VIROLOGY 213, 386–394 (1995)

Characterization of a CD4-Expressing Macaque Cell Line That Can Detect Virus after a Single Replication Cycle and Can Be Infected by Diverse Simian Immunodeficiency Virus Isolates

BRYCE CHACKERIAN, NANCY L. HAIGWOOD,* and JULIE OVERBAUGH[1]

Department of Microbiology, Box 357242, University of Washington, Seattle, Washington 98195; and
**Bristol–Myers Squibb Pharmaceutical Research Institute, Seattle, Washington 98121*

Received June 23, 1995; accepted August 24, 1995

Primate lentiviruses such as human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) are phenotypically diverse, and virus isolates vary in cytopathicity, replication rate, and cell tropism. While all virus isolates infect primary peripheral blood lymphocytes, only a subset of strains infect established CD4-expressing T-cell lines. Here, we describe the development and characterization of a macaque cell line that can be infected by all of the strains of SIV that we have tested, including macrophage- and T-cell-tropic strains, primary and cell-line adapted strains, and SIVmac, SIVMne, and SIVsm isolates. The cells can be infected by strains of HIV type 2 (HIV-2) to varying degrees, but not by either cloned or primary isolates of HIV type 1 (HIV-1). This cell line is a derivative of a rhesus macaque mammary tumor cell line (CMMT) engineered to express human CD4. For these studies, a CMMT-CD4 clone expressing an integrated copy of a truncated HIV-1 long terminal repeat fused to the *β-galactosidase* gene (LTR-*β-gal*) was established to allow detection of infectious SIV after a single round of replication. Here, we demonstrate the ability of the CMMT-CD4-LTR-*β-gal* cell line to rapidly and quantitatively detect infectious SIV. Using these cells to assay virus, we could readily measure neutralizing antibody activity in animals infected with different SIV isolates. Neutralizing activity was detected against the homologous virus and lower, but detectable, activity was measured against heterologous virus. Thus, this system, which is highly sensitive and can detect infection by all of the SIV isolates we tested, is a rapid method for detecting infectious virus and quantitating neutralizing antibody activity.

INTRODUCTION

Lentiviruses such as human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) are characterized by a high degree of genotypic and phenotypic diversity (reviewed in Cheng-Mayer, 1990; Fenyö *et al.,* 1989; Zack and Overbaugh, 1994). For example, in human T-cell lines, primate lentiviruses display distinct differences in host cell specificity, replication rate, and cell killing. Standard methods for quantitatively measuring infectious HIV and SIV typically require infection and spread of the virus in T-cell lines, and replication differences can make the comparison of phenotypically distinct strains problematic. Similarly, protocols that measure infectious virus by cytopathic effect or syncytia, for example, neutralization assays, will be biased against viral isolates that are noncytopathic or nonsyncytia inducing. Other than the viral receptor, the cellular factors that determine viral tropism and syncytia-inducing ability are largely uncharacterized.

Human cell lines engineered to express human CD4 can be infected by HIV type 1 (HIV-1) to varying degrees (Cheng-Mayer, 1990; Clapham *et al.,* 1991). Similarly, assays that require only a single round of infection to detect infectious HIV-1 in CD4 cell lines (Chesebro and Wehrly, 1988; Felber and Pavlakis, 1988; Nara *et al.,* 1987) are each effective with only a limited subset of HIV-1 isolates. Rocancourt *et al.* (1990) and, more recently, Kimpton and Emerman (1992) developed a rapid method of detecting HIV-1 based on the viral activation of an integrated LTR-*β-galactosidase* (LTR-*β-gal*) gene in $CD4^+$ HeLa cells. This assay exploits the ability of the HIV Tat protein to transactivate the *β-gal* gene driven by the HIV-1 long terminal repeat (LTR) promoter. The nuclei of infected cells stain blue after incubation with X-gal, and individual infected cells are counted *in situ* with the aid of a light microscope. Unfortunately, this assay also has limitations; the HeLa-CD4-LTR-*β-gal* cell line developed by Kimpton and Emerman [called the multinuclear activation of a galactosidase indicator (MAGI) cell line] is not sensitive to infection by most primary HIV-1 isolates (Kimpton and Emerman, 1992). In addition, one report indicates that HeLa-CD4 cells are moderately susceptible to SIVagm and, to a lesser extent, SIVmac infection (Rocancourt *et al.,* 1990), but we have found that the HeLa-CD4 (MAGI) cell line is not susceptible to SIVMneCL8 infection (Chackerian and Overbaugh, unpublished observations).

Recently, Goldstein *et al.* (1995) reported that a rhesus macaque cell line expressing human CD4 (CMMT-CD4) is sensitive to SIVsm, SIVmac, and SIVagm infection (Goldstein *et al.,* 1995). In addition, previous studies had shown the SIV Tat protein can efficiently transactivate

[1] To whom correspondence and reprint requests should be addressed.

0042-6822/95 $12.00

the HIV-1 LTR (Viglianti and Mullins, 1988). On the basis of these observations, we engineered the CMMT cell line to carry the human CD4 gene and an HIV-1-LTR-*β-gal* reporter gene. This cell line, the simian MAGI (sMAGI) line, is highly sensitive to infection by T-cell- and macrophage-tropic SIV strains, as well as primary and laboratory-adapted isolates. Using these indicator cells the titer of neutralizing antibody activity in macaque plasma against three commonly used SIV isolates was determined. Our analysis showed that this approach can be used to define neutralizing antibody titers to homologous and heterologous virus isolates. We also tested the sensitivity of the sMAGI cell line to isolates of HIV-1 and HIV-2. The cell line was not susceptible to infection by either cloned or primary isolates of HIV-1. But, the sMAGI cells were sensitive to infection by some strains of HIV-2.

MATERIALS AND METHODS

Cell lines

CMMT cells (a rhesus macaque mammary tumor cell line) were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, 100 U per milliliter penicillin, 100 μg per milliliter streptomycin, and 300 μg per milliliter glutamine (complete DMEM). CMMT-CD4 cells were constructed by infecting CMMT cells with the amphotropic retroviral vector PA317/LT4SN (Kimpton and Emerman, 1992; McElrath *et al.,* 1994). Cells were selected by growth in complete DMEM plus 0.2 mg per milliliter gentamycin (G418, at an active concentration of 700 μg per milligram; GIBCO, Grand Island, NY). From the G418-resistant cell population, we used fluorescence-activated cell sorting (FACS) to segregate the top 15–30% of cells that expressed the highest surface levels of CD4, as was done with the original MAGI cells (Kimpton and Emerman, 1992). To further purify this high CD4-expressing subpopulation, the cells were resorted for high CD4 expression by FACS after culturing for an additional 2 and 4 weeks under G418 selection.

