JOURNAL OF VIROLOGY, Oct. 1997, p. 7895–7902 Vol. 71, No. 10
0022-538X/97/$04.00+0

Neutralization Sensitivity of Cell Culture-Passaged Simian Immunodeficiency Virus

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Received 21 April 1997/Accepted 19 June 1997

CEMx174- and C8166-45-based cell lines which contain a secreted alkaline phosphatase (SEAP) reporter gene under the control of a *tat*-responsive promoter derived from either SIVmac239 or HIV-1_{NL4-3} were constructed. Basal levels of SEAP activity from these cell lines were low but were greatly stimulated upon transfection of *tat* expression plasmids. Infection of these cell lines with simian immunodeficiency virus (SIV) or human immunodeficiency virus type 1 (HIV-1) resulted in a dramatic increase in SEAP production within 48 to 72 h that directly correlated with the amount of infecting virus. When combined with chemiluminescent measurement of SEAP activity in the cell-free supernatant, these cells formed the basis of a rapid, sensitive, and quantitative assay for SIV and HIV infectivity and neutralization. Eight of eight primary isolates of HIV-1 that were tested induced readily measurable SEAP activity in this system. While serum neutralization of cloned SIVmac239 was difficult to detect with other assays, neutralization of SIVmac239 was readily detected at low titers with this new assay system. The neutralization sensitivities of two stocks of SIVmac251 with different cell culture passage histories were tested by using sera from SIV-infected monkeys. The primary stock of SIVmac251 had been passaged only twice through primary cultures of rhesus monkey peripheral blood mononuclear cells, while the laboratory-adapted stock had been extensively passaged through the MT4 immortalized T-cell line. The primary stock of SIVmac251 was much more resistant to neutralization by a battery of polyclonal sera from SIV-infected monkeys than was the laboratory-adapted virus. Thus, SIVmac appears to be similar to HIV-1 in that extensive laboratory passage through T-cell lines resulted in a virus that is much more sensitive to serum neutralization.

Infection of rhesus monkeys with simian immunodeficiency virus (SIV) recapitulates many of the features of human immunodeficiency virus (HIV) infection of humans (17, 20, 36, 55). Study of neutralizing antibody responses is relevant for understanding the ability or inability of the immune system to control infection by these viruses and for vaccine development. While some studies have looked at the nature of neutralizing responses to SIV and HIV type 2 (HIV-2) (5, 8–10, 13, 33–35, 37, 44, 58), considerably more information has accumulated regarding neutralization of HIV-1 (2, 3, 7, 9, 12, 25, 29–31, 45, 53, 58, 60, 63). Laboratory-adapted strains of HIV-1 that have been extensively passaged in cell culture are readily neutralized by a variety of sera (27, 50, 54, 57, 61, 66). In contrast, primary isolates of HIV-1 are quite resistant to neutralization, even by sera from HIV-1-infected individuals (3, 21, 43, 61, 66). A variety of assay conditions have shown little or no neutralizing activity with most primary HIV-1 isolates. Antibodies with neutralizing activity are generally slow to develop in HIV-1-infected people (38, 51).

Many of the currently available neutralization assays have limitations, particularly when examination of a variety of virus strains and sera is desired. Assays that measure the inhibition of virus-induced cell killing (24, 34, 39, 49, 56) are convenient and easy to perform but are generally useful with only a limited panel of viruses. Measurement of the inhibition by antisera of the production of p24 or p27 Gag antigen (3, 22, 26, 64, 68) has the advantage of being adaptable to any virus that grows in cultured cells, including primary peripheral blood mononuclear cells (PBMC). However, it is difficult to control for the presence of anti-Gag antibodies that can interfere with the detection of p24 or p27 (14, 42). Convenient assays that use reporter genes engineered into recombinant virus have been developed (28, 62). For this approach, analysis of each different envelope sequence requires a separate recombinant clone construction. Furthermore, analysis of viral swarms in uncloned stocks or clinical isolates is difficult or impossible with this approach.

In this report, we describe an assay system for measuring neutralization that uses cell lines harboring a secreted alkaline phosphatase (SEAP) gene under the transcriptional control of SIV or HIV-1 long terminal repeat (LTR) sequences. We demonstrate the utility of this assay system by showing differences in neutralization sensitivity between primary and laboratory-passaged SIV and its use with primary isolates of HIV-1.

MATERIALS AND METHODS

Viruses. Viruses SIVmac239 (36), SIVmac251 primary stock (18, 40), SIVmac251 laboratory-adapted stock (19), and HIV-1_{NL4-3} (1) have been described previously. The primary HIV-1 isolates used, BP, CC, DF, DR, RT, and EP, were obtained from patients at the University of Massachusetts Medical School. The primary HIV-1 isolates clinical 25 and clinical 36 were the gift of Martin Hirsch (Massachusetts General Hospital).

Sera. All rhesus macaque sera were from monkeys housed at the New England Regional Primate Research Center. The chimpanzee sera were the gift of Larry Arthur (National Cancer Institute-Fredrick Cancer Research Facility, Frederick, Md.). All sera were heat inactivated at 56°C for 30 min before use.

