Replication of an Acutely Lethal Simian Immunodeficiency Virus Activates and Induces Proliferation of Lymphocytes

PATRICIA N. FULTZ

*Department of Microbiology, University of Alabama at Birmingham,
Birmingham, Alabama 35294*

Received 31 January 1991/Accepted 30 May 1991

A variant of simian immunodeficiency virus from sooty mangabey monkeys (SIVsmm), termed SIVsmm-PBj14, was previously identified and shown to induce acute disease and death within 1 to 2 weeks of inoculation of pig-tailed macaques and mangabey monkeys (P. N. Fultz, H. M. McClure, D. C. Anderson, and W. M. Switzer, *AIDS Res. Hum. Retroviruses* 5:397-409, 1989). SIVsmmPBj14 differed from its parent virus, SIVsmm9, not only in pathogenicity but also in multiple *in vitro* properties. As a first approach to understanding the biological and molecular mechanisms responsible for the acute disease and death induced by this variant, virus-host cell interactions of SIVsmmPBj14 and SIVsmm9 were studied. Initial rates of replication of the two viruses were identical in primary peripheral blood mononuclear cells (PBMC) from normal pig-tailed macaques and mangabey monkeys, but SIVsmmPBj14 infection always resulted in higher yields of virus than did SIVsmm9 infection, as assessed by levels of reverse transcriptase activity in culture supernatants. Surprisingly, despite its cytopathicity for macaque and mangabey CD4⁺ cells, replication of SIVsmmPBj14 was accompanied by up to 10-fold increases in number of viable cells compared with cell numbers in uninfected or SIVsmm9-infected cultures. Furthermore, SIVsmmPBj14 was shown to infect and replicate in resting PBMC just as efficiently as in mitogen-stimulated PBMC, irrespective of whether exogenous interleukin-2 (IL-2) or antibodies that neutralized IL-2 were added to culture media. Accumulation of virus in culture supernatants of resting PBMC preceded by several days the appearance of activated cells which expressed the IL-2 receptor alpha subunit (CD25), suggesting that activation of cells was not essential for replication. The ability to activate and to induce simian PBMC to proliferate appeared specific for the acutely lethal variant because incorporation of [³H]thymidine by PBMC from naive animals was observed only upon incubation with concentrated, heat-inactivated SIVsmmPBj14 and not with other viruses. Both CD4⁺- and CD8⁺-enriched cell populations proliferated in response to SIVsmmPBj14. These results are consistent with *in vivo* observations and suggest that the abilities both to replicate in resting cells and to induce lymphocytes to proliferate may contribute to the extreme virulence of SIVsmmPBj14.

Lentiviruses, as the prefix "lenti-" implies, are retroviruses that are associated with slowly progressive disease and, in general, replicate more efficiently in activated or mature cells, such as macrophages, than in monocytes (9, 21, 28). Among the newest members of the lentivirus subfamily are the human and simian immunodeficiency viruses (HIV and SIV), both of which induce in their respective hosts the slowly progressive disease known as AIDS. There is substantial evidence that while HIV can infect nonactivated resting lymphocytes, activation of cells is required before virus production can be detected (21, 33, 36). Because of this, it has been proposed that AIDS ultimately develops in HIV-infected persons due to a succession of multiple insults on the immune system, each of which may activate lymphocytes, some bearing the HIV provirus, with the result that there is increased production of virus and concomitant cell destruction (4, 38). Recent findings that HIV-infected persons and SIV-infected monkeys have both a higher frequency of HIV- or SIV-infected cells and higher titers of cell-free virus in plasma as disease progresses support this idea (2, 7, 10, 31, 39).

We previously reported (6) the isolation and characterization of a variant of SIVsmm, designated SIVsmmPBj14 (hereafter referred to as smmPBj14), that, unlike all other known lentiviruses, uniformly induces in pig-tailed macaques (*Macaca nemestrina*) an acute disease syndrome characterized by bloody mucoid diarrhea and death of most

animals within 1 to 2 weeks. A pathologic finding associated with acute death was extensive lymphoid hyperplasia, especially prominent in gut-associated lymphoid tissue such as Peyer's patches but also found in other lymphoid tissues, including the spleen. Zack and colleagues (37) showed that in animals dying acutely from smmPBj14 infection, infiltrating cells in the lamina propria of the gastrointestinal tract consisted of lymphoblasts with either a CD4⁺ or CD8⁺ phenotype, in approximately equal numbers, and that SIV antigens could be detected not only in lymphocytes but also in syncytial cells. In addition, large numbers of lentiviral particles were detected in gut-associated lymphoid tissue by electron microscopy. The evidence that acute lethal disease is caused, either directly or indirectly, by smmPBj14 appears incontrovertible since a virtually identical syndrome was induced by molecularly cloned viruses derived from a biological clone of smmPBj14 (3).

Because of the rapid death induced by smmPBj14 in association with extensive lymphoid hyperplasia and the fact that smmPBj14 and its parent virus, SIVsmm9 (smm9), differed in multiple biological properties *in vitro* (6), including replication potential in cells of different origins, the interaction of smmPBj14 with peripheral blood mononuclear cells (PBMC) from different species was investigated. The results of the studies presented herein suggest that replicative properties of smmPBj14 may be responsible in part for its extreme virulence.

