INFECTION OF MONOCYTE-DERIVED MACROPHAGES WITH HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 (HIV-1)

Monocyte-tropic and Lymphocyte-tropic Strains of HIV-1 Show Distinctive Patterns of Replication in a Panel of Cell Types

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Several of the animal lentiviruses, including visna virus of sheep and caprine arthritis-encephalitis virus of goats, are known to infect cells of the monocyte/macrophage lineage. Studies with visna, the prototype lentivirus, have demonstrated that monocyte/macrophages play a critical role in pathogenesis, since these cells act as a reservoir for virus and disseminate infection to target organs such as the lung and central nervous system (1–3). A similar role has been proposed for monocyte/macrophages in HIV infection of humans (4, 5), since these cells have been shown to be infected in HIV-positive subjects (6–11), and can be experimentally infected in vitro (12–18).

Seminal studies of visna-maedi of sheep demonstrated that different strains of visna virus exhibited marked differences in their cellular tropism, some isolates being monocyte tropic while others were fibroblast tropic (1, 4, 19, 20). Furthermore, visna virus strains that showed different tropism in cell culture had different patterns of virulence when injected into sheep (20). Likewise, it appears that HIV-1 isolates vary in their ability to replicate in monocyte/macrophages, and this may correlate in part with the site and method of isolation (6, 16, 21–24). It is plausible that these differences may be important in the persistence, dissemination, pathogenesis, or transmission of HIV infection (25).

This study was designed to address several aspects of HIV-1 infection of monocyte/macrophages. (*a*) We wished to confirm the existence of monocyte-tropic strains and to determine their relative ability to replicate in primary cultures of monocyte/macrophages and of lymphocytes. (*b*) Since the use of primary cells is fraught with difficulties, we wanted to determine whether promonocyte cell lines or lymphoid cell lines

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could be used as surrogates for primary cultures in studies of viral tropism. (*c*) In addition, we addressed several related issues. Does HIV-1 infection of monocyte-derived macrophages (MDM)[1] involve the majority of the cell population or is infection associated with a subpopulation of MDM or with a few residual contaminating $CD4^+$ lymphocytes? Does HIV-1 produce any cytopathic effect on MDM?

The results indicate that the host range of HIV is a complex phenomenon best characterized by using a matrix of several different cell types, and that HIV infection of monocyte/macrophages differs markedly from infection of lymphocytes justifying extensive independent study.

Materials and Methods

Cell Isolation and Culture of MDM. Monocyte-enriched populations were prepared from PBMC of HIV-seronegative volunteers as described (26) and cultured in vitro as MDM. PBMC obtained by Ficoll-Hypaque separation of whole blood were suspended at 2–4 × 10^6 cells/ml in DME (Gibco Laboratories, Grand Island, NY) and 20 ml was placed in 75-cm^2 tissue culture flasks (Corning Glass Works, Corning, NY) previously coated with 2% gelatin. After incubation for 1 h at 37°C, nonadherent cells were removed, and the adherent cells were washed four times with serum-free DME and once with 10 ml of a 1:1 mixture of 10 mM EDTA in PBS and DME with 20% horse serum (Gibco Laboratories). Adherent cells were detached by incubation for 15 min with 10 ml of the EDTA–horse serum mixture, washed in DME, and suspended at 5 × 10^5 cells/ml in DME with 10% FCS (HyClone Laboratories, Logan, UT), 10% horse serum (Gibco Laboratories), glutamine (600 μg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml), granulocyte-macrophage CSF (GM-CSF, 40 U/ml; Amgen, Thousand Oaks, CA [27]), and macrophage CSF (M-CSF, 100 U/ml; kindly provided by Genetics Institute, Cambridge, MA [28]). Suspended cells were seeded at 2–5 × 10^5 cells/well in 24- or 48-well plastic tissue culture plates (Flow Laboratories, McLean, VA; or Gibco Laboratories) and maintained at 37°C in 5% CO_2. Once weekly, cultures were washed with PBS to remove nonadherent cells, and refed with media containing CSFs.

Preliminary studies showed that in combination, M-CSF and GM-CSF improved the viability of uninfected MDM cultures (27, 29) from ~50% at 4 wk and ~25% at 8 wk to ~80% and ~60%, respectively (data not shown). Therefore, CSFs were added at the initiation of macrophage cultures and replaced each time medium was changed. Selected batches of fresh medium were tested periodically and shown to be free of endotoxin by the limulus amoebocyte lysate assay (QCL-1000; Whittaker M. A. Bioproducts, Walkersville, MD).

After initial purification, cells were ≥90% monocytes as determined by surface markers, latex phagocytosis, and nonspecific esterase (Fig. 1, Table I), and after 7 d in culture were ≥97% monocyte/macrophage cells by the same criteria (Table I).