Plasmid construction. Plasmids pSIV-SEAP and pHIV-SEAP were constructed by insertion of a PCR fragment from the SIVmac239 or HIV-1_{NL4-3} LTR (Fig. 1) into the pCMV/SEAP expression vector (Tropix, Bedford, Mass.),

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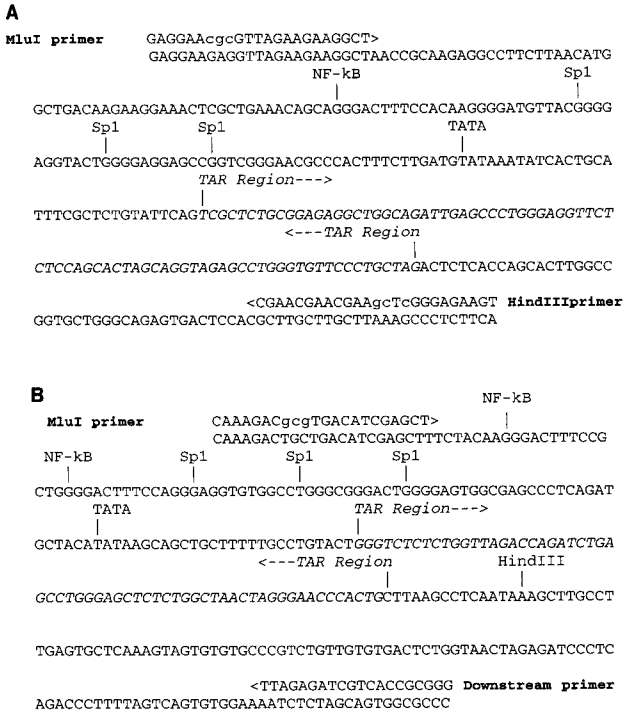

FIG. 1. Locations of amplifying primers within the SIVmac239 and HIV-1_{NL4-3} LTRs. The primers Mlu-SIV and Hind-SIV for amplification of sequences within the SIVmac239 LTR (nucleotides 592 to 927) (A) flank the NF-κB and SP1 binding sites as well as the TATA box and the entire TAR region. The Mlu-HIV and Hind-HIV primers amplify a similar region of the HIV-1_{NL4-3} genome (nucleotides 320 to 643) (B).

using *Mlu*I and *Hin*dIII restriction sites. A minimal promoter/enhancer element of SIVmac239 was amplified by using primers which inserted an *Mlu*I and a *Hin*dIII site flanking the NF-κB and SP1 binding sites as well as the TATA box and the transactivation-responsive region (TAR) (Fig. 1A). A similar region (Fig. 1B) was amplified from HIV-1_{NL4-3} by using an upstream primer which introduced an *Mlu*I site and a nonmutagenic downstream primer. Insertion of the minimal promoter/enhancer elements into the pCMV/SEAP plasmid by using the introduced *Mlu*I and *Hin*dIII sites eliminated the cytomegalovirus promoter. Each of the constructs was completely sequenced through the PCR product. The pLNLTR-SEAP plasmids (Fig. 2) were created by PCR amplification of the pLTR-SEAP plasmids with mutagenic primers which introduced *Bst*BI and *Cla*I sites. The PCR products and the pLNSX retroviral vector plasmid (47) were cut with *Bst*BI and *Cla*I and then ligated. This eliminated the simian virus 40 (SV40) promoter from the final product, leaving only the SIV or HIV LTR sequence to drive SEAP expression (Fig. 2).

Primers. All primers were prepared on a DNA synthesizer (model 8400; Milligen/Biosearch Inc., Burlington, Mass.). Oligonucleotide sequences were as follows: for introduction of an *Mlu*I site into the SIV LTR, Mlu-SIV (GAGGA ACGCGTTAGAAGAAGGCT); for introduction of a *Hin*dIII site into the HIV LTR, Hind-SIV (CGAACGAACGAAGCTTGGGAGAAGT); for introduction of an *Mlu*I site into the HIV LTR, Mlu-HIV (CAAAGACGCGTGACATCG AGCT); for amplification across the *Hin*dIII site of the HIV LTR, Hind-HIV (TTAGAGATCGTCACCGCGGG); for introduction of a *Bst*BI site into pLTR-SEAP, Bst-SEAP (TTCTTCGAAAAGTGCCACCTGACGTCGAC); and for introduction of a *Cla*I site into pLTR-SEAP, Cla-SEAP (TAATCGATAGGTG ACACTATAGAATAGG).

