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Effect of 3′-Azido-3′-Deoxythymidine on Human Immunodeficiency Virus Type 1 Replication in Human Fetal Brain Macrophages

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We investigated whether cells derived from the fetal central nervous system can support productive infection by a human immunodeficiency virus type 1 (HIV-1) isolate termed UHC-1, produced by a cellular clone derived from HIV-1 strain HIV-III$_B$ chronically infected U-937 promonocytic cells, and what the effect of nucleoside analogs might be on viral replication in this system. Fractionation of human fetal brain tissue into two different populations, enriched for either astrocytes or macrophages, showed that only the latter were able to support productive UHC-1 replication and generation of detectable progeny virus. Pretreatment of fetal brain macrophages with either of two nucleoside analogs, 3′-azido-3′-deoxythymidine (AZT) or the (−) enantiomer of 2′-deoxy-3′-thiacytidine, efficiently blocked production of progeny virus. Generation of unintegrated proviral DNA and HIV-1 transcripts were inhibited by pretreatment of fetal brain macrophages with 1 μM AZT. Administration of AZT at 24 h postinfection led to a slight reduction in viral transcript levels and viral progeny production by day 15 postinfection; however, brain macrophages under these conditions did not contain detectable amounts of unintegrated viral DNA. These results suggest that AZT may interfere with the accumulation of unintegrated HIV-1 DNA in brain macrophages. This is the first demonstration that nucleoside analogs are able to block HIV-1 replication in primary cultures of brain cells.

Neurological dysfunction is frequently encountered in advanced cases of AIDS. Complications associated with replication of the human immunodeficiency virus type 1 (HIV-1) in neural tissue may include myelopathy, peripheral nerve disease, meningitis, and a progressive deterioration of mental function, termed AIDS dementia complex (30, 41). AIDS-associated neuropathology is mainly attributed to replication of HIV-1 in cells of the central nervous system rather than to opportunistic infections. HIV-1 proteins, nucleic acids, and virions have often been detected in cells of monocyte-macrophage lineage in the brains of AIDS patients (18, 36, 41, 42). Other cell types, such as capillary endothelial cells (41, 42) and to a lesser extent glial cells (36, 41, 42), have also been shown to express viral products.

Several reports have shown that AIDS dementia may be reversed by the administration of 3′-azido-3′-deoxythymidine (AZT) (29, 32, 43). These observations suggest that the brains of patients with AIDS encephalopathy were not irreversibly damaged, implying that indirect viral effects rather than cytopathology and cell death were the cause of neurological abnormalities. AZT, as well as other 2′,3′-dideoxynucleosides in the triphosphate form, effectively block reverse transcription (8, 35) and are not believed to possess antiviral activity following proviral DNA synthesis (22). Therefore, the effects of AZT on AIDS encephalopathy do not directly conform with the hypothesized mechanism of action of this drug.

To examine this subject, we have established primary cultures derived from human fetal brain, enriched for either macrophages or astrocytes. We challenged these cultures with a viral clone, termed UHC-1, derived from a U-937 promonocytic cell line chronically infected with the HIV-III$_B$ strain of HIV-1 (2). Three factors prompted us to investigate the susceptibility of fetal brain macrophages and astrocytes to infection and replication by HIV-III$_B$ produced by U-937 promonocytic cells. First, several cell lines derived from malignant gliomas are permissive to a latent or low-level infection by HIV-III$_B$ (4, 5, 11, 12); equally susceptible to this virus are primary cultures of glial cells derived from fetal dorsal root ganglia (20). Second, both adult peripheral blood and cord blood monocyte-derived macrophages (MDM) are susceptible to a low-level infection by HIV-III$_B$ (14, 28, 40). Third, supernatants from HIV-III$_B$-infected U-937 cells but not HIV-III$_B$-infected T lymphocytes were shown to contain neurotoxins (10). In addition, direct cocultivation of infected U-937 cells with primary cultures of human spinal cord and cortex led to adhesion and neurotoxicity as well as astrocyte destruction (38). No such effect was observed with HIV-infected lymphocytes. These results suggest that HIV-III$_B$ may have an affinity for cells of the central nervous system and the ability, albeit at a low level, of replicating in macrophages.

We report here that only fetal brain macrophage cultures can become productively infected by the UHC-1 laboratory strain of HIV-1. Treatment of brain-derived macrophages with either of two nucleoside drugs, AZT and the (−) enantiomer of 2′-deoxy-3′-thiacytidine (3TC), effectively prevented viral particle release as judged by p24 antigen in culture supernatants. Both addition of 1 μM AZT at 24 h postinfection and pretreatment of fetal brain macrophages with this drug inhibited accumulation of unintegrated viral DNA, whereas pretreatment with AZT was necessary to prevent generation of viral mRNA.

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MATERIALS AND METHODS

Cell cultures derived from human fetal brain. Cultures of macrophages and astrocytes were established from human fetal brains (14 to 20 weeks of gestational age) of legally induced aborted fetuses. This study was approved by the Committee on Medical, Dental and Pharmaceutical Evaluation of McGill University and the Montreal Children's Hospital.

