A murine monoclonal antibody (MAb) with human CD4 specificity was tested for the ability to inhibit primary human immunodeficiency virus type 1 (HIV-1) isolates clades A through E. Human peripheral blood mononuclear cells (PBMC) were used as target cells for infectivity. The HIV-1 primary isolates were examined for the capacity to infect PBMC targets in the presence or absence of the anti-CD4 MAb, designated P1. P1 broadly inhibited clade A, C, D, and E isolates, based on a reduction of HIV-1 p24 antigen concentrations compared with untreated controls. Little to no virus-inhibiting activity was observed with a primary HIV-1 clade B isolate, designated BZ167. Additionally, a second primary clade B isolate was efficiently inhibited from infecting PBMC targets by P1. The data indicate that P1 exhibits group-specific inhibiting activity against non-clade B primary HIV-1 isolates in vitro.

Materials and Methods

Virus stocks. HIV-1 primary isolates were obtained from Quality Biologicals (Gaithersburg, MD), the Aaron Diamond AIDS Research Center (New York), and the AIDS Research and Reference Reagent Program (Rockville, MD). Stocks were expanded in fresh human peripheral blood mononuclear cell (PBMC) blasts by weekly coculture. Briefly, 2 × 10^6 blasts were washed in saline and pelleted. Cell pellets were infected with 200 μL of virus stock for 2 h at 37°C with shaking every 15 min. After 2 h, the virus inoculum was washed away with saline, and the infected cells were resuspended in 2 mL of RPMI/interleukin-2 medium and plated into an individual well of a 24-well tissue culture plate. On day 4, 1 mL of the culture was removed and replaced with 1 mL of fresh medium. On day 7, 1 mL of culture was removed and replaced with 1 mL of 2 × 10^6 fresh blasts. The following HIV-1 isolates were used in neutralization assays: UG029 (Uganda) subtype A, A284 (Argentina) subtype B, IN905 (India) subtype C, UG001 (Uganda) subtype D, and KH005 (Thailand) subtype E. Each of the isolates induces syncytia in PBMC, and the stock titers ranged from 10^3 to 10^5 TCID₅₀/mL.

Antigen-capture ELISA. To quantify p24 levels in our experiments, we used a modification of an HIV-1 p24 antigen assay (Coulter, Miami). Briefly, individual wells of 96-well microtiter plates were coated with 50 μL of MAb anti-p24 (10 μg/mL) diluted in borate-buffered saline (BBS) for 1 h at 37°C. The unbound sites were blocked by addition of 200 μL of 5% goat serum diluted in BBS (GSBBS) for 30 min at 37°C. The wells were then washed five times with BBS containing 0.05% Tween 20 (BBST). Samples (50 μL) were loaded and incubated for 1 h at 37°C and washed five times with BBST. The anti-p24 biotin reagent from the kit was then diluted 1:4 in GSBBS, and 50-μL volumes were added to the wells for 1 h at 37°C. Unbound material was washed away with five washes with BBST. Finally, 50 μL of a predetermined dilution of streptavidin–horseradish peroxidase diluted in GSBBS was added for 30 min at 37°C. Unbound streptavidin–horseradish peroxidase was washed away with BBST, and the assay was developed by addition of 50 μL of tetramethylbenzidine. The development was stopped after 30 min by addition of 20 μL of 1 M HCl, and the assay was read at 450 nm in a microplate reader. Each point was assayed in duplicate, and the linear range of this modified HIV-1 p24 assay, based on optical density at 450 nm, was 0.12–
Results

Kinetics of viral replication. Before examining the ability of P1 to inhibit the various primary isolate clades, it was necessary to determine the kinetics of HIV-1 replication in human PBMC. We infected phytohemagglutinin-blasted PBMC as described above. Inocula of 1, 10, and 100 TCID₅₀/mL were tested. Samples were taken at days 4, 7, and 10 and assayed by p24 antigen capture. In figure 1, the kinetics of HIV-1 (UG001) replication are depicted. On the basis of HIV-1 p24 levels, at 100 TCID₅₀/mL inoculum, virus titers are maximum and constant from days 4, 7, and 10. At 10 TCID₅₀/mL, the virus titers steadily increase from day 4 to day 10. At 1 TCID₅₀/mL, the titers are not sufficient even at day 10. For evaluating the inhibitory activity of the anti-CD4, a 100 TCID₅₀/mL inoculum was selected for further studies. We performed similar studies with the other HIV-1 isolates (data not shown) and also found 100 TCID₅₀/mL to be optimal.