Cells. Human $CD4^+$ CEMx174 cells and C8166-45 (NIH AIDS Research and Reference Reagent Program, Rockville, Md.) cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. PA317 cells (for retroviral packaging) were maintained in Dulbecco's modified Eagle's medium with 10% fetal calf serum. PA317 pLNLTR-SEAP cell lines were constructed by transfection of the pLNLTR-SEAP plasmids into the PA317 (ATCC CRL 9078) retroviral packaging cell line (46) by the calcium phosphate method. Cell lines were selected by growth in complete Dulbecco's modified Eagle's medium plus 0.5 mg of gentamicin (G418; GIBCO, Grand Island, N.Y.) per ml. The CEMx174 LTR-SEAP and C8166-45 LTR-SEAP cell lines were constructed by infection of either CEMx174 cells or C8166-45 cells with the amphotropic retrovirus produced by the PA317/pLNLTR-SEAP packaging cell lines. Cell lines were selected by growth in complete RPMI 1640 with 0.5 mg of G418 per ml.

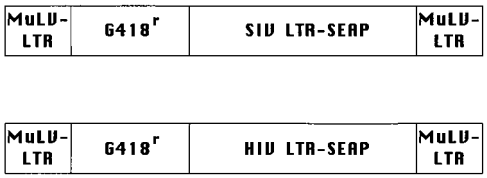

FIG. 2. Diagrams of the pLNLTR-SEAP plasmids. The pLNLTR-SEAP plasmids were created by inserting an SIV LTR-SEAP cassette (top) or an HIV LTR-SEAP cassette (bottom) into the pLNSX amphotropic retrovirus vector. In the final plasmid, the murine leukemia virus LTR (MuLV-LTR) drives expression of the *neo* gene and the SV40 promoter has been eliminated.

Responsiveness of cell lines to *tat*. Potential PA317/pLNLTR-SEAP cell lines were transfected with HIV or SIV *tat* constructs by the calcium phosphate method and assessed for SEAP production. C8166-45 LTR-SEAP and CEMx174 LTR-SEAP cell lines were examined in a similar way by transfection using the DEAE-dextran method. SEAP in the culture supernatant was detected 16 to 18 h later with the Phospha-Light chemiluminescent reporter gene assay system (Tropix) according to the manufacturer's recommendations. Transfection efficiency was normalized by cotransfection with the pSVB-βgal plasmid (Clontech, Palo Alto, Calif.), and β-galactosidase expression was measured with the Galacto-Light Plus system (Tropix) according to the manufacturer's recommendations.

Neutralization assay. Ninety-six-well plates were set up as follows. To the first three columns, 25 μl of RPMI 1640–10% fetal calf serum containing a 1:20 dilution of mock serum was added. To each of the other columns, 4 through 12, 25 μl of successive twofold dilutions of test sera in full RPMI 1640 were added. Next, 75 μl of test virus at the appropriate concentration was added to each well in columns 3 through 12. Virus-free complete RPMI 1640 was added to columns 1 and 2. The plate was incubated for 1 h at room temperature with occasional mixing. After incubation, 40,000 cells in a volume of 100 μl were added to each well. The first column received the parental cell line without the SEAP reporter construct. All of the other columns received the appropriate pLNLTR-SEAP cell line. The plate was then placed into a humidified chamber within a CO_2 incubator at 37°C for 52 to 72 h. For measurement of SEAP activity, 10 μl of medium was harvested and placed into a fresh 96-well plate. To each well, 30 μl of 1× dilution buffer from the Phospha-Light assay kit was added. The plate was covered and incubated for 30 to 40 min at 65°C. At the end of the incubation, the plate was cooled to room temperature. Then 33 μl of room temperature Phospha-Light assay buffer was added to each well, and the plate was incubated at room temperature with occasional shaking for 5 min. Finally, 33 μl of 1× room temperature Phospha-Light reaction buffer was added to each well. The plate was incubated at room temperature for 5 min, and chemiluminescence was quantitated as counts per second with a Wallac micro-beta plate reader (Wallac Oy, Turku, Finland). After collection, data was processed by subtracting the endogenous phosphatase activity as measured in the first well of each row. The

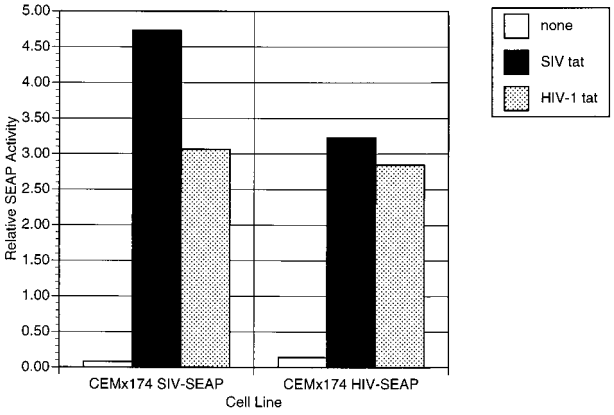

FIG. 3. Transfection of CEMx174 LTR-SEAP cell lines with SIV or HIV-1 *tat*. CEMx174 LTR-SEAP cells were either transfected with plasmid pSVB-βgal alone or cotransfected with pSVB-βgal together with either SIV *tat* or HIV-1 *tat* under the control of the SV40 promoter. Cells and supernatant were harvested 52 h posttransfection, and the levels of β-galactosidase and SEAP activity were determined for each transfection. SEAP activity was normalized for transfection efficiency by dividing by the β-galactosidase expression and reporting it as the relative SEAP activity.