Macrophage cultures. Brain-derived macrophage cultures were established by a modification of a previously described method (17). Mononuclear cells, obtained by Ficoll-Hypaque gradient centrifugation of dispersed single-cell suspensions from fetal brain tissue, were seeded into either plastic tissue culture flasks or 9-mm plastic coverslips, in RPMI 1640 supplemented with 20% heat-inactivated fetal calf serum (hiFCS), 10% pooled human serum (ICN, Montreal, Canada), 2 mM glutamine, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Nonadherent cells were removed by washing with phosphate-buffered saline (PBS) 4 days after culture initiation, and adherent cells were maintained in the same medium but without human serum. Infections were done with 7- to 10-day macrophage cultures. Nonspecific esterase staining was performed on cells from 8-day cultures that had been fixed in situ with Sigma kit No. 90 (Sigma Inc, St. Louis, Mo.). Indirect immunofluorescence assays (IFA) for cellular and viral antigens were performed as described previously (2).

Astrocyte cultures. To establish cultures of human fetal astrocytes, a modification of a previously published protocol was employed (44). After human fetal brain tissue dispersion, the resulting cell suspension was filtered through sterile nylon mesh (100 μm) and centrifuged at 1,000 × *g* for 10 min. Cells were resuspended in a hormonally defined D-MEM medium (23), plated onto 9-mm poly-L-lysine-coated (Sigma) coverslips or tissue culture flasks, and maintained in this medium for 5 to 7 days. The vessels were shaken to dislodge nonadherent cells, which were removed by pipetting. The adherent population consisted almost entirely of astrocytes and was grown in D-MEM/HAMF-12 medium supplemented with 10% hiFCS (23). IFA was performed on cells grown on coverslips at 8 days after culture initiation, using a polyclonal rabbit anti-glial fibrillary acidic protein (GFAP) antiserum (kindly supplied by Hyman Schipper, Jewish General Hospital).

PBL cultures. Peripheral blood lymphocytes (PBL) were isolated by centrifugation over Ficoll-Hypaque (Pharmacia) and seeded at 7×10^5 cells per ml in the same medium as that used for brain macrophages, in the presence of 2 μg of phytohemagglutinin (PHA) per ml, for 3 days. Only nonadherent cells were harvested; these were seeded at 5×10^5 cells per ml in the same medium, without PHA but supplemented with 10 U of interleukin-2 (Boehringer-Mannheim) per ml.

Viral isolates and infection of cell cultures. For most experiments, virus was produced by a cellular clone termed UHC-1, derived by limiting dilution of a HIV-III_B chronically infected U-937 cell line (2). The JRFL macrophage-tropic viral isolate (19) was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health; it had been deposited by Irvin S. Y. Chen. Both the JRFL viral isolate and UHC-1 were propagated on PHA-stimulated human peripheral blood mononuclear cells to titers of 8.9×10^4 and 7.6×10^4 50% tissue culture infective doses, respectively. For each of these viral isolates, the concentration of viral p24 antigen in culture fluids of infected cells was determined by enzyme-linked immunosorbent assay (Abbott Laboratories) according to the manufacturer's instructions. All culture supernatants were kept at −70°C until used. Since the viral strains used display variation in ability to replicate in different cell types, we standardized viral inocula on the basis of p24 antigen, as suggested by other workers (24, 33).

Cell cultures were initially pretreated with polybrene (2 μg/ml) (Sigma) for 20 to 30 min. The cells were then inoculated with filtered (0.45-μm-pore-size filter) DNase I-treated culture supernatant containing equal amounts of p24 antigen from the viral isolates. Virus was allowed to adsorb for 6 h at 37°C in the presence of drug where indicated, following which the cells were washed three times with PBS and refed with fresh medium in the presence or absence of drugs. Culture supernatants were entirely removed at each indicated time point for p24 antigen measurement and replaced with fresh medium with or without drug.

Polymerase chain reaction (PCR). Macrophage monolayers were washed twice with PBS, and low-molecular-weight (LMW) DNA was extracted by a modification of the Hirt technique (13). One milliliter of Hirt lysis buffer (10 mM Tris-Cl, pH 7.4, 10 mM EDTA, pH 8.0, 0.6% sodium dodecyl sulfate) was used to lyse 10^6 cells. Following precipitation of high-molecular-weight DNA and cellular proteins by the addition of 1 M NaCl, the LMW DNA was obtained by centrifugation of the lysate followed by ethanol precipitation of the supernatant. One-fifth of the LMW DNA fraction was used in PCR amplification. Primers in this study were derived from the nucleotide sequence of the BH10 molecular clone, described by Ratner et al. (31). Primers S1 (nucleotide position 496 to 516) and A13 (nucleotide position 635 to 614) were derived from primers M667 and M661, respectively (45). Primer SG4 (nucleotide position 704 to 730) corresponds to the previously described LA8 primer, while AG4 (nucleotide position 804 to 784) was adapted from the LA9 primer (45) (see Fig. 5A). Primers SG4 and S1 were end labelled and added with cold AG4 and A13 to the LMW DNA. Dilutions of *Xho*I-linearized pHXB-2D (31), which contains a full-length HIV-1 genome, were used as a standard for PCR amplification in order to establish the template copy number. The samples were subjected to 27 cycles of denaturation for 1 min at 94°C, annealing for 1.5 min at 60°C, and polymerization for 1 min at 72°C. The PCR product was run on a 8% denaturing polyacrylamide gel, which was then dried and autoradiographed.