CMMT-CD4-LTR-*β-gal* indicator cells were constructed by transfecting CMMT-CD4 cells with plasmid pEQ447 (Harrington and Geballe, 1993) using Lipofectin (GIBCO BRL, Gaithersburg, MD). pEQ447 contains the hygromycin resistance gene under the control of the cytomegalovirus major immediate-early promoter, the *β-gal* gene downstream of a truncated HIV-1 LTR, and the simian virus 40 T antigen nuclear localization signal. Cells were grown in complete DMEM plus 0.2 mg per milliliter of G418 (at an active concentration of 700 μg per milligram) and 50 U per milliliter hygromycin (Calbiochem, La Jolla, CA) in order to isolate single cell clones. Thirty of the G418 and hygromycin-resistant colonies were expanded in culture, and subsequently the two clonal cell lines that we chose for further study were sorted for high CD4 expression one additional time (as described above).

Viruses and infections

SIV stocks were prepared by infecting CEM × 174 cells, C8166 cells, or *Macaca nemestrina* peripheral blood mononuclear cells (PBMCs) with cell-free virus and culturing for 2 to 4 weeks. Cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 100 U per milliliter penicillin, 100 μg per milliliter streptomycin, 1 m*M* sodium pyruvate, and 100 μ*M* nonessential amino acids; 1% phytohemagglutinin-M and 50 U per milliliter interleukin-2 were added to PBMCs in culture. Cell-free virus was harvested from the cultures by low-speed centrifugation for 5 min to remove the majority of cells followed by filtration through a 0.22 μ*M* filter. Viral stocks harvested from CEM × 174 cells include the following: SIVMneCL8 (Morton *et al.,* 1989; Overbaugh *et al.,* 1991), a cloned cytopathic and syncytia-inducing variant of SIVMneCL8, SIVMne170 (Kimata and Overbaugh, unpublished observations), a chimeric virus M87004 35wk, which was constructed by inserting the majority of gp120 from clone 35wk:1-1 (Overbaugh *et al.,* 1991) into an SIVMneCL8 background (Rudensey and Overbaugh, unpublished observations), SIVmac239 (Kestler *et al.,* 1990), and an uncloned virus pool, SIVsmE660 (Hirsch and Johnson, 1994). SIVmac1A11 (Marthas *et al.,* 1989, 1990) and uncloned SIVMne (Benveniste *et al.,* 1986) were grown in macaque PBMCs. Primary isolates derived from the PBMCs of an infected *M. nemestrina* (M87004) 170 weeks postinoculation and passaged through CEM × 174 cells, C8166 cells, or macaque PBMCs were described previously (Rudensey *et al.,* 1993).

Cell free HIV-2 EHO, HIV-2 KR, and HIV-2 ROD stocks were grown in U937 cells, CEM × 174 cells, and human PBMCs, respectively. HIV-1 LAI was grown in CEM × 174 cells and human PBMCs. HIV-1 BAL was grown in human PBMCs. WH91-330 was passaged through human PBMCs (White-Scharf *et al.,* 1993). Primary isolates MM1-2201 and BB2-2847 from a Kenyan woman and infant, respectively, were obtained by coculturing their PBMCs with donor human PBMCs for 4 weeks (Welch, Kreiss, and Overbaugh, unpublished observations). The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: p239SpE3′ and p239SpSp5′ from Dr. Ronald Desrosiers (Kestler *et al.,* 1990), HIV-2 CBL-20/H9 from Dr. Robin Weiss (Schulz *et al.,* 1990), and HIV-2 D194/HUT-78 from Dr. Hagen von Briesen (Kuhnel *et al.,* 1989; von Briesen *et al.,* 1990).

Virus assays

The CMMT-CD4-LTR-*β-gal* indicator cells were plated in 12-well plates at 4×10^4 cells per well in complete DMEM the day before infection. The cells were infected by removing the media from each well and adding dilutions of

virus in a total volume of 300 μl of complete DMEM with 15 μg per milliliter DEAE-Dextran. Some infections were also performed in the presence of 5 μM AZT (Sigma, St. Louis, MO). Two hours after infection, an additional 1.5 ml of complete DMEM was added to each well. Three days later, the medium was removed and cells were fixed with 1–2 ml of 1% formaldehyde–0.2% glutaraldehyde in phosphate-buffered saline (PBS) for 5 min. Cells were then washed twice with PBS and stained with 600 μl of 4 mM potassium ferrocyanide, 4 mM potassium ferricyanide, 1 mM magnesium chloride, 0.4 mg per milliliter 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) in PBS for 50 min at 37° in a non-CO_2 incubator. The staining was stopped by removing the staining solution and washing thoroughly at least two times with PBS.

The infectious titer of some viral supernatants was also determined by endpoint dilution of virus in CEM $\times$ 174 cells. CEM $\times$ 174 cells were infected with serial threefold dilutions of virus in triplicate. Productive virus infection was detected by assaying for SIV $p27^{gag}$ antigen 2 weeks after infection by Enzyme Immunoassay as described by the manufacturer (Coulter, Hialeah, FL). A tissue culture infectious dose (TCID) was defined as the maximum dilution of virus where one of the CEM $\times$ 174 cultures became infected.

Neutralization assays

Plasma samples were obtained from two *M. nemestrina* (animals 93100 and 92263) infected with SIVMne, an uncloned mixture from which SIVMneCL8 was derived (Benveniste *et al.,* 1986), and a *Macaca mulatta* (animal 176) infected with SIVsmE660 (Hirsch and Johnson, 1994) (grown in macaque PBMCs). Plasma samples were obtained both prior to infection (prebleed) and 6 to 8 months postinoculation. All plasma samples were heat-inactivated for 30 min at 56°. sMAGI cells were added to a 96-well plate 24 hr prior to infection at a density of 3.5 $\times$ 10^3 cells per well. The following day, plasma was serially diluted twofold in complete DMEM to a final volume of 25 μl and added to an equal volume of virus. Typically, virus stocks were diluted in complete DMEM to 100–200 infectious particles per 25 μl. The virus/plasma mixture was incubated at 37° in a 5% CO_2 incubator. After 45 min, medium was removed from the sMAGI cells, and then the sMAGI cells were infected with the virus/plasma mixture. At the time of infection, DEAE-Dextran was added to the virus/plasma mixture at a final concentration of 15 μg per milliliter. After 2 hr of infection, an additional 200 μl of complete DMEM was added. Three days after infection, medium was removed and the cells were fixed and stained as described above. Neutralization was scored by comparing the average number of blue infectious centers in two wells in the presence of test plasma (V_n) with the number of infectious centers in the absence of plasma (V_0). In each experiment, replicas of eight were used to determine the average V_0. Dilutions were plotted against V_n/V_0, and the dilution that reduced V_n to 10% of V_0 ($V_n/V_0 = 0.1$) was scored as the neutralizing titer, as described by Nara *et al.* (1987). As a negative control, prebleed plasma from all three animals was tested against each virus. Scoring of blue nuclei in a 96-well format was greatly enhanced using an eyepiece reticule (10 $\times$ 10 grid) and a planar lens (Zeiss 2.5$\times$) to visualize the entire well.

