 1. Clinical findings and virus isolation in HIV-2-infected baboons after serial passage of virus in whole blood and bone marrow[a]

$HIV\text{-}2_{UC2/9429}$ serial passage	Baboon no.	Subsp.	Sex	Age (yr)	Virus isolation	Clinical finding(s)
Source	12281	*hamadryas*	F	2	Persistent (1.5 yr)	LAD, atypical lymphocytes
P1	10568	*hamadryas*	M	3	Persistent (2 yr)	LAD, splenomegaly, atypical lymphocytes, warts
P2	11999	*anubis*	M	1	Transient (6 mo)	LAD, babesia, gingivitis, alopecia, diarrhea (*Campylobacter* sp., *Trichuris trichiura*, *Balantidium coli*)
P3	11966	*anubis*	M	2	Persistent (1 yr)	LAD, thrombocytopenia, alopecia, *Babesia*
P4	12741	*hamadryas*	M	2	Persistent (1 yr)	LAD, *Babesia*
P5	12933	*anubis*	M	1	Persistent (1 yr)	LAD, atypical lymphocytes, lymphocytosis, diarrhea (*Entamoeba coli, Iodoamoeba butschlii*), *Babesia*

[a] For the *Babesia* sp., this was possibly the same species (*Babesia bigemina*) originally described by Smith and Kilbourne in 1893 as the tick-borne agent of Texas cattle fever. The *hamadryas* or sacred baboon subspecies originated primarily in Ethiopia, whereas the *anubis* or olive baboon originated in Kenya. The age of the baboon was at the time of the HIV-2 inoculation. Virus isolation indicates the ability to recover HIV-2 from the PBMC on a monthly basis: Transient, not every month; Persistent at every monthly bleed. LAD, lymphadenopathy; P, passage; M, male; F, female.

and did not recover their preinfection levels ($CD4^+$ cells at 800 to 1,400 cells/μl; data not shown). These findings suggest that the animals over time might progress to disease. At 2 weeks postinoculation, both of these baboons also showed a dramatic rise in the number of $CD8^+$ cells (from 350 $CD8^+$ cells/μl at the time of inoculation to 2,036 and 1,255 $CD8^+$ cells/μl for baboons 12741 and 12933, respectively). After 1 year of HIV-2 infection, the ratio of $CD4^+$ to $CD8^+$ T lymphocytes in the majority of the baboons was <0.5:1. Before HIV-2 inoculation, the $CD4^+/CD8^+$ T-lymphocyte ratio was ca. 1:1. No substantial changes were noted in other white blood cell subsets. The overall antibody responses to HIV-2 gp120 ranged from 1:1,000 to 1:10,000 in the baboons inoculated with HIV-2 via blood and bone marrow (data not shown).

Serial passage of HIV-2 increased viral loads in plasma, PBMC, and lymphatic tissues. Plasma viral loads in the baboons were increased as measured by quantitative-competitive PCR. These loads ranged from 50,000 viral RNA copies per ml of plasma at 2 weeks postinoculation at the first serial passage (baboon 10568) to 200,000 viral RNA copies per ml of plasma at the fifth serial passage (animal 12933; Fig. 2). The viral loads in these baboons in plasma reached a set point at between 1,000 (baboons 10568, 11999, and 11966, passages 1 through 3) and 18,000 (baboon 12741, passage 4) viral RNA copies/ml at 20 weeks postinoculation.

To determine the number of HIV-2-infected PBMC in each of the baboons, infected cell titration was performed. As shown in Table 2, the number of HIV-2-infected PBMC was similar in each of the baboons during the acute phase of infection (between 10,000 and 10 HIV-2-infected cells per 10^6 PBMC at 2 to 12 weeks postinoculation, respectively). However, 1 year postinoculation, three of the baboons that received blood and bone marrow at the later serial passages (baboons 11966, 12741, and 12933) had a substantial increase in the PBMC-associated viral load (between 100 and 10,000 HIV-2-infected cells per 10^6 PBMC at 52 weeks postinoculation). In addition, these baboons had substantially higher viral loads within their lymphatic tissues than the baboons receiving earlier passages (10^4 HIV-2-infected cells per 10^6 total cells versus 1 to 10^2 HIV-2-infected cells per 10^6 total cells in the spleen, tonsils, and adenoids from baboons in the first serial passage) (Table 3).