Northern and dot blotting. Northen (RNA) and dot blotting as well as probes were described elsewhere (9). Briefly, for northern blotting, 20 μg of total RNA was electrophoresed through a 1% agarose-formaldehyde gel and transferred to nitrocellulose. Probes which served to reveal HIV-1 (pBH10) and H3 histone (pRAH3-2) mRNA were generated by nick translation (Boehringer-Mannheim) while the β-actin and CD4 probes were generated by random priming of cDNA inserts (Amersham Corp., Chicago, Ill.). RNA levels were quantitated by scanning the autoradiograms with a LKB Ultroscan XL laser densitometer.

RESULTS

Differential susceptibilities of specifically enriched brain-derived cell cultures to UHC-1 infection. We established two types of enriched cultures from human fetal brain: brain-derived macrophages and astrocytes. IFA, using an anti-GFAP antiserum, demonstrated that the astrocyte-enriched

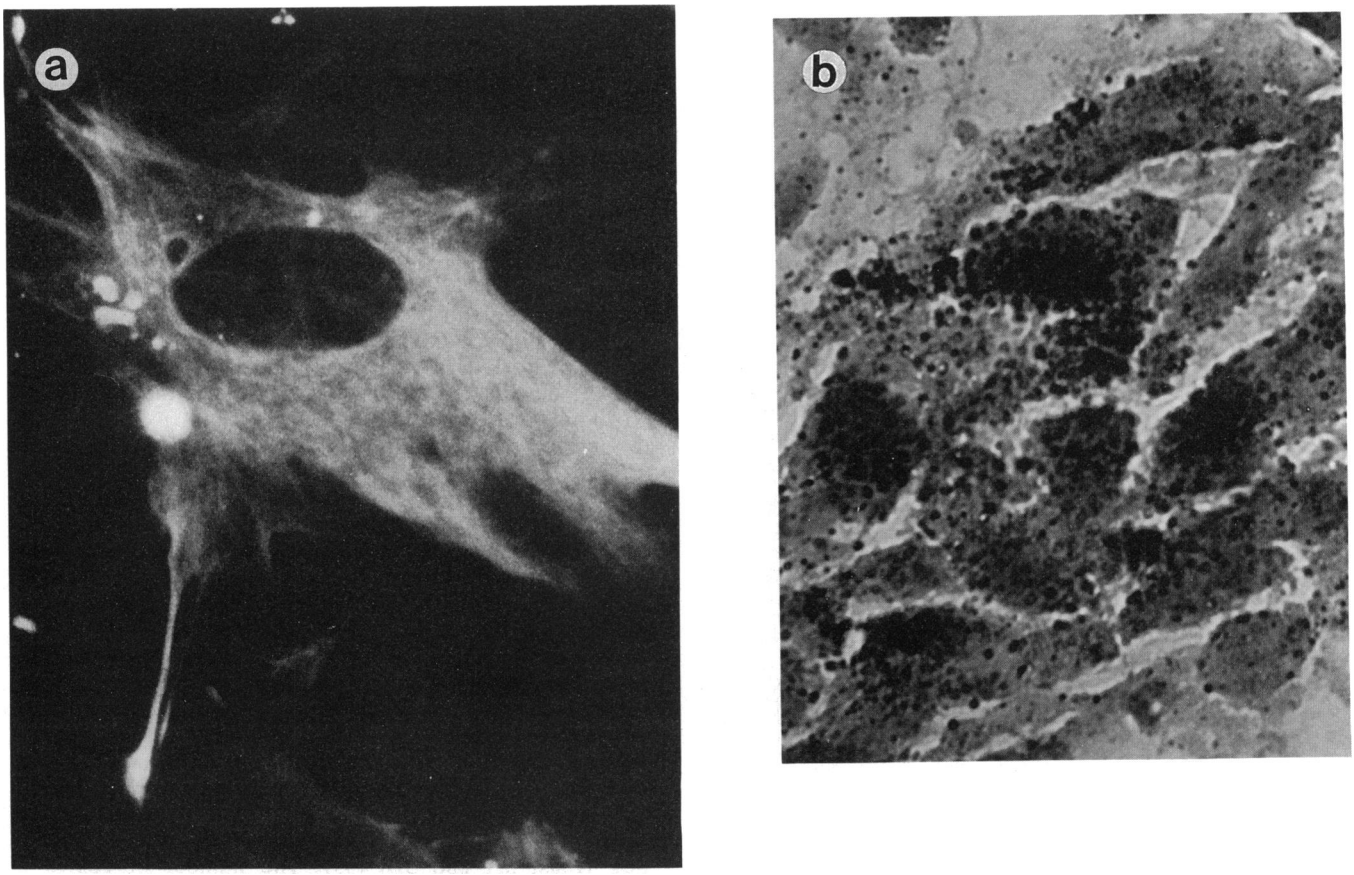

FIG. 1. Enriched fetal brain macrophage and astrocyte cultures. (a) Identification of GFAP-positive cells by IFA in astrocyte-enriched cultures derived from human fetal brain tissue at 8 days after culture initiation. Between 80 and 95% of cells in these cultures were positive for GFAP expression. (b) Nonspecific esterase staining of fetal brain-derived macrophage-enriched cultures at 8 days after culture initiation. Over 95% of cells were positive.

cultures were between 80 and 95% positive for this marker (Fig. 1a). Esterase staining (Fig. 1b) showed that over 95% of cells present in the macrophage-enriched cultures were positive for this activity.

