Enhanced Responsiveness to Nuclear Factor κ B Contributes to the Unique Phenotype of Simian Immunodeficiency Virus Variant SIV_{smmPBj14}

SHEILA C. DOLLARD,¹ SURYARAM GUMMULURU,¹ SOLAM TSANG,¹
PATRICIA N. FULTZ,² AND STEPHEN DEWHURST^{1,3*}

Department of Microbiology and Immunology, University of Rochester Medical Center,¹ and Cancer Center, University of Rochester,³ Rochester, New York 14642, and Department of Microbiology, University of Alabama at Birmingham, Birmingham, Alabama 35294²

Received 25 May 1994/Accepted 31 August 1994

Infection with a variant of simian immunodeficiency virus, SIV_{smmPBj14}, leads to severe acute disease in macaques. This study was designed to investigate the functional significance of previously described mutations in the viral long terminal repeat (LTR) and to elucidate their contribution to the unique phenotype of SIV_{smmPBj14}. LTR-directed transcription was measured by using luciferase reporter constructs that were transiently transfected into cultured cells. In a wide range of cell types, the basal transcriptional activity of the LTR from SIV_{smmPBj14} was found to be 2- to 4.5-fold higher than that of an LTR from a non-acutely pathogenic strain. These LTRs differ by five point mutations and a 22-bp duplication in SIV_{smmPBj14}, which includes a nuclear factor κ B (NF κ B) site. Transcriptional differences between these LTRs were further enhanced by two- to threefold upon treatment of cells with phorbol ester or tumor necrosis factor alpha or by cotransfection with plasmids expressing NF κ B subunits. Mutagenesis studies, and the use of a reporter construct containing an enhancerless promoter, indicate that these transcriptional effects are due principally to the 22-bp sequence duplication and the NF κ B site contained within it. Finally, infectious virus stocks that were isogenic except for the LTR were generated. The LTR from SIV_{smmPBj14} was found to confer an increase in the kinetics of virus replication in cultured cells. Inclusion of this LTR in recombinant SIVs also resulted in a two- to threefold rise in the extent of cellular proliferation that was induced in quiescent simian peripheral blood mononuclear cells. These studies are consistent with the hypothesis that LTR mutations assist SIV_{smmPBj14} in responding efficiently to cellular stimulation and allow it to replicate to high titers during the acute phase of viral infection.

Simian immunodeficiency virus (SIV) infection in experimentally inoculated rhesus macaques typically results in the induction of an AIDS-like disease characterized by wasting, diarrhea, thymic atrophy, depletion of CD4⁺ lymphocytes, opportunistic infections, central nervous system degeneration, and death within 5 to 18 months (39, 45). These properties, together with the close genetic similarity between SIV and human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2), make the SIV/macaque model ideal for the study of AIDS pathogenesis.

The disease induced by HIV-1 and SIV is slow and progressive, and it is preceded by an acute viral syndrome that occurs within the first 2 weeks of infection, prior to seroconversion. Acute-phase HIV-1 disease is marked by a peak of antigenemia, viremia, and widespread viral dissemination throughout the lymphoid organs (14, 15). The clinical presentation includes flu-like symptoms, diarrhea, lymphadenopathy, rash, fever, and malaise (11), followed by a long asymptomatic period in which virus replication continues in the lymphoid sites that were presumably seeded during the acute syndrome (21, 51). In the case of SIV, less is known concerning the acute phase of infection; however within the first 2 weeks of infection, SIV-infected macaques develop many of the same symp-

oms as HIV-1-infected humans, including antigenemia, rash, and lymphadenopathy (39, 45, 54).

One strain of SIV, SIV_{smmPBj14}, induces an especially severe acute-phase infection in experimentally inoculated pig-tailed macaques (*Macaca nemestrina*). This syndrome is marked by very severe gastrointestinal symptoms and diarrhea and by death in most cases between 7 and 10 days postinoculation (25, 32, 40). Gut-associated lymphoid areas are heavily involved in the disease pathogenesis, and there is a profound local inflammatory reaction, marked by lymphoid hyperplasia, tissue damage, and high serum levels of certain cytokines, including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) (8, 59). These findings may be related to SIV_{smmPBj14}'s unique ability, among primate lentiviruses, to trigger proliferation of resting simian peripheral blood mononuclear cells (PBMC) in vitro (24).

Biologic virus clones were derived from SIV_{smmPBj14} by limiting-dilution cloning, resulting in the derivation of stocks with both acutely pathogenic (AP) (bcl-3) and non-acutely pathogenic (NAP) (bcl-1) phenotypes (25). SIV sequences from these stocks were subsequently cloned by PCR amplification, sequenced, and tested in vivo (16). An AP molecular clone of SIV_{smmPBj14}, designated PBj-1.9, was derived (16), and sequence analysis identified a total of fewer than 100 mutational changes (99% sequence identity) between this clone and the NAP bcl-1 virus stock (17). Many of these changes map to the viral long terminal repeat (LTR). Most notably, bcl-3 contains a tandem 22-bp duplication that contains a nuclear factor κ B (NF κ B) site (five of five clones), whereas bcl-1 does not (eight of eight clones).

* Corresponding author. Mailing address: Department of Microbiology and Immunology, University of Rochester Medical Center, Rochester, NY 14642. Phone: (716) 275-3216. Fax: (716) 473-9573. Electronic mail address: DWRT@BPHVAX.BIOPHYSICS.ROCHESTER.EDU.

In the case of SIV_{smmPBj14}, it has been shown that several viral determinants, most notably the *env* gene, contribute to acute disease induction (49). However, the functional significance of the previously described LTR mutations in SIV_{smmPBj14} remains uncertain, although several facts suggest that they may be important. First, these LTR changes are highly conserved among acutely pathogenic clones of SIV_{smmPBj14} (12, 16, 17, 49). Second, there is precedent for similar mutations being important determinants of pathogenesis in other retroviruses (23, 31, 37, 41). Third, many of the cytokines induced during HIV-1 or SIV infection, including IL-6 and TNF- α , are known to activate cellular transcription factors, including NF κ B (4, 8, 9, 20, 33, 42, 50, 53, 59). This interplay between cytokines and transcription factors may be particularly pertinent to SIV_{smmPBj14} in light of the duplicated NF κ B site in the LTR enhancer region of this virus (16).

This study, therefore, evaluates the contribution of LTR changes to the unique phenotype of SIV_{smmPBj14}. Two complementary assay systems were used to determine the functional significance of these LTR mutations. First, transient transcriptional studies were performed in a wide variety of cell lines and primary cells, using LTR-luciferase reporter plasmids. These were carried out in the presence or absence of agents known to stimulate NF κ B, including TNF- α . Second, we constructed infectious SIVs in which LTR elements were exchanged between otherwise isogenic viruses. Infection experiments using these viral stocks allowed us to determine the effects of LTR mutations on viral properties, including replication kinetics and the ability of the virus to trigger proliferation of resting PBMC from pig-tailed macaques. The data presented show that sequence mutations in the SIV_{smmPBj14} LTR result in elevated levels of transcription and faster kinetics of viral replication. Furthermore, the data indicate that these differences are due primarily to the presence of an additional NF κ B site.

