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Dual Tropism for Macrophages and Lymphocytes Is a Common Feature of Primary Human Immunodeficiency Virus Type 1 and 2 Isolates

ANTONIO VALENTIN,[1]† JAN ALBERT,[2]* EVA MARIA FENYÖ,[1] AND BIRGITTA ÅSJÖ[1]‡

Department of Virology, Karolinska Institute, S-171 77 Stockholm,[1] and Department of Clinical Virology, Swedish Institute for Infectious Disease Control/Karolinska Institute, S-105 21 Stockholm,[2] Sweden

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We have investigated the ability of human immunodeficiency virus type 1 (HIV-1) and HIV-2 isolates to infect and replicate in primary human macrophages. Monocytes from blood donors were allowed to differentiate into macrophages by culture in the presence of autologous lymphocytes and human serum for 5 days before infection. A panel of 70 HIV-1 and 12 HIV-2 isolates were recovered from seropositive individuals with different severities of HIV infection. A majority of isolates (55 HIV-1 and all HIV-2) were obtained from peripheral blood mononuclear cells, but isolates from cerebrospinal fluid, monocytes, brain tissue, plasma, and purified $CD4^+$ lymphocytes were also included. All isolates were able to infect monocyte-derived macrophages, even though the replicative capacity of the isolates varied. Interestingly, isolates with a rapid/high, syncytium-inducing phenotype did not differ from slow/low, non-syncytium-inducing isolates in their ability to replicate in monocyte-derived macrophages. Others have reported that rapid/high, syncytium-inducing isolates have a reduced ability to infect and replicate in monocytes. However, different methods to isolate and culture the monocytes/macrophages were used in these studies and our study. Thus, the ability of HIV isolates to replicate in monocytes/macrophages appears to be strongly influenced by the isolation and culture procedures. It remains to be determined which culture procedure is more relevant for the in vivo situation.

Human immunodeficiency virus (HIV) isolates vary in biological properties such as host range, replicative rate, and capacity to induce cytopathic changes. These biological properties correlate with the severity of HIV infection (3, 6, 9, 30). HIV isolates from individuals with mild or no symptoms usually lack the capacity to grow in established cell lines and induce only small or no syncytia; such isolates have been called slow/low (3, 9) or non-syncytium inducing (30). In contrast, many HIV isolates from patients with severe immunodeficiency can replicate in $CD4^+$ cell lines and induce large syncytia in culture; these isolates are referred to as rapid/high (3, 9) or syncytium inducing (30). Furthermore, it has been proposed that HIV variants can be distinguished according to their capacity to infect primary mononuclear phagocytes or T-helper lymphocytes (5, 14, 28, 34). Some HIV variants were shown to lack the capacity to infect monocyte-derived macrophages (MDM), while others were found to be dually tropic for both T lymphocytes and mononuclear phagocytes (14, 34). Passage of HIV isolates through phytohemagglutinin (PHA)-P-stimulated peripheral blood lymphocytes (PBL) yielded progeny virus unable to infect MDM (14). Monocytotropic HIV variants have been reported to have a non-syncytium-inducing phenotype and thus lack the capacity both to replicate in T-cell lines and to induce syncytia in primary lymphocyte cultures (28). In contrast, results obtained by other groups, including ours, suggest that tropism for mononuclear phagocytes is a general property of all primary HIV type 1 (HIV-1) isolates (7, 13, 31, 32) and that repeated passage of an HIV-1 isolate through PBL does not alter its capacity to infect MDM (13).

Differences in replicative capacity in MDM have been suggested to be an important selective factor during transmission of HIV-1 (36). Thus, it is of great importance to clarify if certain HIV-1 variants lack the ability to infect mononuclear phagocytes. In this study, we have tested the ability of a panel of well-characterized HIV-1 and HIV-2 isolates to replicate in MDM that were obtained from peripheral blood mononuclear cells (PBMC) by plastic adherence for 5 days in the presence of autologous lymphocytes and human serum. In addition, we investigated if the tropism of HIV for MDM is related to other biological properties, such as cytopathicity or replicative capacity in established cell lines. Our results show that all HIV isolates can infect and replicate in primary mononuclear phagocytes, regardless of differences in biological phenotype.

