The Inability of Human Immunodeficiency Virus To Infect Chimpanzee Monocytes Can Be Overcome by Serial Viral Passage In Vivo

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Studies of lentivirus infection in ruminants, nonhuman primates, and humans suggest that virus infection of macrophages plays a central role in the disease process. To investigate whether human immunodeficiency virus type 1 (HIV-1) can infect chimpanzee macrophages, we recovered monocytes from peripheral blood mononuclear cells of HIV-1-negative animals and inoculated these and control human monocytes with a panel of four human-passaged monocytotropic virus strains and one chimpanzee-passaged isolate. HIV-1-infected human monocytes synthesized proviral DNA, viral mRNA, p24 antigen, and progeny virions. In contrast, except for the chimpanzee-passaged HIV-1 isolate, chimpanzee monocytes failed to support HIV-1 replication when cultured under both identical and a variety of other conditions. Proviral DNA was demonstrated only at background levels in these cell cultures by polymerase chain reaction for *gag*- and *env*-related sequences. Interestingly, the chimpanzee-passaged HIV-1 isolate did not replicate in human monocytes; viral p24 antigens and progeny virions were not detected. The same monocytotropic panel of HIV-1 strains replicated in both human and chimpanzee CD4+ T lymphoblasts treated with phytohemagglutinin and interleukin-2. The failure of HIV-1 to infect chimpanzee monocytes, which can be overcome by serial in vivo viral passage, occurs through a block early in the viral life cycle.

Chimpanzees and gibbon apes are the only primates other than humans susceptible to human immunodeficiency virus type 1 (HIV-1) infection (2, 7, 12, 14–16, 25). Chimpanzees are readily infected with as little as four tissue culture infectious doses (TCID) of virus (3). Following virus inoculation, similarities between viral replication and the immune response to viral infection in chimpanzees and humans abound. Virus isolation precedes seroconversion to both gp120 and p24 antigen (Ag)-positive status within 4 to 6 weeks of virus challenge (3, 14, 28). Persistent replication is evidenced by sequential virus reisolations over periods of months to years (3, 14) and the emergence of neutralization escape mutants (30). Despite these observations, all clinical and laboratory parameters, including total numbers of CD4+ T cells, for all but 1 of more than 100 animals inoculated worldwide thus far remained within the normal range. However, one research group observed a single animal which developed transiently depressed CD4+ T cells and platelet numbers following inoculation with several strains of HIV-1 (13). Proliferative and variable cytotoxic T-cell responses to HIV-1-specific Ags (7, 37) as well as neutralizing antibody activities (14, 29, 30) have also been reported in HIV-1-infected chimpanzees. Each of these host responses is also found in infected humans.

Studies of lentivirus infection of ruminants, nonhuman primates, and humans suggest that virus infection of macro-

phages plays a major role in the disease process (6, 11, 17, 18–20, 22–24, 31, 35). However, in HIV-1-infected chimpanzees, the identification of monocytes/macrophages as host cells for virus is lacking. Previously, we demonstrated that CD4+ T cells from peripheral blood mononuclear cells (PBMCs) of uninfected donor chimpanzees readily support low levels of virus replication, induce minimal fusion, and serve as targets for laboratory strains of virus passaged in human T-cell lines in the infected host (28). However, monocytes recovered from HIV-1 IIIB-infected chimpanzees given human T-cell-passaged isolates were virus negative both by cocultivation with human phytohemagglutinin-and-interleukin-2 (PHA/IL-2)-stimulated lymphoblasts and by polymerase chain reaction (PCR) for viral DNA (27, 28).

Plasma viremia, central nervous system disease, and lymphatic hyperplasia all characterize human HIV-1 infection but are absent in HIV-1-infected chimpanzees (27, 28). Both central nervous system and lymph node diseases in humans are characterized by a productive and persistent infection of resident macrophages and dendritic cells. Although chimpanzees may develop generalized lymphadenopathy, their lymph nodes show only plasmacytosis, perhaps secondary to the intravenous administration of large inocula of virus preparations containing many foreign human cellular proteins.

The mechanisms surrounding the differences between HIV-1 infection of humans and chimpanzees are of potential importance in understanding disease pathogenesis, in developing effective vaccines, and in developing therapeutic interventions. For example, virus-macrophage interactions for

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binding, penetration, uncoating, or replication in chimpanzee and human cells may differ. To these ends, we studied the ability of HIV-1 to replicate in its two major target cells in chimpanzees, monocytes and CD4$^+$ T lymphocytes. To date, HIV-1 variants that preferentially replicate in human monocytes have not yet been inoculated in chimpanzees, nor has there been a formal in vivo demonstration that macrophages from any organ-specific compartment contain virus. Perhaps, the ability of particular viral strains to productively infect monocytes/macrophages is a necessary event for disease in the chimpanzee.