The levels of antibody specific for SIV gp160 were determined by ELISA, as described previously (Haigwood *et al.,* 1992b; Planelles *et al.,* 1991), with the following modification: recombinant SIVMneCL8 envelope gp160 was purified from vaccinia virus-infected cells infected with Vac-160 (Hu *et al.,* 1995).

RESULTS

Identification of sMAGI cell lines

We engineered the CMMT adherent rhesus macaque mammary tumor cell line to express human CD4 and an HIV-1 LTR-β-*gal* fusion. We examined the susceptibility to SIV infection of randomly chosen G418 (linked to CD4) and hygromycin-B (linked to LTR-β-*gal*) resistant clones by scoring blue nuclei in cells infected with SIVMneCL8. To exclude clones with a high background of cells expressing detectable β-gal activity, we also examined uninfected cells for blue nuclei. Of the 30 CMMT-CD4-LTR-β-*gal* clones that we analyzed, 2 (clones 19 and 21) had reproducibly high numbers of blue nuclei after infection with SIVMneCL8 (Fig. 1A) and a low number of blue nuclei after mock-infection (Fig. 1B). We used clone 21 for subsequent experiments because there was a slightly lower number of background blue nuclei in uninfected cells. Based on the similarities between these cells and the MAGI cells described by Kimpton and Emerman (1992), we renamed the CMMT-CD4-LTR-β-*gal* (clone 21) cells the simian MAGI (sMAGI) cell line.

Sensitivity of the sMAGI cells to SIV infection

We tested the sensitivity of the sMAGI cells to SIV infection by comparing the infectious titer of SIVMneCL8 by sMAGI assay versus limiting dilution in CEM $\times$ 174 cells, a cell line highly susceptible to SIV infection. sMAGI cells were infected with seven dilutions of virus in duplicate. At 3 days, cells were stained and a sMAGI infectious dose was determined by counting the number of blue infectious centers. Clusters of blue nuclei found in syncytia or dense clusters of blue nucleated cells, which probably result from the replication of infected cells, were scored only once. Typically, infections of sMAGI cells with viral dilutions that result in 50 to 1000 blue cells can be counted accurately in 12-well plates and are in the linear range of the assay (data not shown). In 12-well plates, an average of 5 blue nuclei, based on 25 experiments, were observed in uninfected controls (data not shown). The sMAGI infectious titer of this stock

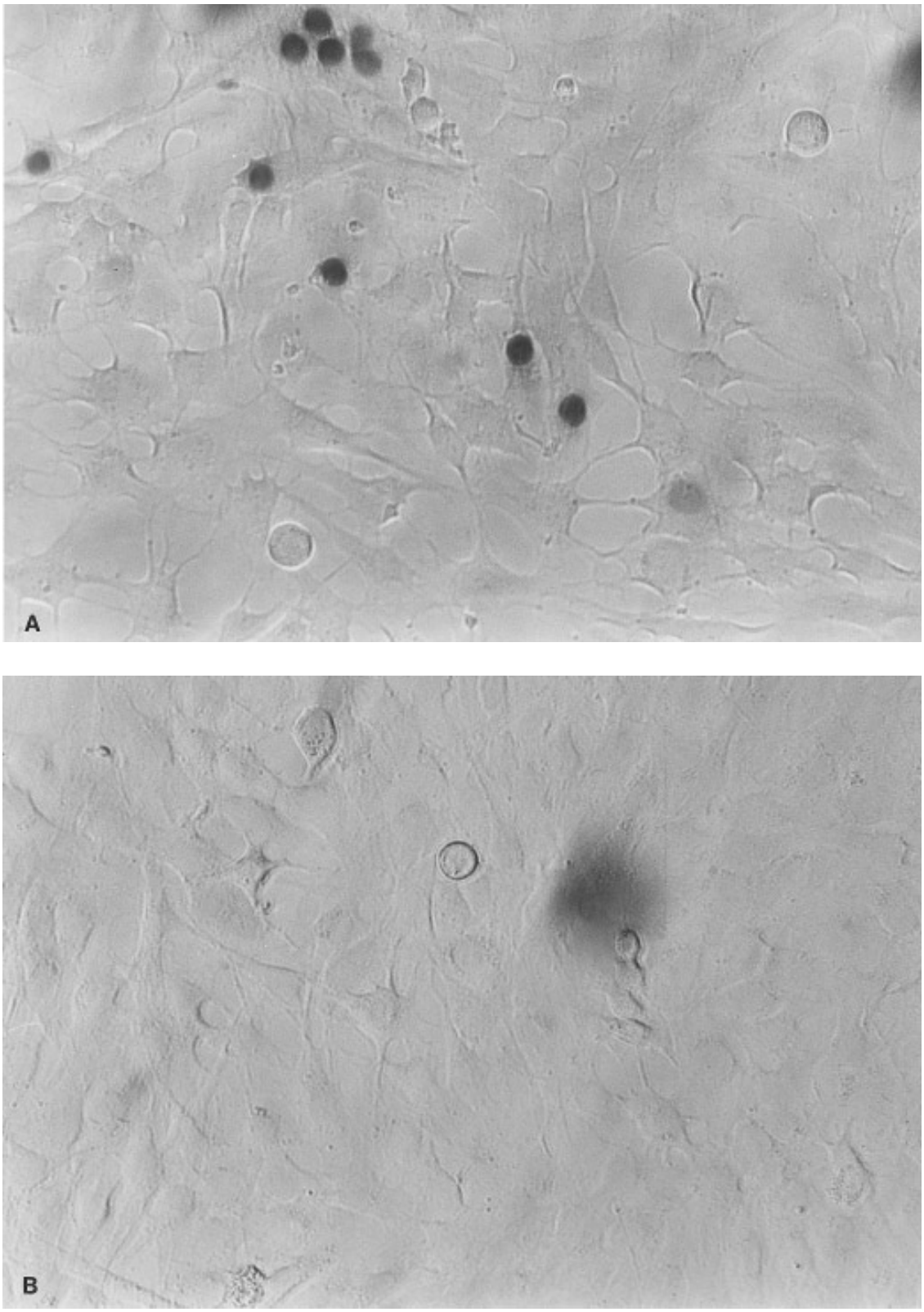

FIG. 1. Activation of β-gal expression in SIV-infected sMAGI cells. Cells were infected, fixed, and stained as described under Materials and Methods. SIVMneCL8- (A) or mock (B)-infected cells are shown. Magnification, ×200.

