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Replication of Macrophage-Tropic and T-Cell-Tropic Strains of Human Immunodeficiency Virus Type 1 Is Augmented by Macrophage-Endothelial Cell Contact

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Macrophages perform a central role in the pathogenesis of human immunodeficiency virus type 1 (HIV-1) infection and have been implicated as the cell type most prominent in the development of central nervous system impairment. In this study, we evaluated the effect of interaction between macrophages and endothelial cells on HIV-1 replication. Upregulation of HIV-1 replication was consistently observed in monocyte-derived macrophages (hereafter called macrophages) cocultured with either umbilical vein endothelial cells or brain microvascular endothelial cells. HIV-1 p24 antigen production of laboratory-adapted strains and patient-derived isolates was increased 2- to 1,000-fold in macrophage-endothelial cocultures, with little or no detectable replication in cultures containing endothelial cells only. The upregulation of HIV-1 in macrophage-endothelial cocultures was observed not only for viruses with the non-syncytium-inducing, macrophage-tropic phenotype but also for viruses previously characterized as syncytium inducing and T-cell tropic. In contrast, cocultures of macrophages with glioblastoma, astrocytoma, cortical neuronal, fibroblast, and placental cells failed to increase HIV-1 replication. Enhancement of HIV-1 replication in macrophage-endothelial cocultures required cell-to-cell contact; conditioned media from endothelial cells or macrophage-endothelial cocultures failed to augment HIV-1 replication in macrophages. Additionally, antibody to leukocyte function-associated antigen (LFA-1), a macrophage-endothelial cell adhesion molecule, inhibited the enhanced HIV-1 replication in macrophage-endothelial cell cocultures. Thus, these data indicate that macrophage-endothelial cell contact enhances HIV-1 replication in macrophages for both macrophage-tropic and previously characterized T-cell-tropic strains and that antibody against LFA-1 can block the necessary cell-to-cell interaction required for the observed upregulation. These findings may have important implications for understanding the ability of HIV-1 to replicate efficiently in tissue macrophages, including those in the brain and at the blood-brain barrier.

Human immunodeficiency virus type 1 (HIV-1) is commonly identified within the central nervous system (CNS), and ample evidence indicates that the CNS becomes infected early in the course of infection (8, 20, 43). A wide range of neurocognitive disorders has been observed in HIV-1-infected individuals, ranging from mild impairment to frank dementia (55). The severity and prevalence of these neurologic syndromes are often related to the stage of HIV-1 infection, with persons experiencing the most advanced disease more likely to exhibit AIDS-related dementia (46, 55).

Despite considerable agreement on the importance of the CNS as a target for HIV-1, there is little consensus on how HIV-1 enters the CNS and what mechanisms are involved in the pathogenesis of neurocognitive disease. Numerous investigators have identified HIV-1 within the brains of persons with AIDS and have observed a correlation between the amount of virus present postmortem and the degree of neurological impairment observed during life (35, 57). However, direct infection of neuronal cells generally has not been found and virus has been identified almost exclusively in cells of macrophage lineage, including brain macrophages, microglia, and multinucleated giant cells (46, 55). Thus, many have postulated that HIV-1-related CNS impairment is a consequence of the cytokines or neurotoxins produced by HIV-1 infection of macrophages rather than a result of direct HIV-1 infection of neural cells (12, 36, 39, 46, 55). Viral structural and regulatory proteins (gp120, Tat, Nef, Rev, etc.) may also contribute to neuronal injury.

How HIV-1 initially enters the brain remains unclear. Two theories of viral entry into the CNS have predominated. The first suggests that HIV-1 enters the brain via latently infected macrophages which spread virus throughout the brain. A second hypothesis, supported by the detection of a low-grade meningitis present in the brains of asymptomatically infected persons (55), is that virus penetrates through a disrupted blood-brain barrier via infected T cells or as free virus. In both cases, the cells ultimately infected are thought to be of macrophage lineage. The importance of T-cell-tropic strains within the CNS in the pathogenesis of CNS disease is unclear. However, because most infected persons have HIV-1 within the CNS early in the course of their infection, it has been proposed that only a subset of viruses may be capable of causing neurological impairment (11, 13, 47).

Abnormalities of the blood-brain barrier may be important for entry of HIV-1 into the brain and may also mediate some of the tissue damage associated with HIV-1 infection (40, 42, 44). Aberrations of endothelial cells lining the blood-brain barrier may be important in the breakdown of the barrier (39, 40). Moreover, endothelial cells frequently come in contact with circulating monocytes and, thus, may modulate monocyte-macrophage differentiation and HIV-1 replication (3, 45). In the current study, we examined the effect of endothelial cell-macrophage interaction on the replication of HIV-1. Our findings indicate that macrophage-endothelial cell contact not only upregulates HIV-1 production of macrophage-tropic strains, but also enables virus strains that would ordinarily be consid-

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ered T-cell tropic to replicate in macrophages. These results have important implications for the pathogenesis of HIV-1 infection in tissue, particularly within the CNS.