MATERIALS AND METHODS

Viruses. Virus stocks of smm9 and smmPBj14 were generated on normal human PBMC by a single passage of primary isolates originally obtained in cocultures of normal human PBMC with PBMC from an SIV-infected mangabey (smm9) or an smm9-infected pig-tailed macaque (smmPBj14). Supernatants from the first passage were cleared of cells by low-speed centrifugation and of cell debris by filtration through a 0.22- μ m-pore-size filter. Partially purified smmPBj14 was generated by infection of human PBMC; after high levels of reverse transcriptase (RT) activity were detected, cell-free supernatant fluids were concentrated by ultracentrifugation and Sepharose 4B column chromatography (22). Aliquots of the concentrated virus were stored at -80°C and were heat inactivated at 56°C for 1 h before use. Infection of human PBMC with the concentrated, heat-inactivated virus preparation showed that all detectable infectious virus was destroyed. The protein concentration of the purified virus preparation was determined to be 0.7 mg/ml, using a Bio-Rad protein assay kit. Sucrose gradient-concentrated, inactivated preparations of HIV-1_{HTLV-IIIb} and HT-PBj14 (see below) were kindly provided by Electronucleonics (Silver Spring, Md.) and Hillcrest Biologicals (Cypress, Calif.), respectively.

Replication experiments. All analyses of replicative properties utilized primary PBMC obtained from normal HIV- and SIV-seronegative pig-tailed macaques, sooty mangabey monkeys (*Cercocebus atys*), chimpanzees (*Pan troglodytes*), or humans. Whole heparinized blood was separated on Ficoll-sodium diatrizoate (LSM; Organon Teknica, Durham, N.C.). PBMC were washed with phosphate-buffered saline and then placed in cultures, where they were either infected immediately with virus or stimulated for 3 days with 0.1% phytohemagglutinin P (PHA) or 10 μ g of concanavalin A (ConA) per ml before infection. Equivalent counts per minute of RT activity of different virus isolates were used to initiate infections. In some experiments, polyclonal rabbit antibodies to human interleukin-2 (IL-2; Genzyme, Boston, Mass.) or human tumor necrosis factor- α (TNF- α ; kindly provided by Genentech, San Francisco, Calif.) were added to culture media to neutralize the biological activity of these cytokines. Antibodies to IL-2 and TNF- α were maintained in cultures at concentrations of 12 μ g/ml and 600 U/ml, respectively.

Proliferation assays. Following infection with virus, PBMC were washed and resuspended in appropriate RPMI 1640 medium containing antibiotics, glutamine, and 10% fetal bovine serum (FBS) (10%-RPMI) or, in addition to the previous supplements, 8 U of recombinant human IL-2 (Boehringer Mannheim, Indianapolis, Ind.) (IL-2-RPMI) per ml. Cultures were maintained in T25 tissue culture flasks in a total volume of 5 or 10 ml. Periodically, aliquots of cell-free supernatant were removed to determine RT activity, as a measure of virus replication, and culture volumes were maintained by the addition of fresh medium. Total number of cells and cell viability were determined by trypan blue dye exclusion. To minimize possible activating effects of FBS, in some experiments autologous serum was obtained from the same animal as PBMC and was used in medium instead of FBS. Activation of PBMC was assessed in two ways: (i) by increases in percentages of cells expressing the CD25 antigen (IL-2 receptor α subunit [IL-2R α]) as determined by FACSscan analysis using the monoclonal antibody anti-TAC (Becton Dickinson, San Jose, Calif.) or (ii) by [^3H]thymidine incorporation into PBMC after incubation with mitogen or

concentrated, heat-inactivated smmPBj14. For the latter assay, 10^5 PBMC per well were incubated for various times in triplicate in 96-well plates in RPMI 1640 medium (containing glutamine and 13% human AB serum) either alone (negative controls) or in medium to which mitogen (ConA) or viral antigen was added; each well was pulsed for 16 h with 1 μ Ci of [^3H]thymidine, and incorporation of radioactivity was determined with a beta counter after harvesting wells with a Skatron cell harvester. As virus controls for induction of proliferation, a gamma-irradiated concentrated preparation of a minimally pathogenic derivative of smmPBj14 that had been adapted to growth in HT cells (HT-PBj14) or β -propiolactone-inactivated concentrated HIV-1 (Electronucleonics, Silver Spring, Md.) was used. To assess proliferative responses of CD4 $^{+}$ - and CD8 $^{+}$ -enriched lymphocytes, PBMC from naive pig-tailed macaques were incubated overnight in T25 flasks to allow monocytes to adhere. After the flasks were washed, the nonadherent cells were divided into two equal aliquots, which were enriched for the two lymphocyte populations by negative selection using magnetic beads (Dynabeads; Dynal Inc., Great Neck, N.Y.) to which were bound antibodies to CD8 or CD4. The efficiency of enrichment was determined by FACSscan analysis.

Determination of CD4/CD8 ratios. To determine percentages of CD4 $^{+}$ and CD8 $^{+}$ lymphocytes in uninfected or infected cultures, aliquots of cells were removed, washed, and incubated with equal amounts of phycoerythrin-labeled Leu3a (CD4) and fluorescein-labeled Leu2a (CD8) (Becton Dickinson). After incubation at 4°C for 30 min, cells were washed and a minimum of 500 cells were counted with a fluorescence microscope.