MATERIALS AND METHODS

Isolation and culture of human and chimpanzee monocytes and T lymphoblast target cells. Human monocytes were recovered from PBMCs of HIV-1- and hepatitis B-seronegative donors after leukapheresis and purified by adherence or by countercurrent centrifugal elutriation of mononuclear leukocyte-rich fractions of blood cells as previously described (18, 23). Chimpanzee monocytes recovered from PBMCs of eight HIV-1-seronegative animals were purified by adherence onto plastic tissue culture dishes. For select experiments, Ficoll-Hypaque-purified PBMCs were infected directly with HIV-1 and subsequently purified into either the T-cell or monocyte fraction by culture techniques outlined below. Combined adherence and antibody- and complement-mediated depletion of T lymphocytes were performed with OKT3 and rabbit complement. As previous studies describing the isolation and culture of human monocytes demonstrated that supplementation of the medium with specific cytokines improved long-term culture (6, 23), optimal culture conditions for chimpanzee monocytes were determined. Monocytes from both species at variable cell concentrations (0.1 × 10^6 to 1.0 × 10^6 cells per 24-mm-diameter tissue culture well) were cultured with or without exogenous cell growth factors. These included: heat-inactivated A$^+$ human serum, autologous chimpanzee serum, granulocyte/macrophage colony-stimulating factor (GMCSF) (Genzyme, Boston, Mass.), recombinant human macrophage CSF (MCSF) (a generous gift from Cetus Corp., Emeryville, Calif.), and conditioned medium from PHA/IL-2-treated chimpanzee PBMCs. Media tested included Dulbecco modified Eagle medium (formula 78-176AJ; GIBCO, Grand Island, N.Y.), RPMI 1640 (GIBCO), and AIM V (GIBCO). Macrophages were removed after 1 week of culture by gentle scraping with a rubber policeman in cold phosphate-buffered saline deficient in magnesium and calcium. Total viable cells were counted by the trypan blue dye exclusion assay. Functional analysis of the specific cell type was determined by their ability to phagocytize latex and carboxylate beads. Human and chimpanzee PBMCs, isolated from whole blood by Ficoll-diatrizoate density gradient centrifugation, were cultured at 10^6 viable cells per ml in RPMI 1640 medium (GIBCO) with 5 μg of PHA (Sigma Chemical Co., St. Louis, Mo.) per ml, 10% purified human IL-2 (Advanced Biotechnologies Inc., Columbia, Md.), and 15% heat-inactivated fetal calf serum (Sterile Systems, Inc., Logan, Utah).

Animals. Housing conditions for the chimpanzees and the serological assays used to detect HIV-1 antibodies were previously described (3, 4). All animals used as blood donors for the virus assays were HIV-1 seronegative. The animals were examined by staff veterinarians every 2 weeks.

HIV-1 infection of monocyte and T-cell targets. PHA/IL-2-treated lymphoblasts and monocytes were exposed to HIV-1 at a multiplicity of infection (MOI) of 0.01 infectious virus

per target cell. Four HIV-1 strains originally isolated and passaged in human primary monocytes (strains ADA, 36, 105, and 24) (18, 23), one strain passaged in T-cell lines (HIV-1 IIIB), and one chimpanzee serial-passaged isolate (HIV-1$_{LAV1b}$) (14, 36) were employed. The TCID was determined by serial dilution of stock virus onto target human monocytes or lymphoblasts. HIV-1 IIIB/H9 (R. Gallo, contributor) was obtained from the AIDS Research and Reference Reagent Program, AIDS Program, National Institute of Allergy and Infectious Diseases, and the virus titer on human and chimpanzee lymphoblasts was determined to be 10^4 50% TCID (TCID$_{50}$). HIV-1 IIIB was passaged through transformed chimpanzee CD4$^+$ cell lines and designated HIV-1 IIIB/CHM-114 (28). PHA/IL-2-treated lymphoblasts previously cultured for 3 days were infected in medium supplemented with either 2 μg of Polybrene (Sigma Chemical Co.) or 25 μg of diethylaminoethyl-dextran (Pharmacia, Piscataway, N.J.) per ml. Monocytes were infected in bulk PBMCs or cultured as adherent monolayers for 0 to 10 days prior to use as target cells. Monocytes or PBMCs were washed twice in complete medium after a 1-h incubation at 37°C with virus. All cultures were refed with fresh medium every 2 to 3 days at which time culture fluids, cells, and/or DNA were removed or isolated for viral quantitation and electron microscopy. Lymphocyte and monocyte cultures were maintained for 14 to 21 days and 20 to 30 days, respectively, following infection. Levels of p24 Ag in culture fluids were determined by enzyme-linked immunosorbent assays (E. I. Dupont de Nemours & Co., Billerica, Mass.). For reverse transcriptase (RT) activity, replicate samples of culture fluids were added to a reaction mixture of Nonidet P-40 (Sigma), poly(rA)· oligo(dT) (Pharmacia), dithiothreitol (Pharmacia), MgCl$_2$, and [α-^{32}P]dTTP (400 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) for 24 h at 37°C. The mixture was applied to chromatography paper, air dried, and washed five times in 0.3 M NaCl–0.03 M sodium citrate (pH 7.4) and twice more in 95% ethanol. The paper was dried and cut, and the radioactivity was counted by liquid scintillation spectroscopy. HIV-1 IIIB served as the positive control for both p24 Ag and RT activity assays.

Detection of HIV-1-specific DNA by PCR amplification. All PCR amplification specimens were coded, and assays were performed in a blind manner. Cellular lysates of HIV-1-infected cells were extracted once each with phenol and then chloroform-isoamyl alcohol (24:1) prior to ethanol precipitation. PCR amplification of HIV-1 proviral DNA was performed using gag- (SK38/39) and env-(SK68/69)-specific primer pairs (1, 9). PCR was performed for 30 cycles, using an automatic thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) and Taq polymerase (Perkin-Elmer Cetus) as previously described (10). All amplifications used 1 μg of chromosomal DNA as an initial target, and each run was accompanied by a positive-control dilution series and known negative and reagent controls. In all cases, <20 copies of HIV-1 proviral DNA were detected. Assessment of specific amplification was determined by liquid hybridization of the PCR products with ^{32}P-end-labeled oligonucleotide detector probes SK19 gag and SK70 env (1, 9), followed by gel electrophoresis (10).

In situ hybridization with HIV-1 RNA probes. Single-stranded HIV-1 [^{35}S]RNA probes were synthesized from a recombinant DNA plasmid containing SP6/T7 promoters (Promega Biotec, Madison, Wis.) (21). Cytosmears or adherent cells grown on glass slides treated with silane were fixed in 4% paraformaldehyde. Specimens were prehybridized in a solution consisting of 10 mM Tris (pH 7.4), 0.3 M NaCl–0.03

M sodium citrate (pH 7.4), Denhardt solution [0.02% poly-vinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin), and 200 μg of yeast tRNA per ml at 45°C for 2 h and hybridized in this solution with 10% dextran sulfate, 5 μM dithiothreitol, and ^{35}S-labeled HIV-1 RNA (10^6 cpm; Oncor, Inc., Gaithersburg, Md.). Slides were serially washed in solutions with RNase to reduce binding of nonhybridized probe. Autoradiography was performed in absolute darkness (21).