MATERIALS AND METHODS

Cells and viruses. Peripheral blood mononuclear cells (PBMC) obtained from HIV-1-seronegative donors were separated by Ficoll-Hypaque density centrifugation as previously described (32). HIV-1 viral stocks were propagated in phytohemagglutinin-stimulated PBMC by methods described previously, unless specified otherwise (56). Monocyte-derived macrophages (referred to herein as macrophages) were obtained from HIV-1-seronegative donor PBMC that were stimulated for 3 days with phytohemagglutinin (3 μg/ml) and then seeded at a concentration of 4×10^6 per well of a 12-well culture plate (Costar, Cambridge, Mass.) in media consisting of RPMI 1640 with glutamine, 20% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and interleukin-2 (5 U/ml). After 5 to 7 days, cells were vigorously washed with phosphate-buffered saline (PBS) to remove nonadherent cells. This procedure was repeated 1 to 2 days later prior to use of the cells in these experiments. We have found that cells obtained by this procedure are 100% esterase positive, >99% CD 11B positive, and <0.1% CD3 positive (32). Elutriated human adult monocytes (unstimulated) were obtained from Advanced Biotechnologies (Columbia, Md.). Following elutriation, cells were characterized by size, differential count by Wright's stain, and immunofluorescence for CD14 and CD15. For further purification, monocytes were adhered to plastic; any nonadherent cells were removed by washing with PBS.

Normal human umbilical vein endothelial cells were obtained from Clonetics (San Diego, Calif.). Pooled single-passage primary human dermal microvascular endothelial cells and human brain microvascular endothelial cells obtained at autopsy were acquired from the Applied Cell Biology Research Institute (Kirkland, Wash.). Human undifferentiated glioblastoma cell line A172 (CRL 1620), human astrocytoma/glioblastoma-derived cell line U373-MG (ATCC HTB17), human cortical neuronal HCN-1 (CRL 10442) cells, and human placental PLC (CRL 7325) cells were obtained from the American Type Culture Collection. Primary human foreskin fibroblasts (HFF) were obtained and propagated as previously described (54). MT-2 cells (a human T-cell line transformed with and continuously producing HTLV-I) (4) were obtained from the NIH AIDS Research and Reference Reagent Program.

Endothelial cells were propagated in endothelial growth cell medium (Clonetics) consisting of epidermal growth factor (10 ng/ml), hydrocortisone (1 μg/ml), and bovine brain extract. The U373-MG, A172, HCN-1, PLC, and HFF cell lines were maintained in Dulbecco's modified Eagle's medium with high-concentration glucose (4.5 g/liter) and 10% fetal bovine serum. The MT-2 cells were grown in RPMI 1640 with 10% fetal bovine serum. All media were supplemented with L-glutamine, and penicillin and streptomycin were added.

HIV-1 viral stocks of Ba-L (15, 16), JR-FL, and JR-CSF (26, 27) were obtained from the NIH AIDS Research and Reference Reagent Program; HIV-1 LAI (originally designated LAV) was obtained from Institut Pasteur (2). HIV-1 clinical isolates were obtained from the blood or cerebrospinal fluid of HIV-1-infected individuals monitored within the HIV Neurobehavioral Research Center or the AIDS Clinical Trials Units at the University of California, San Diego. All clinical strains were initially isolated in PBMC.

Infection of cells. Experiments involving HIV-1 infection of macrophages, endothelial cells, and macrophage-endothelial cocultures were performed with medium consisting of a 1:1 mixture of RPMI 1640 and endothelial growth cell medium. Standard experimental infection conditions consisted of maintenance of 2×10^5 adherent macrophages and 2×10^4 adherent endothelial cells in segregated culture and in coculture for 24 h prior to exposure to 1×10^4 50% tissue culture infective doses of HIV-1. Thus, cultures were typically infected at a multiplicity of infection of 0.05. Following overnight absorption with virus, all cultures were extensively washed with PBS until no p24 antigen was detectable after the cultures were refed with fresh medium. Endothelial cells typically reached confluence ($\sim 6 \times 10^5$ cells per well) within 3 to 4 days postinfection, at which time half of the culture supernatant was exchanged for fresh medium and p24 antigen quantitations were determined by enzyme-linked immunosorbent assay (Abbott Laboratories, Abbott Park, Ill.) from supernatants that were exchanged every 3 to 5 days for 14 to 21 days postinfection.