Human brain was found to express two forms of CD4 mRNA: a 3-kb full-length transcript and a smaller mRNA species of 1.8 kb in length (21). To determine whether either of these mRNA species were expressed in our fetal brain-derived cultures, we probed total RNA from each of the astrocyte and macrophage cultures with a full-length CD4 cDNA (Fig. 2). We found CD4 mRNA in two independent fetal brain macrophage cultures (BM2 and BM3) that comigrated with a 3-kb band present in human PBL (Fig. 2A). No CD4 mRNA was detectable in a fetal astrocyte culture (A1). Quantitation of CD4 mRNA levels in fetal brain macrophages (Fig. 2B), which was performed by establishing individual ratios between CD4 and actin transcripts, revealed some heterogeneity from culture to culture which ranged from 6.5% (BM2) to 18% (BM3) of that found in PBL. These results demonstrate that fetal brain macrophages, but not astrocytes, express a 3-kb CD4 mRNA which comigrates with that of PBL.

We next examined the ability of these enriched cultures to support replication of UHC-1, a viral clone derived from HIV-III_B-infected U-937 promonocytic cells (2), in comparison with replication of the macrophage-tropic JRFL strain (19), which was propagated in peripheral blood mononuclear cells. Neither virus caused productive infection of astrocyte-enriched cultures as determined by p24 antigen release into culture fluids (Fig. 3a). No presence of HIV RNA could be detected in these cells by Northern blotting, nor was there any effect on cell viability (data not shown).

In contrast, the macrophage-enriched cultures clearly displayed productive infection when inoculated with each of UHC-1 or JRFL (Fig. 3b). The kinetics of viral replication were different, with UHC-1 infection leading to a higher daily accumulation of p24 antigen over the 14-day culture period with the exception of day 14, when p24 antigen production was equal for both viruses. Neither viral isolate induced cytopathic changes in fetal brain-derived macrophage cultures. When we inoculated macrophage and astrocyte cultures with herpes simplex virus type 1, even at very low multiplicity of infection for short periods of time, extensive and rapid cytopathic effects culminated in complete destruction of cultures (data not shown). These results demonstrate that fully differentiated and nondividing fetal brain macrophage cultures, grown in the absence of any monocyte-macrophage growth factors, are permissive to a low-level productive and noncytopathic infection by a laboratory strain of HIV-1 (UHC-1), derived from HIV-III_B-infected U-937 promonocytic cells.

Treatment of brain macrophages with AZT or 3TC inhibits

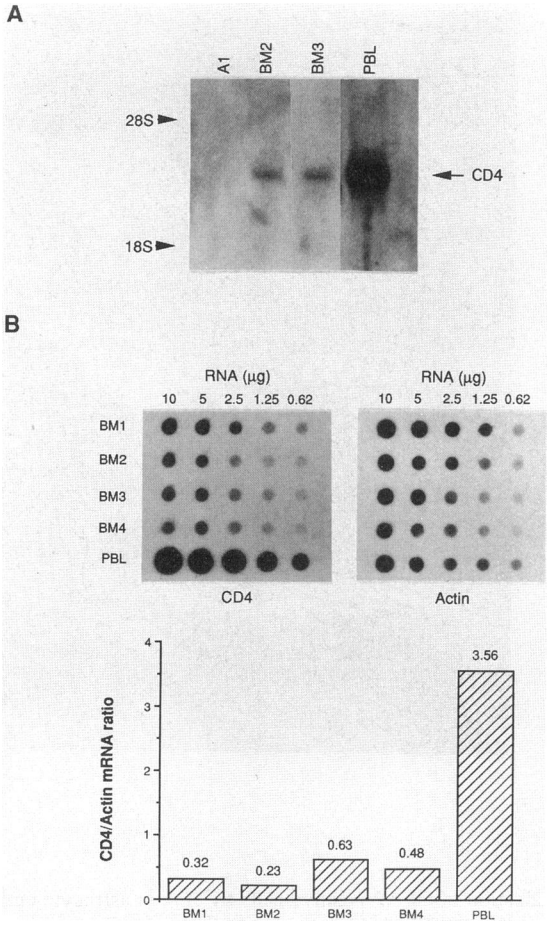

FIG. 2. CD4 mRNA levels in fetal brain macrophages. (A) Northern blot of two independent fetal brain macrophage cultures (BM2 and BM3) showing a CD4 transcript which comigrated with the CD4 mRNA band of normal human PBL. No such band was present in fetal astrocyte cells (A1). The 28S and 18S RNA markers are indicated on the left of the figure. (B) To specifically quantitate CD4 mRNA levels in fetal brain macrophages, serial dilutions of total RNA (0.62 to 10 μg) of four independent cultures (BM1, BM2, BM3, BM4) as well as human PBL were probed for CD4 and actin. The column corresponding to 1.25 μg of RNA was scanned by laser densitometry, and individual ratios of CD4:actin mRNA were plotted as indicated in the graph. The value of each ratio is indicated above each corresponding bar.