PBL. Lymphocyte-enriched cultures were prepared from PBMC by adherence depletion of monocytes. PBMC were suspended at 10^6 cells/ml in RPMI with 10% FCS (Gibco Laboratories), penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (300 μg/ml), nonessential amino acids (1%; Gibco Laboratories), and PHA-L (5 μg/ml; Sigma Chemical Co., St. Louis, MO). After 24 h the nonadherent cells were transferred to a new flask and 2–3 d later the nonadherent cells were resuspended at 10^6 cells/ml in wells in medium (without PHA) supplemented with rIL-2 (600 U/ml; DuPont Co., Wilmington, DE). Lymphocytes were maintained with IL-2 and split 1:2–1:4 every 5 d. At 4 d of culture, these cells were ~75% T lymphocytes and 15% B lymphocytes by surface markers, and ≤2% reacted with surface markers for monocyte/macrophages (Table I).

U937 and SUP-T1 Cells. U937 promonocytic (30) and SUP-T1 T lymphocyte (31) cell lines were maintained at ~10^6 cells/ml in RPMI with 10% FCS, penicillin (100 U/ml), streptomycin (100 μg/ml), and glutamine (300 μg/ml); cells were split 1:4 every 5 d.

[1] *Abbreviations used in this paper:* GM-CSF, granulocyte-macrophage CSF; M-CSF, macrophage CSF; MDM, monocyte-derived macrophages.

Analysis of Cell Populations. Immunofluorescent detection of surface markers was carried out with mAbs OKM1 (anti-CD11b/CR3; Ortho Diagnostic Systems Inc., Westwood, MA), OKT3 (anti-CD3, Ortho Diagnostic Systems, Inc.), OKT4 (anti-CD4; Ortho Diagnostic Systems Inc.), OKT11 (anti-CD2; Ortho Diagnostic Systems Inc.), Leu-M3 (anti-CD14; Becton Dickinson & Co., Mountain View, CA), HD37 (anti-CD19; Boehringer Mannheim Biochemicals, Indianapolis, IN), I2 (anti-Ia; Coulter Electronics, Inc., Hialeah, FL), B73.1 (anti-FcR/NK; provided by G. Trinchieri, The Wistar Institute, Philadelphia, PA [32]), and isotype-matched control antibodies. Cell lines, lymphocytes, and freshly isolated monocytes in suspension were incubated for 30 min at 4°C with mAb at 1:10–1:20 dilution, washed twice with PBS with 2% FCS, incubated for 30 min at 4°C with fluorescein-conjugated goat $F(ab')_2$ anti-mouse IgG (Organon Teknika Corp., Durham, NC) diluted 1:60, washed and analyzed by flow cytometry (Epics; Coulter Electronics, Inc.), or mounted on glass slides and assessed by fluorescent microscopy. For detection of surface markers on adherent MDM, cells were cultured on polylysine-coated glass cover slips, fixed with methanol for 30 min at room temperature, washed twice with PBS, and overlaid with 20% nonimmune rabbit serum and 5% nonimmune goat serum (Organon Teknika Corp.) for 20 min at room temperature to block nonspecific binding. Cells were then incubated for 45 min at room temperature with mAb diluted 1:10 in PBS containing 5% goat and 20% rabbit serum, washed twice with PBS, and incubated for 30 min with fluorescein-conjugated goat $F(ab')_2$ anti-mouse IgG (Organon Teknika Corp.) diluted 1:60 in PBS containing 50% FCS, 10% goat serum, 10% rabbit serum, and 0.5% Evans blue as a counterstain to minimize autofluorescence. Coverslips were mounted on glass slides and examined by fluorescent microscopy.

Monocyte/macrophage cultures were examined by modified Wright-Giemsa stain (Dif-Quik; American Scientific Products, McGaw Pk, IL). Nonspecific esterase was demonstrated cytochemically (diagnostic kit 181B; Sigma Chemical Co. [33]). Latex phagocytosis was detected by incubating cells for 1 h with 1.1-μm latex particles (Dow Chemical Co., Indianapolis, IN); cultures were scored microscopically for intracellular particles (34). Fc receptors were detected by rosette formation with IgG-coated sheep erythrocytes (35). Sheep erythrocytes were incubated with rabbit anti-sheep erythrocyte IgG (Organon Teknika Corp.) for 30 min at 37°C, washed three times with PBS, and incubated with monocyte/macrophages for 1 h at 37°C, which were then washed, fixed, and stained with Wright-Giemsa stain. Cells with three or more adherent erythrocytes were scored positive. Quantification was based on a minimum of 300 cells visualized microscopically or 5,000 cells analyzed by flow cytometry.

Viruses. HIV-I strains HIV-IIIB (36) and HIV-DV (37) were isolated from PBMC, and HIV-SF162 (24) was isolated from cerebrospinal fluid. Virus stocks were grown in SUP-T1 cells or PHA/IL-2-stimulated lymphocytes, and clarified supernatant was frozen at −80°C. Stocks were titered by p24 antigen content and by TCID50 titration on PBL or SUP-T1 cells. The infectious titer per 100 ng p24 antigen was: IIIB, 5.6×10^4 TCID50; DV, 10^5 TCID50; and SF162, 5.3×10^3 TCID50.