MATERIALS AND METHODS

Cells. MDM were prepared from heparinized venous blood from healthy HIV-negative donors by plastic adherence for 5 days according to the method of Gartner et al. (12). Briefly, PBMC obtained by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) separation were washed five times with phosphate-buffered saline (PBS) (the last wash was made at 800 rpm to remove platelets). PBMC were seeded in 25-cm^2 tissue culture flasks (25×10^6 PBMC) or in 9-cm^2 tissue culture slide flasks (9×10^6 PBMC) in the presence of RPMI 1640 culture medium (Gibco Laboratories, Grand Island, N.Y.) supplemented with L-glutamine, penicillin (50 U/ml), streptomycin (50 μg/ml), 20% heat-inactivated fetal calf serum, and 10% heat-inactivated pooled human HIV-1-negative serum; PBMC were cultured at 37°C in 5% CO_2. Five days after initiation of the cultures, nonadherent cells were removed by rinsing the

* Corresponding author. Mailing address: Department of Clinical Virology, Swedish Institute for Infectious Disease Control, S-105 21 Stockholm, Sweden. Phone: 46-8-735 1300. Fax: 46-8-730 3248.

† Present address: National Cancer Institute-FCRDC, ABL-Basic Research Program, Frederick, MD 21701.

‡ Present address: Department of Microbiology and Immunology, Gade Institute, University of Bergen, N-5020 Bergen, Norway.

cultures three times with PBS. Adherent cells were grown in the same medium without HIV-1-negative serum. The cells obtained by this method are esterase positive and trypsin resistant and show a typical monocyte/macrophage phenotype, with expression of receptors for the Fc fragment of immunoglobulin G, complement receptors, and CD14, a marker for monocytes among the mononuclear cell population (12). No cells positive for CD2 or CD3 were present in the cultures.

PBL were obtained from PBMC from healthy blood donors by stimulation for 3 days with 2.5 μg of PHA per ml. PBL were cultured in RPMI medium containing 10% fetal calf serum, interleukin-2, and antibiotics as previously described (1). Purified $CD4^+$ lymphocytes were obtained from PBMC by the use of magnetic beads (Dynal, Oslo, Norway) coated with a monoclonal antibody against CD4 (4). The cells were kindly provided by J. Brinchmann at the Institute of Transplantation Immunology, National Hospital, Oslo, Norway. The human monocytoid cell line U937 and the human T-cell line CEM were cultured in RPMI medium supplemented with 10% fetal calf serum, L-glutamine, and antibiotics.

Viruses. A panel of 70 HIV-1 and 12 HIV-2 primary isolates was used. All HIV-2 and 55 HIV-1 isolates were obtained by cocultivation of patients' PBMC with PHA-stimulated PBL from uninfected donors as described previously (1, 2). Remaining HIV-1 isolates were obtained from the following sources: two from cerebrospinal fluid (recovered in PBL), three from patients' monocytes, two from brain tissue (recovered in PBL), four from plasma (two recovered in PBL and two recovered in MDM), and four directly isolated from purified $CD4^+$ T lymphocytes (two in the absence and two in the presence of the patients' own $CD8^+$ cells) (4). The latter four isolates were kindly provided by J. Brinchmann. Sixteen HIV-1 isolates were obtained from asymptomatic individuals, 11 were obtained from patients with persistent generalized lymphadenopathy, 17 were obtained from patients with AIDS-related complex or constitutional disease, and 10 were obtained from patients with AIDS. The clinical stage of the remaining 16 patients (some of them children from the former USSR accidentally infected with HIV-1-contaminated needles) was unknown. In the case of HIV-2 isolates, three isolates were from asymptomatic individuals, two were from patients suffering from severe asthenia, two were from patients with tuberculosis or pulmonary disease, and five were from patients with AIDS according to the Bangui criteria. A majority of the isolates used in the study have been biologically characterized (2, 9): 22 HIV-1 and 4 HIV-2 isolates grow well in human cell lines and induce syncytia (rapid/high phenotype), whereas 39 HIV-1 and 8 HIV-2 isolates lack the capacity to continuously replicate in established cell lines and induce no syncytia (slow/low phenotype).

