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Cell-to-Cell Transmission of Human Immunodeficiency Virus Type 1 in the Presence of Azidothymidine and Neutralizing Antibody

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Very few peripheral blood lymphocytes of seropositive individuals are presumably actively infected with human immunodeficiency virus type 1 (HIV-1). During coculture of lymphocytes of a seropositive individual with mitogen-stimulated normal peripheral blood lymphocytes, the number of infected cells becomes amplified such that detectable HIV-1 is produced. We report here that in addition to transmission by extracellular virus, cell-to-cell transmission is responsible for spreading HIV-1 infection from infected to uninfected cells. Azidothymidine and virus-neutralizing antibody had no effect on cell-to-cell transmission of HIV-1. Monoclonal antibodies to the CD4 receptor, but not to the CD3 receptor, prevented cell-to-cell transmission, which suggests that CD4 receptor-mediated cell fusion is involved in cell-to-cell transmission. Spread of infection in a cell-to-cell manner may be important in development of drug therapies for HIV-1 infection.

Human immunodeficiency virus type 1 (HIV-1) has been recognized as the etiologic agent of acquired immune deficiency syndrome (AIDS) (2, 7, 9). It is thought that continued virus infection of $CD4^+$ T cells may be responsible for the progressive loss of immune function. In situ hybridization studies suggest that 1 in 10^5 peripheral blood lymphocytes (PBL) is actively infected in patients with AIDS and those in a pre-AIDS status (8). HIV-1 can be recovered in vitro from PBL of seropositive men when infected cells are amplified by coculture with phytohemagglutinin (PHA)-stimulated normal PBL (10). Studies from our laboratory have indicated that HIV-1 can be isolated from more than 80% of seropositive asymptomatic men and 95% of patients with AIDS when the lymphocytes are cocultured with PHA-stimulated normal PBL (1). There are two major ways that HIV-1 infection could spread from cell to cell. First, HIV-1 produced from an infected cell may infect a neighboring, uninfected cell. Second, HIV-1 infection could be transmitted from cell to cell without being exposed to the extracellular environment. Such cell-to-cell transmission has been described for herpes simplex (3), varicella-zoster (17), and rabies (12) viruses. Work with HIV-infected cells has also shown that fusion occurs between infected cells expressing the HIV-1 envelope protein and uninfected cells expressing the CD4 receptor on the cell membrane. In this report, we present findings which indicate that HIV-1 infection can spread from infected cell to uninfected cell by cell-to-cell transmission, regardless of whether infected cells are from seropositive asymptomatic men or from patients with AIDS. Azidothymidine (AZT) and virus-neutralizing antibody were found to have no effect on cell-to-cell transmission of HIV-1.

We investigated the phenomenon of cell-to-cell transmission by examining virus production in infected cells cocultured with PHA-stimulated PBL in the presence of AZT, which blocks the spread of infection by extracellular virus (14). For this purpose, we first determined the conditions under which AZT inhibited infection by cell-free HIV-1. We found that the continuous presence of AZT at a concentration of 0.1, 1.0, or 10.0 μM completely inhibited production of HIV-1 for 24 days in PHA-stimulated PBL infected with cell-free virus isolated from a chronically infected H9 cell line (Fig. 1).

Next, lymphocytes from seropositive men were cocultured with PHA-stimulated normal uninfected PBL at various ratios of lymphocytes to uninfected PBL in the presence of 1 μM AZT. Representative results of cocultivation experiments with lymphocytes from asymptomatic men and patients with AIDS are shown in Fig. 2. HIV-1 was readily detected in all cultures incubated with AZT. However, the kinetics of HIV-1 production in the presence of AZT depended on the ratio of patients' lymphocytes to PHA-stimulated normal PBL. The time of appearance of HIV-1 was shortest (4 to 7 days) in cultures with 10×10^6 lymphocytes from patients with AIDS and longest (18 days) in cultures with 0.8×10^6 lymphocytes, with a constant amount (5×10^6) of uninfected PBL added in each case. In the absence of the drug, however, HIV-1 was readily detected by 4 to 7 days in all cultures regardless of the number of patients' lymphocytes. Although the kinetics of HIV-1 production varied from patient to patient, a delay in virus production in the presence of AZT was observed in all eight experiments. Moreover, similar delayed appearance of HIV-1 was also observed when lymphocytes from a seropositive patient were cocultured with mitogen-stimulated normal PBL in the presence of 10 μM AZT (data not shown).