MATERIALS AND METHODS

Maintenance of cells. Unless otherwise indicated, the cell lines used in this research were obtained from the American Type Culture Collection or the NIH/NIAID AIDS Reagent Repository and were maintained under conditions recommended by the supplier. Human cell lines included SW480 (colon carcinoma), U-87MG (astrocytoma), U-138MG (astrocytoma), J-Jahn (CD4⁺ T cell), Jurkat (CD4⁺ T cell), CEM $\times$ 174 (CD4⁺ B-cell/T-cell hybrid), and U937 (monocytic). Simian cell lines were ChM114 (simian T-cell leukemia virus-transformed chimpanzee T cells) and Si-IIA (human T-cell leukemia virus type II [HTLV-II]-transformed cynomolgus macaque T cells) and were maintained as described previously (46, 62). Primary human PBMC were isolated from human blood by using Ficoll-Paque as instructed by the manufacturer (Pharmacia) and were grown in RPMI 1640 with 10% fetal bovine serum, 5% IL-2, and 1% phytohemagglutinin (PHA) prior to electroporation and without PHA after electroporation.

Plasmid construction. Full-length LTR-luciferase plasmids were generated as follows. PCR amplification was used to generate intact LTR elements from full-length SIV genomic clones PBj-1.9 (AP) and PBj-1.b (NAP). LTR sequences from -560 to +321 were inserted into the luciferase expression vector pXP1 (48) as a *HindIII*-to-*SmaI* cassette and verified by DNA sequencing (57). Truncated LTR-luciferase plasmids were then made by digesting the full-length clones with *HindIII* and *StuI* to remove LTR sequences upstream of the *StuI* site at nucleotide -172 (Fig. 1). A mutated derivative of the trun-

cated NAP LTR-luciferase construct was also generated, in which a second copy of the previously identified 22-bp LTR enhancer element in SIV_{smmPBj14} was inserted by PCR mutagenesis (30) using *Pfu* DNA polymerase (Stratagene); this clone is designated NAP-M. The same 22-bp element was also synthesized as an oligonucleotide, containing one or two copies of either the wild-type or the NF κ B mutant sequence (see Fig. 4A), and cloned into the *SmaI* and *XhoI* sites of pSluc2, which contains the luciferase gene and the simian virus 40 (SV40) enhancerless promoter (48). Clones pWR1 and pWR2 contain one and two copies, respectively, of the wild-type 22-bp element. In clones pMR1 and pMR2, the GGG motif in the NF κ B site was altered to the nonfunctional triplet motif, CTC (47). These clones thus contain one and two copies, respectively, of an NF κ B mutant and a wild-type simian factor 3 (SF3) sequence (shown in Fig. 4A). For Tat expression vectors, the SIV insert in clone pST (64) was replaced with *tat* coding sequences (bases 6015 to 8699) from either clone PBj-3'-9 or clone PBj-3'-b (16, 17) to generate clones capable of expressing AP- or NAP-Tat protein, respectively.

Transient expression assays. SW480, U-87MG, and U-138MG cells were transfected by the low-CO₂ method of Chen and Okayama (13) in 60-mm-diameter plates, using 3 to 6 μ g of total DNA per plate. J-Jahn, Jurkat, U937, and Si-IIA cell lines were electroporated in serum-free RPMI 1640 medium with 10 mM dextrose and 0.1 mM dithiothreitol (5) at a concentration of 2×10^7 cells per ml, using 15 μ g of total DNA in 0.4-cm gap cuvettes at 960 μ F and 300 V. ChM114 cells were transfected by the DEAE-dextran method, using 10^7 cells and 6 μ g of total DNA (1). PBMC were electroporated in cytomix buffer under the conditions used for J-Jahn cells (63). Where noted, 1,3-phorbol myristate acetate (PMA; Sigma) was added to a final concentration of 20 ng/ml at 14 h posttransfection, and cells were harvested 6 h thereafter. TNF- α (Genzyme) was added to a final concentration of 10 ng/ml at 14 h after transfection, and cells were harvested 24 h later. In experiments in which NF κ B expression vectors were used, cells were transfected with 8 μ g of LTR-luciferase plasmid plus 4 μ g of each NF κ B plasmid or 8 μ g of pUC19 (negative control) and harvested for luciferase assays 42 h after transfection. In experiments in which Tat expression vectors were used, cells were transfected with 10 μ g of LTR-luciferase plasmid plus 10 μ g of SIV Tat plasmid or 10 μ g of pUC19 (negative control) and harvested for luciferase assays 36 h after transfection.

All transfections were performed in duplicate. Cell lysates were assayed by using commercially available reagents (Promega) in an LKB series 1250 luminometer. Values presented are the averages of three experiments with similar results, unless otherwise indicated (see figure legends); variability between experimental duplicates was typically <15%.

Protein-DNA binding analysis. Nuclear extracts were prepared by the method of Lee and colleagues (35). Briefly, 3×10^7 to 5×10^7 cells were harvested, allowed to swell on ice, and then lysed rapidly by passage through a hypodermic needle. The nuclear component was lysed, dialyzed against low-salt buffer, snap frozen, and stored at -80°C. The final protein concentration of the extracts was generally 5 to 10 mg/ml. Binding activity was detected by the electrophoretic mobility shift assay (EMSA) (22, 26), in which 10 μ g of nuclear extract was mixed with 10,000 cpm of ³²P-end-labeled DNA, with or without competitor DNA, at 30°C for 20 min and electrophoresed through a 4% Tris-borate gel.

Full-length viral recombinants. LTR recombinant virus stocks were generated by using 5' and 3' SIV_{smmPBj14} half-genome clones as follows. For each recombinant constructed, the 5' and 3' LTR elements were identical, thereby precluding

FIG. 1. Schematic representation of the $SIV_{smmPBj14}$ LTR. (A) The positions of known or putative transcription factor binding sites and of the transactivation responsive element (TAR) are marked. Motifs indicated include putative purine box elements (PuB1 and PuB2; consensus motif, AGGAA), NF κ B, SF3, and Sp1 binding sites. The position at which LTR-luciferase reporter constructs were truncated is also marked (-172). (B) Comparison of the DNA sequences from the enhancer regions from the two SIV LTRs used in our studies. PBj9 corresponds to the AP LTR, while PBjB is the NAP LTR. Dots represent sequence identity, while dashes denote gaps introduced to align the sequences. Transcription factor binding sites are boxed. Note that the truncated NAP and AP LTRs (-172 to +321) differ at one additional residue not shown here; this is located at position +83, in the TAR site.

the possibility of recombinational mutagenesis upon later transfection into human cells. The full-length viruses shown in Fig. 7 were prepared as follows. First, NAP LTR elements (-172 through +102; *StuI-Pf*MI cassette) were substituted for the corresponding AP LTR sequences present in clone PBj-5'-1 (16). Second, analogous LTR recombinant versions of the 3' genome half-clones PBj-3'-9 and PBj-3'-b were constructed. The LTR sequences exchanged in this fashion include the same five point mutations and the 22-bp duplication that were included in the truncated LTR-luciferase reporter constructs used in our transient transfection analyses. For generation of infectious SIV stocks, a *Bcl*II restriction site was used to linearize the desired 5' and 3' SIV genome halves, which were then ligated to one another and transfected directly into U-87MG cells by the method of Lang and colleagues (34). After a single round of viral replication in U-87MG cells, 5×10^6 CEM $\times$ 174 cells were added for cocultivation, and virus stocks were prepared. Virus-containing supernatants were titrated by serial dilution in CEM $\times$ 174 cells, and the 50% tissue culture infectious dose per milliliter was calculated by the method of Spearman and Kärber (19).