of SIVMneCL8 was 5×10^4 per milliliter. The TCID of this virus stock was determined in parallel in CEM × 174 cells and was also 5×10^4 per milliliter (Table 1A).

SIV isolates differ in tropism and replication rate in different cell lines. To assess the infectivity of the sMAGI cell line, we tested six CEM × 174-derived virus stocks,

TABLE 1

Quantitative Analysis of SIV Infectious Dose by sMAGI Assay Compared to Endpoint Dilution Infection in CEM × 174 Cells

Virus	sMAGI titer[a]	CEM × 174 titer[b]	sMAGI titer / CEM titer
A			
SIVMneCL8	5×10^4	5×10^4	1
SIVMne170	1×10^4	5×10^3	2
M87004 35wk	2×10^5	5×10^4	4
M87004 170wk	2×10^6	5×10^5	4
SIVsmE660	2×10^5	1×10^5	2
SIVmac239	4×10^4	5×10^3	8
B			
M87004 170wk[CEM × 174]	2×10^6	5×10^5	4
M87004 170wk[C8166]	2×10^6	5×10^5	4
M87004 170wk[PBMC]	3×10^4	3×10^4	1
SIV mac1A11[PBMC]	2×10^5	1×10^5	2

[a] Number of blue nucleated cells per milliliter of cell-free virus stock.
[b] TCID per milliliter, computed as described under Materials and Methods.

representing a wide variety of SIV isolates from the SIVsm/SIVmac/SIVMne group of primate lentiviruses, for their ability to infect the sMAGI cell line. The viruses included T-cell [SIVmac239 (Banapour *et al.,* 1991a,b) and M87004 170wk (Rudensey *et al.,* 1995)]- and macrophage-tropic [SIVmac1A11 (Banapour *et al.,* 1991a,b) and SIVMneCL8 (Rudensey *et al.,* 1995)] SIV isolates. We compared the sMAGI infectivity titer of the six CEM × 174-derived virus stocks with the TCID obtained by limiting dilution infection of the CEM × 174 cell line. These results are summarized in Table 1A. The sMAGI infectious dose was always the same or higher (up to eightfold) than the TCID in CEM × 174 cells. We consistently observed an eightfold difference between assays with SIVmac239.

In order to determine whether viruses expressed in different cells are equally infectious for sMAGI cells, the infectious titer of stocks of the T-cell-tropic M87004 170wk SIVMneCL8 variant mixture grown in either C8166 cells or macaque PBMCs was determined using the sMAGI assay and CEM × 174 endpoint dilution (Table 1B). These stocks (as well as the M87004 170wk pool grown in CEM × 174 cells) contain very similar populations of virus (Rudensey *et al.,* 1993). We also tested the macrophage-tropic clone SIVmac1A11 grown in macaque PBMCs. Again, the sMAGI assay shows similar sensitivity in comparison with the endpoint dilution method for each of these virus isolates; sMAGI infectious titers were equal to or up to fourfold greater than the TCID in CEM × 174 cells.

Titers of rapidly replicating SIV strains may be slightly inflated using the sMAGI assay

The sMAGI assay measures the number of infectious particles during a 3-day infection. The possibility exists that rapidly replicating strains of SIV, like SIVmac239 and the M87004 170wk pool, may spread in the sMAGI cells during the 3-day infection and inflate calculations of virus titer. To test this, we infected sMAGI cells with similar numbers of infectious particles (approximately 100) of SIVmac239, SIVMneCL8, or the M87004 170wk pool. Some sMAGI cells were infected in the presence of 5 μM AZT, which inhibits virus replication at the step of reverse transcription and thus limits virus spread. As a control, AZT was added prior to infection, and only one to three blue nuclei were observed in these cells (data not shown), indicating that the amount of AZT added was sufficient to inhibit infection. AZT was added at 24 or 48 hr after infection to prevent virus spread. Cells were stained and counted 72 hr after infection. Based on studies of HIV-1 kinetics, most of the infectious particles in the original inoculum infect cell lines during the first 12 hr of infection (Kim *et al.,* 1989; Pellegrino *et al.,* 1991). Thus, at 24 hr we would expect most of the original virus that was infectious to have entered the cell. Treatment with AZT at this point would be predicted to inhibit virus spread, but not affect infection of the original inoculum. To analyze virus spread in the sMAGI cells, we compared the number of infected cells when AZT was added at 24 hr with the number of infected cells when AZT was added later. The number of blue nuclei were counted in infections treated with AZT at 24 and 48 hr and in the untreated culture. In Fig. 2 these data are normalized by dividing the number of blue nucleated (infected) cells scored in each case by the number of blue cells scored in wells in which infected cells were treated with AZT 24 hr after infection. Infection with SIVmac239 resulted in a steady increase in the number of blue cells; there were approximately 1.5 times more blue nucleated cells after 2 days of infection (treatment with AZT at 48 hr) and 2.5 times more blue nucleated cells after 3 days of infection (no AZT) (Fig. 2), indicating that SIVmac239 spread in the

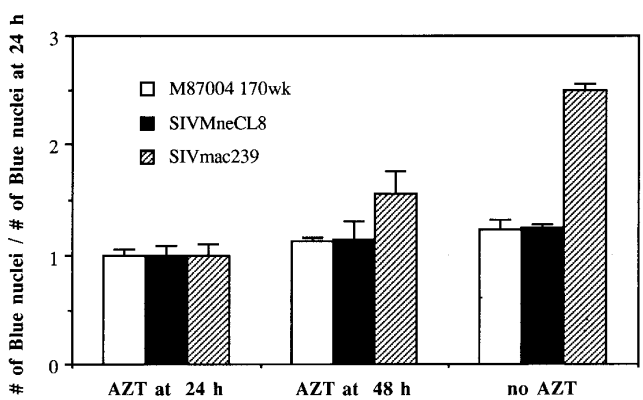

FIG. 2. Comparison of the infection kinetics of SIVMneCL8, M87004 170wk [CEM × 174], and SIVmac239 in sMAGI cells. Cells were infected in triplicate for 3 days. To compare the extent of virus spread, we normalized the data by dividing the number of blue nucleated cells scored in each case by the number of blue cells scored in wells in which infected cells were treated with AZT 24 hr after infection. Error bars indicate the SD of the data.