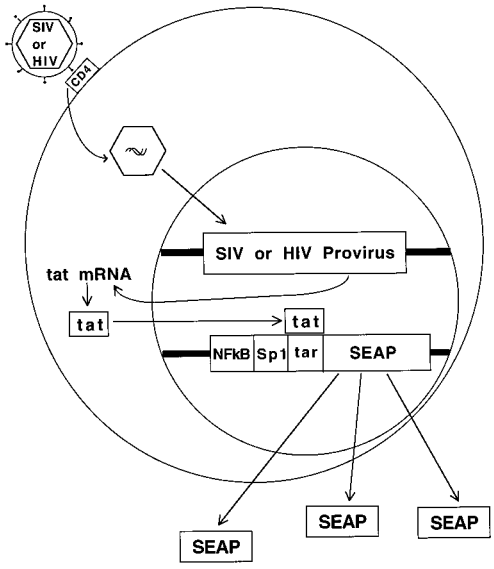

FIG. 4. Diagram of the SEAP neutralization assay. Neutralized or mock-neutralized SIV or HIV is used to infect LTR-SEAP cell lines which are engineered to contain the LTR from either SIVmac239 or HIV-1_{NL4-3} driving the expression of SEAP in a *tat*-dependent manner. After integration of the virus, *tat* mRNA and Tat protein are produced. The Tat protein interacts with the TAR region of the LTR-SEAP construct, causing increased SEAP expression, which can then be measured in the cell-free culture supernatant.

numbers were then converted to percent neutralization by dividing the counts per second of each well in columns 4 through 12 by the average SEAP values in column 3. Those wells contained mock-neutralized virus and represented maximal SEAP expression for the concentration of virus used. The average values of the wells in column 2 were used as a measure of basal activity from the LTR-SEAP cell line used for that experiment.

Nucleotide sequence accession numbers. The sequences of the SIVmac239 LTR (nucleotides 592 to 927) and the HIV-1_{NL4-3} genome (nucleotides 320 to 643) have been assigned GenBank accession numbers M33262 and M19921, respectively.

RESULTS

Construction of TCE-SEAP plasmids. A *tat*-responsive SEAP reporter gene was constructed by exchanging the cytomegalovirus promoter of the pCMV/SEAP expression vector with a minimal transcriptional control element (TCE) from SIVmac239 or HIV-1_{NL4-3}. The minimal TCEs contained the NF-κB and SP1 binding sites as well as the TATA box and TAR regions of the respective viruses (Fig. 1). In past experiments it has been shown that the presence of the TAR region results in suppression of expression, which can be overcome by the inclusion of tat (41). Using PCR, unique restriction sites were engineered at the ends of the TCEs. After introduction into the pCMV/SEAP vector, the newly created plasmids, pSIV-SEAP and pHIV-SEAP, were tested for SEAP expression and *tat* responsiveness. Transfection of the SEAP expression plasmids alone into CEMx174 cells resulted in only a low level of detectable SEAP activity in the supernatant. However, cotransfection of either plasmid with a *tat* expression vector increased the level of SEAP activity in the culture medium approximately 50- to 100-fold (data not shown). To facilitate production of stable cell lines, the SEAP expression cassette was transferred into the pLNSX amphotropic retrovirus vector. The SV40 promoter was removed during cloning to avoid high basal expression levels of SEAP activity. The resulting plasmids, pLNSIV-SEAP and pLNHIV-SEAP (Fig. 2), were analyzed for SEAP expression and *tat* responsiveness by tran-