HIV infection. The MDM cultures were infected 7 days after initiation. Prior to infection, the MDM were washed once with PBS. In addition, in the experiments with the laboratory strains IIIB and BaL, the cells had been vigorously trypsinized 5 days after initiation of the cultures (2 days before infection). Virus inocula for all infections ranged between 3×10^4 and 5×10^4 cpm of reverse transcriptase (RT) activity. After addition of the virus, the cells were incubated overnight at 37°C in 5% CO_2. The next day, the cells were washed two or three times with PBS and once with RPMI 1640 to remove unadsorbed virus, and fresh medium was added. The culture medium was changed once weekly in the MDM cultures and twice weekly in the PBL and cell line cultures; the latter were also split when required. Supernatants were stored for determinations of RT activity and levels of p24 antigen. If the cultures were negative by RT assay and p24 antigen assay after 4 weeks, attempts were made to rescue virus production by adding 10×10^6 PHA-stimulated blood donor PBL to the cultures.

RT assay. For the RT assay, 1.0 ml of cell-free culture supernatant was collected and centrifuged (15,000 × *g*, 1 h) (29). The pellet was resuspended in 100 μl of lysis buffer containing 0.3% Triton X-100, 75 μM Tris-HCl (pH 8.0), 50 mM KCl, and 6 mM dithiothreitol. Fifty microliters of this mixture was analyzed. The RT reaction mixture (100 μl) contained 75 μM Tris-HCl (pH 8.0), 50 mM KCl, 6 mM dithiothreitol, 6 mM $MgCl_2$, 100 μg of bovine serum albumin per ml, 2.5 μg of oligo$(dT)_{12-14}$ (Pharmacia) per ml, 2.0 μg of poly(A) (Pharmacia) per ml, and 25 μCi of [^{3}H]dTTP per ml (50 Ci/mmol; DuPont). Cultures were considered RT positive if two consecutive samples gave RT assay values above the background (>3,000 cpm).

HIV antigen assay. HIV-1 p24 antigen levels were determined by an in-house enzyme-linked immunosorbent assay (ELISA) (29) as previously described. Microtiter plates (Nunc I, Roskilde, Denmark) were coated with immunoglobulins (20 μg/ml) prepared from the serum of an asymptomatic HIV-1-infected person with high levels of anti-HIV-1 antibodies. Two mouse monoclonal antibodies reacting with different epitopes of the HIV-1 p24 antigen were used as tracing antibodies. The tracing antibodies had been directly conjugated to horseradish peroxidase. The detection limit of the p24 antigen assay is 50 to 100 pg of p24 antigen per ml (29). The sensitivity of the RT assay has been shown to be 5 to 10 times lower than that of the p24 antigen assay (29). Cultures were considered HIV antigen positive if two consecutive samples gave increasing absorbance values above the background ($A_{490} > 0.3$). HIV-2 antigen samples were assayed by a commercial HIV-1 antigen ELISA (Abbott) which cross-reacts with HIV-2.

RESULTS

Infection of MDM with HIV isolates. MDM were infected with a panel of well-characterized HIV isolates obtained from patients with different severities of HIV infection and from different tissue and cell origins. Sixty HIV-1 and 9 HIV-2 isolates were primary isolates, whereas 10 HIV-1 and 3 HIV-2 isolates had been passaged in PHA-stimulated PBL. Virus replication was monitored by RT activity and the presence of p24 antigen in culture supernatants. In two cultures, which did not show any detectable virus production, PHA-stimulated PBL were added to the MDM cultures after 4 weeks. As shown in Table 1, all HIV isolates could infect the MDM cultures, even if they had been previously passaged in PBL. However, the rates of replication varied among the isolates. Virus production could be demonstrated by RT activity in supernatants of 52 cultures and by the more sensitive p24 antigen assay in an additional 28 cultures. Only two MDM cultures were negative by both assays, but virus infection could also be demonstrated in these two cultures by cocultivation with PBL.