Since HIV-1 was produced at normal levels after a lag period in drug-treated cultures, we examined whether resistant virus was generated in these cocultures. For this purpose, 1 ml of virus sample recovered from lymphocytes of a patient with AIDS at 21 days after cocultivation in the presence of 1.0 μM AZT was inoculated into PHA-stimulated PBL. After infection, cells were cultured for 14 days in the absence or presence of 1.0 μM AZT. The drug completely inhibited the production of HIV-1 as determined by the antigen capture test.

The phenomenon of cell-to-cell transmission of HIV-1 was further investigated by inhibiting extracellular virus with neutralizing antibody during cocultivation. To determine the amount of antibody required to neutralize extracellular HIV-1, we first determined the level of HIV-1 production from

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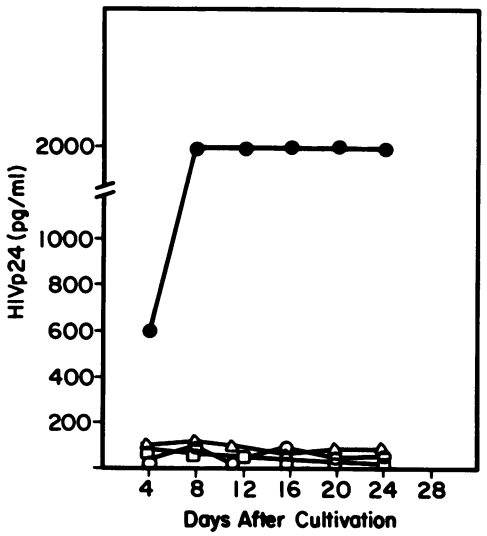

FIG. 1. Effect of AZT on infection of PHA-stimulated PBL by cell-free HIV-1. PHA-stimulated normal PBL (5×10^6) were infected with 1 ml of cell-free HIV-1 (2×10^5 cpm/ml of reverse transcriptase activity) for 1 h at 37°C as described previously (1). Cells were then washed and cultured in the absence (●) and presence of 0.1 μM (□), 1 μM (△), and 10 μM (○) AZT. Incubation was continued for 25 days. Virus production was monitored by measuring the HIV p24 antigen in the culture supernatant, using the antigen capture test.

patients' lymphocytes cultured alone. For this purpose, 0.8×10^6, 3×10^6, and 10×10^6 lymphocytes from a patient with AIDS and from a seropositive asymptomatic man were cultured in the absence of PHA-stimulated normal PBL. No significant levels of virus were produced by 0.8×10^6 to 10×10^6 lymphocytes from the asymptomatic man. Although no HIV-1 was produced from 0.8×10^6 lymphocytes from the patient with AIDS, significant levels of HIV-1 were produced from 3×10^6 to 10×10^6 lymphocytes from the same patient.

Second, we measured the virus-neutralizing antibody titer of a number of sera from seropositive asymptomatic men enrolled in the Multicenter AIDS Cohort Study in Pittsburgh. The virus-neutralizing antibody titer was determined as the reciprocal of the highest serum dilution that neutralized more than 90% of infectivity of 2.5×10^5 RT units of HIV-1 in PHA-stimulated PBL. The serum with a virus-neutralizing titer of more than 500 was used in this experiment.