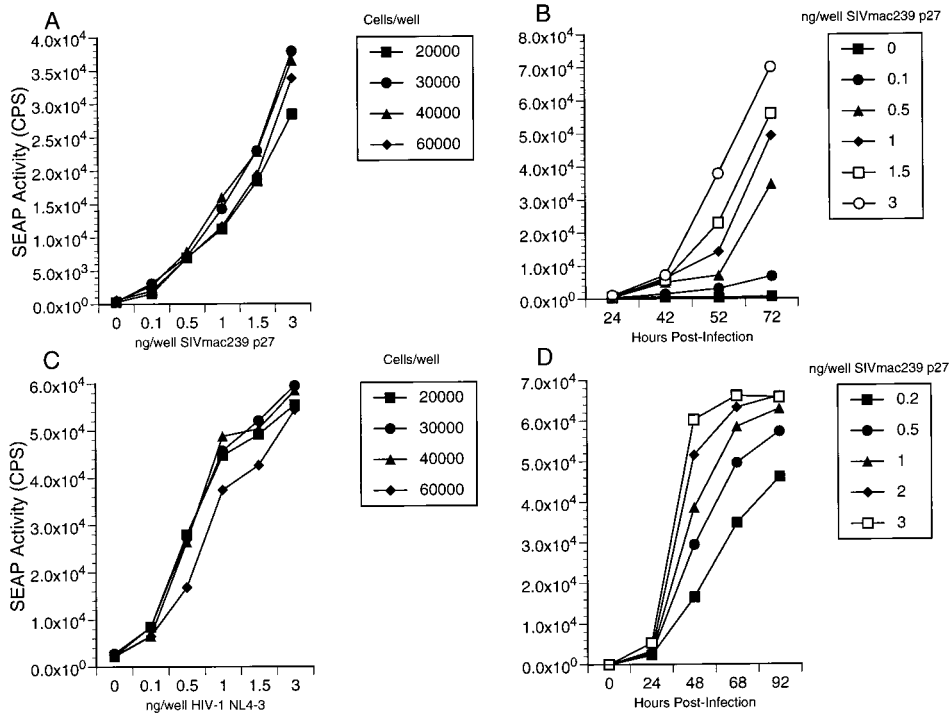

FIG. 5. SEAP activity from various LTR-SEAP cell lines. Various numbers of CEMx174 SIV-SEAP cells were exposed to the indicated amounts of SIVmac239, and SEAP activity was determined at 72 h postinfection as described in Materials and Methods (A). In panel B, CEMx174 SIV-SEAP cells (40,000/well) were infected with increasing amounts of SIVmac239, and SEAP activity was determined at the indicated times postinfection. C8166-45 SIV-SEAP cells were tested in a similar manner (D). CEMx174 HIV-SEAP cells (C) were tested by using the indicated amounts of HIV-1_{NL4-3}.

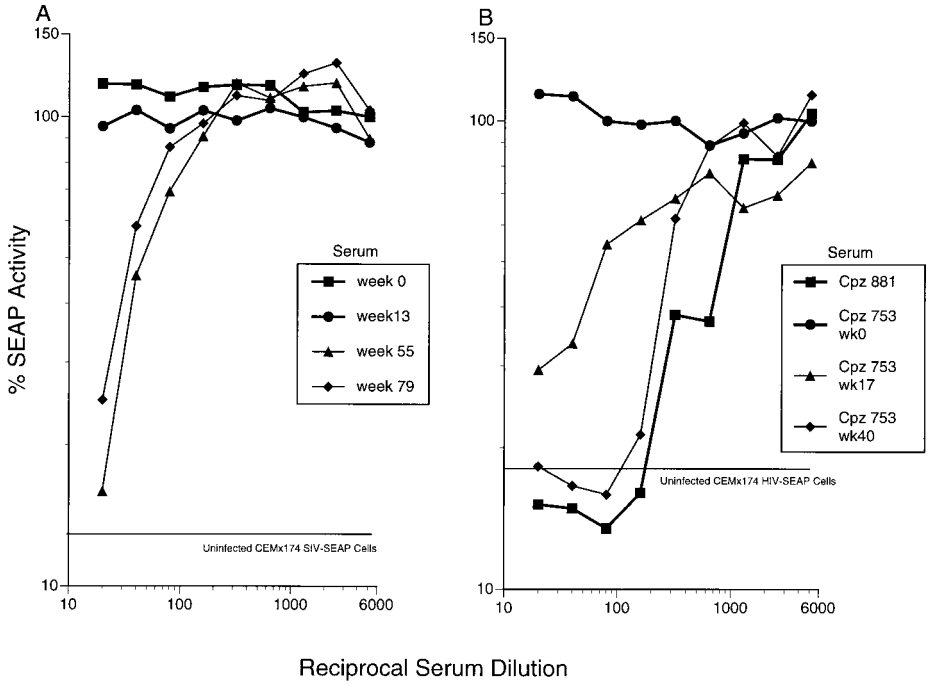

FIG. 6. Neutralization of SIVmac239 and HIV-1_{NL4-3}. Neutralization sensitivity of SIVmac239 was tested by using CEMx174 SIV-SEAP cells and sera taken from rhesus macaque Mm243-86 at various weeks postinfection with SIVmac239 (A). A similar assay was performed using CEMx174 HIV-SEAP cells to test the neutralization sensitivity of HIV-1_{NL4-3} with a panel of sera taken from chimps infected with HIV-1 NL4-3 or IIIB.

sient transfection into CEMx174 cells with and without *tat* expression plasmids; high SEAP expression was seen only with *tat* cotransfection (data not shown).

Construction of TCE-SEAP cell lines. PA317 packing cells were transfected with the pLNLTR-SEAP plasmids and selected for G418 resistance. CEMx174 and C8166-45 cells were engineered to contain the SEAP reporter gene under the control of either a SIV or HIV TCE by infection with the retroviral vectors and G418 selection. Upon transfection of a reporter plasmid for either HIV-1 or SIV *tat*, supernatant from the G418-resistant cells showed dramatically increased SEAP activity (Fig. 3) compared to mock-transfected cells. As has been shown in previous studies (23, 59, 65), both Tat proteins were able to transactivate their own and the heterologous TCE (Fig. 3).