Replication studies. Equivalent amounts of viral stocks (50% tissue culture infectious dose units) were used to initiate infection of 5×10^6 CEM $\times$ 174 and C8166 cells (multiplicity of infection [MOI] of 10^{-4}). Following initial infection, kinetics of viral replication were monitored by removing cell-free supernatants at 3- to 4-day intervals. Reverse transcriptase activity in these supernatants was determined by previously described procedures (27).

Proliferation studies. Pig-tailed macaque PBMC were obtained by layering heparinized blood from SIV-seronegative

animals over Ficoll-sodium diatrizoate density gradients (Pharmacia). Cells were then resuspended in RPMI 1640 medium supplemented with 10% human AB serum (R/Hs medium) and used immediately for proliferation studies. Briefly, viral supernatants (MOI of 0.003), mitogen (1% PHA plus 5% IL-2), or medium alone was added to 10^5 PBMC in R/Hs medium in triplicate, and cells were incubated for 6 days at 37°C in 5% CO_2 . On day 6, each well was pulsed for 16 h with 1 μ Ci of [3 H]thymidine, and the radioactivity incorporated into cellular DNA was quantitated.

RESULTS

Elevated basal transcriptional activity of the AP LTR from $SIV_{smmPBj14}$. We constructed LTR-luciferase reporter plasmids in which either intact (-560 to +321) or truncated (-172 to +321) SIV LTR sequences from AP or NAP viral clones were inserted upstream of a luciferase reporter gene (Fig. 1). These constructs were then transfected transiently into a variety of cell lines, and luciferase activity was determined. Initial experiments confirmed reports that upstream SIV LTR elements are dispensable for near normal levels of basal, mitogen-enhanced, or Tat-transactivated LTR transcription (2, 3, 55) (data not shown). Therefore, all subsequent analyses were performed with the truncated LTR reporter constructs. The AP and NAP LTR sequences contained in these reporter constructs (-172 to +321) differ by only five point mutations and a 22-bp duplication (Fig. 1B). In addition, a third LTR clone, designated NAP-M, was constructed and tested. This clone is identical to the NAP LTR except that it contains the 22-bp duplication from the AP LTR.

FIG. 2. Basal LTR activity. The indicated cell lines and primary human PBMC were transiently transfected with AP and NAP luciferase plasmids. The values of all NAP LTR transfections were normalized to 1, so as to account for variations in transfection efficiency between different cells. The fold increase in luciferase activity of the AP LTR compared with the NAP LTR in each cell type is noted. Values presented are the averages of two or three experiments performed in duplicate with similar results. The standard error of mean values are marked by bars for experiments that were performed three times; error bars are not shown for experiments performed only twice (ChM114, Si-IIA, and U937).

Basal LTR-directed luciferase activity was measured in cell lines of T-cell, non-T-cell, human, and simian lineage, as well as in primary human PBMC. The LTR from the AP strain was consistently more active than the LTR from the NAP strain (Fig. 2), while the NAP-M construct had basal activity that was only very slightly less than that of the AP LTR (Fig. 3). The difference in transcriptional activity ranged from 2- to 4.5-fold, depending on the cell type, but there was no evidence of either species or tissue specificity.

Effect of cytokine and phorbol ester on transcription. To assess whether LTR mutations result in different levels of responsiveness to cellular stimulation, LTR-luciferase constructs were transiently transfected into Jurkat T cells, which were then treated with PMA or TNF- α prior to determination of luciferase activity. PMA and TNF- α both activate NF κ B (20, 42, 47, 50); however, PMA is a less specific activator of NF κ B since it also regulates a variety of other cellular transcription factors, including AP-1 and the *c-ets* gene products (28, 36, 65, 66).

In these experiments, differences in basal transcription between the LTRs were considerably enhanced under conditions of cellular stimulation. The SIV LTRs were stimulated 2.2- to 7.8-fold over basal levels by PMA and 1.9- to 3.6-fold over basal levels by TNF- α (Fig. 3). This level of stimulation is slightly less than that observed when an HIV-1 LTR reporter construct was used (this clone was derived by subcloning LTR sequences from pNL432 into pXP1). The HIV-1 LTR was stimulated 10.6-fold or 5.5-fold over basal levels when cells were treated with PMA or TNF- α , respectively (data not shown). In all cases, however, transcription from the AP SIV LTR was preferentially activated by cellular stimulation, by approximately two- to threefold relative to the NAP LTR (Fig. 3). Furthermore, the NAP-M LTR exhibited transcriptional properties quite similar to those of the AP LTR under conditions of cellular stimulation and in fact exhibited an enhanced responsiveness to PMA treatment relative to the AP LTR (Fig. 3). These findings suggest strongly that the 22-bp sequence duplication confers both increased basal transcrip-

FIG. 3. LTR response to cellular stimulation and NF κ B. Jurkat cells were transfected with the AP, NAP, or NAP-M SIV LTR-luciferase reporter plasmid (the NAP-M clone is identical to the NAP LTR except for the inclusion of the 22-bp enhancer sequence duplication from the AP LTR). Cells were then treated with PMA, TNF- α , or nothing prior to enzyme assays as described in Materials and Methods. In addition, some cells were cotransfected with expression vectors for the Rel family proteins p50 and p65 (RelA), which together comprise NF κ B (p50-p65). Numbers shown correspond to either the fold increase in luciferase activity relative to the unstimulated NAP LTR (Basal) or to the fold stimulation over the uninduced level of luciferase activity for each of the LTRs tested (PMA, TNF α , and p50/p65). Values shown are the averages of three experiments performed in duplicate with similar results; the standard error of mean values are marked by bars.

tional activity and an enhanced ability to respond to cellular stimuli.

The data obtained with PMA and TNF- α were confirmed by additional studies using expression plasmids encoding the Rel family proteins p50 and p65 (RelA), which together comprise NF κ B (p50-p65 heterodimer [58]). When p50 and p65 expression vectors were cotransfected into Jurkat cells along with the LTR-luciferase reporter constructs, there was a 6.2- to 22-fold induction of LTR activity for the SIV LTRs and a 16.7-fold induction of the HIV-1 LTR activity. Once again, the AP SIV LTR was preferentially activated, by approximately threefold relative to the NAP LTR (Fig. 3). The NAP-M LTR behaved similarly to the AP LTR, although it was activated somewhat less efficiently by the cloned NF κ B subunits p50 and p65 (Fig. 3).