To examine the phenomenon of cell-to-cell transmission in the presence of virus-neutralizing antibody, various numbers of patients' lymphocytes were cocultured with 5×10^6 PHA-stimulated PBL in the presence of the neutralizing antibody at a final concentration of 1:50, which was 10-fold higher than that required to neutralize HIV-1 produced from 10×10^6 lymphocytes cultured alone from a patient with AIDS. Results for lymphocytes from a patient with AIDS and from a seropositive asymptomatic man are shown in Fig. 4. Neutralizing antibody delayed the production of HIV-1 in cultures with 1×10^6 to 3×10^6 lymphocytes from the patient with AIDS and from the asymptomatic man. Moreover, a shift in the kinetics of HIV-1 production with the increase in the number of these lymphocytes was observed regardless of whether the culture was initiated with lymphocytes from a patient with AIDS or from an asymptomatic man. The virus-neutralizing antibody had very little effect on HIV-1 production in cultures initiated with 10×10^6 lymphocytes from the patient with AIDS and from the asymptomatic man (data not shown).

We examined whether HIV-1 produced in the presence of virus-neutralizing antibody represented nonneutralizable HIV-1 variants. For this purpose, 1 ml of HIV-1-containing supernatant fluid, recovered from an experiment in which lymphocytes from a patient with AIDS were cocultured for 22 days in the presence of neutralizing antibody, was microfuged for 2 h to separate unbound antibody from virus particles. The HIV-1 particles thus recovered were found to be neutralizable by the virus-neutralizing antibody used in the coculture experiment (data not shown).

The fusion process (syncytium formation), which is known to occur between HIV-1-infected and uninfected cells, is presumably responsible for bringing uninfected and infected cells together and for eventual cell-to-cell transmission of HIV-1. Since the fusion depends on expression of the CD4 receptor in uninfected cells and on expression of the HIV envelope protein on the surfaces of infected cells (4, 11), we examined the effect of antibodies to the CD4 receptor on HIV-1 production in a patient's lymphocytes cocultured with PHA-stimulated PBL. For this purpose, 2×10^6 lymphocytes from a patient with AIDS were cocultured with 5×10^6 mitogen-stimulated PBL in the presence of a mouse monoclonal antibody to the CD4 or the CD3 receptor. The anti-CD4 antibody completely suppressed production of HIV-1 during a 25-day incubation period (Fig. 5). However, the anti-CD3 antibody did not inhibit production of HIV-1, although it delayed the appearance of HIV-1 in the culture supernatant in comparison with results for the untreated control culture. Monoclonal antibody at the concentration used in this experiment was not toxic for PBL. In a separate experiment, we found that pretreatment of PHA-stimulated PBL with either anti-CD4 (1:10) or anti-CD3 (1:10) antibody inhibited infection of these cells by a cell-free virus preparation. During coculture of lymphocytes from patients with AIDS with PHA-stimulated normal PBL, both cell-to-cell transmission and infection by extracellular virus are presumably responsible for the spread of infection to uninfected cells. The fact that the anti-CD4 antibody inhibited HIV-1 production in lymphocytes from patients with AIDS indicates that the antibody not only inhibits infection by extracellular virus but probably also blocks virus production resulting from cell-to-cell transmission of HIV-1. The anti-CD3 antibody, on the other hand, blocked infection by cell-free virus but probably had no effect on cell-to-cell transmission. This finding is in agreement with the data reported by Dalgleish et al. (4), who found that anti-CD3 did not inhibit the HIV-1-induced fusion process. However, it is not clear why anti-CD3 antibody inhibited infection by cell-free virus. McDougal et al. (13) also reported such an inhibition of HIV-1 infection of PHA-stimulated PBL by anti-CD3 antibody at a high concentration (1:5). It is possible that this antibody recognizes antigen-bearing molecules other than CD3 that may be involved in HIV-1 adsorption and penetration but may not participate in cell-to-cell transmission of HIV-1.