The lymphatic tissues that had the highest viral loads were the tonsils, cervical lymph nodes, and the spleen (Table 3). The oral mucosal-associated lymphatic tissue has been shown to be an active site both for chronic HIV-1 infection (15, 16) and primary SIV infection (41). The present studies indicated it was also an efficient anatomic site for HIV-2 replication in baboons (see below). The higher viral load in the spleen may reflect viral particle trapping rather than active virus replication although the spleen of one baboon contained a large amount of RNA^+ cells and did not show viral particle trapping by in situ hybridization (see below).

Lymphoid tissue pathogenesis. A hallmark of lymphadenopathy in HIV infection is the breakdown of the germinal centers in lymph nodes known as follicular lysis (35, 36, 42). This finding can occur in a relatively early phase of HIV infection and corresponds to dendritic trapping of HIV particles in the germinal center. Later in the disease there is generally lymphoid depletion. We have observed such lymphoid depletion in HIV-2-infected baboons with an AIDS-like illness (3). In the baboons receiving serial passages of HIV-2, the beginning of lymphoid tissue disruption was observed with follicular lysis at 6 months postinoculation. At the time of necropsy, the most pronounced HIV-related disease was observed after three serial passages in baboons 11966 and 12933) (Table 4). In these $HIV\text{-}2_{UC2}$ inoculated baboons, widespread vascular and endothelial cell proliferation, follicular lysis, and lymphoid depletion were observed. The process was generalized because florid hyperplasia was readily observed in many of the lymphoid tissues from each of the baboons. Moreover, fibrosis, hyalinization, and eosinophilic infiltrates were also observed.

Virus replication in lymphatic tissues. To assess viral replication within lymphatic tissues, in situ hybridization was performed on two of the $HIV\text{-}2_{UC2}$-inoculated animals, baboons 12281 and 10568 (data not shown). Active virus replication was found primarily within the germinal center, as well as in the paracortical T-cell zones in peripheral lymph nodes and in the marginal sinus lying beneath the capsule. In the spleen, active virus replication and very fine viral particle trapping was present in the germinal center. In contrast, the gut-associated lymphoid tissue, including the Peyer's patches, showed virus replication within the germinal center without viral particle trapping. In the tonsil tissues, HIV-2-infected cells were seen throughout the germinal centers and the extrafollicular regions. In addition, virus replication was noted in the lymphoepithelium of the tonsil near the lumen. No virus infection was detected in the liver, kidney, heart, pancreas, adrenal gland,