Virologic assays. The amount of HIV-1 p24 antigen was determined by an HIV-1 enzyme immunoassay (Abbott Laboratories). Values greater than 25 pg/ml were considered positive. Quantitative assays for infectious viral yield used procedures previously described (25). The MT-2 cell assay was used to determine the syncytium-inducing capacity of HIV-1 strains (4). Immunofluorescence assays using anti-HIV p24 antigen monoclonal antibody (Dupont, Wilmington, Del.), anti-CR3 antibody (Coulter, Hialeah, Fla.), and anti-factor VIII (Biomeda, Foster City, Calif.) were performed.

Adhesion-blocking antibodies. Adhesion-blocking monoclonal antibodies to leukocyte function-associated antigen (LFA-1; CD11a) were obtained from both Pharmingen (San Diego, Calif.) and Biosource International (Camarillo, Calif.). Adhesion-blocking monoclonal antibodies to intercellular adhesion molecule 1 (ICAM-1; CD54) were obtained from both AMAC (Westbrook, Maine) and R&D Systems (Minneapolis, Minn.).

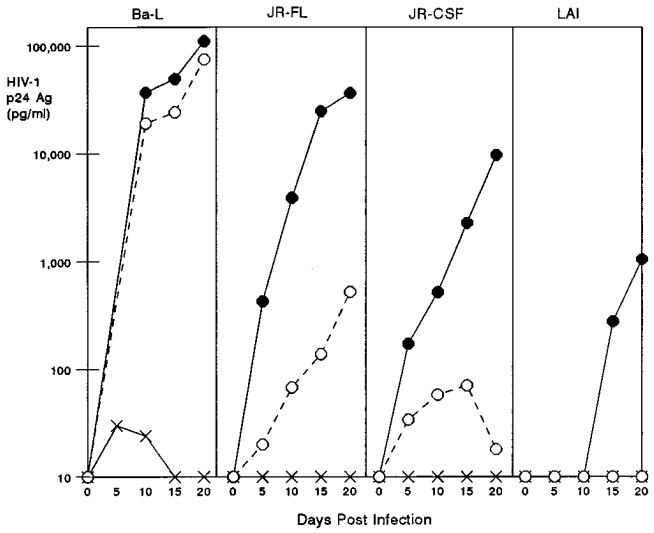

FIG. 1. Augmentation of HIV-1 replication in macrophage-endothelial cell cocultures. Macrophages (○), endothelial cells (×), and macrophage-endothelial cell cocultures (●) were simultaneously infected with four laboratory-adapted HIV-1 strains at a multiplicity of infection of 0.05 as described in Materials and Methods. The amount of HIV-1 p24 antigen production was determined from culture supernatants at the indicated times. The results shown are of representative experiments from at least three to five replicate experiments performed for each HIV-1 strain (Ba-L, JR-FL, JR-CSF, and LAI).

RESULTS

Infection of macrophages by macrophage-tropic laboratory HIV-1 strains is augmented by coculture with endothelial cells. To study the effect of endothelial cells on HIV-1 replication in macrophages, umbilical vein endothelial cells and macrophage-endothelial cocultures were infected at a multiplicity of infection of 0.05 with macrophage-tropic non-syncytium-inducing (NSI) strains of HIV-1, $HIV_{Ba\text{-}L}$, $HIV_{JR\text{-}FL}$, and JR_{CSF} (Fig. 1). As previously observed (7), $HIV_{Ba\text{-}L}$ replicated with high efficiency, producing more than 10^5 pg of p24 antigen per ml in culture supernatants of macrophage-only cultures. A moderate to low degree of replication in macrophages infected with $HIV_{JR\text{-}FL}$ and $HIV_{JR\text{-}CSF}$, resulting in less than 10^3 pg of p24 antigen per ml, was observed. Infection of endothelial cultures consistently resulted in low (below 50 pg/ml) to undetectable levels of p24 antigen production with each of the macrophage-tropic HIV-1 strains evaluated (Fig. 1).

Cocultures of macrophages and endothelial cells uniformly resulted in an upregulation of HIV-1 replication compared with replication in macrophage cultures alone (Fig. 1). Increases in replication were obtained consistently in three separate experiments for rapidly replicating $HIV_{Ba\text{-}L}$, in which a twofold increase was observed at days 5 and 10 postinfection (mean ± standard error fold increase at day 5 was 2.1 ± 0.2 and at day 10 was 1.8 ± 0.1). There was a 10- to >100-fold increase for $HIV_{JR\text{-}FL}$ and $HIV_{JR\text{-}CSF}$. For $HIV_{JR\text{-}FL}$, the mean increase in p24 antigen (± standard error) produced at day 15 postinfection was 18.6 (± 9.5)-fold greater in macrophage-endothelial cell cultures than in macrophages alone. For $HIV_{JR\text{-}CSF}$, no p24 antigen was produced in three experiments in macrophage-only cultures versus a mean of 1,550 ± 559.5 pg in macrophage-endothelial cell cultures. In two additional experiments, a small amount of p24 antigen was detected when macrophages alone were infected with $HIV_{JR\text{-}CSF}$ (203 ± 13 pg) but macrophage-endothelial cell cultures produced 19-fold-greater quantities of p24 antigen.