HIV-1 Infection. Monocyte/macrophages were infected as MDM after 7–14 d in culture. Cells were washed four times with PBS, and the viral inoculum was added. After incubation for 2 h at 37°C, cells were washed four times with PBS to remove unadsorbed virus, and fresh medium was added. Lymphocytes were cultured for 4 d before infection. Virus was added to 10^6 cells in 1 ml for 2 h at 37°C, washed four times with PBS, and cells were resuspended at the same density in medium containing IL-2. U937 and SUP-T1 cells were infected by the same protocol. In each experiment the final wash was tested for viral p24 antigen and shown to be free of residual inoculum.

Virus Detection. All samples were stored at −80°C until assayed. p24 antigen was determined on tissue culture supernatant or on cell pellets lysed with 1% Triton X-100 (and resuspended to the original culture volume) by ELISA (Coulter Electronics, Inc.). Tissue culture infectious dose 50% endpoint (TCID50) was determined by inoculating replicate microtiter wells of SUP-T1 cells (10^4 cells/well) or PHA-stimulated lymphocytes (5×10^4 cells/well, with IL-2) with serial dilutions of cell-free supernatant. Lymphocyte wells were split 1:5 once weekly and fed with fresh medium containing IL-2. After 3 wk, each well was scored for the presence of syncytia (SUP-T1 cells) or for p24 antigen production (lymphocytes) by ELISA (Chiron Corp., Emeryville, CA). MDM cultured on polylysine-coated glass

cover slips were stained 22 d after infection. Coverslips were washed twice with PBS, fixed with methanol for 30 min at room temperature, and incubated with 20% nonimmune rabbit serum and 5% nonimmune goat serum for 20 min at room temperature. They were then overlaid for 45 min at room temperature with IgG purified from HIV-seropositive human serum (Affi-gel; Bio-Rad Laboratories, Richmond, CA) at a concentration of 0.35 mg/ml diluted in PBS containing 5% goat serum and 5% rabbit serum. Coverslips were washed three times with PBS and incubated for 30 min with fluorescein-conjugated goat $F(ab')_2$ anti-human IgG (Boehringer Mannheim Biochemicals) diluted 1:60 in PBS with 50% FCS, 10% goat serum, 10% rabbit serum, and 0.5% Evans blue as a counterstain to minimize autofluorescence.

Results

MDM. Monocyte/macrophages were prepared from peripheral blood of normal human subjects and were infected as MDM after 7–14 d in culture. On the day of preparation, >95% of these cells exhibited monocyte markers, as they did at the time of infection, and very few cells showed T cell markers (Table I and Fig. 1). These cells expressed very low levels of CD4 by flow cytometry on the day of preparation (Fig. 1), and CD4 could not be detected by fluorescent microscopy on adherent MDM at the time of infection (Table I).

HIV-1 Replication in Monocyte-derived Macrophages and Lymphocytes. We selected three HIV-1 strains as prototypes, IIIB, DV, and SF162, and compared their replication in MDM, PBL, a promonocytic line (U937), and a T cell line (SUP-T1). Virus inocula were standardized by their content of p24 antigen, and cultures were infected

TABLE I

Characteristics of Cell Populations Used for HIV Infection

Marker	Monocyte/macrophage 0 d	7 d	60 d	PBMC (0 d)	PBL (4 d)	U937	SUP-T1
OKM1	99	82	63	20	2	0	0
Leu-M3	95	43	ND	19	0	5	0
OKT3	2	0	ND	52	78	0	1
OKT11	7	0	0	64	75	0	98
OKT4	4	0	ND	35	31	96	97
HD37	0	1	0	5	15	0	0
NSE	92	97	65	ND	ND	95	ND
Latex phagocytosis	91	98	88	ND	ND	<5	ND
Fc receptors	96	98	ND	ND	ND	85	ND

Values are the percentage of positive cells. Monocytes were characterized immediately after separation from PBMC (day 0), and after culture as adherent MDM at day 7 and 60. PBMC fraction was characterized immediately after Ficoll-Hypaque isolation before further separation. PBL cultures were obtained by culturing PBMC for 4 d, with PHA stimulation and serial depletion of plastic-adherent cells. U937, promonocytic cell line; SUP-T1, T lymphocyte cell line. Binding of mAbs to macrophage markers (OKM1 and Leu-M3), T lymphocyte markers (OKT3 and OKT11), B lymphocyte marker (HD37), and CD4 (OKT4) was assessed by fluorescence microscopy. Nonspecific esterase (NSE), latex phagocytosis, and Fc receptors were also assessed by microscopy, as described in Materials and Methods. Percentage of cells positive is based on a minimum of 300 cells counted.