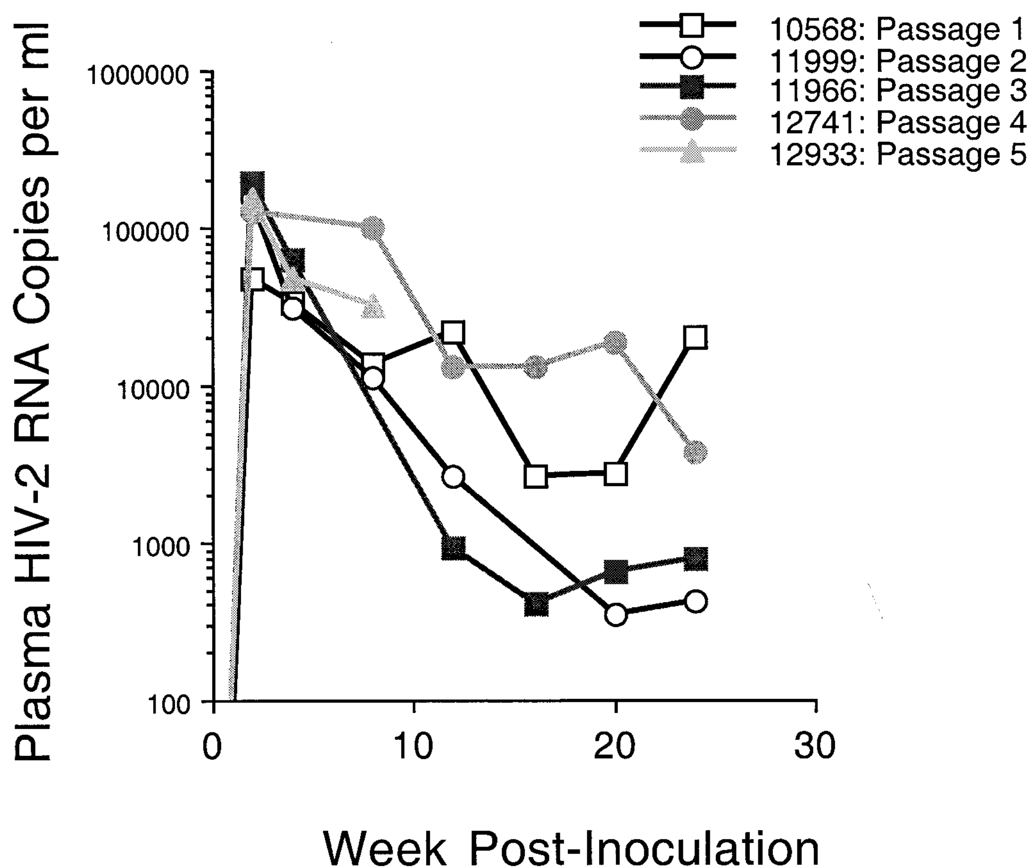

FIG. 2. RNA viral loads in plasma in HIV-2-infected baboons inoculated with serial passages in virus in blood and bone marrow. HIV-2 RNA was measured by quantitative PCR amplification of the long terminal repeat region. The highest levels of plasma viremia were noted after the third serial passage (baboon 11966).

lamina propia of the intestine, or ovary. However, virus replication was observed in four cells in the testis of baboon 10568.

To determine the extent of HIV-2-infection within the lymphoid tissues, we counted the number of HIV-2-infected cells, measured the area of the section and calculated the frequency of RNA^+ cells per mm^2 of tissue section (Table 5). The highest number of infected cells was found in baboon 10568 that had received HIV-2 after serial passage. Most of the virus replication was present within the tonsils, supporting our viral load determination by infection center assay. The number of virus-infected cells was highest in the T-cell zone, followed by the

TABLE 2. Viral load in PBMC of baboons after serial passage of virus in whole blood and bone marrow transfer[a]

No. of wk postinfection	Viral load in baboon no. (passage):					
	12281 (source)	10568 (1)	11999 (2)	11966 (3)	12741 (4)	12933 (5)
2	NT	10,000	10	10,000	10,000	1,000
4	1,000	100	10	1,000	NT	100
8	100	100	10	NT	10	10
12	100	10	1	100	100	10
16	10	1	NT	10	10	10
20	NT	1		1		10
24	NT	100	1		10	NT
52	1	1		100	10,000	1,000

[a] The number of HIV-2-infected cells in baboon PBMC was determined by using the infected cell titration assay as previously described (5). Five- to ten-fold serial dilutions of 10^6 baboon PBMC were cocultured in a 24-well tissue culture plate with 10^6 human PBMC that had been stimulated with PHA. The infected cell titration cultures were monitored over a 28-day period for virus production twice weekly by using the RT assay (23). NT, not tested.