Effect of P1 on PBMC viability. Before testing for inhibition, it was necessary to test whether P1 itself had any toxic effect on the PBMC blasts. To evaluate toxicity, we used the MTT cell viability assay. No significant decrease in viability was observed in the presence of P1 at 10 and 100 μg/mL (data not shown). The results indicate that P1 is not toxic to the PBMC blasts that will serve as targets for subsequent infectivity assays.

Inhibition of HIV-1 primary isolates by MAb anti-CD4. Levels of HIV-1 p24 in cultures of human PBMC infected with representative primary isolates in the presence or absence of anti-CD4 MAb P1 are shown in table 1. On days 4, 7, and 10, the clade A isolate was inhibited with 10 μg/mL by 91%, 7%, and 0, and with 100 μg/mL by 91%, 100%, and 98%, respectively. Clade B (BZ167) was inhibited with 10 μg/mL by 54%, 0, and 0, and with 100 μg/mL by 91%, 15%, and 12%, respectively. The clade C isolate was inhibited with 10 μg/mL by 76%, 97%, and 91%, and with 100 μg/mL by 84%, 95%, and 98%. Similarly, 10 μg/mL decreased clade D by 100%, 93%, and 90%, and 100 μg/mL by 100%, 100%, and 100%, respectively. The clade E isolate was inhibited by 91%, 99%, and 98% and by 90%, 98%, and 98% with 10 μg/mL and 100 μg/mL, respectively. In replicate cultures that contained the same concentrations of a control MAb specific for hepatitis B surface antigen, inhibition of HIV-1 infectivity was <15%. To address the issue regarding the lack of efficient inhibitory activity against the clade B isolate, we examined a second clade B isolate, along with other primary isolates.
representing the other clades. P1 efficiently inhibited the second clade B isolate (A284), similar to the levels of inhibition observed with the representative clade A, C, D, and E isolates (data not shown).

Discussion

In this study, we examined the inhibition potential of an anti-CD4 MAb, P1, on HIV-1 primary isolates. We first determined, in vitro, that P1 did not affect the viability of the PBMC preparations that were used as target cells in the inhibition assay. The inhibition results demonstrate that the anti-CD4 MAb, P1, exhibits broad inhibitory activity against HIV-1 clade A, C, D, and E isolates, based on a reduction of HIV-1 p24 antigen concentrations compared with untreated controls and controls treated with an irrelevant MAb. Inhibitory concentrations of P1 (100 µg/mL) are well within the range of anti-CD4 MAbs that have been infused into patients and maintained for weeks at a time with no deleterious effects [10]. Interestingly, little to no inhibition activity was observed with the primary HIV-1 clade B isolate BZ167. This lack of inhibitory activity against the BZ167 isolate did not appear to reflect infectious titer. Other clades that exhibited titers higher than or equivalent to that of BZ167 were efficiently inhibited by P1. If the input titer of BZ167 was reduced to 10 TCID₅₀, P1 was capable of neutralizing infectivity (data not shown). The reasons for the inability of P1 to effectively inhibit this primary HIV-1 clade B isolate at titers at which other non–clade B isolates are neutralized is unknown. It is possible that BZ167 is not representative of other clade B primary isolates. Indeed, a second clade B primary isolate, designated A284, along with other non–clade B isolates, were inhibited from infecting PBMC by P1, similar to the data presented in table 1 (data not shown). This suggests that BZ167 is not representative of other clade B primary isolates. These data suggest that BZ167 may infect PBMC without the need for a CD4 interaction or may interact with a CD4 epitope not recognized by P1 and different from the CD4 epitope used by the other primary isolates of HIV-1.

References