UHC-1 replication. 3TC, the (−) enantiomer of 2′-deoxy-3′-thiacytidine, as well as the racemic mixture of both enantiomers, termed BCH-189, has potent anti-HIV replication activity in several cell types (6, 34). Pretreatment of brain-derived macrophages or simultaneous addition of 4.3 μM 3TC with the viral inoculum prevented viral p24 antigen secretion into culture fluids (Fig. 4a).

Pretreatment of brain-derived macrophages with 1 μM AZT also prevented viral p24 antigen production (Fig. 4b), as well as the synthesis of the three major species of HIV-1 mRNA (Fig. 4c). Similar results were obtained with the JRFL macrophage-tropic variant of HIV-1 (data not shown). Addition of AZT to brain macrophage cultures at 24 h postinfection led to only a slight reduction in p24 antigen levels and HIV-1 mRNA synthesis, when compared to those in nontreated UHC-1-infected brain macrophages (Fig. 4b and 4c). When AZT was used to pretreat brain macrophages but was removed at 6 days postinfection, the decline in p24 antigen and HIV-1 mRNA production was identical to that in cultures in which the drug was maintained throughout the 15-day period (Fig. 4b and 4c). Production of cellular H3 histone and β-actin mRNA was not affected by such nucleoside treatment (Fig. 4c). These results show that AZT antagonizes UHC-1 replication in fetal brain macrophages and rule out the possibility that the low-level production of p24, observed in our macrophage cultures, is due to residual inoculum virus. AZT (1.0 μM) and 3TC (4.3 μM) were both nontoxic for brain-derived macrophages during the culture periods studied, as assessed by trypan blue exclusion (data not shown). These results are consistent with previous studies showing the lack of AZT toxicity in the range of 0.2 to 20 μM in MDM (27).

We next examined the ability of 1 μM AZT to inhibit UHC-1 replication in actively dividing PBL and compared this with UHC-1 replication in brain macrophages. UHC-1 successfully infected PHA-stimulated PBL, leading to high-level p24 production (Fig. 4d). Pretreatment of PBL and subsequent maintenance in 1 μM AZT inhibited viral production only until 12 days (Fig. 4d). In contrast, breakthrough was not apparent in brain macrophages treated with either 4.3 μM 3TC (Fig. 4a) or 1 μM AZT (Fig. 4b) after 18 and 15 days, respectively.

To further characterize this inhibitory effect, we compared unintegrated HIV-1 DNA by PCR amplification of LMW DNA fractions of PBL and brain macrophages at 15 days after infection, using the S1-A13 primer pair to amplify an early segment in R-U5 of the long terminal repeat and the SG4-AG4 primer pair to amplify a late-replicating segment in *gag* (Figs. 5A and 5B) (45). The number of copies of unintegrated viral genomes was clearly more abundant in infected PBL than brain macrophages and parallelled levels of p24 antigen in culture fluids (Figs. 4b and 4d).

Treatment of PBL with 1 μM AZT preferentially affected the late PCR product (SG4-AG4), reflecting chain termination (22, 35). The fact that complete inhibition was not achieved (Fig. 5B) is consistent with the presence of p24 Ag after 15 days in culture supernatants of these cells (Fig. 4d). In contrast, neither early nor late PCR products were detected in brain macrophages treated with 1 μM AZT (Fig. 5B), suggesting an absence of unintegrated viral DNA. Removal of drug from continuously treated cultures, at 6 days after infection, did not restore either p24 antigen production or viral mRNA synthesis by 15 days (Fig. 4b and 4c). However, unintegrated viral DNA could be amplified from Hirt extracts (Fig. 5B), showing that continuous AZT presence in brain macrophages was necessary to prevent accumulation of unintegrated viral DNA. Finally, addition of 1 μM AZT after 24 h of infection of brain-derived macrophages by UHC-1 failed to prevent generation of p24 antigen or HIV-1 specific mRNA (Fig. 4b and 4c), at the same time as it prevented accumulation of unintegrated viral DNA (Fig. 5B).