Transmission electron microscopy. Monocytes or lympho-blast cultures in microtiter plates were fixed in 2% glutaral-dehyde in cacodylate buffer and treated with osmium tetrox-ide (23). The fixed cells were dehydrated, embedded in Epon, and mounted for sectioning parallel to the culture surface. Blocks were first thick sectioned and stained with toluidine blue for light microscopic observations. Those areas of interest were further thin sectioned, stained with uranyl acetate, and examined with a Zeiss electron micro-scope. Approximately 1,000 chimpanzee monocytes from three different experiments were evaluated for evidence of cell surface or vacuolar budding of HIV-1 particles.

RESULTS

Maintenance and cultivation of human and chimpanzee monocytes. Previous studies demonstrated that cell differen-tiation affects the susceptibility of monocytes to lentiviral infection (22). In this regard, an evaluation of numerous culture conditions that could affect monocyte/macrophage differentiation was performed. Optimal cell viabilities were obtained at cell concentrations of 7.5×10^5 cells per ml in each well of a 24-mm-diameter tissue culture plate. Signifi-cant differences between cells cultured in Dulbecco modified Eagle medium and RPMI 1640 were not demonstrated (data not shown). In numerous assays, monocytes from humans and chimpanzees cultured without cytokines and with MCSF, GMCSF, or combinations of MCSF and GMCSF yielded 20, 70, 70, and 85%, respectively, viable cells after 1 week in culture. Cells maintained in the absence of CSFs were flattened and pyknotic. Human and chimpanzee mono-cytes cultured in AIM V demonstrated ~75 and 95% cell viability after 1 week with or without the addition of CSFs.

Morphologically, chimpanzee monocyte cultures demon-strated a heterogeneous appearance, with fusiform cells, cells with fibroblastlike features, and rounded cells with diffuse cytoplasm (Fig. 1A and B). Ultrastructurally, mono-cytes had highly irregular cell surfaces with numerous mi-crovillar lamellipodia and subplasmalemma densities. The cytoplasm contained numerous mitochondria, bundles of microfilaments, moderate amounts of rough endoplasmic reticulum, free ribosomes, and multiple well-developed Golgi complexes. In addition, numerous phagocytic vacu-oles, granules with electron-dense homogeneous contents (azurophil granules), secondary lysosomes, residual bodies,
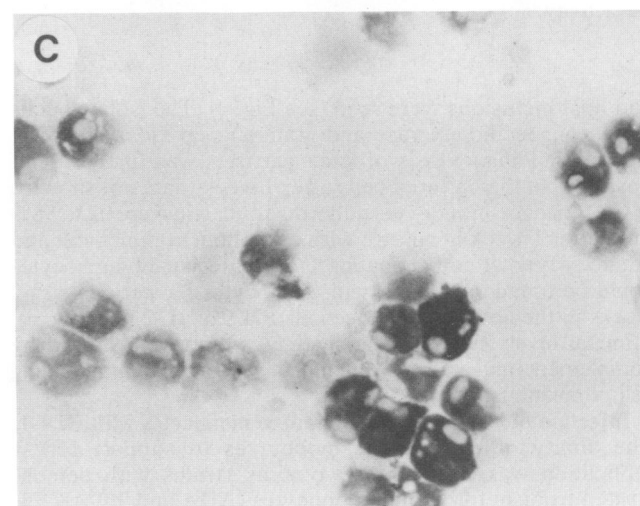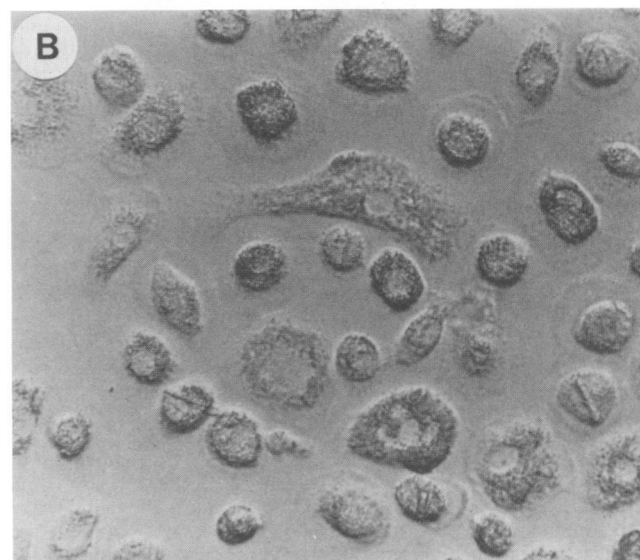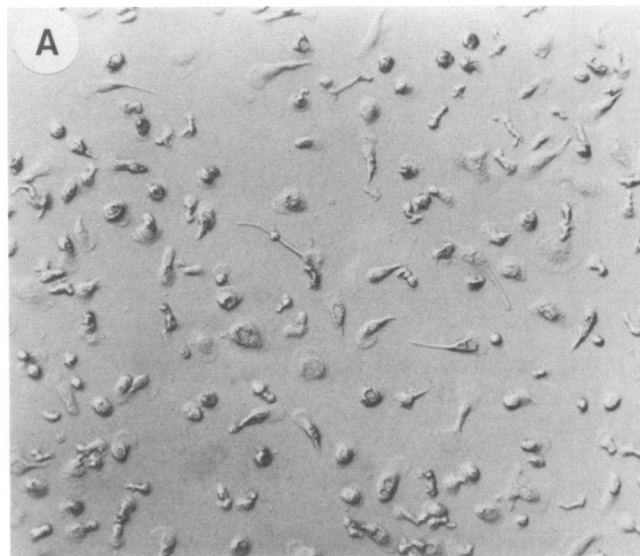

FIG. 1. Morphology of cultured chimpanzee monocytes. Chim-panzee PBMCs were purified to >94% monocytes and cultured for 7 days as adherent monolayers in medium with human serum and GMCSF. All cultures were refed with fresh medium every 2 to 3 days. Studies of the morphology of cultured monocytes demon-strates a heterogeneous monocyte population, with fusiform cells, cells with fibroblastlike features, and rounded cells with diffuse cytoplasm. Low-power (×250; Hoffman modulation) (A) and high-power (×450; Hoffman modulation) (B) magnifications. Nonspecific esterase reactivity at day 14 of culture (C) is illustrated.