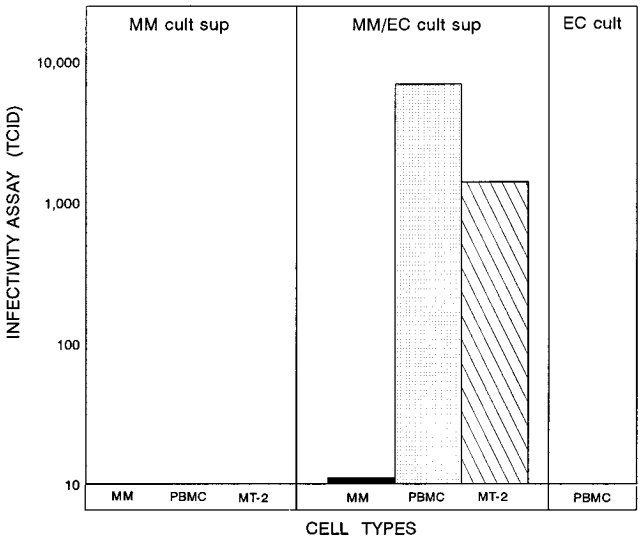

FIG. 2. Replication of supernatant virus from T-cell-tropic SI HIV-1 strains in macrophage-endothelial cell cocultures and maintenance of their original phenotype. Supernatant (sup) virus from macrophages, endothelial cells (EC), and macrophage-endothelial cell cocultures previously infected with HIV_{LAI} was used to infect macrophages (MM), PBMC, and MT-2 cells. Supernatant virus from macrophage-endothelial cell coculture replicated in PBMC and MT-2 cells; supernatant from both macrophages and endothelial cells failed to produce detectable p24 antigen in the cells evaluated. cult, culture; TCID, tissue culture infective dose.

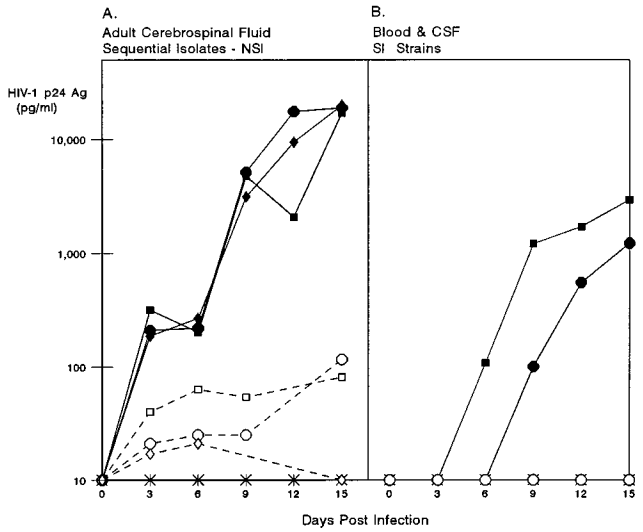

FIG. 3. Increased replication of patient-derived HIV-1 strains in macrophage-endothelial cell cocultures. (A) HIV-1 replication of three sequential isolates (CSF-6589, -6590, and -6591) cultured from the cerebrospinal fluid of an adult AIDS patient with neurocognitive impairment. The isolates were cultured in macrophages (CSF-6589 [◇], CSF-6590 [□], and CSF-6591 [○]), endothelial cells (×), and macrophage-endothelial cell (CSF-6589 [◆], CSF-6589 [■], and CSF-6591 [●]) cocultures. (B) Replication of T-cell tropic SI HIV-1 strains cultured from the PBMC of a child with AIDS and the cerebrospinal fluid (CSF) of an adult with neurocognitive impairment. Cultures in macrophages (○, □), macrophages-endothelial cells (●, ■), and endothelial cells (×) are represented.

In order to eliminate the possibility that the increased replication of HIV-1 resulted from low-grade contamination with $CD4^+$ lymphocytes, unstimulated monocytes were obtained by a three-step purification procedure which included initial Ficoll-Hypaque separation followed by elutriation and adherence in culture prior to the addition of endothelial cells and infectious virus. Similar results were obtained for each of the viruses studied and were not dependent on the method used for obtaining peripheral blood monocyte-derived macrophages (data not shown).

Replication of T-cell-tropic (SI) HIV-1 strains is facilitated in macrophage-endothelial cocultures. To determine if the upregulation of HIV-1 in macrophage-endothelial cocultures was specific for macrophage-tropic HIV-1 strains, macrophages, endothelial cells, and macrophage-endothelial cell cocultures were infected with HIV_{LAI}. As expected (7, 30), HIV_{LAI} failed to replicate in macrophages or endothelial cultures. However, approximately 10^3 pg of p24 antigen per ml was consistently detected in macrophage-endothelial cell cocultures, indicating that HIV-1 replication was induced in the cocultures despite the inability of the virus to replicate in either of the cells alone (Fig. 1).