The ability of the isolates to replicate in MDM was analyzed semiquantitatively by dividing them into two groups: isolates that were positive by RT assay and isolates that were positive only by the more sensitive p24 antigen assay and coculture techniques. Interestingly, there were no significant differences in the replicative capacities in MDM between HIV isolates with a slow/low phenotype (31 of 47 RT-positive cultures) and isolates with a rapid/high phenotype (15 of 26 RT-positive cultures) ($\chi^2 = 0.2$, not significant) (Table 2). Similarly, replicative capacities of isolates obtained from patients with mild or no symptoms did not differ significantly (23 of 32 RT-positive cultures) from those of isolates derived from patients with more severe disease (18 of 34 RT-positive

TABLE 1. Infection of MDM by primary HIV isolates and PBL-passaged viruses

Virus	No. of positive cultures detected by:			Total no. positive
	RT assay	p24 assay[a]	Cocultivation[b]	
HIV-1				
Primary isolates	35	23	2	60
PBL passaged	9	1		10
HIV-2				
Primary isolates	5	4		9
PBL passaged	3			3
Total	52	28	2	82

[a] Number of HIV isolates in which virus replication was detected by the p24 antigen ELISA but not by the less sensitive RT assay.
[b] Number of HIV isolates in which virus replication was detected by cocultivation with PHA-stimulated PBL but not by the less sensitive RT assay or by the p24 antigen ELISA.

cultures) (χ^2 = 1.77, not significant) (data not shown). It is noteworthy that infection with most of the PBL-passaged isolates (12 of 13) could be detected by RT activity in culture supernatant, while this was the case for only 40 of 69 primary isolates.

MDM infection with HIV isolates from purified CD4$^+$ lymphocytes. Next we investigated if HIV isolates obtained from purified T-helper lymphocytes and selectively passaged in this cell type retained the capacity to infect and replicate in human mononuclear phagocytes. These isolates were passaged four times in monocyte-free CD4 lymphocytes purified from blood donors. Biological characterization of these viruses demonstrated that isolates 1 and 2 have a slow/low phenotype and isolates 3 and 4 have rapid/high properties (data not shown). All isolates could infect and replicate in MDM (Table 3). There were no significant differences in replication kinetics among the isolates, except that isolate 4 was positive only by p24 antigen assay 3 weeks after infection.

MDM infection with HIV-1$_{IIIB}$ and HIV-1$_{BaL}$. In the literature, there are conflicting data about the capacity of HIV-1$_{IIIB}$ to infect human mononuclear phagocytes (15, 19, 28). Therefore, we compared the capacities of HIV-1$_{IIIB}$ and HIV-1$_{BaL}$

TABLE 2. Infection of MDM by HIV isolates with different biological properties

Virus	No. of positive cultures detected by:			Total no. positive
	RT assay[a]	p24 assay[b]	Cocultivation[c]	
Slow/low				
HIV-1	26	11	2	39
HIV-2	5	3		8
Rapid/high				
HIV-1	12	10		22
HIV-2	3	1		4
Total	46	25	2	73

[a] Number of HIV isolates that replicated to high levels in MDM as detected by RT activity in culture supernatant. The replicative capacities of the different isolates varied but with no significant differences between HIV-1 and HIV-2 isolates. The mean value for maximal RT activity for slow/low isolates was 73 × 10^3 cpm/ml, with a range from 520 × 10^3 to 8 × 10^3 cpm/ml. In the case of rapid/high isolates, the mean was 67 × 10^3 cpm/ml, and the range was from 206 × 10^3 to 8 × 10^3 cpm/ml.
[b] See footnote *a* in Table 1.
[c] See footnote *b* in Table 1.

TABLE 3. Replication of CD4$^+$ lymphocyte-derived HIV-1 isolates in MDM

Virus	RT activity (10^3 cpm/ml)		
	Day 7	Day 14	Day 21
1	96	135	85
2	78	87	94
3	43	67	38
4	32	17	—[a]

[a] —, a value lower than five times the background level (0.5 × 10^3 cpm/ml). This culture was positive for p24 antigen (10 ng/ml).