RESULTS

Replication in simian PBMC. Because smmPBj14 appeared to be more virulent for pig-tailed macaques than for other simian species tested (3, 6), replication and cytopathic effects of smmPBj14 and its parent, smm9, which induces classic AIDS-like disease in pig-tailed and rhesus macaques (20), were compared in primary pig-tailed macaque PBMC that had been stimulated for 3 days with PHA (Fig. 1A). Initial rates of replication (accumulation of RT activity with time) of the two viruses were equivalent, but higher yields, as reflected by higher levels of RT activity, of smmPBj14 than of smm9 were always obtained, irrespective of the donor animal. To assess cytopathic effects of the viruses, total numbers of viable cells and cell viability were determined. Although overall cell viabilities were similar, accumulation of smmPBj14 virions in the culture medium, as assessed by RT activity, preceded slightly or coincided with increased numbers of viable cells (Fig. 1A and B). In a representative experiment (Fig. 1), ratios of CD4 $^{+}$ to CD8 $^{+}$ cells present in the cultures on day 16 were 1.5, 0.75, and 0.44 for the uninfected control, smm9-infected, and smmPBj14-infected cultures, respectively, suggesting that replication of both viruses in PBMC from pig-tailed macaques resulted in selective loss of CD4 $^{+}$ lymphocytes.

Since smmPBj14 had been shown to induce lethal disease in a majority of normal mangabey monkeys but not in SIVsmm-infected mangabeys (6), the experiment described above was performed in primary PBMC from both uninfected and infected mangabeys. Essentially the same results as with PBMC from pig-tailed macaques were obtained with cells from uninfected mangabeys, including apparent proliferation of cells in the smmPBj14-infected culture (Fig. 2B). Interestingly, superinfection and replication of both smm9

FIG. 1. Replication of smmPBj14 (▲) and smm9 (●) in primary PBMC from a pig-tailed macaque. (A) Virus replication as monitored by RT activity in cell-free culture supernatants. (B) Total number of viable cells in SIV-infected and uninfected (■) cultures. (C) Viability of PBMC in SIV-infected and uninfected cultures.

and smmPBj14 were suppressed in PBMC from the naturally SIV-infected mangabey, but replication of smmPBj14 was detected by day 16 postinfection, which also coincided with increased numbers of viable cells in the culture (Fig. 2D to F). In a similar experiment (not shown) involving infection of PBMC from an uninfected mangabey, CD4/CD8 ratios determined at the time of maximum virus production were 2.0, 2.0, and 0.11 in the uninfected control, smm9-infected, and

smmPBj14-infected cultures, respectively. This result is consistent with those of previous studies which showed that smmPBj14, but not smm9, was cytopathic for mangabey PBMC (3). These data suggested that smmPBj14 not only was cytopathic for CD4⁺ cells from both pig-tailed macaques and mangabeys but also induced simian lymphocytes to proliferate. In different experiments, the proliferation usually resulted in a 3- to 4-fold increase in viable cell

FIG. 2. Replication of smmPBj14 (▲) and smm9 (●) in primary PBMC from an uninfected (A to C) or naturally SIV-infected (D to F) sooty mangabey monkey. (A and D) Virus replication as monitored by RT activity in cell-free culture supernatants. (B and E) Total number of viable cells in SIV-infected or uninfected (■) cultures. (C and F) Viability of PBMC in SIV-infected or uninfected control cultures.

FIG. 3. Replication of smmPBj14 in resting PBMC from a normal pig-tailed macaque. The culture was infected immediately after PBMC were separated from heparinized blood, and infected cells were maintained in RPMI 1640 medium containing 10% FBS and IL-2. Note large number of balloonlike cells observed in clumps of proliferating lymphocytes late in infection.

numbers compared with uninfected control cultures; in some experiments, however, as much as a 10-fold difference was observed. Although smmPBj14 replicated in PBMC from humans and, to a lesser extent, in those from chimpanzees, obvious increases in numbers of viable cells, indicative of

proliferation, were not observed with PBMC from either of these species (data not shown).

Replication in resting lymphocytes. Because of the apparent induction of cell proliferation by smmPBj14, we tested whether the virus shared with HIV-1 the requirement that cells be activated or mitogen stimulated before efficient replication occurred. Initially, infections of resting or quiescent lymphocytes were performed with PBMC from pig-tailed macaques in medium containing exogenous recombinant human IL-2. When smmPBj14 repeatedly replicated to high titers under these conditions (Fig. 3), subsequent experiments used 10%-RPMI to which no IL-2 was added. Irrespective of whether resting PBMC were obtained from uninfected pig-tailed macaques or mangabeys, whether they were activated, or whether exogenous IL-2 was in the medium, smmPBj14 replicated efficiently and to high levels. Replication of virus was accompanied not only by increases in cell number but also by cytopathic effects characteristic of T-lymphotropic lentiviruses. In contrast, smm9 either produced no detectable virus or replicated at a much slower rate and to significantly lower levels (see below) under comparable conditions. Although significant differences were not observed in the replication of smmPBj14 in resting PBMC from different simian donors, the observed lack of replication or low-level replication of smm9 in these cells could have resulted from differences in the resting state of individual donor animals.