To ascertain that the p24 antigen detected in coculture supernatants represented infectious virus, supernatants obtained from macrophages, endothelial cells, and macrophage-endothelial cell cocultures were used to infect PBMC (Fig. 2). The supernatants from both the macrophages and endothelial cells infected with HIV_{LAI} failed to produce p24 antigen in PBMC. In contrast, extensive infection of PBMC in cultures infected with supernatants from the macrophage-endothelial cell cocultures was observed. Additionally, the same supernatants, when used to infect MT-2 cells, indicated that virus produced in the original cocultures remained T-cell tropic and was still capable of inducing syncytia.

To determine the cell type(s) responsible for replicating HIV-1 in macrophage-endothelial cell cocultures, cultures were infected with HIV-1 and evaluated by immunofluorescence. HIV-1 Gag proteins were detected in macrophages which were colabeled with antibodies to CR3. In contrast, Gag proteins were not detected in endothelial cells which were factor VIII positive (data not shown).

Replication of patient-derived HIV-1 isolates is enhanced in macrophage-endothelial cell cocultures. Because comparisons of findings with laboratory-adapted strains and patient-derived strains of HIV-1 may yield misleading results (38), we performed experiments similar to those described above using low-passage-number clinical isolates. Isolates used for these studies were derived from adults and children and were originally isolated from either patient PBMC or cerebrospinal fluid. Results obtained for the clinical isolates were similar to those observed for the laboratory-adapted strains of both macrophage-tropic NSI isolates and T-cell-tropic SI strains (Fig. 3).

Three HIV-1 strains examined in these studies were originally isolated from the cerebrospinal fluid of an AIDS patient with progressive neurocognitive impairment (designated CSF-6589, -6590, and -6591). Each of these isolates replicated to a moderate extent in macrophages. However, when these isolates were grown in macrophage-endothelial cocultures, more than a 100-fold increase in viral replication was detected (Fig. 3A). Other clinical isolates obtained from a child perinatally infected with HIV-1, which were originally characterized by our laboratory as SI and T-cell tropic (and unable to replicate in macrophages), were consistently found to replicate efficiently in macrophage-endothelial cell cocultures as well (Fig. 3B).

In total, we examined five macrophage-tropic NSI isolates and three T-cell-tropic SI isolates grown from cerebrospinal fluid and three macrophage-tropic NSI and two T-cell-tropic SI strains isolated from PBMC. For each virus, replication was

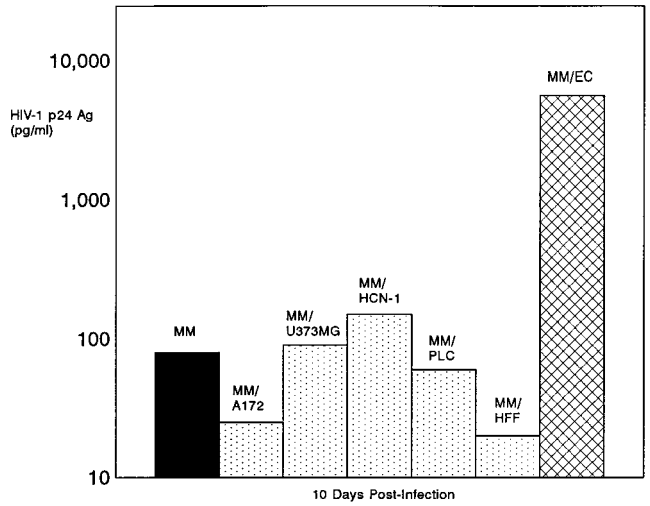

FIG. 4. Augmentation of HIV-1 replication limited to macrophage-endothelial cell cocultures. Monocyte-derived-macrophages (MM) were maintained in segregated culture and in coculture with a glioblastoma cell line (A172), an astrocytoma line (U373MG), a human cortical neuronal cell line (HCN-1), a primary placental culture (PLC), human foreskin fibroblasts (HFF), and endothelial cells (EC). The amount of HIV-1 p24 antigen was determined 10 days postinfection.