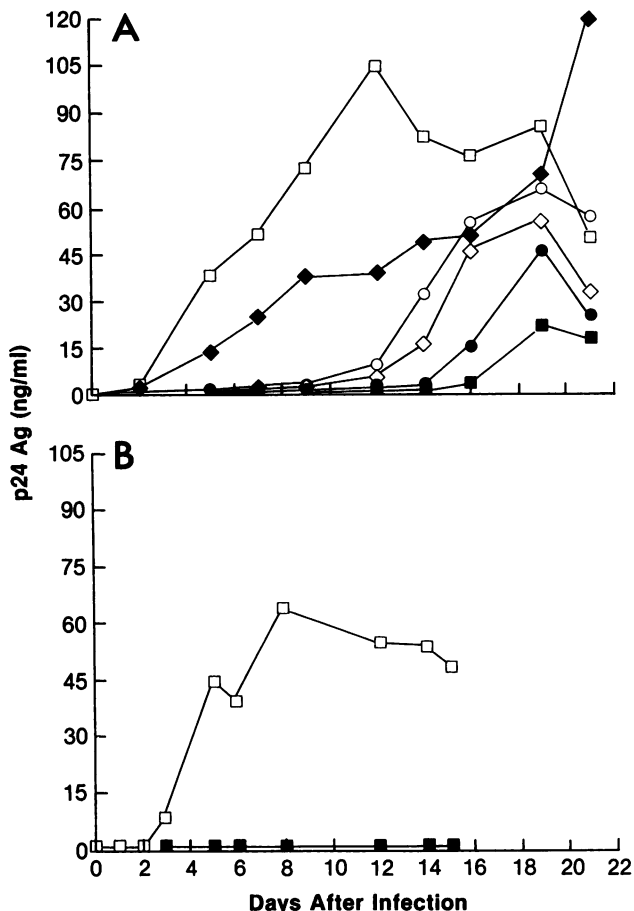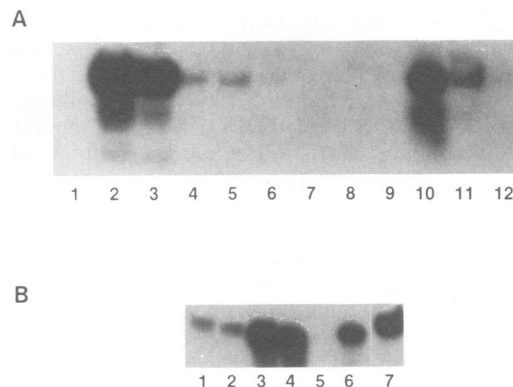FIG. 3. HIV-1 proviral DNA in human and chimpanzee mono-cytes following virus inoculations. PBMCs purified to >94% and 98% monocytes for chimpanzee and human cells, respectively, were cultured for 7 days as adherent monolayers in medium with human serum and infected with isolates ADA (A) or 36 (B) at a MOI of 0.01. Autoradiographs show samples from HIV-1-infected monocytes from chimpanzee and human PBMCs cultured for 8 h or 7 or 14 days. (A) Samples from HIV-1 ADA-infected chimpanzee and human monocytes. Lane 1, Chimpanzee cells with no virus cultured for 14 days; lanes 5 and 6, 8 h and 14 days, respectively, following HIV-1 inoculation of chimpanzee monocytes; lanes 2, 3, and 4, 14 days, 7 days, and 8 h, respectively, following HIV-1 inoculation of human monocytes. The lambda DNA negative (lanes 7 to 9) and proviral DNA positive controls (lanes 10 to 12) are illustrated. (B) Samples from HIV-1 36-infected chimpanzee and human mono-cytes. Lanes 1 and 2, 8 h and 7 days, respectively, following virus inoculation of chimpanzee monocytes; lanes 3 and 4, 7 and 14 days, respectively, following virus inoculation of human monocytes; lane 5, uninfected human monocytes; lane 6, ADA-infected human PBMCs; lane 7, HIV proviral DNA positive control.

FIG. 2. Infection of HIV-1 ADA monocytotropic stock virus onto human and chimpanzee monocytes. PBMCs purified to >98% monocytes and cultured for 7 days as adherent monolayers in medium with human serum and MCSF were exposed in duplicate determinations to HIV-1 isolate ADA. (A) Human monocytes infected with 10-fold dilutions of viral inoculum (□, undiluted; ◆, 1:10; ○, 1:100; ◇, 1:1,000; ●, 1:10,000; ■, 1:100,000). (B) Viral replication following the inoculation of human (□) and chimpanzee (■) monocytes with HIV-1 ADA at a MOI of 0.01. All cultures were refed with fresh medium every 2 to 3 days, and the culture fluids were saved at the indicated times and later assayed for p24 Ag and RT activity.

and lipid inclusions were seen (see Fig. 4). The isolated cells were nonspecific esterase and granular peroxidase positive (Fig. 1C). Phagocytosis of latex particles was further dem-onstrated in the cultured cells after 1 week (data not shown).

Chimpanzee monocytes adhering to plastic were 92 to 98% pure after 1 week in culture with or without complement and antibody lysis of contaminating T cells. Residual lymphocytes could be found associated with monocytes for periods of >2 weeks in the absence of exogenous PHA or IL-2 (see Fig. 4). Minimal levels (≤2% contaminating cells) were achieved by complement- and antibody-mediated lysis of the contaminating cell populations.