TABLE 3. Viral load in baboon PBMC and lymphatic tissues after serial passage of virus in blood and bone marrow from HIV-2-infected baboons[a]

$HIV\text{-}2_{UC2/9429}$ serial passage	Baboon no.	No. of HIV-2 infected cells/million uninfected cells			
		PBMC	Lymph node	Tonsils	Spleen
Source	12281	1	1	10	100
P1	10568	1	10	10	100
P2	11999	ND	ND	ND	ND
P3	11966	100	100	10,000	10,000
P4	12741	10,000	1,000	10,000	10,000
P5	12933	1,000	ND	10,000	10,000

[a] The number of HIV-2-infected cells in baboon PBMC and lymphatic tissues was determined at the time of necropsy by using the infected cell titration assay as previously described (5). Five- to ten-fold serial dilutions of 10^6 baboon PBMC were cocultured in a 24-well tissue culture plate with 10^6 human PBMC that had been stimulated with PHA. The infected cell titration cultures were monitored for virus production twice weekly over 28 days by using the RT assay (23). The lowest number of cells added that showed presence of infected cells is presented. P, passage; ND, not done.

TABLE 4. HIV-associated pathogenesis in lymphatic tissues of HIV-2_{UC2}-infected baboons after serial passage of virus in blood and bone marrow[a]

Baboon no.	Pathogenesis findings								
	Cer	Ax	Ing	Med	Mes	Tonsil	Adenoid	Spleen	Thymus
12281	1GC	2, FH, florid	2, FH	Med Fib	Ragged GC	2, FH, florid	FL	Ragged GC	NL
10568	Mix	Mix, hyalin	1GC mix	Mix	Mix, SH	Mix	Mix	NL	NL
11999	HED, GC, PE, EH	1 GC	Mix	PE, VP	Early mix	EH, VP, mix	Mix	NL	CM blur
11966	LD, F	–	LD, F	LD, focal F	LD	–	LD, F	–	Medullary HED
12741	2GC	–	2GC	2GC	2GC	–	2GC	–	NL
12933	HD (eos)	–	F	LD, VP	F, Hemorr	–	NL	–	–

[a] Tissues were conventionally processed by fixation in 10% neutral buffered formalin and embedded in paraffin. Sections were then cut to 5 μm and stained with hematoxylin and eosin. Abbreviations: Cer, cervical; Ax, axillary; Ing, inguinal; Med, mediastinal; Mes, mesenteric; GC, germinal center (primary [1] or secondary [2]); FH, follicular hyperplasia; Med Fib, medullary fibrosis; NL, normal; mix, mixed follicular and paracortical expansion; HED, hyaline or eosinophilic (eos) deposits; SH, sinus histiocytosis; PE, paracortical expansion; VP, vascular proliferation; CM, corticomedullary; LD, lymphoid depletion of germinal center; F, fibrosis; Hemorr, hemorrhagic; –, not examined.

germinal centers and finally in the sinuses of the lymph node. A large number of these tissues also exhibited germinal center trapping of virus particles.

Changes in the biological phenotype of HIV-2. We found that HIV-$2_{UC2/12741}$, recovered from the fourth serial passage, replicated to higher levels in baboon PBMC than the original human isolate HIV-2_{UC2} and the HIV-$2_{UC2/9429}$ isolate that was recovered from the infected baboon with an AIDS-like disease. In two separate studies, HIV-$2_{UC2/12741}$ replicated more rapidly and showed peak RT activity of up to 500,000 cpm/ml of culture fluid between 7 and 10 days after inoculation in vitro. In contrast, cells infected with HIV-2_{UC2} had a mean RT activity of 80,000 cpm/ml of culture fluid, and those infected with HIV-$2_{UC2/9429}$ had a mean RT activity of 200,000 cpm/ml. These data indicated that the virus isolated from baboon 12741, after serial passage, was better adapted to replication in baboon PBMC than the original infecting HIV-2 isolate. In addition, when inoculated onto the MT-2 T-cell line, the serially passaged isolate, HIV-$2_{UC2/12741}$ was more cytotoxic than the HIV-$2_{UC2/9429}$ isolate. However, human macrophages infected with HIV-$2_{UC/12741}$ did not demonstrate any difference from the parental virus isolate (HIV-2_{UC2}) in virus replication (~200,000 cpm/ml of RT activity).