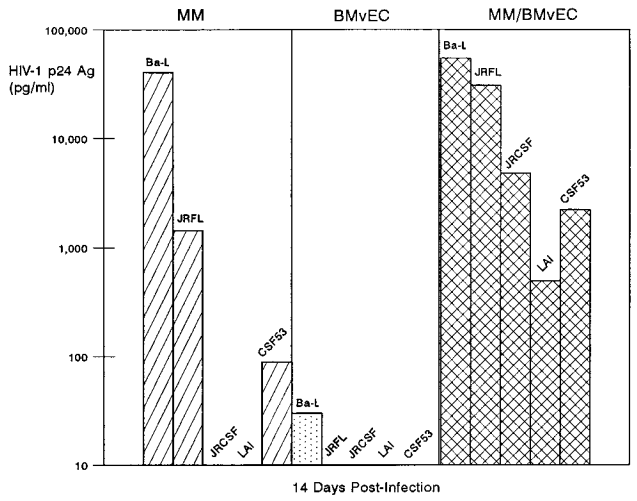

FIG. 5. Replication enhancement by brain microvascular endothelial cells of HIV-1 in macrophage-brain microvascular endothelial cell cocultures. The levels of replication of strains $HIV_{Ba\text{-}L}$, $HIV_{JR\text{-}FL}$, $HIV_{JR\text{-}CSF}$, HIV_{LAI}, and HIV_{CSF53} (a T-cell-tropic SI isolate from cerebrospinal fluid) in macrophages (MM), brain microvascular endothelial cells (BMvEC), and macrophage-brain microvascular endothelial cell cocultures are shown.

enhanced in macrophage-endothelial cocultures, an enhancement ranging from 5- to >100-fold the replication rate observed for macrophage cultures alone.

Augmentation of HIV-1 replication is limited to macrophage-endothelial cell cocultures. To determine if the enhancement of HIV-1 replication was specific to the interaction between macrophages and endothelial cells or was a general phenomenon that occurred when macrophages came in contact with other cells, human undifferentiated glioblastoma-derived cell line A172 (CRL 1620), human astrocytoma/glioblastoma-derived cell line U373-MG (ATCC HTB17), human cortical neuronal HCN-1 (CRL 10442) cells, human placental PLC (CRL 7325) cells, and human fibroblast cells were cocultured with macrophages and infected with different strains of HIV-1. There was no increase in HIV-1 replication observed for any of these cell lines when supernatants from macrophage cocultures were examined for p24 antigen production (Fig. 4). Thus, the augmentation of HIV-1 production appears to be specific for macrophage-endothelial cell interaction and is not generalizable to other macrophage cocultures.

HIV-1 replication is enhanced by brain and dermal microvascular endothelial cells. The experiments described above demonstrate that HIV-1 production can be augmented when macrophages are cocultured with umbilical vein endothelial cells. However, it was unknown whether endothelial cells of different origin would similarly enhance viral replication in macrophages. Because the interaction of endothelial cells with macrophages may be important in the pathogenesis of HIV-related CNS disease, the next series of experiments were designed to determine if brain microvascular endothelial cells would augment HIV-1 infection of macrophages. Findings for the macrophage-brain endothelial cell cocultures were similar to those previously described for endothelial cells derived from umbilical veins. Replication of $HIV_{Ba\text{-}L}$ was increased at least 2-fold in cocultures, while $HIV_{JR\text{-}FL}$, $HIV_{JR\text{-}CSF}$, and HIV_{LAI} replication rates were enhanced from 10-fold up to 1,000-fold (Fig. 5). Little or no replication of HIV-1 was observed in brain endothelial cells infected with HIV-1. Further experiments were performed with dermal microvascular endothelial cells; similar enhancement of HIV-1 replication was observed for macrophage-dermal endothelial cell cocultures (data not shown). Thus, endothelial cell-mediated enhancement of HIV-1 infection in macrophages was not limited to endothelial cells obtained from a single site but was a general property of vascular endothelial cells.

Enhanced HIV-1 replication in macrophage-endothelial cell cocultures requires cell-to-cell contact and can be blocked by monoclonal antibody to LFA-1. Although in previous experiments we established that the interaction of macrophages with endothelial cells results in an upregulation of HIV-1 replication, the interaction necessary between macrophages and endothelial cells that mediates this enhancement had not been established. Thus, subsequent experiments were designed to determine if the enhancement observed in cocultures requires endothelial cell-macrophage contact or if certain as-yet-unidentified soluble factors mediate the augmented viral infection. In the initial experiments, conditioned medium obtained from endothelial cells, macrophages, or macrophage-endothelial cell cocultures was added to macrophages infected with $HIV_{JR\text{-}FL}$. No consistent differences between any of these cultures and standard macrophage cultures were observed during multiple experiments (Fig. 6A). In similar experiments, macrophages and endothelial cells were cultured in upper and lower *trans*-well chambers, which allowed for interchange of media between the two chambers but prevented contact between the two cell types, but these conditions also failed to alter the HIV-1 replication rate from that of macrophages cultured alone.