to infect and replicate in MDM and PHA-stimulated PBMC. Stocks of both viruses were serially diluted (10-fold) in RPMI 1640 culture medium. Table 4 shows that HIV-1$_{BaL}$ replicated to higher levels than HIV-1$_{IIIB}$ in MDM but the end-point dilution for a productive infection of MDM was the same for both viruses in two independent experiments and one step lower for HIV-1$_{BaL}$ in a third experiment (data not shown). We also tested if HIV-1$_{IIIB}$ passaged in PBL or cell lines (H9 and Jurkat) differed in the ability to infect human macrophages. MDM were infected with 4 × 10^4 cpm of RT activity of HIV-1$_{IIIB}$ obtained from PBL and H9 and Jurkat cells. In our hands, all three HIV-1$_{IIIB}$ stocks could infect and replicate in MDM irrespective of the cellular origin (data not shown). In order to exclude cross-contamination of the HIV-1$_{IIIB}$-infected MDM cultures, we determined the RNA sequence of virus particles produced by a MDM culture infected by HIV-1$_{IIIB}$ derived from PBL. Thus, virion-associated RNA was extracted, reverse transcribed into cDNA, PCR amplified, and sequenced by previously described methods (25). As expected, the V3 loop sequence of the virus produced by the HIV-1$_{IIIB}$-infected MDM culture was identical to the published sequence of this virus isolate (data not shown).

Biological characterization of HIV strains after passage in PBL and MDM cultures. HIV-1 has been reported to consist

TABLE 4. Infection of MDM and PBL with serial dilutions of HIV-1$_{IIIB}$ and HIV$_{BaL}$[a]

Strain and dilution	PBL		MDM	
	Max RT activity (10^3 cpm/ml)	p24[b]	Max RT activity (10^3 cpm/ml)	p24
IIIB				
None	560		43	
10^{-1}	222		34	
10^{-2}	148		14	
10^{-3}	112		7	
10^{-4}	66		—[c]	+
10^{-5}	54		—	+
10^{-6}	—	−	—	−
BaL				
None	119		201	
10^{-1}	103		179	
10^{-2}	106		66	
10^{-3}	37		19	
10^{-4}	26		12	
10^{-5}	—	−	—	+
10^{-6}	—	−	—	−

[a] MDM and PBL were infected with serial 10-fold dilutions of supernatants containing 100 × 10^3 cpm of RT activity of HIV-1$_{IIIB}$ and HIV$_{BaL}$ per ml.
[b] Only cultures considered negative for RT activity were tested for the presence of the p24 antigen by ELISA.
[c] —, a value considered negative for RT activity.

TABLE 5. Biological characterization of HIV strains after passage in PBL and MDM

Passage cell type and virus[a]	Max RT activity (10^3 cpm/ml) in:		
	PBL	U937	CEM
PBL			
IIIB	608	413	167
A692	1,018	238	4
6669	417	29	—[b]
MDM			
IIIB	650	460	83
A692	397	93	7
6669	387	376	—

[a] The infectious supernatants used in these experiments were obtained after in vitro propagation of the different HIV strains in PBL and MDM. Syncytium formation, which was observed in all strains regardless of passage cell type, was evaluated in PBL cultures.

[b] —, a value considered negative for RT activity.

of a mixture of variants with different biological properties (27); therefore, it is conceivable that passage of an HIV isolate in MDM may select for a minor virus population with properties distinct from those of the original isolate. To test this possibility, PHA-stimulated PBL and MDM were infected in parallel with three HIV strains (HIV-1_{IIIB}, HIV-1_{A692}, and HIV-$2_{SBL6669}$) which all replicate in $CD4^+$ cell lines and induce syncytium formation. Progeny virus from these cultures were used as cell-free supernatant to infect PBL, the monocytoid cell line U937, and the T-cell line CEM. Results from these experiments are shown in Table 5. Phenotypical changes were not observed for any of the HIV strains after in vitro passage in MDM or PBL. All three strains were also still able to replicate in cell lines and induce syncytium formation after passage in MDM.

DISCUSSION

The major targets for HIV infection are bone marrow-derived cells expressing CD4 on their surfaces. HIV infection in vivo has been demonstrated in T-helper lymphocytes (17) and mononuclear phagocytes (8, 18, 22, 23, 35). However, there is considerable controversy about whether all or only certain HIV isolates have the ability to replicate in monocytes/macrophages (5, 7, 13, 14, 28, 31, 32, 34). For this reason and since monocytes/macrophages are the main target cells for other lentiviruses (20), we systematically investigated the capacity of well-characterized HIV-1 and HIV-2 isolates to infect and replicate in primary human MDM. The MDM were derived from blood donor PBMC by plastic adherence for 5 days in the presence of autologous lymphocytes and human serum. Our results show that all HIV isolates could infect these cells, although the levels of replication varied.