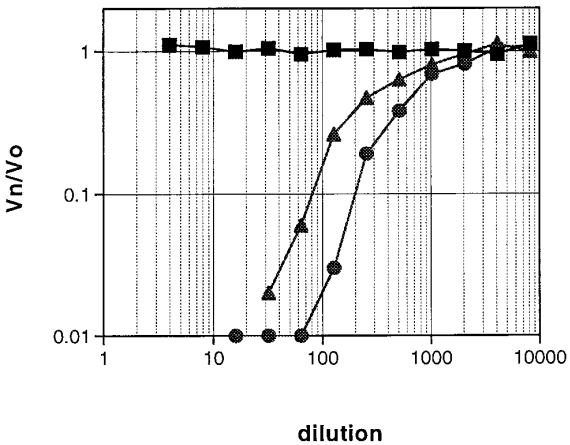

FIG. 3. Neutralization of SIVMne with diluted plasma from macaques 92263, 93100, and 176. Neutralization was scored for each sample by determining the value V_n/V_0, where V_n is the average number of blue nuclei in the presence of test plasma, and V_0 is the number of blue nuclei in the absence of plasma. Plasma from (▲) Macaque 92263, (●) Macaque 93100, and (■) Macaque 176.

sMAGI cells during the 3-day assay period. Thus, the infectious titer of SIVmac239 may be slightly inflated when using the sMAGI assay. If this inflation was taken into account, sMAGI infectious titers of SIVmac239 were about 3 times higher than the TCID on CEM × 174 cells, which is in the range (i.e., 1- to 4-fold) of the other isolates that we tested. Thus, accurate sMAGI infectious titers of SIVmac239 may be obtained by treating infected sMAGI cells with AZT 24 hr after infection. SIVMneCL8 and the M87004 170wk pool spread slowly in the sMAGI cell line, as indicated by the fact that the number of infected cells stayed fairly constant whether AZT was added at 24 hr or not (Fig. 2). Thus, viral spread did not dramatically influence our calculations of the sMAGI infectious titer of these virus isolates.

Neutralization assay using sMAGI cells

We were interested in determining the extent of neutralizing activity of plasma from macaques infected with different strains of SIV against homologous and heterologous virus. Plasma from two *M. nemestrina* infected with uncloned SIVMne or a *M. mulatta* infected with SIVsmE660 was tested for virus neutralization activity against SIVMne, SIVsmE660, and SIVmac239 (all grown on CEM × 174 cells) using the sMAGI assay. Plasma was tested in duplicate, using twofold dilutions from 1:4 to 1:8192. Figure 3 demonstrates the range of the assay for neutralization of SIVMne. SIVMne was strongly neutralized by plasma from both of the macaques infected with SIVMne, at titers of 1:200 and 1:80, but not neutralized by plasma from the SIVsmE660-infected macaque (titer of <1:4). Plasma neutralization of all three virus strains is shown in Table 2. While neutralization of SIVMne was the highest in the SIVMne-infected macaques, both sets of plasma also cross-neutralized SIVsmE660 at a low level (1:8 and 1:14) and both neutralized SIVmac239 at a low but detectable level (1:4). Plasma from the SIVsmE660-infected macaque neutralized SIVsmE660 at a titer of 1:140, but failed to show any neutralizing activity against SIVMne or SIVmac239. Prebleed plasma from all three animals failed to neutralize any of the SIV isolates tested (titer of <1:4), demonstrating that neutralizing antibodies are specific to SIV. All three of the animals tested had no clinical signs of AIDS at the time of testing and the pattern of antibody production represented typical humoral immune responses (data not shown).

HIV-1 and HIV-2 infection of sMAGI cells

Because the sMAGI cells were susceptible to infection by all isolates of SIV examined, we tested the sensitivity of the sMAGI cell line to infection by other primate lentiviruses, HIV-1 and HIV-2. We determined the infectivity of several strains of HIV-1 and HIV-2 in the simian CMMT-CD4 (sMAGI) cells compared to the human HeLa-CD4 (MAGI) cells (Kimpton and Emerman, 1992), CEM × 174 cells, or human PBMCs (Table 3). The sMAGI cells were not sensitive to infection by either of the laboratory-adapted HIV-1 strains that we tested, HIV-1 BAL (grown in CEM × 174 cells) and HIV-1 LAI (grown in both CEM × 174 cells and human PBMCs), nor were they susceptible to infection by several primary isolates of HIV-1. Cells were infected with 5 to 100 μl of two uncharacterized mixtures of virus isolated from HIV-1-seropositive Kenyan individuals (MM1-2201 and BB2-2847) and one uncharacterized mixture isolated from an HIV-1-seropositive American (WH91-330). We observed background numbers of blue nuclei when we infected with any of these virus populations, even if the sMAGI cells were passaged for a week after infection before staining.

In contrast, the sMAGI cell line was susceptible to HIV-2 infection, although the infectivity varied among the strains that we tested (Table 3B). The infectivity titers of HIV-2 strains KR and CBL-20 were approximately 100-fold lower by the sMAGI assay compared to the MAGI assay or endpoint dilution on human PBMCs. However, sMAGI cells were relatively more sensitive to infection by HIV-2 strains EHO, ROD, and D194; infectivity titers were about 5- to 10-fold less by the sMAGI assay compared to the MAGI assay or endpoint dilution on human PBMCs. The infectivity for the sMAGI cells correlates with their relative ability to infect macaque PBMCs, and both HIV-2 EHO and HIV-2 ROD have been shown to persistently infect rhesus macaques (Livartowski *et al.,* 1992).