To determine the efficiency of smmPBj14 replication in resting versus mitogen-stimulated PBMC and in the presence or absence of exogenous IL-2, four parallel cultures of resting PBMC from a pig-tailed macaque were infected with smmPBj14 for 2 h in 10%-RPMI. The cells were then washed and resuspended in 10%-RPMI or IL-2-RPMI alone or 10%-RPMI containing 10 μ g of ConA per ml (two cultures). After 2 days, PBMC from all four cultures were washed and

FIG. 4. Comparative replication of smmPBj14 in PBMC maintained under different growth conditions. (A) Resting PBMC from a normal pig-tailed macaque were infected with smmPBj14 and were placed in 10%-RPMI (▲) or IL-2-RPMI (●) with no mitogen stimulation or in 10%-RPMI (◆) or IL-2-RPMI (■) following ConA stimulation. (B and C) PBMC were either unstimulated (ConA -) or stimulated (ConA +) and then were maintained in RPMI 1640 medium with (+) or without (-) IL-2, as indicated below panel C.

FIG. 5. Induction of expression of IL-2R α during replication of smmPBj14 in resting pig-tailed macaque PBMC. Uninfected (□) and smm9-infected (■) or smmPBj14-infected (▨) cultures were maintained in RPMI 1640 medium containing 10% autologous macaque serum. Periodically, RT activity and percentage of cells expressing IL-2R α in the cultures were determined. Percentages of cells with IL-2R α were determined by FACScan analysis with fluoresceinated antibodies to CD25 (anti-TAC; Becton Dickinson).

resuspended in the same medium as before except that one of the ConA-stimulated cultures was placed in 10%-RPMI and the other one was placed in IL-2-RPMI. Surprisingly, both cultures lacking exogenous IL-2 initially produced more virus than did their companion cultures (Fig. 4A). This was more obvious when total RT activity per viable cell was calculated and normalized to the activity of the culture with the lowest virus output per cell, i.e., the ConA-stimulated culture in IL-2-RPMI medium (Fig. 4C). These results were not related to the donor animal, since similar results were obtained when the experiment was repeated with PBMC from a different pig-tailed macaque. In general, initial production of smmPBj14 was always higher in cultures to which no exogenous IL-2 was added, irrespective of whether PBMC were or were not lectin stimulated.

Cell activation. Although the data presented above indicated that smmPBj14 did not require cell activation for replication, it was possible that the virus itself activated the cells upon binding or entry. This was suggested by the fact that cell numbers increased concomitantly with virus accumulation in culture supernatants. Because resting (G_0) lymphocytes generally have low or undetectable levels of IL-2R on their surface, we determined whether infection of resting lymphocytes with smmPBj14 would result in increased expression of IL-2R α . To minimize stimulation of PBMC, RPMI medium was supplemented with 10% autologous serum from the same animal from which PBMC were obtained. Significant increases in IL-2R α were not detected until several days after substantial amounts of virus had accumulated in the medium (Fig. 5). These data suggested that measurable activation of PBMC did not occur prior to replication of smmPBj14. Since the multiplicity of infection

TABLE 1. Effects of antibodies to IL-2 on replication of smmPBj14^a

Virus	Antibody	RT (cpm)	Viable cells (10 ⁶)
—	None	788	2.5
+	None	437,400	7.8 (3.1)
—	IL-2	941	0.95
+	IL-2	297,500	4.1 (4.3)
+	TNF- α	242,000	7.0

^a Data are for day 16 after infection, the time of maximum RT activity in all cultures. Values in parentheses represent the magnitude of increase in viable cells in smmPBj14-infected versus uninfected parallel cultures. Polyclonal rabbit neutralizing antibodies to human IL-2 and TNF- α were used at concentrations of 12 μ g/ml and 600 U/ml, respectively. Data are from a representative experiment.

was less than 0.001, it is possible that individual cells infected by the virus might have been activated, but at a level insufficient to induce detectable increases in IL-2R α . Given the high levels of smmPBj14 replication and the extended delay in IL-2R α expression, however, this possibility seems unlikely. Furthermore, increases in cell numbers were not observed until increases in IL-2R were detected (data not shown). In the experiment depicted in Fig. 5, smm9 was able to replicate to low levels (maximum RT activity was 9% of that achieved by smmPBj14), which may be due to the fact that approximately 11% of the lymphocytes were expressing IL-2R α (and therefore were activated) at the time the culture was initiated. The limited replication of smm9, however, was not accompanied by increased numbers of IL-2R α ⁺ cells significantly different from that observed in the uninfected culture.

To explore further the possible requirement for IL-2 in replication of smmPBj14, the effect of adding antibodies against IL-2 to the culture media (10%-RPMI) was tested (Table 1). Irrespective of whether antibodies to IL-2 were present in the culture medium, there was no difference in virus production. As a control, rabbit antiserum to TNF- α was used since we had established previously that antibodies to TNF- α had little if any effect on replication of smmPBj14 in simian PBMC. Although the total cell numbers in smmPBj14-infected and uninfected cultures containing antibodies to IL-2 were lower than in those without anti-IL-2, the relative increases in cell number due to replication of the virus were similar (Table 1). This suggested that some cells may have expressed and secreted IL-2, which affected overall cell number, but that IL-2 was not required for the virus-induced activation and proliferation of lymphocytes.