Increased binding to the NF κ B site after treatment with phorbol ester. The transient transfection analyses demonstrated that PMA and TNF- α treatment led to elevated transcription from both the AP and NAP SIV LTRs (Fig. 3). EMSAs were performed to confirm that these increases in transcription were accompanied by a rise in nuclear levels of NF κ B. Parallel cultures of Jurkat T cells were harvested after treatment with either PMA, TNF- α , or nothing. EMSAs were then performed on nuclear extracts by using a radiolabeled oligonucleotide representing the 22-bp duplication found in the SIV_{smmPBj14} LTR (Fig. 4A). This 22-bp DNA fragment contains an NF κ B binding site as well as an overlapping site for the transcription factor SF3 (67). To differentiate between these factors, we designed a second oligonucleotide, which contains a mutated NF κ B site and a wild-type SF3 site (Fig. 4A). The wild-type and NF κ B mutant oligonucleotides were then used as competitors in gel shift experiments.

FIG. 4. EMSA. (A) Double-stranded oligonucleotides corresponding to the 22-bp enhancer sequence that is in tandem duplication in *SIV_{smmPBj14}* were synthesized. Wild-type DNA was end labeled, and unlabeled wild-type or mutant DNAs were used for competition experiments; the latter has been shown not to bind NFκB (47). Underlined sequences denote the binding site for NFκB, brackets mark the SF3 binding site (67), and mutated bases are shown in outline form. (B) EMSA reaction mixtures contained 10 μg of protein from nuclear extracts prepared simultaneously from PMA- or TNF-α-stimulated or unstimulated Jurkat cultures, 10,000 cpm of radiolabeled DNA, and, in some cases, unlabeled competitor DNA. Arrows indicate NFκB and (putative) SF3 complexes; N.S., a nonspecific DNA-protein complex that was not competed for by unlabeled DNA. Lane 1 contains no nuclear extract; lanes 2, 5, and 8 have no competitor; lanes 3, 6, and 9 have a 25-fold molar excess of wild-type (W) competitor; lanes 4, 7, and 10 have a 25-fold molar excess of mutant (M) competitor.

In PMA- and TNF-α-stimulated cultures, two shifted bands were observed (Fig. 4B). The lower band had the same mobility as a band that was detected in unstimulated cultures and was competed for by both the wild-type and NFκB mutant oligonucleotides. The upper band was detected only in nuclear extracts from stimulated cells and was competed for with wild-type DNA but not with NFκB mutant DNA. Thus, it seems likely that the lower band corresponds to the constitutive transcription factor SF3, while the upper band represents NFκB binding activity (Fig. 4B). It is intriguing that the putative SF3 binding activity appears to be induced in Jurkat cells by TNF-α treatment but not by PMA. EMSA analysis using nuclear extracts from SW480 and J-Jahn cells suggest that the basal levels of this factor may also vary between different cells (data not shown).

Transactivation by viral Tat. Tat-coding sequences from both AP and NAP clones of *SIV_{smmPBj14}* were cloned into a eukaryotic expression vector to examine whether there is a difference in the extent of transactivation mediated by the two SIV Tat proteins. A second goal of these experiments was to determine whether there was any difference in the responses of the AP and NAP LTRs to Tat-mediated transactivation, as was described for the LTRs from *SIV_{mac142}* and *SIV_{mac239}* by Anderson and Clements (3).

LTR-luciferase reporter plasmids were transfected into Jur-

FIG. 5. LTR transactivation by SIV Tat. Human T cells (Jurkat) were transiently transfected with the AP and NAP LTR luciferase plasmids and either the AP or NAP Tat expression vector or the vector with no insert (pUC19). The luciferase activity values for the NAP LTR were normalized to 1 in all experiments; other datum points are expressed as multiples of this. Numbers shown correspond to the fold stimulation over the uninduced level of luciferase activity for each of the LTR-Tat combinations tested. Values are the averages of three experiments performed in duplicate with similar results; the standard error of mean values are marked by bars.

kat cells together with one of the Tat expression plasmids or with the pUC19 vector. The two SIV Tat protein expression vectors conferred essentially indistinguishable levels of transactivation on the SIV LTR reporter constructs, and the two LTRs were equally Tat responsive, despite the presence of a point mutation at residue +83 in the AP LTR (Fig. 5).

Enhancer activity of the 22-bp LTR repeat. To determine whether the duplicated LTR sequences in *SIV_{smmPBj14}* have intrinsic enhancer activity, oligonucleotides similar to those used in EMSA experiments (Fig. 4A) were cloned into the pSluc2 luciferase reporter plasmid (48). This plasmid contains an enhancerless SV40 promoter which has multiple Sp1 binding sites, like the SIV LTR. Both one and two copies of the 22-bp LTR repeat from *SIV_{smmPBj14}* were cloned upstream of the SV40 promoter in both wild-type and NFκB mutant versions. The constructs were then transfected into SW480 cells, and luciferase assays were performed.

A single copy of the 22-bp LTR sequence, either wild type or NFκB mutant, had transcriptional activity approximately fivefold higher than that of the vector alone (Fig. 6). Presumably, the modest enhancer activity from these single-copy clones reflects SF3-mediated transcriptional enhancement, since the SF3 binding motif is intact in both the wild-type and NFκB mutant oligonucleotides. When two copies of the 22-bp *SIV_{smmPBj14}* LTR motif were placed upstream of the SV40 promoter, an increase in the level of transcriptional enhancement was detected (approximately fivefold higher than for the single-copy clone; compare pWR1 and pWR2 in Fig. 6). This additional increase in transcriptional activity was completely abolished, however, when the NFκB binding site was mutated (compare pMR1 and pMR2 in Fig. 6).

Replication of LTR recombinant SIVs in continuous cell lines. We constructed two pairs of SIVs that were isogenic except for their LTR elements (-172 through +102). These virus pairs were designed to allow us to test the effects of LTR mutations in the context of *env* (and accessory) genes from both AP and NAP derivatives of *SIV_{smmPBj14}* (Fig. 7). The LTR sequences that were exchanged in these SIV recombinants were carefully chosen so as to include all of the differences between the NAP and AP LTRs in the U5, R, and promoter-proximal half of U3 (there are no differences between these LTRs in the region +102 through +321). Thus, the full-length SIVs were designed to allow comparison of the

FIG. 6. Transcriptional activity of the 22-bp enhancer element in SW480 cells. Clones pWR1 and pWR2 contain one and two copies, respectively, of the 22-bp LTR element from SIV_{smmPBj14}, cloned upstream of the SV40 enhancerless promoter and luciferase reporter gene in pSluc2. Clones pMR1 and pMR2 contain one and two copies, respectively, of a derivative of this sequence in which the NF κ B site(s) has been mutated so as to abolish its function (GGG $\rightarrow$ CTC), while the adjacent SF3 site(s) has been left intact (see Fig. 4). Numbers shown correspond to the fold stimulation over the level of luciferase activity for the vector alone. Values are the averages of three experiments performed in duplicate with similar results; the standard error of mean values are marked by bars.

biologic effects of the same LTR mutations that were previously compared in our truncated LTR-luciferase reporter constructs (these contain LTR sequences from -172 through +321).