DISCUSSION

We have developed a rhesus macaque cell line that has broad sensitivity to infection by numerous SIV isolates as well as some sensitivity to HIV-2 infection and is useful in detecting infectious virus after a single cycle

TABLE 2

Neutralization Titers of Plasma versus SIVMne, SIVsmE660, and SIVmac239

Animal (infected with)	Time of sample	Env ELISA titer[b]	Neutralization titer against[a]		
			SIVMne	SIV smE660	SIVmac239
93100	Prebleed	<100	<4	<4	<4
(SIVMne)	6 months p.i.[c]	16,000	200	14	4
92263	Prebleed	<100	<4	<4	<4
(SIVMne)	6 months p.i.	15,000	80	8	4
176	Prebleed	<100	<4	<4	<4
(SIV smE660)	6 months p.i.	2500	<4	140	<4

[a] 90% neutralization, as described in the text.
[b] As described under Materials and Methods.
[c] p.i., postinoculation.

of viral replication. The CMMT line was initially chosen because previous reports indicated that a CMMT-CD4 line is permissive for SIV infection (Goldstein *et al.,* 1995; Hirsch *et al.,* 1994). The sMAGI cells are sensitive to infection by all the SIV strains that we tested, including (1) virus propagated in CEM × 174 cells, C8166 cells, and macaque PBMCs, (2) molecular clones and uncloned mixtures, (3) cell-line-adapted and primary isolates, and (4) T-cell- and macrophage-tropic isolates. sMAGI infectious titers were consistently one- to eightfold higher than the TCID defined by endpoint dilution in CEM × 174 cells. In the case of the virus (SIVmac239) which yields an eightfold higher sMAGI infectious titer, a portion of this difference could be attributed to virus spread in the sMAGI cells over the 3-day incubation period. The tendency for moderately inflated infectious titers with some viruses may be circumvented by the addition of AZT to sMAGI cells 24 hr after infection. Thus, for all the SIV isolates that we tested, the determination of infectious titer by the sMAGI assay is a highly sensitive and reproducible alternative to endpoint dilution in CEM × 174 cells.

TABLE 3

Sensitivity of Detection of HIV-1 and HIV-2 Isolates by the sMAGI Assay versus Other Assays

Virus	sMAGI Titer	Titer
A		
HIV-1 BAL	0	1×10^6 [a]
HIV-1 LAI [PBMC]	0	1×10^6 [b,c]
HIV-1 LAI [CEM]	0	1×10^7 [b,c]
HIV-1 (MM1-2201)	0	nd[d]
HIV-1 (BB2-2847)	0	nd[e]
HIV-1 WH91-330	0	1×10^7 [a]
B		
HIV-2 KR	8×10^2	2×10^5 [a,c]
HIV-2 CBL-20	5×10^3	2×10^5 [c]
HIV-2 EHO	2×10^5	1×10^6 [a,c]
HIV-2 ROD	2×10^3	2×10^4 [a,c]
HIV-2 D194	2×10^3	1×10^4 [c]

[a] TCID per milliliter in human PBMC.
[b] TCID per milliliter in CEM × 174 cells.
[c] Infectious dose by MAGI assay (blue cells per milliliter).
[d] Approximately 300 ng $p24^{gag}$ per milliliter.
[e] Approximately 70 ng $p24^{gag}$ per milliliter.

A key advantage of the sMAGI cells is that detection of virus requires only one round of viral replication. Because these cells allow us to directly compare a broad spectrum of viral isolates that have different replication rates, the sMAGI assay is ideally suited to determine neutralizing titers against different SIV isolates. Neutralization assays described to date (reviewed by D'Souza *et al.,* 1993) have utilized human cell lines or hybrid cell lines, such as CEM × 174, and readouts that are indirect, such as antigen production (Haigwood *et al.,* 1992a; Planelles *et al.,* 1991) or cytopathic effects (Montefiori *et al.,* 1988). Another widely applied assay that measures the inhibition of syncytium induction by SIVmac251 is limited to studies with that virus isolate (Langlois *et al.,* 1991). Here, we demonstrate the ability of the sMAGI neutralization assay to quantitate neutralizing antibody titers. We show the reactivity of neutralizing antibody from a macaque infected with one SIV strain against other common SIV strains. We can detect homologous as well as heterologous neutralizing activity because the sMAGI cells are equally susceptible to infection by diverse SIV isolates. Thus, this single-cycle infection assay circumvents previous difficulties in detecting the breadth of neutralizing activity in SIV-infected macaques to different variants.

Clearly, the sMAGI neutralization assay is adaptable for testing neutralizing activity using all three of the commonly utilized SIV isolates, as well as subclones of these isolates. In addition, this assay maintains several of the advantages of a commonly used assay to detect HIV-1 neutralization (Nara *et al.,* 1987). The sMAGI neutralization assay allows direct measurements of infectivity that are scored visually, the 3-day assay period is relatively short, and the assay yields statistically significant results.

The sMAGI neutralization assay will be useful in quantitatively comparing the homologous and heterologous neutralizing activity in macaques at various stages of disease progression.

The primate lentiviruses SIV, HIV-2, and HIV-1 share the same cellular receptor, CD4 (Dalgleish *et al.,* 1984; Klatzmann *et al.,* 1984; Sattentau *et al.,* 1988). However, these three viruses have distinct infectivity requirements in many human and nonhuman $CD4^+$ cell lines. For example, the CMMT-CD4 cell line is susceptible to SIV infection, but it is only partially sensitive to HIV-2 infection and is not sensitive to HIV-1 infection. In contrast, the HeLa-CD4 cell line is susceptible to infection by HIV-2 and many T-cell-tropic HIV-1 strains, but not macrophage-tropic HIV-1 or some SIV strains. SIV and HIV-2 have a similar ability to infect or induce cell fusion in a number of engineered $CD4^+$ primate and nonprimate cell lines, including U87/$CD4^+$ glioma cells (Clapham *et al.,* 1991; Dragic and Alizon, 1993) and $CD4^+$ cat and mink cell lines (McKnight *et al.,* 1994). Thus, these and our results support the idea that SIV and HIV-2 share similar requirements for infectivity that are distinct, in some cases, from HIV-1. SIV, however, appears to have a more restricted host range than HIV-2 on HeLa-CD4 cells and a number of other human cell lines (Clapham *et al.,* 1991; Koenig *et al.,* 1989), but, SIV is more infectious for CMMT-CD4 cells. Because of differences in sensitivity to SIV, HIV-2, and HIV-1, the CMMT-CD4 cell line may be useful for assessing viral and cellular determinants of infectivity.