To further determine if cell-to-cell contact was required for enhanced HIV-1 replication, endothelial cells, macrophages, and macrophages-endothelial cells were infected with HIV-1 in the presence of monoclonal antibodies directed against adhesion molecules LFA-1 (anti-CD11a) and ICAM-1 (anti-CD54) (Fig. 6B). Antibodies were added 24 h before infection and were maintained at a concentration of 0.5 μg/ml throughout the course of the experiment. In the cultures treated with the monoclonal antibody directed against LFA-1, no effect on

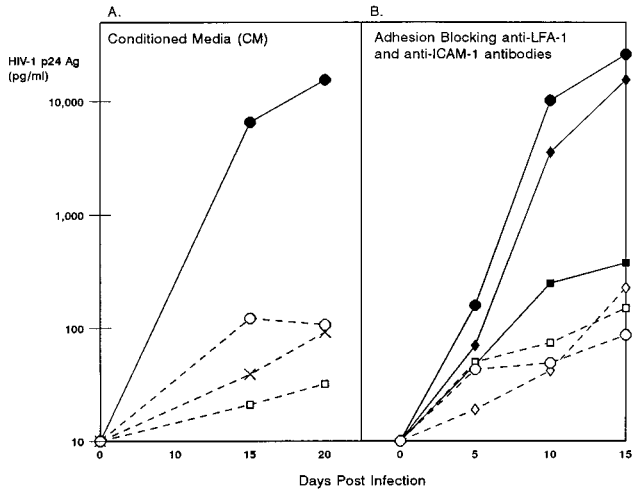

FIG. 6. Monoclonal antibody to LFA-1 inhibition of enhanced HIV-1 replication in macrophage-endothelial cell cocultures. (A) HIV-1 p24 antigen production from macrophage-endothelial coculture (●) and from macrophage cultures that were continuously supplemented with conditioned media from either macrophages (○), endothelial cells (×), or macrophage-endothelial cocultures (□). (B) HIV-1 p24 antigen production from macrophages (open symbols) and macrophage-endothelial cocultures (solid symbols) maintained in culture media (○ and ●) and in media in which adhesion-blocking monoclonal antibodies anti-LFA-1 (□ and ■) or anti-ICAM-1 (◇ and ◆) were added and maintained at a concentration of 0.5 μg/ml.

viral replication was observed in macrophage cultures. However, the upregulation of HIV-1 replication in macrophage-endothelial cell cocultures was almost completely inhibited by the addition of the anti-LFA-1 monoclonal antibody. In contrast, the antibody directed against ICAM-1 failed to alter HIV-1 replication in either the macrophage cultures or macrophage-endothelial cell cultures even at antibody concentrations of up to 2.5 μg/ml.

DISCUSSION

Overwhelming evidence indicates the importance of macrophages in the pathogenesis of HIV-1-related disease (19, 33). Although peripheral blood monocytes have been identified as harboring and replicating HIV-1, tissue macrophages are far more important sites of infection and serve as critical reservoirs of HIV-1 (19). Considerable data support the argument for persistent productive infection of macrophages without true latent infection (18), and HIV-1-infected macrophages continue to replicate virus for prolonged periods without cell lysis or apparent cytopathicity (18, 19). Several HIV-1 strains determined to be macrophage tropic or T-cell tropic have been characterized phenotypically and genotypically by a large number of investigators. Although there is no full consensus, several overriding principles have been proposed by a number of groups. Phenotypic analyses of macrophage- versus T-cell-tropic viruses indicate that whereas macrophage-tropic viruses will infect both macrophages and T cells, T-cell-tropic viruses will productively infect only T cells (18). T-cell-tropic viruses may also induce syncytium, whereas syncytium formation is usually not observed with macrophage-tropic viruses. Many data also support the hypothesis of selection for T-cell-tropic SI virus with prolonged HIV-1 infection and progression of related symptoms (50). The genotypic basis for macrophage versus T-cell tropism has also been investigated extensively (1, 6, 9, 10, 14, 24, 29, 34, 51). Most studies have indicated that the viral determinants for cellular tropism map to a domain of gp120 that includes the third variable region (V3) of gp120, which contains a disulfide-linked loop of about 35 amino acids and also has a major HIV-1-neutralizing epitope (21, 31). In one study, as few as three amino acid changes could alter the tropism of a viral strain (52). In other reports, nonoverlapping regions of gp120 involving V4, V5, and the CD4 binding domain were found to be responsible for different tropisms (5). In another study, V1-to-V2 and V3 regions were found to contain the major determinants for SI capacity, although the V3-to-V4 regions were also found to contribute independently to syncytium induction (22). Although the majority of these data support the hypothesis that cellular tropism of HIV-1 is related to virus entry, more recent findings suggest that defects in late events of the viral life cycle may, actually, be responsible (24, 41). In particular, T-cell-tropic viruses have been found to efficiently fuse (41), enter, and synthesize viral DNA in macrophages (24, 48).