Infectious SIVs were generated by transfection of ligated SIV genome halves into U-87MG cells, followed by cocultivation with CEM $\times$ 174 cells to generate virus stocks. SIV stocks were then used to infect the SIV-susceptible lymphoid cell lines CEM $\times$ 174 (a B-cell/T-cell hybrid) and C8166 (an HTLV-I-transformed T cell) at an MOI of 10^{-4} . In CEM $\times$ 174 cells (Fig. 8A), all four recombinant SIVs replicated to similar levels at later time points (days 18 through 25). However, at early time points (day 10), there was a significant increase in replication by the two SIVs which contained the AP LTR elements (DEW-1 and DEW-3) compared with their otherwise isogenic counterparts (DEW-2 and DEW-4, respectively). Similar results were obtained for C8166 cells (Fig. 8B). In these cells, however, 3' genome half sequences from NAP virus clones conferred a significant growth advantage (compare clones DEW-3 and DEW-4 with clones DEW-1 and DEW-2). This difference presumably reflects an alteration in virus cell tropism, mostly likely due to differences in the *env* gene.

Ability of LTR recombinant SIVs to trigger proliferation in resting cells. The ability of SIV_{smmPBj14}, and molecular clones derived from it, to induce proliferation in resting PBMC from

pig-tailed macaques is unique among SIVs and is a highly predictive correlate of acute pathogenicity in vivo (24, 49). Infection of resting pig-tailed macaque PBMC with the recombinant SIVs was therefore performed, and cellular proliferation was measured at 6 days after infection by the method of Novembre et al. (49). Two of the four recombinant SIVs tested, DEW-1 and DEW-2, induced cellular proliferation (Fig. 9). These contain both *gag* and *env* elements from AP clones of SIV_{smmPBj14}, whereas clones DEW-3 and DEW-4 lack the AP *env* elements previously shown to be necessary for the induction of cellular proliferation (49). Interestingly, we also observed that DEW-1 induced a level of cellular proliferation two- to threefold higher than that produced by the DEW-2 virus. This finding indicates that AP LTR elements can significantly enhance an important phenotypic property of SIV_{smmPBj14}.

DISCUSSION

We previously reported isolation and sequence analysis of a variant of SIV referred to as SIV_{smmPBj14} that is unlike any other primate lentiviruses in causing acute disease and death 1 to 2 weeks postinfection (25). One of the most striking genetic differences between SIV_{smmPBj14} and other viruses belonging to the SIV_{smm}/HIV-2 group is in the LTR enhancer element; SIV_{smmPBj14} has a tandem sequence duplication that contains a binding site for transcription factor NF κ B (12, 16, 17, 49). Studies of other molecular clones of SIV_{smmPBj14} have determined that the viral determinants of acute disease induction map to multiple genetic loci, including the *env* and *gag* genes (49). These findings are analogous to those for the feline leukemia virus model for AIDS, in which viral determinants of pathogenicity were also mapped to several gene segments, including the *env* gene and the LTR (18). Because SIV_{smmPBj14} has unique replicative properties, it was important to define precisely the effects that the two NF κ B sites had on the functional activity of this virus's LTR.

We show here that the basal transcriptional activity of an LTR derived from an AP molecular clone of SIV_{smmPBj14} is higher than that of a prototype SIV_{smm} LTR, derived from an NAP virus stock. These LTRs differ by only five point mutations, four of which map to the LTR enhancer, as well as a 22-bp sequence duplication that contains an NF κ B binding site. Basal transcription from the AP SIV LTR was consistently 2- to 4.5-fold higher than that from the NAP LTR, regardless of cell type or species of origin. This difference was found in primary cells (human PBMC) as well as in cell lines. Furthermore, the increased level of basal transcription was shown to be due in large part to the 22-bp enhancer sequence duplication, since addition of this element to the NAP LTR resulted

FIG. 7. LTR recombinant SIVs. Full-length recombinant SIVs with the indicated genomic composition were derived as described in Materials and Methods.

FIG. 8. Replication kinetics of recombinant SIVs. Human lymphoid cell lines (CEM $\times$ 174 and C8166) were infected with recombinant SIVs at an MOI of 10^{-4} , and reverse transcriptase assays were performed on cell-free culture supernatants at timed intervals thereafter. The filled symbols represent viruses with AP LTR elements (DEW-1 [■] and DEW-3 [▲]), while the corresponding open symbols represent isogenic viruses with NAP LTRs (DEW-2 [□] and DEW-4 [△]). Dark lines are used to further distinguish the DEW-1/DEW-2 virus pair from the DEW-3/DEW-4 virus pair (lighter lines). The open circles represent negative control cultures (no virus added). Data shown are mean reverse transcriptase data for three (CEM $\times$ 174) or two (C8166) experiments with similar results.

in a clone (NAP-M) with basal transcriptional activity that was only slightly less than that of the AP LTR.

Experiments designed to test the effect of cellular stimulation on SIV LTR-directed transcription showed that when cells were treated with PMA or TNF- α , or when they were cotransfected with p50/p65 NF κ B subunit expression vectors, there was a significant rise in LTR transcription relative to basal levels. The AP LTR was induced to a greater (two- to threefold) extent than the NAP LTR by all of these treatments. We determined whether the duplicated enhancer element in the AP LTR from SIV_{smmPBj14} was required for responsiveness to cellular stimulation by using the NAP-M LTR clone. This clone was induced to a somewhat higher level by PMA than either of the parental LTRs, while its responsiveness to the cloned NF κ B subunits p50 and p65 was slightly reduced relative to the AP LTR. The minor difference in PMA responsiveness may reflect the binding of PMA-induced factors, such as the Elf-1 protein (36), to the putative PuB2 element (AGGAA consensus motif) in the NAP LTR, since this element is absent in the AP LTR (Fig. 1). The fact that the AP and NAP-M LTRs responded almost equally to TNF- α , but not to the cloned NF κ B subunits p65 and p50, was somewhat unexpected. Presumably, this reflects (i) an interaction between NF κ B subunits p65 and p50 and an unknown cellular factor which binds preferentially to either the NAP or AP LTR and (ii) the fact that TNF- α activates several NF κ B proteins in addition to p65 and p50 (6).