The cell lines were next characterized for expression of SEAP in response to viral infection (Fig. 4). Plates were seeded with four concentrations of cells to which five dilutions of either SIVmac239 or HIV-1_{NL4-3} were added. SEAP activity was monitored by harvesting the supernatant at several time points postinfection. Expression of SEAP in the medium was directly correlated with the amount of input virus, either HIV-1 or SIV, for all concentrations of cells and virus tested (Fig. 5). Input virus containing as little as 0.1 ng of p27 reproducibly induced measurable SEAP activity from the CEMx174 SIV-SEAP and C8166-45 SIV-SEAP cell lines by 72 h postinfection (Fig. 5B and D). The overall level of SEAP activity from all cell lines increased with time (Fig. 5B and D) but not with increasing numbers of cells (Fig. 5A and C). The independence of cell number is consistent with the low multiplicities of infection that were used. The induction of SEAP activity at early times is likely to reflect *tat* production and LTR transactivation principally from the initial round of infection.

LTR-SEAP cells can be used to measure the neutralizing potential of serum. Several sera from SIV-infected monkeys and HIV-1-infected chimpanzees were tested for the ability to neutralize viral infectivity. Briefly, a fixed amount of either

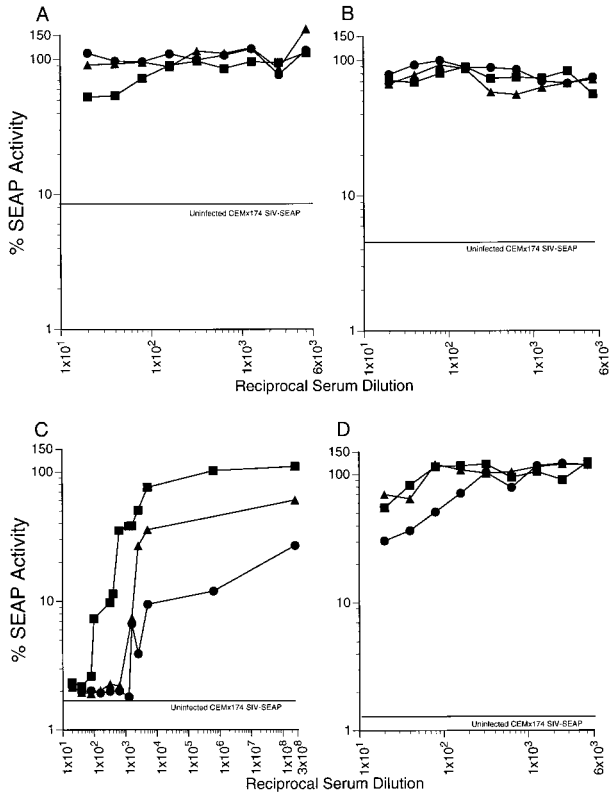

FIG. 7. Neutralization of primary HIV-1 isolates. Primary isolates BP (A), DF (B), EP (C), and HIV-1_{NL4-3} (D) were grown for one passage in CEMx174 cells and then tested for sensitivity to neutralization by homologous and heterologous sera (squares, from BP; circles, from CC; triangles, from EP) on CEMx174 HIV-SEAP cells. For each assay, 350 pg of p27 of the respective virus was added per well. All SEAP values were normalized to a mock-neutralized control as described in Materials and Methods.

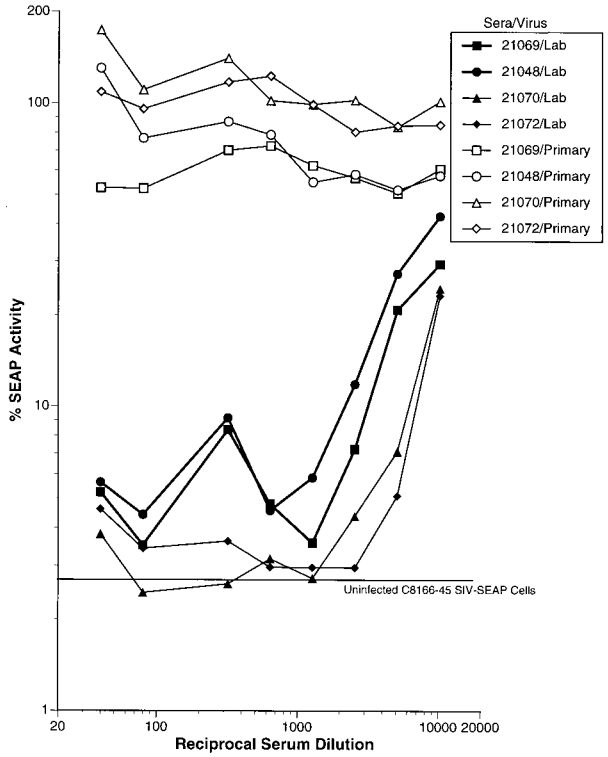

FIG. 8. Neutralization of SIVmac251 stocks with various sera. The SIVmac251 primary stock (open symbols) and SIVmac251 laboratory-adapted stock (closed symbols) were tested for neutralization sensitivity on C8166-45 SIV-SEAP cells. Values were normalized to a mock-neutralized control as described in Materials and Methods.