DISCUSSION

Several reports have shown that cells of monocyte-macrophage lineage are the principal target for HIV-1 replication in the brains of AIDS patients (18, 36, 41, 42) and that HIV-1 isolates derived from brain tissue are macrophage tropic (3, 19). This is the first demonstration of productive infection by HIV-1 of fetal brain macrophage cultures. This work therefore extends previous observations on susceptibility of both

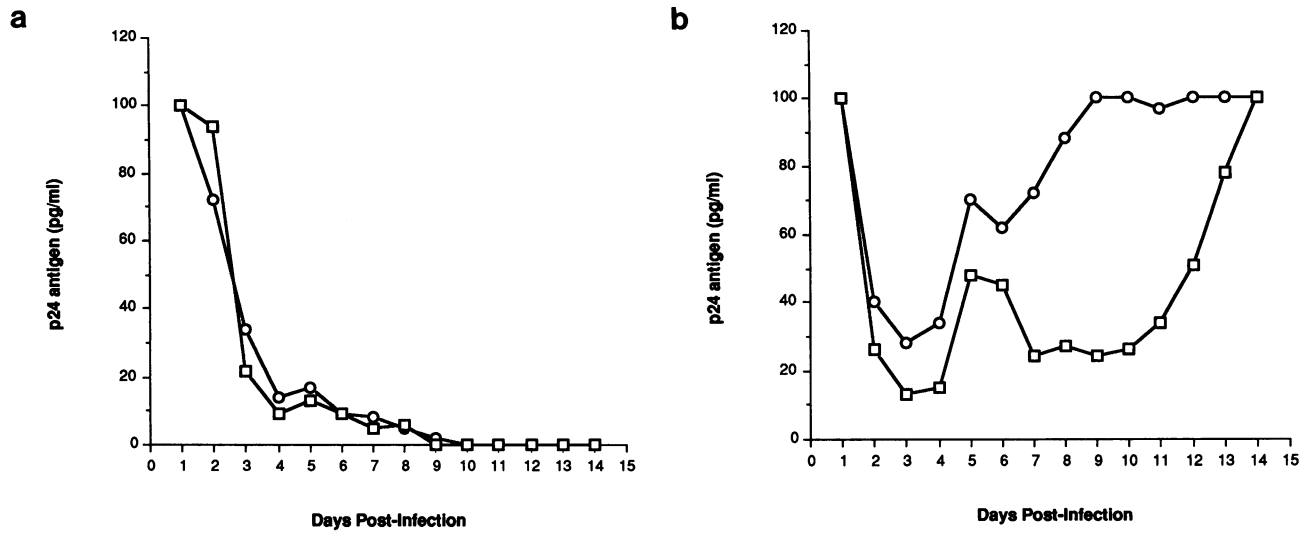

FIG. 3. Productive infection of brain macrophage but not astrocyte-enriched cultures. Fetal astrocyte and brain macrophage-derived cultures (containing approximately 10^7 cells) were inoculated with equal amounts of p24 antigen (150 ng) from culture fluids of either UHC-1 cells (○) or JRFL-infected PBL (□). Culture supernatants were removed each day and replaced with fresh medium to enable measurements of daily production of HIV-1 p24 antigen. Three independent cultures of each type were studied, yielding similar results each time. A representative p24 antigen profile is displayed for each cell type for each virus strain. (a) nonproductive infection of astrocyte-enriched cultures as assessed by p24 antigen release in culture fluids. (b) Production of p24 antigen by infected brain macrophages.

adult MDM (28, 40) and cord blood MDM (14) to infection. We could not demonstrate productive infection of astrocytes, in agreement with others who showed that cocultivation with activated lymphocytes or HIV-1-susceptible cell lines was necessary to induce viral production (5, 12, 19, 39).

Northern blot analysis with total RNA revealed a 3-kb CD4 transcript in fetal brain macrophage cultures which comigrated with CD4 mRNA in PBL; no such band was observed in RNA from astrocytes (Fig. 2). CD4 mRNA has also been detected in cultured adult microglia (15) but generally not in glial cell lines (12); consistent with these results is the observation that HIV-1 infection was blocked in the former cell type (15) but not in the latter (5, 12) by soluble CD4 and/or the anti-CD4 monoclonal antibody Leu3a. In addition, transfection of a CD4 expression vector into glial cells did not enhance infection (4). The lack of CD4 on glial cells may thus be only one factor contributing to the low efficiency of infection; efficient viral production may be dependent on appropriate activation associated with interleukin-1β and tumor necrosis factor alpha (39).

Viral production by fetal brain macrophages, as assessed by p24 antigen presence in culture fluids, was lower than reported for adult brain macrophages (33) or MDM (24). However, we deliberately used a low multiplicity of infection and short incubation period (6 h) in our study, to maximize our chances of observing nucleoside effects on accumulation of unintegrated viral DNA. Higher levels of HIV replication in MDM have been reported by other workers, who treated cultures with granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) (16, 28). The latter cytokine also caused enhanced cytopathicity in MDM (16). This may help to explain the higher levels of viral production and cytopathicity in cultured adult brain macrophages grown in conditioned medium containing both M-CSF and GM-CSF (33). Fetal brain macrophages may also be more refractory than adult brain macrophages to HIV-1 infection.

The following considerations had prompted us to investigate the antiretroviral activity of AZT in human fetal brain-derived macrophages. First, administration of AZT has been shown to improve neurological dysfunction in AIDS patients with dementia (29, 32, 43). Second, quantitative decreases in immunostaining for p24 in brains of AIDS patients, at autopsy, may be associated with AZT treatment (1). Third, AZT achieves the highest CSF/plasma ratio of any nucleoside (7). We showed that pretreatment with either 1 μM AZT or 4.3 μM 3TC can effectively block viral replication by fetal brain-derived macrophages, a result in agreement with previous work on blood MDM, using both HIV-III_B as well as macrophage-tropic variants of HIV-1 (27, 28, 37). Brain macrophages, continuously treated with AZT from 24 h after viral inoculation, did not contain unintegrated viral DNA when examined at 15 days postinfection, despite viral production. In contrast, brain macrophages, which had been pretreated with AZT and from which drug had been removed after 6 days, did contain unintegrated viral DNA after 15 days. This suggests that the continuous presence of AZT is necessary to inhibit accumulation of unintegrated viral DNA.