We also performed parallel studies in which the transcriptional properties of the duplicated 22-bp LTR sequence from the AP LTR from SIV_{smmPBj14} were analyzed. These included experiments designed to differentiate the relative contributions of the two overlapping transcription factor binding sites (SF3 and NF κ B) contained within the 22-bp DNA fragment. Two copies of the 22-bp LTR element had significantly (fivefold) elevated transcription-enhancing activity, when placed upstream of an enhancerless promoter, relative to a single copy of the same element. This level of enhancement is very similar to the difference in basal transcriptional activity from the intact AP and NAP SIV LTRs. These studies also showed that two

copies of the NF κ B binding site are absolutely required for enhancement of transcription, since enhancement was abolished upon mutation of the NF κ B binding sites. The more-than-additive transcriptional effect of the 22-bp enhancer duplication may result from one of several mechanisms. These include synergistic interactions between bound NF κ B proteins and other components of a single transcription complex, including other transcription factors such as SF3 (67) or Sp1 (52, 61), which is present both in the SV40 promoter used in these experiments and in the native SIV LTR. Alternatively, there may be kinetic synergism between NF κ B complexes bound to the two tandem NF κ B sites (29) or cooperative DNA binding by some NF κ B proteins (52).

It is also interesting that binding of the constitutive transcription factor SF3 alone can provide a considerable level of transcriptional enhancement in T cells (fivefold greater than

FIG. 9. Induction of cellular proliferation by recombinant SIVs. Quiescent pig-tailed macaque PBMC were cultured in RPMI 1640 medium without PHA or IL-2, and human AB serum was substituted for fetal bovine serum. Cells were then infected with recombinant SIVs, and cellular proliferation was quantitated as described in Materials and Methods. Values are the averages of three experiments with similar results in which PBMC from two different macaques were used; the standard error of mean values are marked by bars.

background levels). This finding might help to explain why derivatives of SIV_{mac239} that contained a mutated NF κ B LTR but an intact SF3 site replicated to normal levels in T cells (7). Overall, the transcriptional data presented here suggest that NF κ B plays a significant role in both basal and cytokine/mitogen-inducible transcription from the SIV_{smmPBj14} LTR.

Transient transfection analyses do not, however, always reveal the effects of LTR mutations when they are introduced into intact clones of HIV-1 or SIV (7, 38). Two pairs of recombinant SIVs, which were isogenic except for LTR elements from -172 to +102, were constructed. These permitted a comparison of the phenotypic properties conferred on intact SIVs by the same five LTR point mutations and 22-bp duplication that were contained in our LTR-luciferase reporter constructs. Molecular clones DEW-1 and DEW-3 contain AP LTR sequences, whereas clones DEW-2 and DEW-4 contain NAP LTRs. These LTRs were paired with different 3' genome halves (i.e., *env* and accessory genes) in DEW-1 and DEW-2 versus DEW-3 and DEW-4 to determine whether the biologic properties conferred by LTR elements were influenced by other SIV genetic determinants.

Replication studies using these recombinant SIVs revealed that LTR sequences do in fact influence viral replication in continuous cell lines. Inclusion of AP LTR sequences in otherwise isogenic SIVs consistently resulted in faster kinetics of virus replication in continuous cell lines, although final levels of virus production were not affected. We also determined the ability of each of these viruses to trigger proliferation of resting simian PBMC in vitro. This property has consistently proved to be the most reliable in vitro correlate of acute disease induction in vivo (24, 49), and viral genetic determinants in both *gag* and *env* are required for induction of proliferation (49). Our studies confirmed that 3' genome half sequences (presumably *env*) are indeed crucial for the induction of cellular proliferation by SIV_{smmPBj14}. Furthermore, our data showed that SIVs containing AP LTR elements were significantly (two- to threefold) more efficient inducers of cellular proliferation compared with viruses containing NAP LTR elements. These findings demonstrate that LTR elements can strongly influence a crucial biologic property of SIV_{smmPBj14}, possibly as a result of increasing the kinetics of virus replication during the initial phase of infection.

On the basis of these data, we propose that the viral determinants of pathogenesis for individual strains of SIV and HIV-1 may be multifactorial and reflect coevolution at multiple genetic loci. Thus, LTR mutations in SIV_{smmPBj14} may reflect an adaptation that allows the virus to more efficiently exploit cellular responses to the elevated cytokine levels that occur during acute immunodeficiency virus infection. A better understanding of the transcriptional regulation of SIV and HIV-1 may provide important insights into possible strategies to control viral infection and disease. For example, antioxidants, antitranscriptional drugs, and antagonists of NF κ B activation might prove effective in limiting the initial spread of HIV-1 as well as the reactivation of latent proviruses (10, 43, 56, 60).

ACKNOWLEDGMENTS

We are grateful to David Markovitz and Frank Novembre for helpful discussions, to Alanna Ruddell for critical review of the manuscript, to Daria Krenitsky for excellent technical assistance, to Richard Raubertas for advice on statistics, and to Becky Schwiebert for obtaining blood samples from macaques, which were housed at the University of Alabama at Birmingham according to Animal Welfare Act guidelines. We also thank the following for generous gifts of plasmids or cells: Gary Nabel (NF κ B p50 and p65 expression vectors),

Peter Nara (ChM114 cells), Arnold Rabson (SW480 cells), Neil Perkins (Jurkat cells), and Kanji Miyamoto (Si-HIA cells).

This work was supported by NIH grants to S.D. (RO1 AI31342 and KO4 AI01240) and to S.C.D. (F32 AI08894) and by a Howard Hughes Memorial Institute summer research fellowship to S.T.