SIVmac239 or HIV-1_{NL4-3} was incubated at room temperature with serial dilutions of heat-inactivated sera in a 96-well plate. After 1 h, 40,000 LTR-SEAP cells were added per well, and the plate was incubated for 50 to 72 h. SEAP activity was then measured by transferring an aliquot of cell-free supernatant to another 96-well plate and performing a modified SEAP detection assay as described in Materials and Methods. Representative results are shown in Fig. 6. Sera with neutralizing activity decreased the amount of SEAP activity relative to a mock-neutralized control. As has been noted previously (13, 15, 67), antibodies with neutralizing activity to SIVmac239 were somewhat slow to develop in macaques infected with SIVmac239 (Fig. 6A). Neutralizing activity toward HIV-1_{NL4-3} was also observed with sera from HIV-1 infected chimpanzees (Fig. 6B).

We next investigated the utility of the assay system for primary isolates of HIV-1. Eight primary HIV-1 strains isolated on human PBMC and the NL4-3 laboratory strain were passaged to CEMx174 cells. NL4-3 and one primary isolate from an individual with AIDS (the EP isolate) replicated with rapid kinetics and to high titer in CEMx174 cells. The seven additional primary HIV-1 isolates also replicated in CEMx174 cells but to lower levels (20 to 80 ng versus 2,500 to 3,300 ng of p24) and with slower kinetics (8- to 12-day delay). These seven primary isolates again yielded low levels of virus with slow kinetics when passed from the CEMx174 supernatants onto fresh CEMx174 cells. These results suggest inefficient but definite use of a second receptor present on CEMx174 cells by these primary HIV-1 isolates. All eight of the primary isolates reproducibly induced SEAP activity upon infection in the CEMx174 LTR-SEAP cell lines. The EP isolate was readily neutralized by both homologous and heterologous antisera (Fig. 7C). The two other primary isolates that were examined for neutralization sensitivity were relatively resistant (Fig. 7A and B). However, significant neutralization of the BP isolate by the homologous sera was observed (Fig. 7A).

SIVmac neutralization sensitivity changes with T-cell line adaptation. The SEAP assay was used to examine the neutralization sensitivities of two stocks of SIVmac251 with different cell culture passage histories. The primary stock of SIVmac251 was passaged only twice in rhesus monkey PBMC cultures. A second stock of SIVmac251 was extensively passaged through the MT4 immortalized T-cell line. Sera from animals that had been infected with SIVmac251 primary stock were tested for neutralizing activity against the two virus stocks. A representative experiment is shown in Fig. 8. The same amount of input p27 (3 ng per well) was used for both the laboratory-adapted and primary stocks. The SEAP activity per ng of p27 differed by less than 20% between the two stocks, and the neutralization curves were normalized to a mock-neutralized control standardized as having 100% SEAP activity. As a mock-neutralized control, sera from six SIV-negative animals were pooled. These pooled sera (Fig. 8) and sera from each of the individual negative animals (data not shown) showed no neutralizing activity against either of the virus stocks. With all of the SIV-positive sera tested, as summarized in Table 1, markedly higher levels of neutralizing activity were observed against the laboratory-adapted stock. To determine whether this difference in neutralization sensitivity existed only with sera from animals infected with SIVmac251, sera from animals infected with cloned SIVmac239 and SIVmac239ΔNef were also tested

TABLE 1. Summary of 50% neutralization titers with sera from monkeys infected with SIVmac251[a]

Serum no.	50% Neutralization titer	
	Primary stock	Lab-adapted stock
21046	<1:40	1:2560
21069	<1:40	>1:5,120
20671	<1:40	>1:5,120
20706	1:320	>1:5,120
21048	<1:40	>1:5,120
21070	<1:40	>1:5,120
20708	<1:40	>1:5,120
21072	<1:40	>1:5,120
21364	1:320	>1:5,120
22160	<1:40	1:2,560
22161	<1:40	1:2,560
21705	1:80	1:5,120
22166	<1:40	1:2,560
23103	1:40	>1:10,240
23334	1:1,280	>1:10,240
21850	1:160	>1:10,240
21849	1:320	>1:10,240
21335	1:640	>1:10,240
21334	1:80	>1:10,240
21846	<1:40	>1:10,240
21851	1:80	>1:10,240
22626	<1:40	>1:10,240
21852	<1:40	1:40
21428	<1:40	1:40
21439	<1:40	1:80
22165	<1:40	1:2,560

[a] Neutralization of SIVmac251 primary stock and laboratory-adapted stock was measured as described in Materials and Methods, using sera from rhesus macaques that had received SIVmac251. The 50% neutralization titer is the dilution of serum which yielded 50% of the SEAP activity of nonneutralized virus.