As indicated above, several authors have previously reported that only certain HIV-1 isolates can productively infect monocytes/macrophages (5, 14, 28, 34). Thus, Schuitemaker et al. (28) reported that most syncytium-inducing HIV isolates do not replicate in macrophages. Furthermore, Gendelman et al. (14) reported that serial passage of an HIV isolate in PBL selects for virus variants lacking the capacity to infect mononuclear phagocytes. In contrast, other groups, including ours, have found that all HIV-1 isolates can productively infect MDM (7, 13, 31, 32). In this study, we have directly tested if the biological phenotype and passage history of HIV isolates influence the ability to replicate in MDM. In contrast to Schuitemaker et al. (28) and Gendelman et al. (14), we found that isolates with a rapid/high, syncytium-inducing phenotype can infect and replicate in MDM and that the biological phenotype of HIV isolates remains unchanged after passage in MDM and purified $CD4^+$ lymphocytes. In support of our findings, Connor et al. (7) recently reported that all 28 HIV-1 isolates sequentially obtained from four individuals replicated in macrophages, despite differences in their ability to replicate in tumor cell lines. Similarly, Gartner and Popovic (13) reported that repeated passage of an HIV-1 isolate through MDM does not generally result in the loss of the ability to infect normal T cells. In agreement with results from earlier reports, our results were that the BaL isolate of HIV-1 differed from the IIIB isolate in that it replicated to higher titers on MDM than on PBL (12, 28).

The seemingly conflicting data on the ability of HIV-1 isolates to replicate in monocytes/macrophages are probably due to technical differences in the methods used to isolate and cultivate the macrophages and monocytes. Gendelman et al. (14) and Schuitemaker et al. (28) isolated blood monocytes by countercurrent centrifugal elutriation and cultured the cells in the presence of macrophage colony-stimulating factor, a cytokine that induces maturation of monocytes into macrophages. In contrast, we isolated the cells by adherence to plastic, and the differentiation process of the monocytes took place in the presence of autologous lymphocytes and human serum over 5 days. Several investigators have shown that the susceptibility of macrophages to HIV infection is greatly influenced by their stage of differentiation (21, 26, 33). It is of special interest that Schrier et al. (26) showed that efficient virus expression in monocyte cultures from HIV-1-infected individuals is dependent on the presence of lymphocytes during the first 24 h of culture. Similarly, Ibanez et al. (16) found that productive infection of MDM with human cytomegalovirus is dependent on in vitro maturation of the blood monocytes in the presence of stimulated lymphocytes. Thus, in view of the importance of the stage of differentiation of monocytes/macrophages, it is not surprising that different isolation and culture procedures may give seemingly contradictory results on the susceptibility of monocytes/macrophages to HIV infection.

Mononuclear phagocytes have been implicated as the primary target for HIV infection and may also be of importance as a site for persistence and dissemination of the infection (13). Furthermore, authors who have reported that only non-syncytium-inducing HIV-1 variants are capable of infecting monocytes/macrophages have suggested that these variants have an essential role during transmission and persistence of HIV-1 (28, 36). However, the present study reemphasizes that such speculations may be premature. It is clear that the ability of individual HIV-1 isolates to replicate in monocytes/macrophages is dependent on how the cells are cultured, but at present it is not clear which culture technique is more representative of the in vivo situation. However, it is likely that a significant proportion of the monocytic phagocytes in an infected individual will be susceptible to infection by rapid/high, syncytium-inducing HIV-1 variants at any given time. In accordance with this, we have found that rapid/high, syncytium-inducing HIV-1 variants can be transmitted both sexually and from mother to child (10, 25).

In conclusion, we have demonstrated that HIV-1 isolates can infect both mononuclear phagocytes and lymphocytes, irrespective of the biological phenotype and passage history of the isolate. The differences between these results and certain previously published studies are likely to be due to technical differences in how the monocytes/macrophages were isolated

and cultured. It remains to be determined which culture procedure is more relevant for the in vivo situation.

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