REFERENCES

1. Aldovini, A., and M. B. Feinberg. 1990. Transfection of molecularly cloned HIV genomes, p. 147-175. In A. Aldovini and B. D. Walker (ed.), Stockton Press, New York.
2. Anderson, M. G., and J. E. Clements. 1991. Comparison of the transcriptional activity of the long terminal repeats of simian immunodeficiency viruses SIV_{mac251} and SIV_{mac239} in T-cell lines and macrophage cell lines. *J. Virol.* 65:51-60.
3. Anderson, M. G., and J. E. Clements. 1992. Two strains of SIV_{mac} show differential transactivation mediated by sequences in the promoter. *Virology* 191:559-568.
4. Aukrust, P., N.-B. Liabakk, F. Muller, E. Lien, T. Espevik, and S. S. Froland. 1994. Serum levels of tumor necrosis factor- α (TNF- α) and soluble TNF receptors in human immunodeficiency virus type 1 infection—correlations to clinical, immunologic, and virologic parameters. *J. Infect. Dis.* 169:420-424.
5. Barry, P., E. Pratt-Lowe, and P. A. Luciw. 1989. Electroporation of T-cell and macrophage cell lines. Bio-Rad bulletin 1349. Bio-Rad Laboratories, Richmond, Calif.
6. Beg, A. A., and A. S. Baldwin, Jr. 1994. Activation of multiple NF- κ B/Rel DNA-binding complexes by tumor necrosis factor. *Oncogene* 9:1487-1492.
7. Bellas, R. E., N. Hopkins, and Y. Li. 1993. The NF- κ B binding site is necessary for efficient replication of simian immunodeficiency virus of macaques in primary macrophages but not in T cells in vitro. *J. Virol.* 67:2908-2913.
8. Birx, D., M. G. Lewis, M. Vahey, K. Tencer, P. Zack, C. R. Brown, P. B. Jahrling, G. Tosato, D. Burke, and R. Redfield. 1993. Association of interleukin-6 in the pathogenesis of acutely fatal SIV_{smmPBj14} in pigtailed macaques. *AIDS Res. Hum. Retroviruses* 9:1123-1129.
9. Birx, D., R. R. Redfield, K. Tencer, A. Fowler, D. S. Burke, and G. Tosato. 1990. Abnormally elevated interleukin 6 levels in individuals with HIV infection. *Blood* 76:2303-2310.
10. Biswas, D. K., C. M. Ahlers, B. J. Dezube, and A. B. Pardee. 1993. Cooperative inhibition of NF- κ B and Tat-induced superactivation of human immunodeficiency virus type 1 long terminal repeat. *Proc. Natl. Acad. Sci. USA* 90:11044-11048.
11. Cooper, D. A., P. Maclean, R. Finlayson, H. M. Michelmores, J. Gold, B. Donovan, T. G. Barnes, P. Brooke, and R. Penny. 1985. Acute AIDS retrovirus infection. Definition of a clinical illness associated with seroconversion. *Lancet* i:537-545.
12. Courgnaud, V., F. Laure, P. N. Fultz, L. Montagnier, C. Brechot, and P. Sonigo. 1992. Genetic differences accounting for evolution and pathogenicity of simian immunodeficiency virus from a sooty mangabey monkey after cross-species transmission to a pig-tailed macaque. *J. Virol.* 66:414-419.
13. Chen, C., and H. Okayama. 1987. High-efficiency transformation of mammalian cells by plasmid DNA. *Mol. Cell. Biol.* 7:2745-2752.
14. Clark, S. J., M. S. Saag, W. D. Decker, S. Campbell-Hill, J. L. Roberson, P. J. Veldkamp, J. C. Kappes, B. H. Hahn, and G. M. Shaw. 1991. High titers of cytopathic virus in plasma of patients with symptomatic primary HIV-1 infection. *N. Engl. J. Med.* 324:954-960.
15. Daar, E. S., T. Moudgil, R. D. Meyer, and D. D. Ho. 1991. Transient high levels of viremia in patients with primary human immunodeficiency virus type 1 infection. *N. Engl. J. Med.* 324:961-964.
16. 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_{smmPBj14}. *Nature (London)* 345:636-640.
17. Dewhurst, S., J. E. Embretson, P. N. Fultz, and J. I. Mullins. 1992. Molecular clones from a non-acutely pathogenic derivative of SIV_{smmPBj14}: characterization and comparison to acutely pathogenic clones. *AIDS Res. Hum. Retroviruses* 8:1179-1187.
18. Donahue, P. R., S. L. Quackenbush, M. V. Gallo, C. M. C.

- deNoronha, J. Overbaugh, E. A. Hoover, and J. I. Mullins. 1991. Viral genetic determinants of T-cell killing and immunodeficiency disease induction by the feline leukemia virus FeLV-FAIDS. *J. Virol.* **65**:4461–4469.
19. Dougherty, R. M. 1964. Animal virus titration techniques, p. 215–221. In R. J. C. Harris (ed.), *Techniques in experimental virology*. Academic Press, New York.
 20. Duh, E. J., W. J. Maury, T. M. Folks, A. S. Fauci, and A. B. Rabson. 1989. Tumor necrosis factor alpha activates human immunodeficiency virus type 1 through induction of nuclear factor binding to the NF- κ B sites in the long terminal repeat. *Proc. Natl. Acad. Sci. USA* **86**:5974–5978.
 21. Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase. 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation of AIDS. *Nature (London)* **362**:359–362.
 22. Fried, M., and D. M. Crothers. 1981. Equilibria and kinetics of lac repressor-operator interactions by polyacrylamide gel electrophoresis. *Nucleic Acids Res.* **9**:6505–6525.
 23. Fulton, R., M. Plumb, R. Shield, and J. C. Neil. 1990. Structural diversity and nuclear protein binding sites in the long terminal repeats of feline leukemia virus. *J. Virol.* **64**:1675–1682.
 24. Fultz, P. N. 1991. Replication of an acutely lethal simian immunodeficiency virus activates and induces proliferation of lymphocytes. *J. Virol.* **65**:4902–4909.
 25. 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.
 26. Garner, M. M., and A. Revzin. 1981. A gel electrophoresis method for quantifying the binding of proteins to specific DNA regions. *Nucleic Acids Res.* **9**:3047–3060.
 27. Goff, S. P., P. Tractman, and D. Baltimore. 1981. Isolation and properties of Moloney murine leukemia virus mutants: use of a rapid assay for release of virion reverse transcriptase. *J. Virol.* **38**:239–248.
 28. Hannibal, M., D. M. Markovitz, N. Clark, and G. J. Nabel. 1993. Differential activation of human immunodeficiency virus type 1 and 2 transcription by specific T-cell activation signals. *J. Virol.* **67**:5035–5040.
 29. Herschlag, D., and F. B. Johnson. 1993. Synergism in transcriptional activation: a kinetic view. *Genes Dev.* **7**:173–179.
 30. Higuchi, R., B. Krummel, and R. K. Saiki. 1988. A general method of *in vitro* preparation and specific mutagenesis of DNA fragments: study of protein and DNA interactions. *Nucleic Acids Res.* **16**:7351–7367.
 31. Holland, C. A., C. Y. Thomas, S. K. Chattopadhyay, C. Koehne, and P. V. O'Donnell. 1989. Influence of enhancer sequences on thymotropism and leukemogenicity of mink cell focus-forming viruses. *J. Virol.* **63**:1284–1292.
 32. Israel, Z. R., G. A. Dean, D. H. Maul, S. P. O'Neil, M. J. Dreitz, J. I. Mullins, P. N. Fultz, and E. A. Hoover. 1993. Early pathogenesis of disease caused by SIV_{smmPBj14} molecular clone 1.9 in macaques. *AIDS Res. Hum. Retroviruses* **9**:277–286.
 33. Kobayashi, S., Y. Hamamoto, N. Kobayashi, and N. Yamamoto. 1990. Serum level of TNF- α in HIV-infected individuals. *AIDS* **4**:169–170.
 34. Lang, S. M., M. Weeger, C. Stahl-Hennig, C. Coulbaly, G. Hunsmann, J. Muller, H. Muller-Hermelink, D. Fuchs, H. Wachter, M. M. Danile, R. C. Desrosiers, and B. Fleckenstein. 1993. Importance of *vpr* for infection of rhesus monkeys with simian immunodeficiency virus. *J. Virol.* **67**:902–912.
 35. Lee, K. A. W., A. Bindereif, and M. R. Green. 1988. A small scale procedure for preparation of nuclear extracts that support efficient transcription and pre-mRNA splicing. *Gene Anal. Tech.* **5**:22–31.
 36. Leiden, J. M., C.-Y. Wang, B. Petryniak, D. M. Markovitz, G. J. Nabel, and C. B. Thompson. 1992. A novel *ets*-related transcription factor, Elf-1, binds to human immunodeficiency virus type 2 regulatory elements that are required for inducible *trans* activation in T cells. *J. Virol.* **66**:5890–5897.
 37. Lenz, J., D. Celandier, R. L. Crowther, R. Patarca, D. W. Perkins, and W. A. Haseltine. 1984. Determination of the leukaemogenicity of a murine retrovirus by sequences within the long terminal repeat. *Nature (London)* **308**:467–470.
 38. Leonard, J., C. Parrott, A. J. Buckler-White, W. Turner, E. K. Ross, M. A. Martin, and A. B. Rabson. 1989. The NF- κ B binding sites in the human immunodeficiency virus type 1 long terminal repeat are not required for virus infectivity. *J. Virol.* **63**:4919–4924.
 39. Letvin, N. L., and N. W. King. 1990. Immunologic and pathologic manifestations of the infection of rhesus monkeys with simian immunodeficiency virus of macaques. *J. Acquired Immune Defic. Syndr.* **3**:1023–1040.
 40. Lewis, M. G., P. M. Zack, W. R. Elkins, and P. B. Jahrling. 1992. Infection of rhesus and cynomolgus macaques with a rapidly fatal SIV (SIV_{smm/PBj}) isolate from sooty mangabeys. *AIDS Res. Hum. Retroviruses* **8**:1631–1639.
 41. Li, Y., E. Golemis, J. W. Hartley, and N. Hopkins. 1987. Disease specificity of nondefective Friend and Moloney murine leukemia viruses is controlled by a small number of nucleotides. *J. Virol.* **61**:693–700.
 42. Lowenthal, J. W., D. W. Ballard, E. Bohnlein, and W. C. Greene. 1989. Tumor necrosis factor alpha induces proteins that bind specifically to κ B-like enhancer elements and regulate interleukin 2 receptor alpha-chain expression in primary human T lymphocytes. *Proc. Natl. Acad. Sci. USA* **86**:2331–2335.
 43. Makonkawkeyoon, S., R. N. R. Limson-Pobre, A. L. Moriera, V. Schauf, and G. Kaplan. 1993. Thalidomide inhibits the replication of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* **90**:5974–5978.
 44. Markovitz, D. M., M. J. Smith, J. Hilfinger, M. C. Hannibal, B. Petryniak, and G. J. Nabel. 1992. Activation of the human immunodeficiency virus type 2 enhancer is dependent on purine box and κ B regulatory elements. *J. Virol.* **66**:5479–5484.
 45. 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.
 46. Miyamoto, K., N. Tomita, K. Hayashi, and T. Akagi. 1990. Transformation of animal cells with human T-cell leukemia virus type II. *Jpn. J. Cancer Res.* **81**:720–722.
 47. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (London)* **326**:711–713.
 48. Nordeen, S. K. 1988. Luciferase reporter gene vectors for analysis of promoters and enhancers. *BioTechniques* **6**:454–460.
 49. Novembre, F. J., P. R. Johnson, M. G. Lewis, D. C. Anderson, S. Klumpp, H. M. McClure, and V. M. Hirsch. 1993. Multiple viral determinants contribute to pathogenicity of the acutely lethal simian immunodeficiency virus SIV_{smmPBj} variant. *J. Virol.* **67**:2466–2474.
 50. Osborn, L., S. Kunkel, and G. J. Nabel. 1989. Tumor necrosis factor alpha and interleukin 1 stimulate the human immunodeficiency virus enhancer by activation of the nuclear factor κ B. *Proc. Natl. Acad. Sci. USA* **86**:2336–2340.
 51. Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. Orenstein, D. P. Kotler, and A. S. Fauci. 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature (London)* **363**:355–358.
 52. Perkins, N., N. L. Edwards, C. S. Duckett, A. B. Agranoff, R. M. Schmid, and G. J. Nabel. 1993. A cooperative interaction between NF- κ B and Sp1 is required for HIV-1 enhancer activation. *EMBO J.* **12**:3551–3558.
 53. 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**:151–158.
 54. Reimann, K. A., K. Tenner-Racz, P. Racz, D. C. Montefiori, Y. Yasutomi, W. Lin, B. J. Ransil, and N. L. Letvin. 1994. Immunopathogenic events in acute infection of rhesus monkeys with simian immunodeficiency virus of macaques. *J. Virol.* **68**:2362–2370.
 55. Renjifo, B., N. A. Speck, S. Winandy, N. Hopkins, and Y. Li. 1990. *cis*-acting elements in the U3 region of a simian immunodeficiency virus. *J. Virol.* **64**:3130–3134.