Breakthrough in PBL treated with 1 μM AZT occurred after 15 days, consistent with the presence of a *gag* gene PCR product at this time (Fig. 5B). An early PCR product was not affected by AZT treatment of PBL. In contrast, no such breakthrough was apparent in fetal brain macrophages (Fig. 4b) under similar circumstances (Fig. 5B). Even though phosphorylation of AZT is apparently lower in MDM than in T cells, suppression of HIV-1 replication in MDM can be achieved with drug concentrations betwen 0.1 and 10 μM (27). The antiretroviral activity of AZT in these cells has been attributed to reduced levels of endogenous dTTP, thus favoring the utilization of AZT-triphosphate by the HIV-1 reverse transcriptase (27). By extension, lower endogenous pools of such molecules in macrophages may retard reverse transcription (25) and permit earlier incorporation of AZT into nascent viral DNA than occurs in activated PBL. This may explain why the early PCR product amplified by the

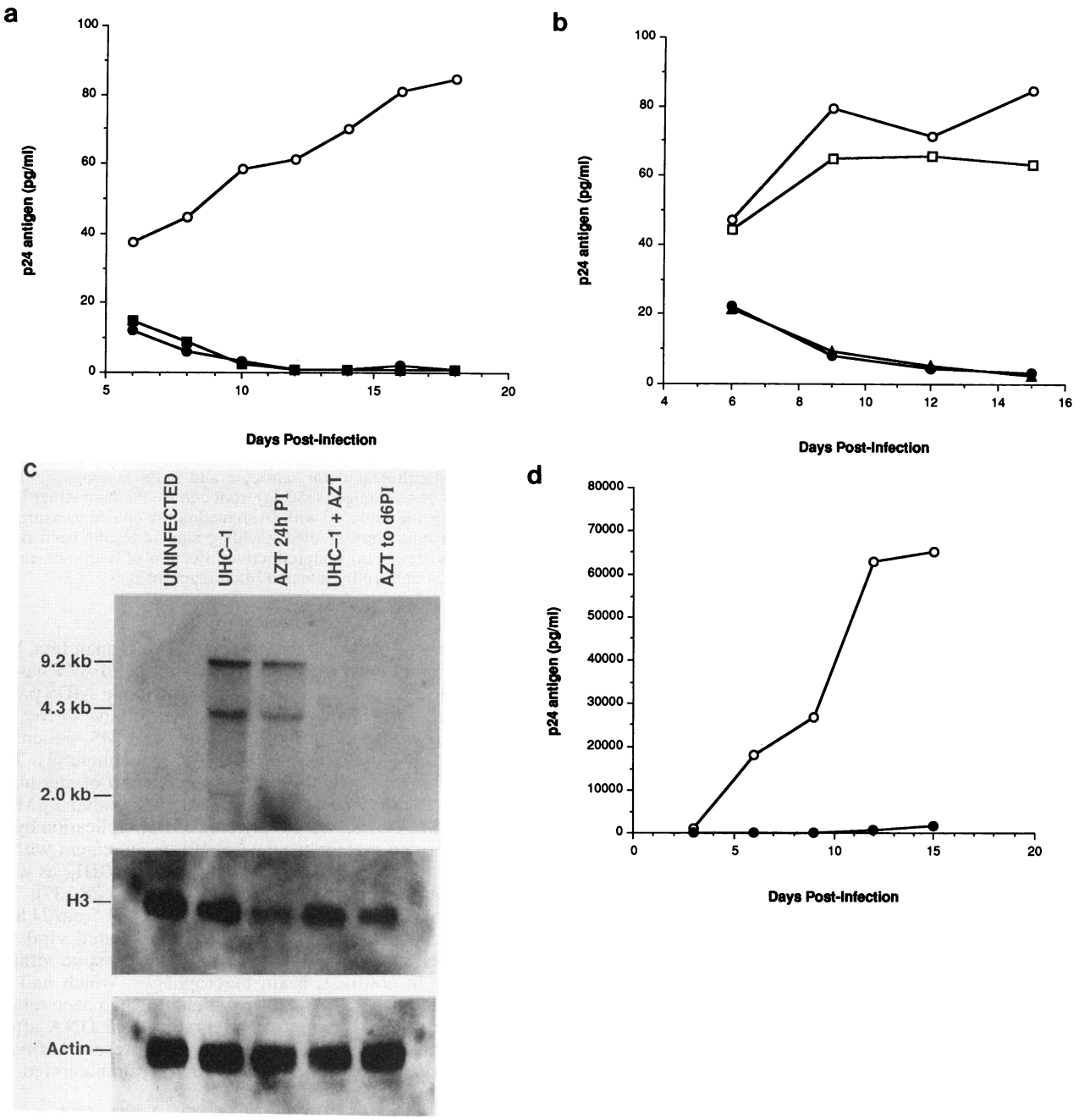

FIG. 4. Inhibition of UHC-1 production in brain macrophages treated with 3TC and AZT. (a) Pretreatment (18 h) of brain macrophages (●) or simultaneous addition of 4.3 μM 3TC (■) with viral inoculum prevented production of p24 antigen in comparison with untreated brain macrophages (○). (b) AZT (1 μM) was either used to pretreat brain macrophage cultures for 18 h prior to UHC-1 exposure (●), added 24 h postinfection (□), or removed at 6 days postinfection (▲). Also shown are nontreated cultures (○). Infections were carried out by incubating cell monolayers enriched for brain macrophages (approximately 10^7 cells) in 75-cm^2 tissue culture flasks with UHC-1 supernatant containing 150 ng of p24 antigen for 6 h. Each drug was tested with brain macrophage cultures derived from three different fetuses, generating similar results each time. A representative experiment is shown for each of 3TC and AZT. (c) Northern blot showing absence of HIV-1 mRNA in AZT-pretreated brain macrophages at 15 days postinfection (UHC-1 + AZT and AZT to d6PI). In contrast, all three major HIV-1 mRNA species (indicated on the left) were detectable at 15 days postinfection in brain macrophage cultures treated with AZT at 24 h postinfection (AZT 24h PI) or that did not receive AZT (UHC-1). Blots were also probed for H3 histone and actin mRNA. (d) UHC-1 infection of PBL cultures (○) and inhibition of UHC-1 production by pretreatment of PBL with 1 μM AZT (●).