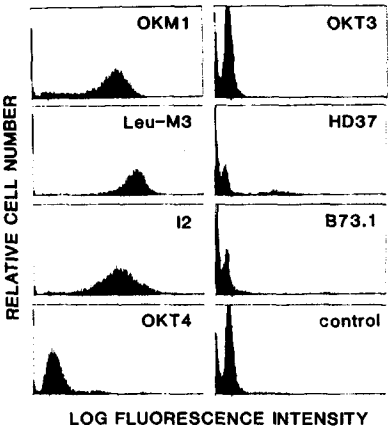

FIGURE 1. Purity and surface characteristics of monocytes immediately after isolation from PBMC. Flow cytometry profiles represent the entire population of cells isolated. Monocytes were stained with mAbs for monocyte markers (OKM1, Leu-M3), class II expression (I2), CD4 expression (OKT4), T lymphocyte marker (OKT3), B cell marker (HD37), and NK cell marker (B73.1). An IgG2a control antibody is shown.

with ~100 ng/10^6 cells. Infection was followed by examining supernatants for concentration of p24 antigen and infectious virus, by p24 assay of cell lysates for cell-associated antigen, and by immunohistochemical staining of viral antigens for the proportion of cells infected.

The three virus strains showed sharply different patterns of replication in MDM (Figs. 2 and 3); strain SF162 produced moderate levels of p24 antigen and infectious virus, while strain III B produced little or no antigen or infectious virus, and strain DV showed an intermediate pattern. All three strains of HIV-1 replicated to a high titer in PBL, with similar kinetics of antigen and infectious virus production (Figs.

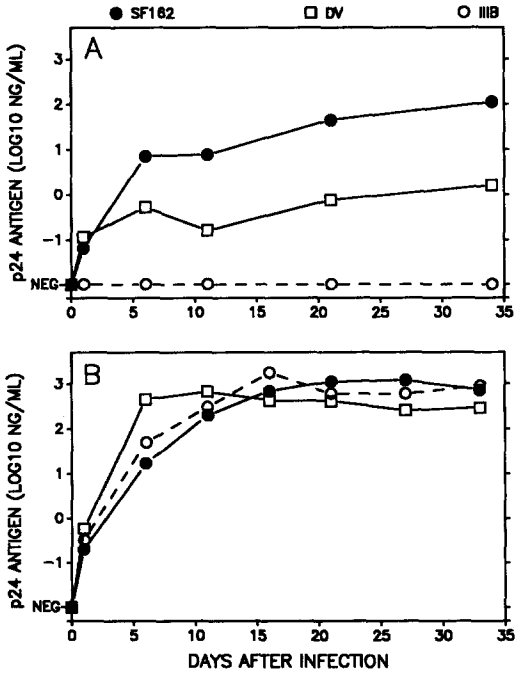

FIGURE 2. Antigen production by three strains of HIV-1 (SF162, DV, and IIIB) in MDM (*A*) and PBL (*B*). MDM cultured for 7 d and lymphocytes cultured for 4 d were infected with equal amounts of virus (100 ng p24 antigen per 10^6 cells) for 2 h, washed, and maintained as described. The culture supernatant was periodically tested for p24 antigen.

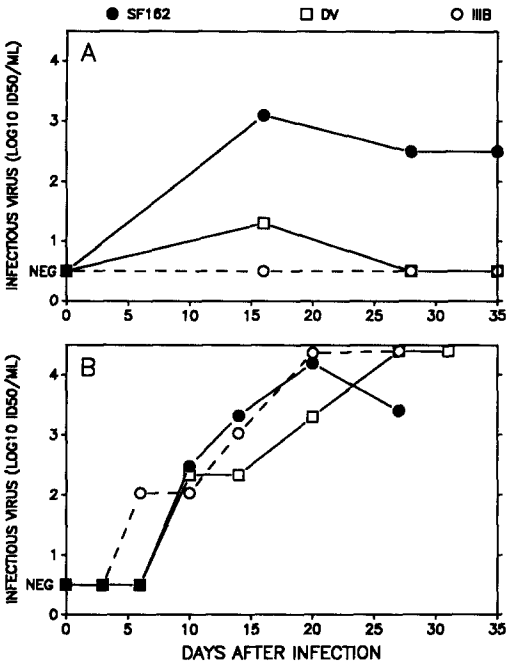

FIGURE 3. Replication of three strains of HIV-1 (SF162, DV, and IIIB) in MDM (*A*) and PBL (*B*). MDM cultured for 7 d and lymphocytes cultured for 4 d were infected with equal amounts of virus (100 ng of p24 antigen per 10^6 cells) for 2 h, washed, and maintained as described. The culture supernatant was tested periodically for infectious virus production by titration on PBL.

2 and 3). Of note, even the monocyte-tropic strain SF162 replicated to titers 10- to 100-fold higher in PBL than in MDM.