56. Roederer, M., F. J. T. Staal, P. A. Raju, S. W. Ela, L. A. Herzenberg, and L. A. Herzenberg. 1990. Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine. *Proc. Natl. Acad. Sci. USA* **87**:4884–4888.
57. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. Sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
58. Schmid, R. M., N. D. Perkins, C. S. Duckett, P. C. Andrews, and G. J. Nabel. 1991. Cloning of an NF- κ B subunit which stimulates HIV transcription in synergy with p65. *Nature (London)* **353**:711–714.
59. Schwiebert, R. S., and P. N. Fultz. 1994. Immune activation and viral burden in acute disease induced by simian immunodeficiency virus SIV_{smmPBj14}: correlation between in vitro and in vivo events. *J. Virol.* **68**:5538–5547.
60. Staal, F. J. T., M. Roederer, L. A. Herzenberg, and L. A. Herzenberg. 1990. Intracellular thiols regulate activation of nuclear factor κ B and transcription of human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **87**:9943–9947.
61. Tjian, R., and T. Maniatis. 1994. Transcriptional activation: a complex puzzle with few easy pieces. *Cell* **77**:5–8.
62. Tsujimoto, H., A. Komuro, K. Iijima, J. Miyamoto, K. Ishikawa, and M. Hayami. 1985. Isolation of simian retroviruses closely related to human T-cell leukemia virus by establishment of lymphoid cell lines from various non-human primates. *Int. J. Cancer* **35**:377–384.
63. van den Hoff, M. J. B., A. F. M. Moorman, and W. H. Lamers. 1992. Electroporation in “intracellular” buffer increases cell survival. *Nucleic Acids Res.* **20**:2902.
64. Viglianti, G. A., and J. I. Mullins. 1988. Functional comparison of transactivation by simian immunodeficiency virus from rhesus macaques and human immunodeficiency virus type 1. *J. Virol.* **62**:4523–4532.
65. Wasylyk, B., C. Wasylyk, P. Flores, A. Begue, D. Leprince, and D. Stehelin. 1990. The *c-ets* proto-oncogenes encode transcriptional factors that cooperate with c-Fos and c-Jun for transcriptional activation. *Nature (London)* **346**:191–193.
66. Wasylyk, C., P. Flores, A. Gutman, and B. Wasylyk. 1989. PEA3 is a nuclear target for transcription activation by non-nuclear oncogenes. *EMBO J.* **8**:3371–3378.
67. Winandy, S., B. Renjifo, Y. Li, and N. Hopkins. 1992. Nuclear factors that bind two regions important to transcriptional activity of the simian immunodeficiency virus long terminal repeat. *J. Virol.* **66**:5216–5223.