The current study supports the hypothesis that HIV-1 strains that would be characterized as SI and T-cell tropic can enter macrophages and, under conditions of macrophage adherence to endothelial cells, can efficiently replicate within macrophages. The upregulation of HIV-1 replication occurred for both macrophage-tropic and T-cell-tropic viral strains and was observed for a wide range of laboratory-adapted and low-passage-number clinical HIV-1 strains. Viruses associated with the highest replication rates in macrophages (i.e., $HIV_{Ba\text{-}L}$) demonstrated a 2- to 5-fold increase in virus production, whereas viruses with the lowest replication rates in macrophages alone were increased by 10- to >1,000-fold in macrophage-endothelial cocultures. No HIV-1 replication in endothelial cells was observed during the course of our studies, which is consistent with most (28, 30, 53) but not all (37) reports. The enhanced replication of HIV-1 observed in macrophage-endothelial cocultures was found in endothelial cells of different origins, including umbilical vein, brain microvascular, and dermal microvascular cells. However, the interaction of macrophages with endothelial cells was required for enhancement and was not observed when macrophages were cocultured with other cells lines, including human undifferentiated glioblastoma-derived cells, human astrocytoma/glioblastoma cells, human cortical neuronal cells, human placental cells, and human fibroblasts.

Our data strongly support the hypothesis that the enhanced replication of HIV-1 observed in macrophage-endothelial cell cocultures is dependent on cell-to-cell contact and is not mediated by soluble factors. The ability of monoclonal antibodies directed against a β_2-integrin molecule, LFA-1, to block increased replication in macrophage-endothelial cell cocultures supports the contention that macrophage-endothelial cell contact is required for the upregulation of HIV-1 infection. It is of interest that a monoclonal antibody against ICAM-1, for which LFA-1 is a ligand, failed to interrupt the HIV-1 enhancement in our experiments. An explanation for this apparent discrepancy is that LFA-1 is recognized by the first immunoglobulin domain of ICAM-1 (23), and that the monoclonal antibody used in our experiments is directed against a domain further toward the carboxy terminus or that other ligands on endothelial cells are involved. Our data are consistent with recent findings of Schrier et al., who demonstrated that antibodies directed against LFA-1 blocked macrophage–T-cell infection, thereby preventing HIV-1 expression in macrophages (49).

The findings reported here have important implications for the pathogenesis of HIV-1 infection. Our data suggest that although certain HIV-1 strains are more macrophage tropic than others, it is likely that most, if not all, viral strains can

enter and replicate efficiently in macrophages if the infected macrophage comes in contact with an endothelial cell. Because monocytes exist within the peripheral circulation for only 1 to 3 days (19), egress of monocytes from the peripheral circulation to extravascular tissue is an important route for dissemination of HIV-1 throughout body organs. An important component for the transit of monocytes into tissue is adherence and subsequent passage through endothelium. The transit of circulating monocytes to organ tissues occurs under noninflammatory conditions and increases during periods of inflammation. The adhesiveness of endothelial cells increases during periods of inflammation (3). Thus, increased recruitment of infected macrophages into tissues with increased macrophage-endothelial cell contact may promote enhanced HIV-1 replication and progressive disease.

The upregulation of HIV-1 replication in macrophages that are in contact with endothelial cells may be of particular importance in the pathogenesis of HIV-1-related CNS impairment. Epstein and Gendelman (12) have discussed the apparent paradox of the relatively small number of infected macrophages within the brain compared with the widespread CNS impairment that may be observed. They hypothesized that a cellular mechanism may be necessary to amplify the replication of persistent HIV-1 infection. They further suggested that interactions between macrophages and astroglia are important in the generation of neurotoxins which might be associated with diffuse CNS impairment. The finding that infected macrophages that adhere to endothelial cells may produce large amounts of HIV-1 provides further support for the hypothesis that cells of macrophage origin are critical in the progressive cascade of events which lead to neurocognitive disorders associated with HIV-1 infection. Additionally, the interaction of HIV-1-infected macrophages and endothelial cells may be important in the abnormalities observed at the blood-brain barrier. Enhanced HIV-1 replication in macrophages may contribute to the diffuse blood-brain barrier leak observed in patients with AIDS. Additionally, increased viral replication could promote inflammation and vascular endothelial adhesiveness with recruitment of increased numbers of infected monocytes as well as cytotoxic T lymphocytes from the peripheral circulation.

In summary, the data presented in this report indicate that HIV-1 infection of macrophages is enhanced by cell-to-cell contact with endothelial cells. Moreover, in addition to the augmented replication of macrophage-tropic HIV-1 strains, viruses that traditionally would be considered SI, T-cell tropic, and incapable of replicating productively in macrophages are induced to replicate efficiently in macrophage-endothelial cell cocultures. These findings suggest that most, if not all, strains of HIV-1 are capable, under certain conditions, of replicating in macrophages and that this replication may play an important role in the pathogenesis of HIV-1-related disease, particularly in the CNS.

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