TABLE 2. Proliferative responses of PBMC from a normal pig-tailed macaque to heat-inactivated smmPBj14

PBMC incubated with:	[³ H]thymidine incorporation (cpm) ^a	
	Day 2	Day 3
Medium	1,785	331
ConA	117,646 (66)	54,473 (165)
smmPBj14		
3.5 μ g	7,936 (4.4)	4,937 (14.9)
7.0 μ g	10,832 (6.0)	1,790 (5.4)
HT-PBj14, 7.0 μ g	1,452 (0.8)	542 (1.6)
HIV-1 _{IIIb} , 7.0 μ g	2,627 (1.4)	965 (2.9)

^a Values are mean counts per minute of triplicate wells pulsed for 16 h after incubation for the indicated times. Values in parentheses are the stimulation indices; values greater than 3 are considered positive. Data are from a representative experiment.

TABLE 3. Effect of heat-inactivated smmPBj14 on enriched populations of macaque lymphocytes

Expt	Lymphocyte population	³ H]thymidine incorporation (10 ³ cpm) following addition of ^a :		
		No mitogen	ConA	smmPBj14
1	CD4 (96) ^b	1.8	513 (283)	7.2 (4.0)
	CD8 (98)	0.92	370 (399)	19.9 (21.6)
2	CD4 (72)	7.4	212 (28.7)	9.9 (1.3)
	CD8 (93)	4.6	206 (44.8)	24.6 (5.4)

^a Data are from two representative experiments in which PBMC were obtained from two different pig-tailed macaques. Values are mean counts per minute for six replicate wells to which were added the indicated mitogens; stimulation indices are given in parentheses.

^b Percentage of lymphocytes expressing the indicated antigen is given in parentheses.

Induction of proliferation. To determine whether the virus itself could induce cells to proliferate, resting PBMC from pig-tailed macaques were tested for their ability to incorporate [³H]thymidine during incubation with purified heat-inactivated smmPBj14 (Table 2). Several experiments, in which PBMC were pulsed with [³H]thymidine 2 to 6 days after incubation with virus, showed that maximum incorporation occurred on days 2 and 3. The induction of proliferation appeared specific for smmPBj14, since little or no proliferation was observed following incubation with HIV-1 or, more importantly, HT-PBj14. This virus pool, which was obtained after long-term passage of smmPBj14 in the human HT cell line, does not induce acute disease and death in macaques. When CD4⁺- and CD8⁺-enriched macaque lymphocyte populations were tested for the ability to incorporate [³H]thymidine in response to inactivated smmPBj14, both cell populations responded. It is of particular interest that in all experiments (Table 3 and data not shown), CD8⁺ cells appeared to proliferate to a greater extent than did CD4⁺ cells, for which the responses were more variable.

DISCUSSION

The smmPBj14 isolate was shown previously (6) to be atypical of lentiviruses with respect to its pathogenicity in both mangabey monkeys and pig-tailed macaques. Data presented here and elsewhere (3) indicate that the pathogenicity of this virus for mangabey monkeys may be due in part to its cytopathicity for CD4⁺ lymphocytes from this species, a property not shared by its parent, smm9. In this report, additional unique properties of this T-lymphotropic lentivirus were identified. Unlike smm9 and HIV strains tested to date, smmPBj14 replicated with equal efficiency in resting and in mitogen-stimulated lymphocytes and, more importantly, induced lymphocytes to proliferate. The latter phenomenon was documented both by [³H]thymidine incorporation in quiescent lymphocytes in response to heat-inactivated smmPBj14 and also by an increase in cell numbers in cultures of primary simian PBMC that were actively producing virus. This paradox—cytopathicity for CD4⁺ cells accompanied by increased cell numbers—can be explained in part by the fact that the virus is cytopathic only for CD4⁺ cells but induces both CD4⁺ and CD8⁺ cells to proliferate. Because B cells were not removed from the CD4⁺- and CD8⁺-enriched cell populations prior to the proliferative assays, it is possible that smmPBj14 was also activating B cells. Although direct polyclonal activation of normal B cells by multiple HIV-1 isolates has been reported (27, 32), this is unlikely to have

occurred in the assays used in this study because (i) no proliferative responses were observed with the HIV-1 and HT-PBj14 preparations; (ii) no B-cell growth factors were added to cultures in which increased numbers of cells were observed, a phenomenon seen only in cultures infected with smmPBj14 and not in cultures infected with HIV-1, HIV-2, or other SIV isolates; and (iii) both the proliferative response and increases in cell numbers in smmPBj14-infected cultures occurred only with simian PBMC and was not observed in either assay with human or chimpanzee PBMC (data not shown). The B-cell stimulatory activity of HIV-1 has been localized to the carboxyl terminus of the transmembrane portion of the *env* protein, gp41 (1).