Infection of human and chimpanzee monocytes with HIV-1. The ability of chimpanzee monocytes to support HIV-1 replication was analyzed. Two virus strains with demon-strated tropism for human monocytes (ADA and 36) (18, 23) and one chimpanzee-passaged HIV-1 isolate were used in

initial studies in attempts to infect chimpanzee monocytes. The titers of the viral stocks in human monocytes were 10^5, 10^4, and 0 $TCID_{50}$ for strains ADA, 36, and HIV-1_{LAV1b}, respectively. A representative titration of HIV-1 ADA (10-fold serial dilutions of virus stock inoculated onto 7-day-old human monocytes) is illustrated in Fig. 2A. Following infec-tion of human monocytes with ADA (MOI = 0.1), p24 Ag levels were initially detected in culture fluids at 3 to 5 days, with maximum levels of ≥50 ng/ml and RT levels in excess of 60 × 10^6 cpm/ml. Typical cytopathic effects, including multinucleated giant cell formation and cell lysis, are seen beginning by day 7 following infection in the human cells.

Chimpanzee monocytes from four different donors were inoculated with the three virus strains at an MOI of 0.1 or 0.01. The cells were cultured in media supplemented with MCSF (1,000 U/ml), GMCSF (100 U/ml), or MCSF and GMCSF (1,000 and 100 U/ml, respectively). Monocytes were inoculated with virus after 7 days of culture. A repre-sentative experiment is shown in Fig. 2B. Productive viral replication could not be demonstrated for either ADA or 36 in any of the four chimpanzee monocyte populations. p24 Ag and RT activity assays performed on culture supernatants produced negative results, and there was no evidence of syncytium formation or cell death upon microscopic evalu-ation. The lack of productive virus replication in chimpanzee monocytes with human-passaged monocytotropic HIV-1 strains was evident regardless of culture conditions (includ-ing infection of monocytes directly in the bulk PBMCs), timing of infection, or the age or sex of the animal donor (data not shown).
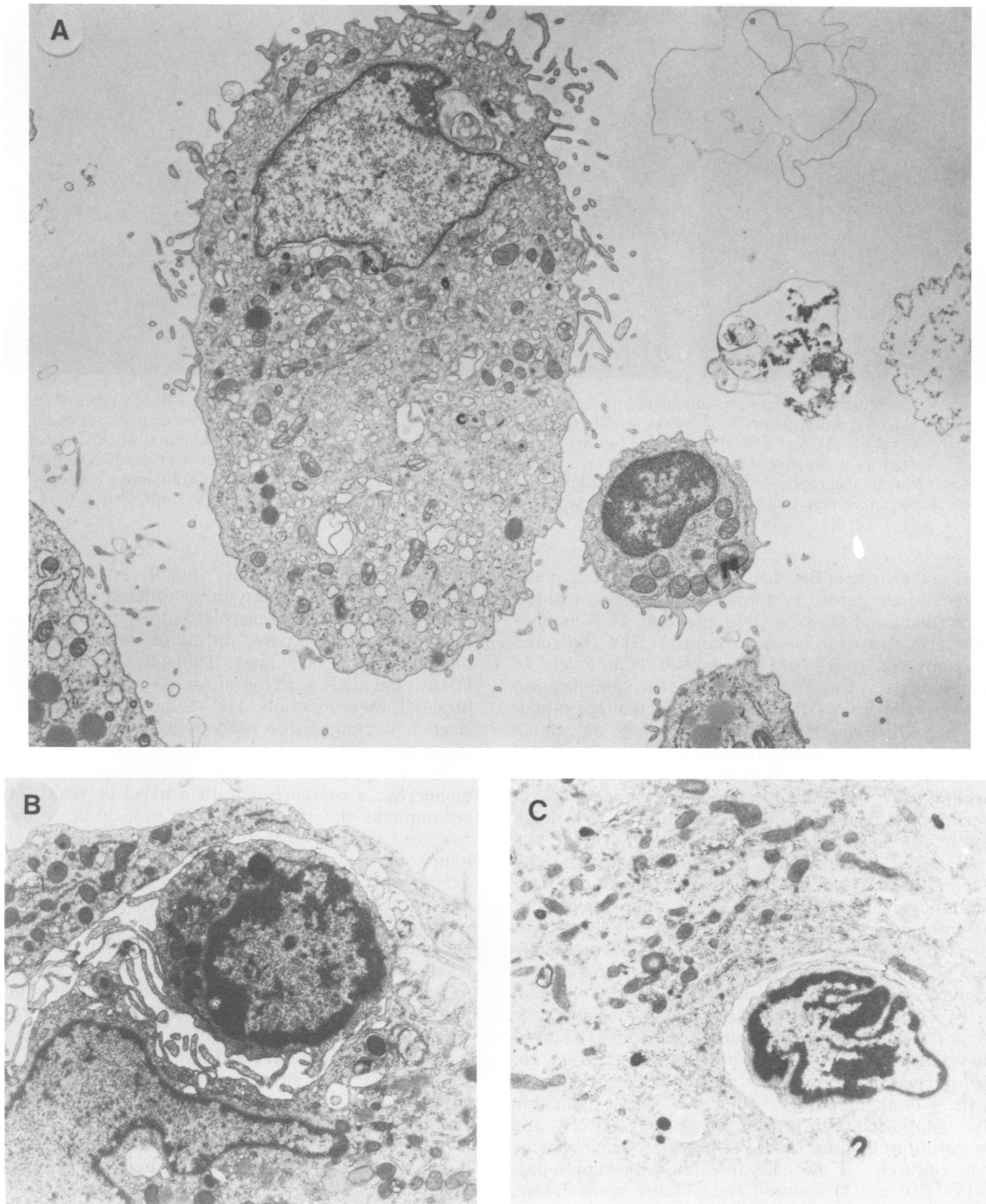
FIG. 4. Electron microscopic observations of HIV-1-inoculated chimpanzee monocytes. Chimpanzee PBMCs purified to >94% mono-cytes were cultured 7 days as adherent monolayers in medium with chimpanzee serum and GMCSF and infected with isolate ADA at a MOI of 0.01 for up to 21 days. Progeny virions were not demonstrated, but contaminating T cells are shown phagocytized by these monocytes. (A) A 5-day-old culture of a typical macrophage with a irregular lamellipodia surface and eccentric nucleus; adjacent small lymphocytes are seen. Magnification, ×13,200. (B) A monocyte-associated lymphocyte in a 14-day-old culture. Note the apparent normal ultrastructural appearance of the lymphocyte. Magnification, ×12,000. (C) A lymphocyte within a cytoplasmic vacuole of a monocyte.
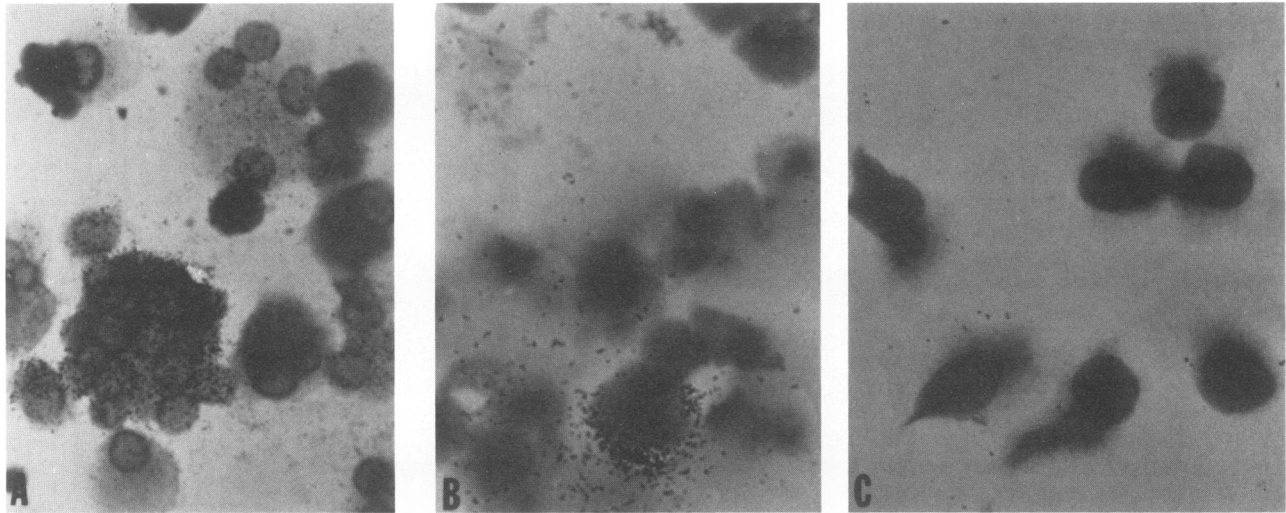
FIG. 5. HIV-1-specific mRNA in virus-inoculated human and chimpanzee cells. Human and chimpanzee PBMCs purified to 94 and 98% monocytes, respectively, were cultured for 7 days as adherent monolayers in medium with chimpanzee or human serum and MCSF. Cells were infected with isolate ADA at a MOI of 0.01 for 14 days. Human PHA/IL-2-stimulated lymphoblasts infected with ADA for 7 days at a MOI of 0.01 served as a positive control for this assay. Autoradiography revealed silver grains over HIV-1 mRNA-producing cells. HIV-1-infected human macrophages (A) and human PBMCs (B) demonstrate abundant HIV-1 mRNA-containing cells following virus inoculations. Chimpanzee macrophages cultured and infected as the human cells show no measurable HIV-1 mRNA (C).