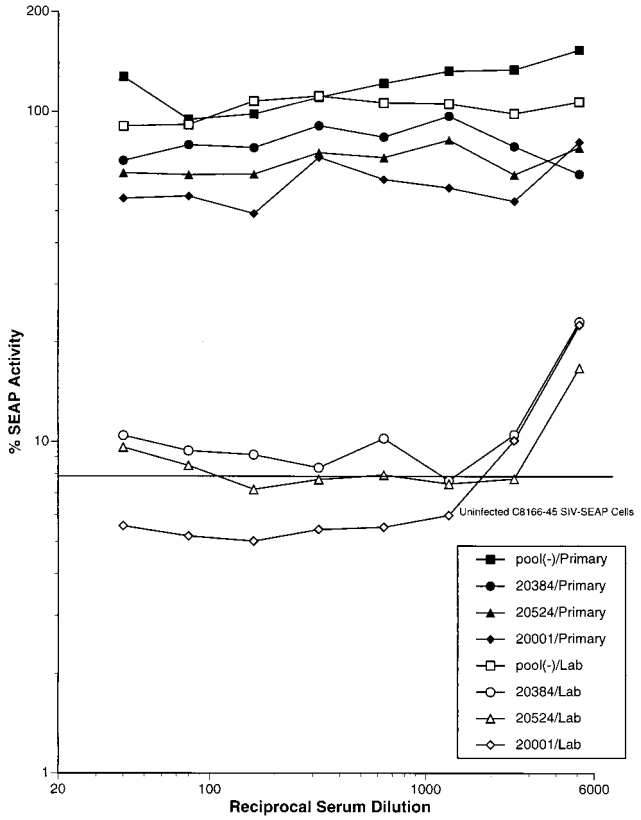

FIG. 9. Neutralization of SIVmac251 stocks with various sera. SIVmac251 stocks were tested for neutralization sensitivity as described in the legend to Fig. 8 with a panel of sera from rhesus macaques infected with SIVmac239 or SIVmac239ΔNef.

(Fig. 9 and Table 2). Again, the primary stock consistently showed greater resistance to neutralization, consistent with previous observations (48, 67).

DISCUSSION

We have described in this report the utility of a new assay system for measuring SIV and HIV infection and neutralization. The SEAP gene was selected as the reporter in the design of the assay for several distinct reasons. Since SEAP is a secreted product, harvesting and lysing of cells are not required, thus reducing manipulations and error. Harvesting small aliquots of the culture medium also allows convenient analysis of time course in a single well. Currently available chemiluminescent methods for measuring SEAP activity have dramatically increased the sensitivity of detection over the colorimetric methods used in the early descriptions of SEAP reporter gene activity (6, 16). The chemiluminescent detection system used for measuring SEAP activity in our studies has a lower limit of detection of about 3 fg (11). Infection of the LTR-SEAP cell lines with 10 to 100 50% tissue culture infective doses of SIV or HIV produced readily measurable SEAP activity in the supernatant by 2 to 3 days postinfection. The time needed to complete the assay is thus short, within the period of single cycle infection events.

Perhaps the most commonly used assay for neutralization measures inhibition of viral p24/p27 antigen production (3, 22, 26, 52, 64, 68). However, sera from infected individuals contain antibodies to p24/p27 at various levels, and these can interfere with the detection of p24/p27 antigen (14, 42). While extensive washing has been used to alleviate this problem (4, 52), efficient removal of interfering antibody is difficult in 96-well plates. Furthermore, since the levels of anti-p24/p27 antibodies will vary with the individual sera, controls need to be run with each serum sample being tested. The SEAP reporter gene system described in this report circumvents any problems associated with antibodies to p24/p27 or to reverse transcriptase interfering with the readout.

The assay system described in this report may also have utility for antiviral drug or natural inhibitor testing. In fact, a similar system has already been used to screen for *tat* antagonists (32). Induction of SEAP activity is dependent on virus binding to receptors, entry, uncoating, reverse transcription, integration, transcription of *tat* mRNA, synthesis of *tat*, and *tat* transactivation. A drug, cytokine, or other such compound that inhibits any of these steps should result in decreased SEAP reporter activity.

TABLE 2. Summary of 50% neutralization titers with sera against heterologous viruses[a]

Serum no.	50% Neutralization titer		Inoculum
	Primary stock	Lab-adapted stock	
20528	1:40	>1:5,120	239
20398	1:320	>1:5,120	239
21133	1:40	>1:5,120	239
20041	<1:40	<1:40	239
20121	1:40	>1:5,120	239
20001	<1:40	>1:5,120	239
20399	<1:40	>1:5,120	239
20526	1:80	>1:5,120	239
20055	<1:40	>1:5,120	239
20517	1:40	>1:5,120	239
21124	1:40	>1:5,120	239
20384	<1:40	>1:5,120	239Δ Nef
20524	<1:40	>1:5,120	239Δ Nef
21218	<1:40	>1:5,120	239Δ Nef
21291	<1:40	>1:5,120	239Δ Nef

[a] The 50% neutralization titer of sera from animals infected with either SIVmac239 or SIVmac239ΔNef was measured as described in the footnote to Table 1.

ACKNOWLEDGMENTS

We thank L. Arthur and M. Hirsch for their generous gifts. We thank John Sullivan for continued support. We also thank Susan Czajak and Daniel Silva for technical assistance and Joanne Newton for help in preparing the manuscript.

This work was supported by PHS grants A125328, A138559, AI01382, HL42257, and RR00168.

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