The results presented here indicate that infected lymphocytes cultured alone do not produce a significant level of HIV-1, presumably because of the presence of very low numbers of productively infected cells. Amplification of infected cells by cocultivation with mitogen-stimulated PBL is necessary to produce high levels of HIV-1. This conclusion is in accordance with the data reported by Levy and Shimabukuro (10), which indicate that cocultivation is nec-

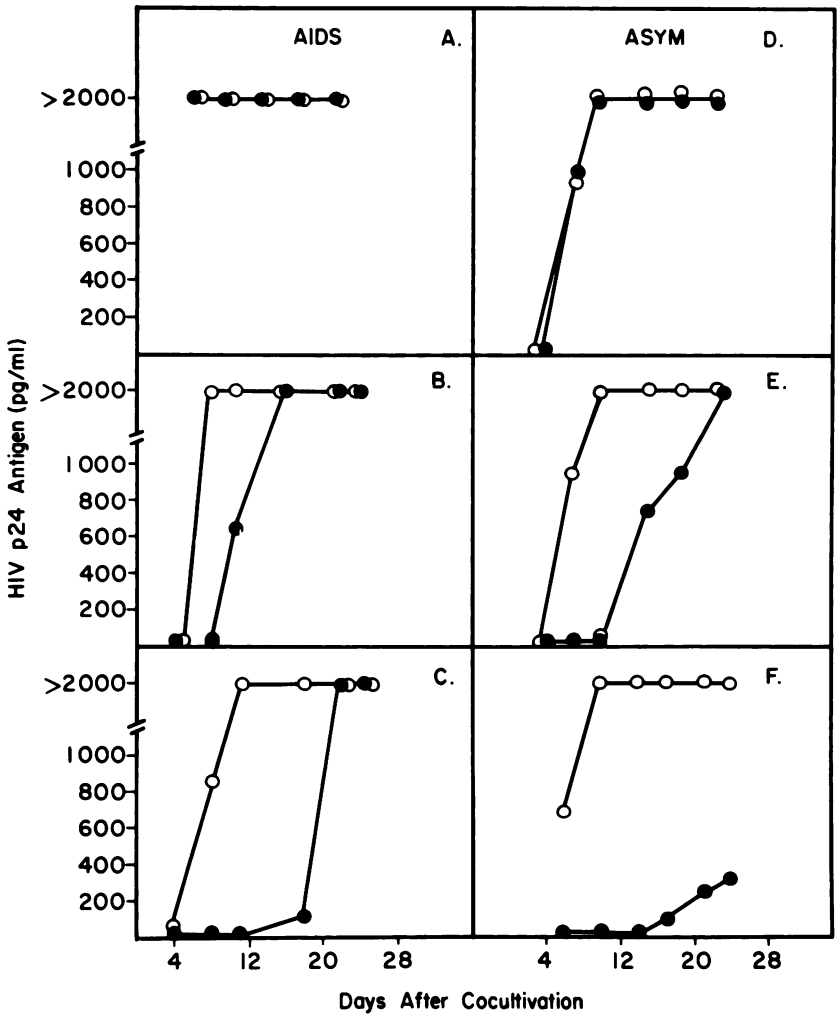

FIG. 2. Kinetics of HIV-1 production from lymphocytes of patients with AIDS (A through C) and of asymptomatic men (D through F), cocultured with PHA-stimulated normal donor PBL in the presence (●) and absence (○) of AZT. Lymphocytes (0.80×10^6 in panels C and F; 3.0×10^6 in panels B and E; 10×10^6 in panels A and D) from seropositive subjects were cocultured with 5×10^6 PHA-stimulated PBL in the presence and absence of 1 μm AZT as previously described (1). The medium was changed twice a week thereafter, and 2×10^6 to 3×10^6 PHA-stimulated normal PBL were added to the culture. Incubation was continued for 24 days in the presence or absence of AZT. Virus production was monitored by measuring the HIV p24 antigen in the culture supernatant, using the antigen capture test.

essary to obtain optimal HIV isolation from lymphocytes of seropositive patients. Since the addition of AZT or virus-neutralizing antibodies in culture presumably prevented spread of infection by extracellular virus from lymphocytes of infected patients, the production of HIV-1 in the presence of these antiviral agents is most likely due to the spread of virus infection from lymphocytes to uninfected PBL by cell-to-cell transmission. This hypothesis of cell-to-cell transmission of HIV would predict that the efficiency of transmission will increase with an increase in the ratio of infected to uninfected cells. Indeed, the data presented here indicate that the time of appearance of HIV-1 in the presence of AZT or virus-neutralizing antibodies is shortest with the highest numbers of infected lymphocytes and longest with lowest numbers. Our data also show that cell-to-cell spread of infection occurred irrespective of whether infected lymphocytes were from a patient with AIDS or from a seropositive asymptomatic man.