Since infected MDM consistently produced lower levels of supernatant p24 antigen than did infected PBL, a search was made for sequestration of p24 antigen in infected MDM. Thoroughly washed cells were lysed and the p24 content of the lysates compared with p24 in the supernatants from the same cultures (Table II). Of the total p24 antigen, ~3:4 was associated with the supernatant and 1:4 with the cell, and this ratio was similar for infected MDM and PBL.

TABLE II

Supernatant and Cell-associated Viral Antigen in HIV-1-infected MDM and Lymphocyte (PBL) Cultures

		IIIB		DV		SF162	
Cell	Day	Supernatant	Cellular	Supernatant	Cellular	Supernatant	Cellular
				ng			
MDM	6	0.15	<0.01	10.1	2.8	13.3	0.8
	13	0.06	0.06	29.2	50.0	63.0	18.2
	21	0.17	0.09	47.0	8.9	35.8	9.7
PBL	7	14	2	187	58	228	32
	13	29	22	71	39	118	11
	22	131	33	58	15	104	10

MDM and lymphocyte cultures were infected as described. At each time point, culture was divided into supernatant and cellular component, and p24 antigen levels were determined. Values are expressed as the total p24 antigen content in each compartment of the culture.

Donor Variability in MDM. Replicate experiments with MDM are shown in Table III. Although strain IIIB usually produced no antigen or only trace levels (0.01–0.1 ng/ml), titers of 2–4 ng/ml were occasionally seen; conversely, strain SF162 usually produced titers of 50–150 ng/ml, but occasionally did not exceed 1 ng/ml. There appeared to be both experiment-to-experiment and donor-to-donor variation. Despite this biological variation, within each experiment there was a consistent gradient of replication, from SF162 (highest) to IIIB (lowest).

Viral Antigen in HIV-1-infected MDM. To determine the proportion of infected cells and the morphological effects of infection, MDM, inoculated 22 d previously with SF162, were examined for viral antigen by immunofluorescence (Fig. 4). Many cells contained viral antigen, and these cells were readily identified as macrophages because of their large size, abundant cytoplasm, and multiple nuclei. Antigen was seen in the cytoplasm and not in nuclei and was usually concentrated in multiple focal accumulations. A striking feature of infected cells was the localization of antigen at sites of cell-to-cell contact (Fig. 4 *B*).

Viral antigen was clearly associated with multinucleated MDM. Cultures were mononuclear at the time of preparation, but during cultivation (in the absence of infection) the cells gradually fused to form small syncytia. However, infected companion cultures demonstrated much more pronounced syncytia formation (Fig. 5), and the largest syncytia were almost always antigen positive (Fig. 4 *C*). These multinucleated giant cells expanded over time and formed cellular "lakes" with >100 nuclei, yet remained viable and continued to produce virus for >8 wk. The association of syncytia formation with infection is demonstrated in Table IV, which compares the number of nuclei in antigen-positive and antigen-negative MDM within a single infected culture. Antigen-positive MDM form much larger syncytia, while antigen-negative MDM form smaller syncytia very similar to the size of those seen in uninfected cultures. Table IV also shows that in infected cultures, ~2:3 of nuclei (representing 2:3 of the initial monocytes in the original culture) were in antigen-positive cells.

TABLE III

Variability of HIV-1 Replication in MDM

Exp.	Donor	IIIB	DV	SF162
1	A	–	+	+ + +
2	B	–	+ +	+ + + +
3	B	–	+	+ + +
4	C	+	+	+ + + +
5	C	+	+ +	+ + +
6	D	±	+ +	+ + +
7	D	±	+ +	+
8	E	+	+ + +	+ + +
9	E	+ +	+ + +	+ + + +
10	F	+ +	+ + +	+ + + +
11	G	+	+ +	+ +

MDM were prepared and infected as described in Materials and Methods. All cultures were followed for at least 30 d after infection and peak titers of p24 antigen in supernate are recorded in this table. Titer of p24 antigen: + + + + (100–1,000 ng/ml); + + + (10–100 ng/ml); + + (1–10 ng/ml); + (0.1–1 ng/ml); and ± (<0.1 ng/ml).