To analyze the stage of the virus life cycle restricted by the chimpanzee monocyte, we performed a systematic analysis of HIV-1 replication following virus infection. PCR amplification for HIV proviral DNA in control HIV-1-infected human monocytes (ADA or 36) showed high levels of amplified products at 7 and 14 days. However, amplification of DNA extracted from HIV-1-infected chimpanzee monocytes at these time intervals produced barely detectable signals (<1% of human control monocytes) following liquid hybridization analyses (Fig. 3). The low levels of proviral DNA seen in HIV-1-inoculated chimpanzee monocyte cultures were typical of that demonstrated in other viral stock preparations (8). In an attempt to resolve whether the low levels of HIV-1 DNA were due to input DNA or contaminating lymphocytes in the monocyte preparations, we treated monocyte cultures with OKT3 and complement prior to virus infection. Although a reduced PCR signal was demonstrated, amplification products could still be visualized (data not shown). This suggested that the viral inocula was a source of the contaminating HIV DNA. Indeed, analysis by electron microscopy of chimpanzee monocyte cultures not treated with OKT3 and complement showed rare but viable T cells in the cell preparations (Fig. 4). No budding viral particles were ever seen by electron microscopy in these contaminating lymphocytes. This explanation is further supported by the absence of viral RNA and progeny virion production in HIV-1-inoculated chimpanzee monocyte cultures. Of the cells infected with human-passaged HIV-1, 10 and 70% after 7 and 14 days, respectively, were demonstrated by in situ hybridization to be expressing HIV-1-specific mRNAs (Fig. 5A). In contrast, no viral RNAs could be detected in either ADA- or 36-infected chimpanzee monocytes (Fig. 5C). Production of progeny HIV-1 particles was evaluated at 7, 14, and 21 days by transmission electron microscopy. In human cultures, virions were easily identified in intracytoplasmic vacuoles and via budding from the plasma membrane (Fig. 6). However, a survey of over 1,000 chimpanzee monocytes in independent

laboratories failed to identify vacuolar or membrane-associated virus particles at any point following infection. These results were obtained regardless of whether the chimpanzee monocytes were prepared for culture immediately following phlebotomy or 18 h later. Thus, by analyses of proviral DNA, viral mRNA, p24 viral Ag, RT activity, and ultrastructural virion production, HIV-1 infection was not demonstrated in chimpanzee monocytes with these human-passaged monocytotropic virus strains.