TABLE 5. HIV-2-infected cells in lymphatic tissues from baboons after serial passage of virus in whole blood and bone marrow[a]

Baboon no.	Tissue	No. of RNA$^+$ cells/mm^2 of GC	No. of RNA$^+$ cells			Germinal center trapping
			ETL	GC	Sinus/LE	
12281	Lymph node	10.18	4	11	1	No
	Spleen	6.15	114	31	24	No
	Peyer's patches	9.09	7	7	0	No
10568	Lymph node	17.41	15	18	5	Yes
	Lymph node	13.67	18	16	0	Yes
	Tonsil	31.50	35	23	1	Yes

[a] The number of HIV-2-infected cells was determined by in situ hybridization of lymphatic tissues. The antisense HIV-2 probe was synthesized from DNA templates that represent 90% of the viral genome (14). See Materials and Methods for details. The area occupied by all germinal centers (GC) together is <1 mm^2, and the value given was the extrapolated value from the examination of 10 fields. ETL, extrafollicular T lymphocytes; LE, lymphatic endothelium.

DISCUSSION

In the present study, we have found that serial passage of virus in whole blood and bone marrow cells during the acute phase of infection was effective at developing a more virulent HIV-2_{UC2} variant (Tables 1 to 4). In the inoculated baboons, a higher viral load was noted in most lymphatic tissues (particularly spleen and tonsils) and the peripheral blood after the third serial passage (Table 2 and 3). In addition, isolation of HIV-$2_{UC2/12741}$ after the fourth serial passage showed enhanced replication kinetics in baboon PBMC in vitro. Moreover, the lymphoid tissues from animals receiving serially passaged virus showed more AIDS-associated pathology (vascular proliferation and lymphoid depletion) at an earlier time period than observed with the parental virus (Table 4). Finally, a more substantial CD4 T-cell decline, lymphopenia, and thrombocytopenia were observed in the virus-infected baboons after the fourth and fifth serial passages of HIV-2. These findings indicate that this HIV-2 isolate was better adapted to replicate in baboons and has the potential to cause an AIDS-like disease within a shorter amount of time than the original parent virus isolate (3).

The lymph node pathology in HIV-2-infected animals was similar to that seen in some HIV-2-infected macaques and in HIV-1-infected humans (4, 11, 12, 32, 34, 35) and was most progressive after the third serial passage (i.e., baboons 11966, 12741, and 12933). In addition, the uneven distribution of follicular dendritic cell-bound HIV is also often seen in cases of HIV-1 infection. Similar to human HIV-1 infection, a spectrum of lymphoid tissue pathology was observed (Table 3) (4, 9), some of which has been noted in baboons with an AIDS-like disease after 3 years of HIV-2_{UC2} infection (3, 26, 31).

The baboons in the present study were monitored for only 1 year. Future studies may show that the baboon-isolated HIV-2 (e.g., HIV-$2_{UC2/12741}$) gives higher viral loads and a larger proportion of baboons developing an AIDS-like disease within a shorter amount of time than the initial HIV-2 isolate (3). Since the virulence of HIV-2 was increased by serial passage in blood and bone marrow cells, the new HIV-2 baboon-tropic variant may be useful in new studies of viral pathogenesis and as a challenge virus in immunization strategies with candidate vaccines.

ACKNOWLEDGMENTS

We thank Kaylynn Peter and Ann Murai for assistance with manuscript preparation.

C.P.L. was the recipient of a postdoctoral training grant provided by the George Williams Hooper Foundation, San Francisco, Calif. This work was supported by National Institutes of Health grants AI-34704 and P30 MH 59037, as well as by the University of California CFAR program.

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