Since HIV production in PBL from patients was detected in the presence of AZT, it seems that cell-to-cell transmission of HIV-1 did not involve the transfer of an intact HIV virion from infected cell to uninfected cell. In this respect, cell-to-cell transmission of HIV-1 differs from that of herpesviruses and rabies virus; in the latter cases, the intact virion is transmitted from cell to cell (3, 12, 17). The finding that the anti-CD4 antibody blocked cell-to-cell transmission suggests that the fusion between HIV-1-infected and uninfected $CD4^+$ cells was involved in the spread of HIV-1 into uninfected cells by an unknown mechanism.

In a phase 2 efficacy trial of AZT in patients with AIDS-related complex and AIDS (6, 18), the drug had striking effects in preventing both opportunistic infections and death. Virus was recovered, however, from the patients throughout the period of drug treatment. The failure of AZT to block cell-to-cell transmission may explain the lack of antiviral effect of AZT in patients treated with the drug. Such a mode of transmission of HIV-1 could create a problem in the use of reverse transcriptase-inhibitory drugs in treatment of AIDS. Cell-to-cell transmission of HIV-1 can also explain the persistence of HIV-1 in the presence of virus-neutralizing

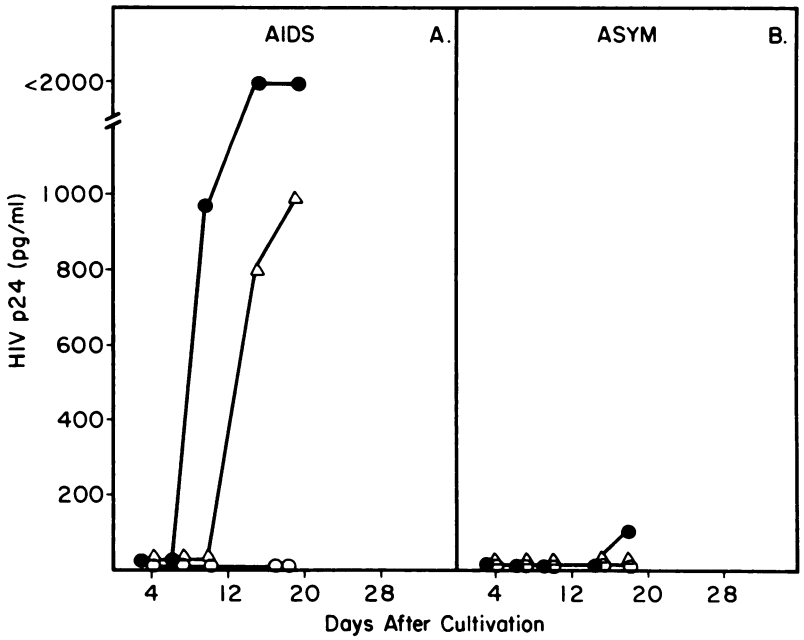

FIG. 3. Kinetics of HIV-1 production from lymphocytes of a patient with AIDS (A) and of an asymptomatic man (B) cultured in the absence of stimulated PBL. A total of 10×10^6 (●), 3×10^6 (△), and 1×10^6 (○) lymphocytes from an asymptomatic man and from a patient with AIDS were cultured in RPMI 1640 containing 20% fetal bovine serum and 5% interleukin-2. Virus production was monitored by measuring HIV p24 in the culture supernatant.