HIV-1 replication in human and chimpanzee PBMCs and monocytes: a comparison with a panel of viral isolates. To substantiate the lack of HIV-1 replication with human-passaged virus in chimpanzee monocytes, virus growth in lymphoblasts and monocytes from identical donor cells was examined. Virus from a panel of four monocytotropic strains and one lymphotropic HIV-1 strain (HIV-1 IIIB) (Table 1) were inoculated at identical MOIs onto human and chimpanzee monocytes or lymphoblasts. Virus titer, viral mRNA levels, and maximum levels of released p24 Ag were determined in the virus-inoculated cells. Both human and chimpanzee lymphoblasts supported productive virus replication when infected with the human- or chimpanzee-passaged monocytotropic or lymphotropic strain (Table 1). Titers of each of the virus stock preparations were similar for the PBMCs of both species and ranged from 10^2 TCID$_{50}$ for isolate 105 to 10^4 for ADA. In each instance, the maximum level of p24 Ag released into culture fluids of infected lymphoblasts was greater in human cells (60 to 80 ng) than in chimpanzees (4 and 3 ng) for ADA and HIV-1 IIIB, respectively. The level of p24 Ag correlated with the numbers of progeny virions observed by electron microscopy (Fig. 7). Human lymphoblasts underwent ultrastructural degeneration and cytolytic changes following human-passaged HIV-1 infection, and approximately 1 in 10 cells examined demonstrated budding or cell-associated virus particles. Progeny virions associated with or budding from human-passaged HIV-1-infected chimpanzee lymphoblasts made up approximately 1 in 100 of total cells examined. No degenerative
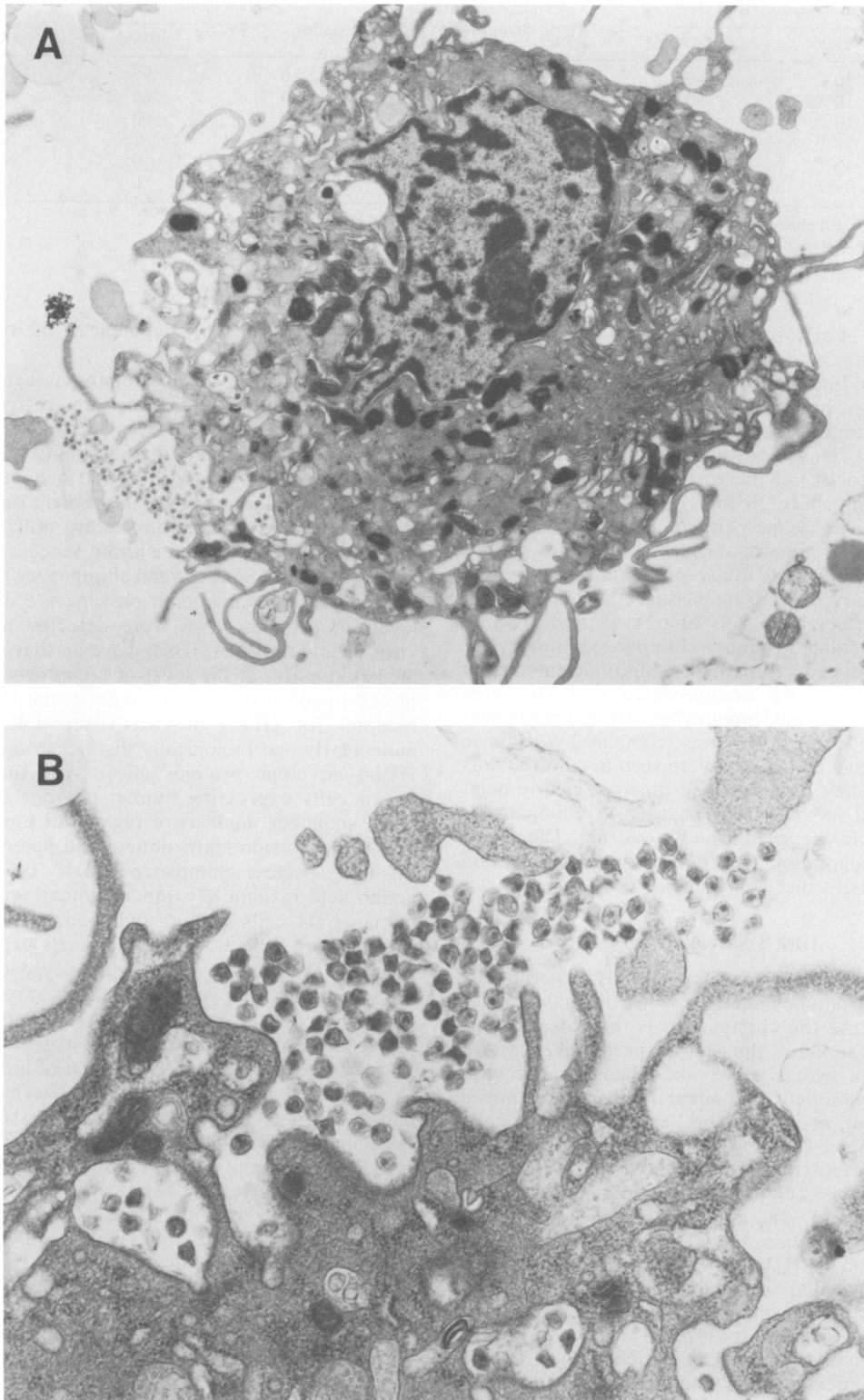
FIG. 6. Electron microscopic observations of HIV-1-infected human monocytes. Human PBMCs were purified to >94 to >98% monocytes, cultured for 7 days in medium supplemented with MCSF, and infected with ADA at a MOI of 0.01 for 14 days. (A) HIV-1 ADA-infected human monocytes; (B) same monocytes magnified to show extracellular and intravacuolar particles.