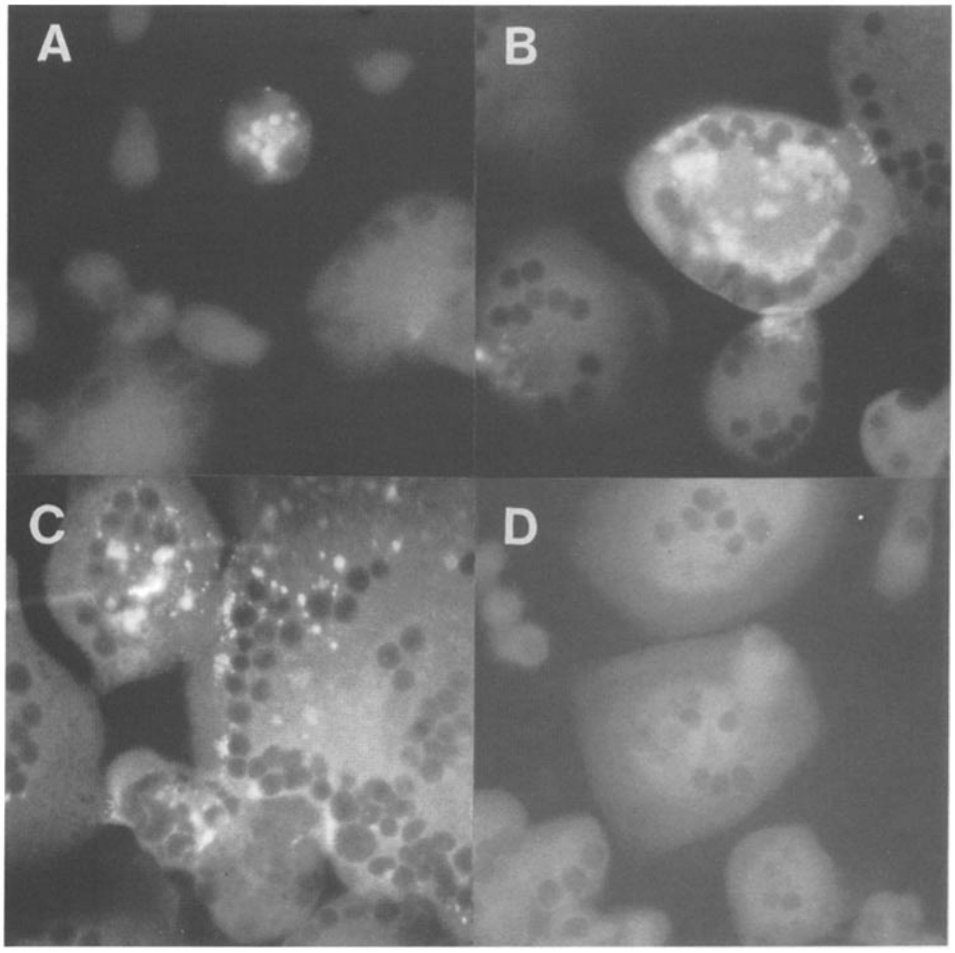

FIGURE 4. Viral antigens in infected MDM. Macrophages cultured for 14 d were infected with HIV-1 SF162 (75 ng of p24 antigen per 6×10^5 cells), and 22 d later were fixed, stained for viral antigens, and examined by immunofluorescence. (*A*) Infected culture showing a single small polykaryon with focal areas of viral antigen. (*B*) Infected culture with antigen-positive polykaryon and antigen at points of contact with other cells. (*C*) Infected culture showing a very large polykaryon with multiple foci of viral antigen. (*D*) Uninfected culture of same age showing a heterogenous population of cells that contain up to several nuclei (×250).

Large syncytia were frequently seen in cultures infected with strain SF162, less commonly with strain DV, and never in MDM infected with strain IIIB. However, among SF162-infected cultures, there was only a general correlation between the degree of giant cell formation and the titer of p24 antigen in the supernate.

U937 and SUP-T1 Cell Lines. Both the promonocytic line, U937, and the T cell line, SUP-T1, supported replication of strains IIIB and DV to high titer, but strain SF162 replicated poorly with only trace amounts of p24 antigen detected (Fig. 6). Similarly, as measured by TCID50, strains IIIB and DV produced high titers of free infectious virus in both U937 and SUP-T1 cells, while no infectious virus was