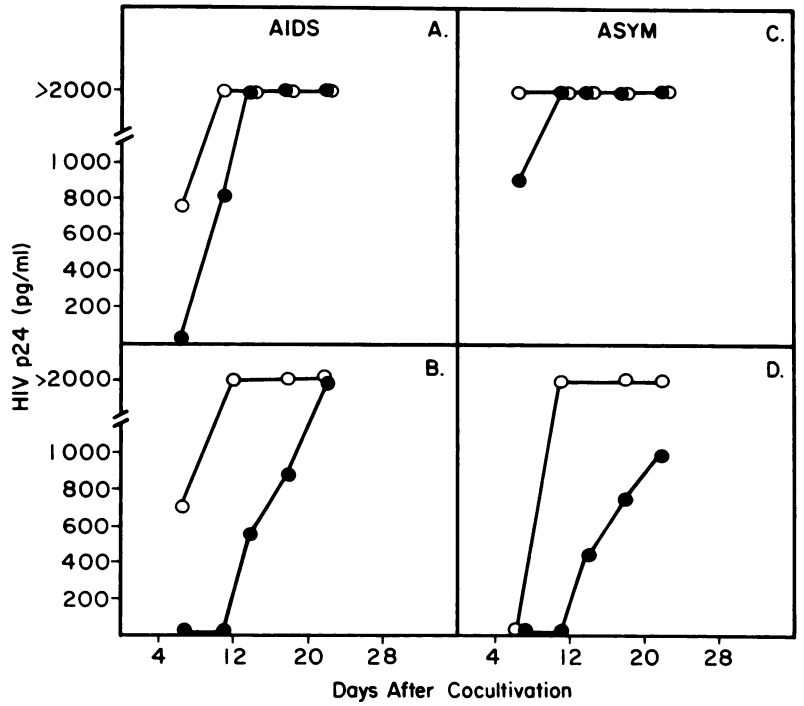

FIG. 4. Kinetics of HIV-1 production from lymphocytes of a patient with AIDS (A and B) and of an asymptomatic man (C and D), cocultured with PHA-stimulated normal donor PBL in the presence (●) and absence (○) of virus-neutralizing antibody. Lymphocytes (1×10^6 to 3×10^6) from a seropositive man were cocultured with 5×10^6 PHA-stimulated PBL in the presence of HIV-1-neutralizing serum (1:50 dilution) or control HIV-1-negative serum at the same dilution as described in the legend to Fig. 1. Virus-neutralizing antibody or the HIV-1-negative control serum was also added to the culture medium every 3 to 4 days throughout the incubation period. Production of HIV in the culture supernatant was monitored by the antigen capture test.

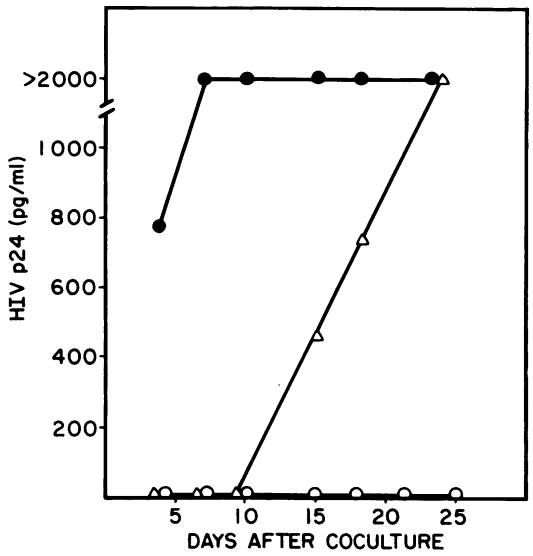

FIG. 5. Effect of the anti-CD4$^+$ antibody on HIV-1 production from lymphocytes of a patient with AIDS, cocultured with PHA-stimulated normal donor PBL. Lymphocytes (2×10^6) were cocultured with 5×10^6 PHA-stimulated normal PBL in the absence (●) and presence of mouse monoclonal antibody to the CD4 receptor (○) or to the CD3 receptor (△) as described in the legend to Fig. 1. Antibodies were added to the culture medium every 3 to 4 days throughout the incubation period. Production of HIV-1 was monitored by the antigen capture test.

antibodies (15, 16). Therefore, a vaccine that elicits only virus-neutralizing antibodies may not be effective in controlling HIV-1 infection.

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