ACKNOWLEDGMENTS

We thank Michael Emerman for many helpful suggestions and discussion, and for comments on the manuscript. We thank Bill Sutton and Lyle Rudensey for assistance with neutralization assays, and Paul Lewis and Vanessa Hirsch for additional comments on the manuscript. We also thank J. Victor Garcia for constructing the PA317/LT4SN cell line, Adam Geballe and Bob Harrington for providing the plasmid pEQ447, Mary Tomas for assistance with FACS analysis, and Lyle Rudensey, Jason Kimata, Michael Agy, Marta Marthas, Paul Luciw, Jan McClure, and Jan North for providing virus isolates. HIV-2 CBL-20/H9, HIV-2 D194/HUT78, p239SpE3′, p239SpSp5′, and the CEM × 174 cell line were obtained through the AIDS Research and Reference Reagent Program, AIDS Program, NIAID. This work was supported by the NIH (ROI AI34251).

REFERENCES

Banapour, B., Marthas, M. L., Munn, R. J., and Luciw, P. A. (1991a). *In vitro* macrophage tropism of pathogenic and nonpathogenic molecular clones of simian immunodeficiency virus (SIVmac). *Virology* 183, 12–19.

Banapour, B., Marthas, M. L., Ramos, R. A., Lohman, B. L., Unger, R. E., Gardner, M. B., Pedersen, N. C., and Luciw, P. A. (1991b). Identification of viral determinants of macrophage tropism for simian immunodeficiency virus SIVmac. *J. Virol.* 65, 5798–5805.

Benveniste, R. E., Arthur, L. O., Tsai, C. C., Sowder, R., Copeland, T. D., Henderson, L. E., and Oroszlan, S. (1986). Isolation of a lentivirus from a macaque with lymphoma: Comparison with HTLV-III/LAV and other lentiviruses. *J. Virol.* 60, 483–490.

Cheng-Mayer, C. (1990). Biological and molecular features of HIV-1 related to tissue tropism. *AIDS* 4(Suppl. 1), s49–s56.

Chesebro, B., and Wehrly, K. (1988). Development of a sensitive quantitative focal assay for human immunodeficiency virus infectivity. *J. Virol.* 64, 4553–4557.

Clapham, P. R., Blanc, D., and Weiss, R. A. (1991). Specific cell surface requirements for the infection of CD4-positive cells by human immunodeficiency virus types 1 and 2 and by simian immunodeficiency virus. *Virology* 181, 703–715.

Dalgleish, A. G., Beverley, P. C., Clapham, P. R., Crawford, D. H., Greaves, M. F., and Weiss, R. A. (1984). The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* 312, 763–767.

Dragic, T., and Alizon, M. (1993). Different requirements for membrane fusion mediated by the envelopes of human immunodeficiency virus types 1 and 2. *J. Virol.* 67, 2355–2359.

D'Souza, M. P., Kent, K. A., Thiriart, C., Collignon, C., and Millman, G. (1993). International collaboration comparing neutralization and binding assays for monoclonal antibodies to simian immunodeficiency virus. *AIDS Res. Hum. Retroviruses* 9, 415–422.

Felber, B. K., and Pavlakis, G. N. (1988). A quantitative bioassay for HIV-1 based on trans-activation. *Science* 239, 184–187.

Fenyo, E. M., Albert, J., and Asjo, B. (1989). Replicative capacity, cytopathic effect and cell tropism of HIV. *AIDS* 3, s5–s12.

Goldstein, S., Hague, B., Montefiori, D., and Hirsch, V. (1995). A macaque adherent cell line that expresses human CD4 is susceptible to SIV: Utility for assessing neutralizing antibody. *J. Virol. Methods* 53, 139–148.

Haigwood, N. L., Misher, L., Chin, S. M., Blair, M., Planelles, V., Scandella, C. J., Steimer, K. S., Gardner, M. B., Yilma, T., Hirsch, V. M., and Johnson, P. R. (1992a). Characterization of group specific antibodies in primates: Studies with SIV envelope in macaques. *J. Med. Primatol.* 21, 82–90.

Haigwood, N. L., Nara, P. L., Brooks, E., Van-Nest, G. A., Ott, G., Higgins, K. W., Dunlop, N., Scandella, C. J., Eichberg, J. W., and Steimer, K. S. (1992b). Native but not denatured recombinant human immunodeficiency virus type 1 gp120 generates broad-spectrum neutralizing antibodies in baboons. *J. Virol.* 66, 172–182.

Harrington, R. D., and Geballe, A. P. (1993). Cofactor requirement for human immunodeficiency virus type 1 entry into a CD4-expressing human cell line. *J. Virol.* 67, 5939–5947.

Hirsch, V., and Johnson, P. (1994). Pathogenic diversity of simian immunodeficiency viruses. *Virus Res.* 32, 183–203.

Hirsch, V. M., Goldstein, S., Hynes, N. A., Elkins, W. R., London, W. T., Zack, P. M., Montefiori, D., and Johnson, P. R. (1994). Prolonged clinical latency and survival of macaques given a whole inactivated simian immunodeficiency virus vaccine. *J. Infect. Dis.* 170, 51–59.

Hu, S.-L., Polacino, P., Stallard, V., Kaniecki, J., Pennathur, S., Travis, B. M., Misher, L., Kornas, H., Langlois, A. J., Morton, W. R., and Benveniste, R. E. (1995). Role of the transmembrane protein and core antigens in the protection against SIV infection. *Vaccines95,* in press.

Kestler, H., Kodama, T., Ringler, D., Marthas, M., Pedersen, N., Lackner, A., Regier, D., Sehgal, P., Daniel, M., King, N., and Desrosiers, R. (1990). Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. *Science* 248, 1109–1112.

Kim, S., Byrn, R., Groopman, J., and Baltimore, D. (1989). Temporal aspects of DNA and RNA synthesis during human immunodeficiency virus infection: Evidence for differential gene expression. *J. Virol.* 63, 3708–3713.

Kimpton, J., and Emerman, M. (1992). Detection of replication-competent and pseudotyped human immunodeficiency virus with a sensitive cell line on the basis of activation of an integrated beta-galactosidase gene. *J. Virol.* 66, 2232–2239.

Klatzmann, D., Champagne, E., Chamaret, S., Gruest, J., Guetard, D., Hercend, T., Gluckman, J. C., and Montagnier, L. (1984). T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature* 312, 767–768.

Koenig, S., Hirsch, V. M., Olmsted, R. A., Powell, D., Maury, W., Rabson, A., Fauci, A. S., Purcell, R. H., and Johnson, P. R. (1989). Selective infection of human $CD4^+$ cells by simian immunodeficiency virus:

Productive infection associated with envelope glycoprotein-induced fusion. *Proc. Natl. Acad. Sci. USA* 86, 2443–2447.