S1-A13 primer pair was absent in AZT-treated brain macrophages but not in activated PBL (Fig. 5B). The slower kinetics of reverse transcription in macrophages grown in the absence of M-CSF and/or GM-CSF may also explain the absence of breakthrough infection in fetal brain macrophages, as opposed to PBL (Fig. 4b and 4d), in the presence of AZT.

Brain tissue from patients with HIV encephalitis contains

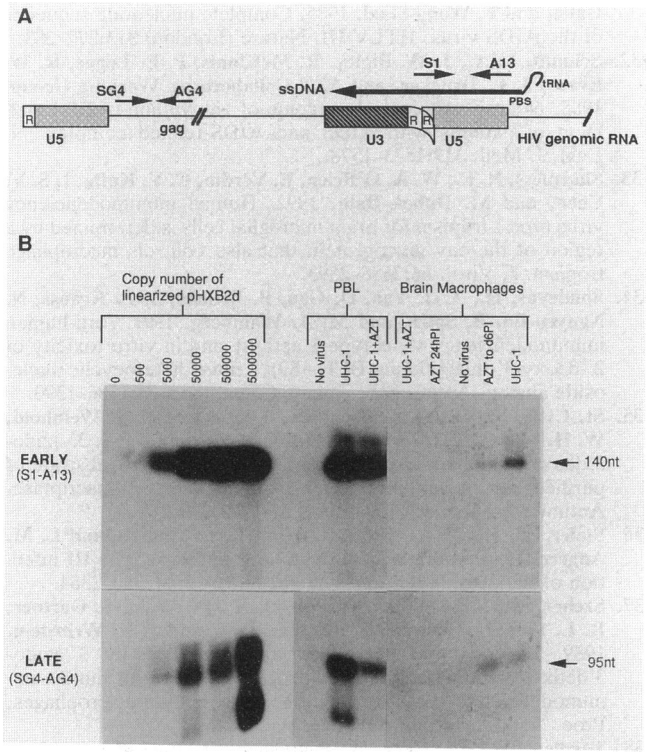

FIG. 5. At day 15 postinfection, the LMW DNA obtained by Hirt extraction from the brain macrophage and PBL cultures, used in Fig. 4b and d, was subjected to HIV *gag* and R-U5 specific quantitative PCR amplification. (A) Schema of the initial steps of HIV-1 replication, showing negative-strand DNA synthesis and the first jump in reverse transcription. Primer pair S1-A13 amplifies an area located in the R-U5 segment of the long terminal repeats, while SG4-AG4 amplifies a DNA fragment near the end of viral negative-strand DNA synthesis in the *gag* gene. (B) The plasmid pHXB-2D, containing a full-length molecular clone of HIV-III_B, was linearized with *Xho*I, and dilutions ranging from 5×10^2 to 5×10^6 copies were PCR amplified along with the LMW DNA from each of the indicated samples. A 140-nucleotide PCR product was amplified by the S1-A13 primer pair, while a 95-nucleotide PCR product was amplified by the SG4-AG4 primer pair. The source of LMW DNA is indicated above each lane and corresponds to the cells used in the previous figure.

higher levels of unintegrated viral DNA compared with blood cells from the same individuals or nonHIV encephalitis samples (26). Since most HIV-infected cells in the central nervous system are of monocyte-macrophage lineage (41), it is likely that unintegrated viral DNA accumulates in these cells. Our finding that AZT can antagonize accumulation of unintegrated HIV-1 DNA in fetal brain macrophages may be related to the effectiveness of this compound in neurological disease (29, 32, 43).

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