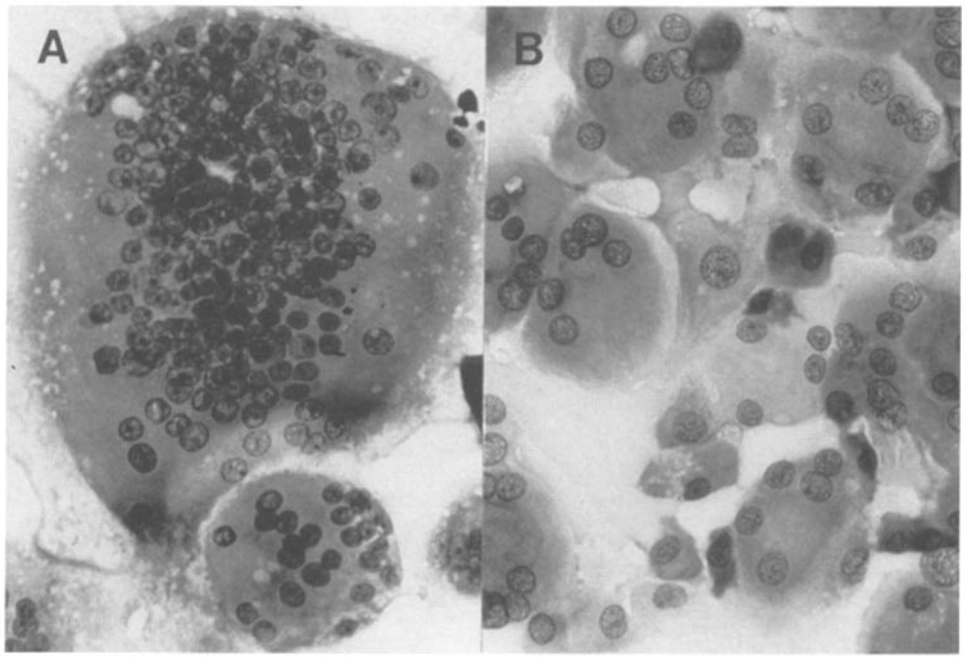

FIGURE 5. Morphology of infected MDM. Macrophages cultured for 14 d were infected with HIV-1 SF162 (75 ng of p24 antigen per 6 × 10^5 cells), and 22 d later cells were fixed and stained by a modified Wright-Giemsa method. (*A*) Infected culture showing very large syncytia. (*B*) Uninfected culture showing smaller multinucleated cells (×250).

TABLE IV

The Distribution of Polykaryocytes in HIV-1-infected and Uninfected Cultures of MDM

			Infected			
	Uninfected		Without antigen		With antigen	
Nuclei/cell	Nuclei	Percent	Nuclei	Percent	Nuclei	Percent
1	98	24	163	33	4	0
2–5	200	48	135	28	64	7
6–10	52	13	85	17	102	11
11–25	66	16	74	15	151	16
26–50	0	0	32	7	218	23
>50	0	0	0	0	415	44
	416*	100	489	100	954	100

MDM cultured for 14 d were infected with HIV-1 strain SF162 (75 ng p24 antigen per 6 × 10^5 cells), incubated for 22 d, and stained for viral antigen by immunofluorescence. Individual cells were classified according to the number of nuclei and presence of antigen; the total nuclei in the cells in each category is recorded.

* Totals of each column.

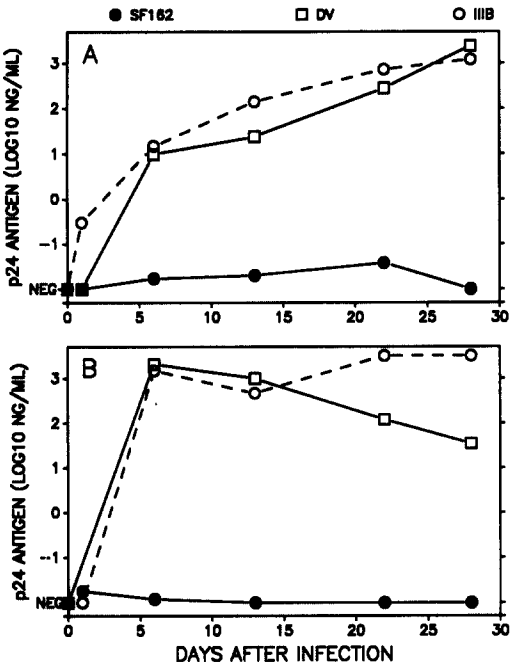

FIGURE 6. Antigen production by three strains of HIV-1 (SF162, DV, and IIIB) in U937 cells (*A*) and SUP-T1 cells (*B*). Cells were infected with equal amounts of virus (50 ng of p24 antigen per 10^6 cells) for 2 h, washed, and maintained as described. Supernatant was tested periodically for p24 antigen.

produced in these two cell lines after infection with strain SF162 (data not shown). Infection of U937 cells was noncytopathic, with no morphological changes or cell death, and viral antigen was distributed diffusely throughout the cytoplasm of virtually 100% of cells at peak infection. Infected SUP-T1 cells were also uniformly positive for cytoplasmic viral antigen but, in contrast, formed extensive syncytia with rapid cell killing. After variable periods of time, however, a non-syncytia-forming chronically HIV-IIIB-infected population of SUP-T1 cells emerged (data not shown).

The pattern of tropism of the three strains of HIV-1 in four different cell types is summarized in Table V. Strains SF162 and IIIB showed reciprocal patterns, since SF162 replicated in MDM but poorly in U937 or SUP-T1 cells, while strain IIIB replicated in U937 and SUP-T1, but poorly or not at all in MDM. PBL were the

TABLE V

Antigen Production by Selected Strains of HIV-1 in Primary Cultures and Transformed Lines of MDM and Lymphocytes

Cell type	Strain IIIB	Strain DV	Strain SF162
MDM	+	+ +	+ + +
PBL	+ + + +	+ + + +	+ + + +
U937	+ + + + +	+ + + + +	±
SUP-T1	+ + + + +	+ + + + +	±

U937, promonocytic cell line; SUP-T1, T lymphocyte cell line. Titers of p24 antigen in supernatant are reported as: + + + + + (>1,000 ng/ml); + + + + (100–1,000 ng/ml); + + + (10–100 ng/ml); + + (1–10 ng/ml); + (0.1–1 ng/ml); ± (<0.1 ng/ml). Values are the peak titers and represent the medians of 3–11 experiments.

only one of the four cell cultures in which all strains replicated to high titer. It is noteworthy that the promonocyte line, U937, showed a totally different pattern of permissiveness than did MDM, and that the T cell line, SUP-T1, showed a totally different pattern of permissiveness than did PBL.