Kuhnel, H., von Briesen, H., Dietrich, U., Adamski, H., Mix, D., Biesert, L., Kreutz, R., Immelmann, A., Henco, K., Meichsner, C., Andreesen, R., Gelderblom, H., and Rubsamen-Waigmann, H. (1989). Molecular cloning of two West African human immunodeficiency virus type 2 isolates that replicate well in macrophages: A Gambian isolate from a patient with neurologic acquired immunodeficiency syndrome, and a highly divergent Ghanian isolate. *Proc. Natl. Acad. Sci. USA* 86, 2383–2387.

Langlois, A. J., Weinhold, K. J., Matthews, T. J., and Bolognesi, D. P. (1991). In vitro assays for detecting neutralizing and fusion-inhibiting antibodies to SIVMAC251. *AIDS Res. Hum. Retroviruses* 7, 713–720.

Livartowski, J., Dormont, D., Boussin, F., Chamaret, S., Guetard, D., Vazeux, R., Lebon, P., Metivier, H., and Montagnier, L. (1992). Clinical and virological aspects of HIV-2 infection in rhesus monkeys. *Cancer Detect. Prev.* 16, 341–345.

Marthas, M. L., Banapour, B., Sutjipto, S., Siegel, M. E., Marx, P. A., Gardner, M. B., Pedersen, N. C., and Luciw, P. A. (1989). Rhesus macaques inoculated with molecularly cloned simian immunodeficiency virus. *J. Med. Primatol.* 18, 311–319.

Marthas, M. L., Sutjipto, S., Higgins, J., Lohman, B., Torten, J., Luciw, P. A., Marx, P. A., and Pedersen, N. C. (1990). Immunization with a live, attenuated simian immunodeficiency virus (SIV) prevents early disease but not infection in rhesus macaques challenged with pathogenic SIV. *J. Virol.* 64, 3694–3700.

McElrath, M. J., Rabin, M., Hoffman, M., Klucking, S., Garcia, J. V., and Greenberg, P. D. (1994). Evaluation of human immunodeficiency virus type 1 (HIV-1)-specific cytotoxic T-lymphocyte responses utilizing B-lymphoblastoid cell lines transduced with the CD4 gene and infected with HIV-1. *J. Virol.* 68, 5074–5083.

McKnight, A., Clapham, P. R., and Weiss, R. A. (1994). HIV-2 and SIV infection of nonprimate cell lines expressing human CD4: Restrictions to replication at distinct stages. *Virology* 201, 8–18.

Montefiori, D. C., Robinson, W. E., Schuffman, S. S., and Mitchell, W. M. (1988). Evaluation of antiviral drugs and neutralizing antibodies to human immunodeficiency virus by a rapid and sensitive microtiter infection assay. *J. Clin. Virol.* 26, 231–235.

Morton, W. R., Kuller, L., Benveniste, R. E., Clark, E. A., Tsai, C. C., Gale, M. J., Thouless, M. E., Overbaugh, J., and Katze, M. G. (1989). Transmission of the simian immunodeficiency virus SIVmne in macaques and baboons. *J. Med. Primatol.* 18, 237–245.

Nara, P. L., Hatch, W. C., Dunlop, N. M., Robey, W. G., Arthur, L. O., Gonda, M. A., and Fischinger, P. J. (1987). Simple, rapid, quantitative, syncytium-forming microassay for the detection of human immunodeficiency virus neutralizing antibody. *AIDS Res. Hum. Retroviruses* 3, 283–302.

Overbaugh, J., Rudensey, L. M., Papenhausen, M. D., Benveniste, R. E., and Morton, W. R. (1991). Variation in simian immunodeficiency virus *env* is confined to V1 and V4 during progression to simian AIDS. *J. Virol.* 65, 7025–7031.

Pellegrino, M. G., Li, G., Potash, M. J., and Volsky, D. J. (1991). Contribution of multiple rounds of viral entry and reverse transcription to expression of human immunodeficiency virus type 1. *J. Biol. Chem.* 266, 1783–1788.

Planelles, V., Haigwood, N. L., Marthas, M. L., Mann, K. A., and Scandella, C. (1991). Functional and immunological characterization of SIV envelope. *AIDS Res. Hum. Retroviruses* 7, 889–898.

Rocancourt, D., Bonnerot, C., Jouin, H., Emerman, M., and Nicolas, J-F. (1990). Activation of a beta-galactosidase recombinant provirus: Application to titration of HIV and HIV-infected cells. *J. Virol.* 64, 2660–2668.

Rudensey, L. M., Kimata, J. T., Benveniste, R. E., and Overbaugh, J. (1995). Progression to AIDS in macaques is associated with changes in the replication, tropism, and cytopathic properties of the simian immunodeficiency virus variant population. *Virology* 207, 528–542.

Rudensey, L. M., Papenhausen, M. D., and Overbaugh, J. (1993). Replication and persistence of simian immunodeficiency virus variants after passage in macaque lymphocytes and established human cell lines. *J. Virol.* 67, 1727–1733.

Sattentau, Q. J., Clapham, P. R., Weiss, R. A., Beverley, P. C., Montagnier, L., Alhalabi, M. F., Gluckmann, J. C., and Klatzmann, D. (1988). The human and simian immunodeficiency viruses HIV-1, HIV-2 and SIV interact with similar epitopes on their cellular receptor, the CD4 molecule. *AIDS* 2, 101–105.

Schulz, T. F., Whitby, D., Hoad, J. G., Corrah, T., Whittle, H., and Weiss, R. A. (1990). Biological and molecular variability of human immunodeficiency virus type 2 isolates from the Gambia. *J. Virol.* 64, 5177–5182.

Viglianti, G. A., and Mullins, J. I. (1988). Functional comparison of transactivation by simian immunodeficiency virus from rhesus macaques and human immunodeficiency virus type 1. *J. Virol.* 62, 4523–4532.

von Briesen, H., Andreesen, R., and Rubsamen-Waigmann, H. (1990). Systematic classification of HIV biological subtypes on lymphocytes and monocytes/macrophages. *Virology* 178, 597–602.

White-Scharf, M., Potts, B. J., Smith, L. M., Sokolowski, K. A., Rusche, J. R., and Silver, S. (1993). Broadly neutralizing monoclonal antibodies to the V3 region of HIV-1 can be elicited by peptide immunization. *Virology* 192, 197–206.

Zack, J. A., and Overbaugh, J. (1994). SIV and HIV pathogenesis in animal systems. *AIDS* 8(Suppl. 1), s43–s52.