The structural component of smmPBj14 most likely responsible for induction of proliferation is the envelope glycoprotein. However, since CD8⁺ as well as CD4⁺ cells appear to be activated, the binding site for the CD4 receptor molecule probably is not involved. Induction of both CD4⁺ and CD8⁺ resting lymphocytes to proliferate by the type C retrovirus human T-cell lymphotropic virus type I has been described (8), but whether this results from interaction of the virus with receptors known to function in cell activation, e.g., the CD3/T-cell receptor complex, has not been determined. Activation of erythroblast proliferation by binding of another type C virus, Friend spleen focus-forming virus, glycoprotein to the erythropoietin receptor, however, has been demonstrated (19). Results of several studies to assess the ability of either HIV-1 itself, HIV-1 gp120, or synthetic peptides derived from gp120 to activate or induce proliferation of T lymphocytes are contradictory (11, 14, 16, 26). The varying results are difficult to explain and cannot be attributed to strain differences because the HIV_{HTLV-IIIb} isolate was used in all of the above-referenced studies. With the identification of a molecularly cloned virus derived from smmPBj14 that is acutely pathogenic and induces proliferation of lymphocytes in infected cultures (3), it will be possible to obtain purified proteins that can be used to identify that portion of the smmPBj14 virion responsible for eliciting cell proliferation.

The other property of smmPBj14 documented in this report—the ability to replicate efficiently in resting lymphocytes—appears also to be unique among isolates of HIV-1 and SIV tested to date. While HIV-1 has been shown to infect resting lymphocytes, recent studies indicate either that HIV-1 synthesis is initiated but the entire genome is not reverse transcribed (36) or that the genome is completely reverse transcribed but is maintained as extrachromosomal DNA because of an inability to integrate (33). Because of the problems inherent in analyzing events in a single cell, it is difficult to determine whether binding and internalization of smmPBj14 are sufficient to activate a cell such that replication can be efficiently initiated. At the population level, there is no indication that activation and proliferation occur until virus has accumulated in the culture fluid (Fig. 2). Early events in T-cell activation, as defined by interaction of antigen with the CD3/T-cell receptor complex, include activation of protein kinase C via diacylglycerol and increased concentrations of intracellular calcium. Through different pathways, protein kinase C then plays a major role in induction of IL-2 and IL-2R α expression, both of which are produced within hours of stimulation; their production must be sustained in order for proliferation to follow. Because IL-2, and not IL-2R, is more likely to be the limiting factor in the proliferative response, the fact that proliferation was still observed in smmPBj14-infected cultures to which anti-

IL-2 antibodies were added suggests that the proliferative response induced by smmPBj14 is independent of IL-2. There is evidence for IL-2-independent T-cell proliferation (17, 24) which, in some cases, may be mediated by IL-4 (25).

Although the molecular basis for the ability of smmPBj14 to replicate in resting lymphocytes has not been established, nucleotide sequence analyses have identified a distinct change in the enhancer region of the long terminal repeat (LTR) in this virus in comparison with other members of the HIV-2/SIVmac/SIVsmm group (3). It is intriguing to speculate that this change—duplication of the nuclear factor (NF)- κ B binding site—may be important. Relevant to this, it was demonstrated recently that the HIV-1 LTR, which contains two functional NF- κ B binding sites, responds to T-cell activation signals better than does the HIV-2 LTR, which contains only one NF- κ B binding site (34). Preliminary experiments indicate that replication of smmPBj14 is partially inhibited in simian PBMC pretreated with cyclosporin A (5). This drug acts distal to protein kinase C in activation pathways and inhibits the activity of NF-AT and NF- κ B sites in the IL-2 enhancer region and of NF- κ B in the HIV-1 enhancer following activation with PHA (30).

The acute disease and death induced by smmPBj14 may be related to the ability of the virus to replicate to high titers in and to induce proliferation of resting lymphocytes. As mentioned above, in animals that die within 2 weeks, the gut-associated lymphoid tissues and Peyer's patches contain large numbers of both CD4⁺ and CD8⁺ lymphoblasts, consistent with the *in vitro* results presented here. Because active proliferation of lymphocytes in the presence of high titers of virus is likely to be accompanied by expression and secretion of multiple cytokines, it is possible that cytokines also contribute to acute disease and death. In support of this view, high levels of TNF- α (5) and IL-6 (18) were found in plasma of animals dying of acute infection. Both TNF- α and IL-6 have been shown to influence induction of HIV-1 from latently infected cells and to enhance HIV-1 infection and replication in primary human lymphocyte and monocyte/macrophage cultures (29, 35). In addition, IL-6 has been shown to play a role in T-cell activation (12, 15), and serum levels of TNF and IL-6 were shown to correlate directly with stage of HIV infection and disease in humans (13, 23). Identification of the role of cytokines and the requirements for replication of smmPBj14 at the cellular and molecular levels will help to establish whether a link between replicative properties and pathogenicity of T-lymphotropic lentiviruses exists.

ACKNOWLEDGMENTS

I thank Todd Welch and Chris Horaist for excellent technical assistance, Becky Siegel for graphics, and Eric Hunter for critical review of the manuscript.

This work was supported in part by NIH grants AI-27136 (P.N.F.) and RR-00165 to the Yerkes Primate Research Center, Emory University, where much of this work was performed.