Discussion

HIV strains are heterogeneous in their host range and infect a wide variety of cell types (38–41). Therefore, cell tropism is best defined if viruses and cells are studied in multiple combinations and cell lines are compared with the corresponding primary cells. By systematically examining the replication of a selected panel of prototype HIV-1 strains in primary monocyte-derived macrophages, primary lymphocytes, a promonocytic cell line, and a lymphoid cell line, we have developed a matrix that represents a spectrum of HIV-cell interactions from permissive to highly restricted (Table V). Our results confirm previous work, demonstrating that HIV-1 strains display differing degrees of monocyte tropism, but challenge the widespread use of cell lines as surrogates for primary macrophages and T lymphocytes in studies of tropism.

We found that the three isolates studied exhibit a gradation in their ability to replicate in macrophages; macrophages are most permissive for SF162, intermediate in permissiveness for DV, and least permissive for IIIB. These results confirm previous findings (6, 16, 21–25) of monocyte tropism in HIV isolates and document that some HIV strains may undergo a productive infection in the same macrophages that will only support persistent latent infection of other HIV strains.

The promonocytic U937 cell line is widely used as a surrogate for monocyte/macrophages in studies of HIV infection (42–46). U937 cells resemble promonocytes rather than MDM (47), and there are other significant differences between U937 cells and monocyte/macrophages, including CD4 expression, which is high in U937 cells but very low in MDM. U937 cells are readily infected by the lymphocyte-tropic strain IIIB and do not support replication of the monocyte-tropic strain SF162, which is the inverse of the pattern seen with primary MDM. SUP-T1 is one of a number of cell lines used as a model for T4 lymphocytes (48) and expresses high levels of CD4. However, SUP-T1 cells fail to support replication of strain SF162, which replicates to high titer in primary lymphocytes (24). Thus, the promonocytoid U937 and lymphoid SUP-T1 cell lines demonstrate similar patterns of viral restriction (Table V), and are not adequate surrogates for primary MDM and PBL in studies of viral host cell tropism. It is possible that U937 cells induced to "differentiate" to a more mature phenotype would demonstrate a pattern of permissiveness more representative of macrophages (49–51).

Because T4 lymphocytes are exquisitely permissive for HIV infection, it is critical to document that HIV infection of MDM involves macrophages and is not limited to a contaminating fraction of lymphocytes. Our immunofluorescence observations indicate that a large proportion of cells are antigen positive and that infection is strongly associated with syncytium formation, the best established cytopathic effect of HIV. Since >90% of the cells in these cultures exhibit macrophage markers (Table I), it seems clear that MDM are heavily infected. HIV antigens are concentrated in focal accumulations (Fig. 4), and this could reflect both internal budding of virus (16) and phagocytic accumulation of virions and viral proteins. The immunofluorescent

images also suggest (Fig. 4 *B*) that HIV may spread by cell-to-cell transmission of virus as well as by production of free infectious virions, which could be relevant to the potential blocking effects of soluble T4 or of antiviral antibody.

Summary

To characterize the host range of different strains of HIV-1, we have used four types of cells, primary monocyte-derived macrophages (MDM), primary PBL, a promonocyte cell line (U937), and a $CD4^+$ T cell line (SUP-T1). These cells were infected with three prototype strains of HIV-1, a putative lymphocyte-tropic strain (IIIB), and two putative monocyte-tropic strains (SF162 and DV). Infections were monitored by assays for infectious virus, for cell-free and cell-associated viral antigen (p24), and for the proportion of cells infected by immunohistochemical staining.

It was concluded that: (*a*) the use of four different cell types provides a useful biological matrix for distinguishing the tropism of different strains of HIV-1; this matrix yields more information than the infection of any single cell type. (*b*) A monocyte-tropic strain of HIV-1, such as strain SF162, shows a reciprocal host range when compared with a lymphocyte-tropic strain such as IIIB; strain SF162 replicates well in primary MDM but not in U937 or SUP-T1 cells, while strain IIIB replicates well in both U937 and SUP-T1 cells but not in MDM. (*c*) Both lymphocyte-tropic and monocyte-tropic strains of HIV-1 replicate well in PBL. (*d*) The promonocyte cell line, U937, and the T cell line, SUP-T1, differ markedly from primary cells, such as MDM and PBL, in their ability to support the replication of different strains of HIV-1; these cell lines cannot be used as surrogates for primary cells in host range studies of HIV-1 strains.

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