REFERENCES

- Chirmule, N., V. S. Kalyanaraman, C. Saxinger, F. Wong-Staal, J. Ghayeb, and S. Pahwa. 1990. Localization of B-cell stimulatory activity of HIV-1 to the carboxyl terminus of gp41. *AIDS Res. Hum. Retroviruses* 6:299–305.
- Coombs, R. W., A. C. Collier, J.-P. Allain, B. Nikora, M. Leuther, G. F. Gjeret, and L. Corey. 1989. Plasma viremia in human immunodeficiency virus infection. *N. Engl. J. Med.* 321:1626–1631.
- Dewhurst, S., J. E. Embretson, D. C. Anderson, J. I. Mullins, and P. N. Fultz. 1990. Sequence analysis and acute pathogenicity of molecularly cloned SIV_{SMM-PBj14}. *Nature (London)* 345:636–640.
- Fauci, A. S. 1988. The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. *Science* 239:617–622.
- Fultz, P. N. Unpublished data.
- Fultz, P. N., H. M. McClure, D. C. Anderson, and W. M. Switzer. 1989. Identification and biologic characterization of an acutely lethal variant of simian immunodeficiency virus from sooty mangabeys (SIV/SMM). *AIDS Res. Hum. Retroviruses* 5:397–409.
- Fultz, P. N., R. B. Stricker, H. M. McClure, D. C. Anderson, W. M. Switzer, and C. Horaist. 1990. Humoral response to SIV/SMM infection in macaque and mangabey monkeys. *J. Acquired Immune Defic. Syndr.* 3:319–329.
- Gazzolo, L., and M. DucDodon. 1987. Direct activation of resting T lymphocytes by human T-lymphotropic virus type 1. *Nature (London)* 326:714–717.
- Gendelman, H. E., O. Narayan, S. Kennedy-Stoskopf, P. G. E. Kennedy, Z. Ghotbi, J. E. Clements, J. Stanley, and G. Pezesk-pour. 1986. Tropism of sheep lentiviruses for monocytes: susceptibility to infection and virus gene expression increases during maturation of monocytes to macrophages. *J. Virol.* 58:67–74.
- Ho, D. D., T. Moudgil, and M. Alam. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N. Engl. J. Med.* 321:1621–1625.
- Hofmann, B., P. Nishanian, R. L. Baldwin, P. Insixiengmay, A. Nel, and J. L. Fahey. 1990. HIV inhibits the early steps of lymphocyte activation, including initiation of inositol phospholipid metabolism. *J. Immunol.* 145:3699–3705.
- Holsti, M. A., and D. H. Raulet. 1989. IL-6 and IL-1 synergize to stimulate IL-2 production and proliferation of peripheral T cells. *J. Immunol.* 143:2514–2519.
- Honda, M., K. Kitamura, Y. Mizutani, M. Oishi, M. Arai, T. Okura, K. Igarahi, K. Yasukawa, T. Hirano, T. Kishimoto, R. Mitsuyasu, J.-C. Chermann, and T. Tokunaga. 1990. Quantitative analysis of serum IL-6 and its correlation with increased levels of serum IL-2R in HIV-induced diseases. *J. Immunol.* 145:4059–4064.
- Horak, I. D., M. Popovic, E. M. Horak, P. J. Lucas, R. E. Gress, C. H. June, and J. B. Bolen. 1990. No T-cell tyrosine protein kinase signalling or calcium mobilization after CD4 association with HIV-1 or HIV-1 gp120. *Nature (London)* 348:557–560.
- Houssiau, F. A., P. G. Coulie, and J. VanSnick. 1990. Distinct roles of IL-1 and IL-6 in human T cell activation. *J. Immunol.* 143:2520–2524.
- Kornfield, H., W. W. Cruikshank, S. W. Pyle, J. S. Berman, and D. M. Center. 1988. Lymphocyte activation by HIV-1 envelope glycoprotein. *Nature (London)* 335:445–448.
- Laing, T. J., and A. Weiss. 1988. Evidence for IL-2 independent proliferation in human T cells. *J. Immunol.* 140:1056–1062.
- Lewis, M. G., D. L. Birx, P. M. Zack, M. A. Vahey, R. R. Redfield, D. S. Burke, and P. B. Jahrling. 1990. Elevated levels of circulating interleukin-6 are associated with infection with an acutely fatal simian immunodeficiency virus isolate (SIVsm/pbj). *abstr.* 43, p. 105. *Abstr. 3rd Annu. Meet. NIH Natl. Cooperative Drug Disc. Groups for Treatment of AIDS.*
- Li, J.-P., A. D. D'Andrea, H. F. Lodish, and D. Baltimore. 1990. Activation of cell growth by binding of Friend spleen focus-forming virus gp55 glycoprotein to the erythropoietin receptor. *Nature (London)* 343:762–764.
- McClure, H. M., D. C. Anderson, P. N. Fultz, A. A. Ansari, E. Lockwood, and A. Brodie. 1989. Spectrum of disease in macaque monkeys chronically infected with SIV/SMM. *Vet. Immunol. Immunopathol.* 21:13–24.
- McDougal, J. S., A. Mawle, S. P. Cort, J. K. A. Nicholson, G. D. Cross, J. A. Scheppler-Campbell, D. Hicks, and J. Sligh. 1985. Cellular tropism of the human retrovirus HTLV-III/LAV. I. Role of T cell activation and expression of the T4 antigen. *J. Immunol.* 135:3151–3161.
- McGrath, M., O. Witte, T. Pincus, and I. L. Weissman. 1978. Retrovirus purification: method that conserves envelope glycoprotein and maximizes infectivity. *J. Virol.* 25:923–927.

23. Mintz, M., R. Rapaport, J. M. Oleske, E. M. Connor, M. R. Koenigsberger, T. Denny, and L. G. Epstein. 1989. Elevated serum levels of tumor necrosis factor are associated with progressive encephalopathy in children with acquired immunodeficiency syndrome. *Am. J. Dis. Child.* **143**:771-774.
24. Moldwin, R. L., D. W. Lancki, K. C. Herold, and F. W. Fitch. 1986. An antigen receptor-driven interleukin 2-independent pathway for proliferation of murine cytolytic T lymphocyte clones. *J. Exp. Med.* **163**:1566-1582.
25. Mosmann, T. R., M. W. Bond, R. L. Coffman, J. Ohara, and W. E. Paul. 1986. T-cell and mast cell lines respond to B-cell stimulatory factor 1. *Proc. Natl. Acad. Sci. USA* **83**:5654-5658.
26. Nair, M. P. N., R. Pottathil, E. P. Heimer, and S. A. Schwartz. 1988. Immunoregulatory activities of human immunodeficiency virus (HIV) proteins: effect of HIV recombinant and synthetic peptides on immunoglobulin synthesis and proliferative responses by normal lymphocytes. *Proc. Natl. Acad. Sci. USA* **85**:6498-6502.
27. Pahwa, S., R. Pahwa, C. Saxinger, R. C. Gallo, and R. A. Good. 1985. Influence of the human T-lymphotropic virus/lymphadenopathy-associated virus on functions of human lymphocytes: evidence for immunosuppressive effects and polyclonal B-cell activation by banded viral preparations. *Proc. Natl. Acad. Sci. USA* **82**:8198-8202.
28. Pauza, C. D., J. Galindo, and D. D. Richman. 1988. Human immunodeficiency virus infection of monoblastoid cells: cellular differentiation determines the pattern of virus replication. *J. Virol.* **62**:3558-3564.
29. Poli, G., P. Bressler, A. Kinter, E. Duh, W. C. Timmer, A. Rabson, J. S. Justement, S. Stanley, and A. S. Fauci. 1990. Interleukin 6 induces human immunodeficiency virus expression in infected monocytic cells alone and in synergy with tumor necrosis factor α by transcriptional and post-transcriptional mechanisms. *J. Exp. Med.* **172**:151-157.
30. Schmidt, A., L. Hennighausen, and U. Siebenlist. 1990. Inducible nuclear factor binding to the κ B elements of the human immunodeficiency virus enhancer in T cells can be blocked by cyclosporin A in a signal-dependent manner. *J. Virol.* **64**:4037-4041.
31. Schnittman, S. M., J. J. Greenhouse, M. C. Psallidopoulos, M. Baseler, N. P. Salzman, A. S. Fauci, and H. C. Lane. 1990. Increasing viral burden in CD4⁺ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapidly progressive immunosuppression and clinical disease. *Ann. Intern. Med.* **113**:438-443.
32. Schnittman, S. M., H. C. Lane, S. E. Higgins, T. Folks, and A. S. Fauci. 1986. Direct polyclonal activation of human B lymphocytes by the acquired immune deficiency syndrome virus. *Science* **233**:1084-1086.
33. Stevenson, M., T. L. Stanwick, M. P. Dempsey, and C. A. Lamonica. 1990. HIV-1 replication is controlled at the level of T cell activation and proviral integration. *EMBO J.* **9**:1551-1560.
34. Tong-Starksen, S. E., T. M. Welsh, and B. M. Peterlin. 1990. Differences in transcriptional enhancers of HIV-1 and HIV-2. Response to T cell activation signals. *J. Immunol.* **145**:4348-4354.
35. Vyakarnam, A., J. McKeating, A. Meager, and P. C. Beverley. 1990. Tumour necrosis factors (α , β) induced by HIV-1 in peripheral blood mononuclear cells potentiate virus replication. *AIDS* **4**:21-27.
36. Zack, J. A., S. J. Arrigo, S. R. Weitsman, A. S. Go, A. Haislip, and I. S. Y. Chen. 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* **61**:213-222.
37. Zack, P. M., W. C. Hall, A. P. Vogel, W. R. Elkins, C. R. Brown, M. G. Lewis, and P. B. Jahrling. 1989. Pathology and immunopathology of SIV_{SMM-PBJ} associated gastroenteritis in macaques, abstr. 85, p. 99. Abstr. Symp. Nonhuman Primate Models for AIDS.
38. Zagury, D., J. Bernard, R. Leonard, R. Cheynier, M. Feldman, P. S. Sarin, and R. C. Gallo. 1986. Long-term cultures of HTLV-III-infected T cells: a model of cytopathology of T-cell depletion in AIDS. *Science* **231**:850-853.
39. Zhang, J., L. N. Martin, E. A. Watson, R. C. Montelaro, M. West, L. Epstein, and M. Murphey-Corb. 1988. Relationship of antibody response and viral antigenemia to SIV/Delta-induced immunodeficiency disease in the rhesus monkey. *J. Infect. Dis.* **158**:1277-1286.