TABLE 1. Viral titers[a] and levels of p24 Ag[b] in culture fluids of monocytes and lymphoblasts infected with five HIV-1 isolates

Virus isolate	Human PBMCs		Chimpanzee PBMCs		Human monocytes		Chimpanzee monocytes	
	Titer	p24 Ag	Titer	p24 Ag	Titer	p24 Ag	Titer	p24 Ag
ADA	10^{-4}	80	10^{-3}	5	10^{-5}	95	0	0
36	10^{-3}	55	ND[c]	ND	10^{-4}	65	0	0
24	10^{-3}	50	10^{-2}	1	10^{-4}	60	0	0
105	10^{-2}	12	10^{-2}	0.4	ND	8	ND	ND
HIV-1 IIIB	10^{-4}	60	10^{-2}	3	0	0	ND	ND
HIV-1$_{LAV1b}$	ND	ND	10^{-3}	30	0	0	10^{-3}	9

[a] Titer represents the last dilution positive for viral replication as determined by p24 Ag capture assay.

[b] The p24 Ag levels (in nanograms per milliliter) were measured as described in Materials and Methods.

[c] ND, Not done.

changes were seen at an ultrastructural level in chimpanzee lymphocytes budding human-passaged HIV-1 progeny virions (Fig. 7A and B). Infection with the chimpanzee-passaged HIV-1$_{LAV1b}$ isolate, however, resulted in high levels of viral replication (Table 1). All four human-passaged monocytotropic HIV-1 strains replicated in human monocytes. The TCID$_{50}$s (10^2 to 10^5) of these viral strains were similar in human and chimpanzee PBMCs (Table 1). Productive viral replication was not demonstrated in chimpanzee monocytes with any of the human-passaged viral strains tested. However, and in contrast to these previous observations, HIV-1$_{LAV-1b}$, the only viral isolate passaged in chimpanzees, produced easily detectable levels of p24 Ag and progeny virions in virus-inoculated primary chimpanzee monocytes. Moreover, virus-induced cytopathicity, multinucleated giant cells, and cell death were demonstrated in both virus-infected lymphoblasts and monocytes. In lymphoblasts, significantly higher levels of p24 Ags, compared with those of the human-propagated strains, were seen in culture fluids of infected cells. These results taken together clearly demonstrate that serial in vivo virus passage in chimpanzees results in phenotypic changes of the virus strain. The ability of this host cell adaptation to produce clinical disease in chimpanzees awaits further analyses.

DISCUSSION

Chimpanzees and gibbon apes are the only experimental animal species shown to be infectable with HIV-1 (2–4, 7, 12, 14–16, 25, 28–30). As the chimpanzee is the closest living relative of Homo sapiens, the shared permissiveness for HIV-1 is consistent with a genus- or species-specific relationship usually exhibited by animal lentiviruses. Indeed intravenous, vaginal, or intracerebral experimental inoculation with either culture supernatants of HIV-1-infected cells or plasma, brain, or thymic suspensions of AIDS patients results in a persistent cell-associated viremia in infected chimpanzees (3, 7, 14, 28, 29). Despite the continuous reisolation of virus for years, the CD4$^+$ T-cell tropism, and the development of an HIV-1-specific antibody response, none of the more than 100 virus-inoculated animals reported show clinical, hematological, or pathological signs of disease. Except for a single report (14), the absence of plasma viremia following experimental inoculation with HIV-1 or virus-containing clinical tissues remains a unique feature of chimpanzee infections. These observations are in sharp contrast to the clinical and pathological sequelae observed in humans and demonstrate important differences between the interactions of HIV-1 in humans and chimpanzees. These clinical and pathological differences may correlate with resistance to HIV-1 infection of chimpanzee monocytes in

vivo or with limited involvement of the reticuloendothelial cell system.

This examination of the human-passaged HIV-1-infected chimpanzee monocyte demonstrates restriction of infection at an early stage in the virus life cycle. However, these analyses do not preclude the possibility of in vivo infection, as isolated monocytes from blood behave biologically and functionally differently than tissue macrophages. However, as multiple culture conditions were utilized in attempts to optimize in vitro infection without success, it appears that in vitro susceptibilities between chimpanzee and human monocytes for HIV-1 of human passage are distinct. Only low levels of proviral DNA were detected in the absence of virus-specific mRNA. This suggests that the block may be mediated early, at the level of receptor-virus interaction(s) or cell-to-cell transmission. Indeed, the CD4 molecule, the receptor for HIV-1 in virus-susceptible human cells, is molecularly and biologically distinct in the chimpanzee (5). HIV-1 envelope proteins allow syncytium formation between cells expressing human but not chimpanzee CD4. DNA sequence analysis of regions of the CD4 gene which govern cell fusion show amino acid heterogeneity between the two species; chimpanzee CD4$^+$ cells bearing human amino acid residue 87 support syncytium formation, while human CD4 cells bearing chimpanzee residue 87 do not. Furthermore, infection of human cells expressing the chimpanzee CD4 gene is insensitive to lysosomotropic agents, implying that viral penetration does not require endocytosis as was previously demonstrated with human CD4-expressing cells. The binding affinity of virus to chimpanzee CD4, however, remains equivalent to that of human CD4$^+$ T cells (5, 28). These subtle differences between human and chimpanzee CD4 receptors may well be associated with both resistance to virus-induced cytopathicity and the characteristic low-level virus released from infected lymphocytes (28). Perhaps a low level of CD4 expression in an altered configuration plays a role in the resistance of the chimpanzee monocyte to virus infection. This could explain the permissiveness of the chimpanzee-passaged HIV-1$_{LAV1b}$ isolate. Alternatively, cytokines produced from HIV-1-inoculated chimpanzee lymphocytes and monocytes could effect susceptibility to HIV-1 infection. Indeed, recent reports demonstrate that when ≥5 U of alpha interferon per ml is added to cultures of human monocytes, HIV-1 infection is abrogated. This block in HIV-1 infection is localized at the level of proviral DNA (19, 20).

Macrophages play essential roles in the virus-specific immune response in the host by presenting Ag to lymphocytes during infection and by serving as accessory cells to lymphocytes (32). Further indirect evidence of the in vivo
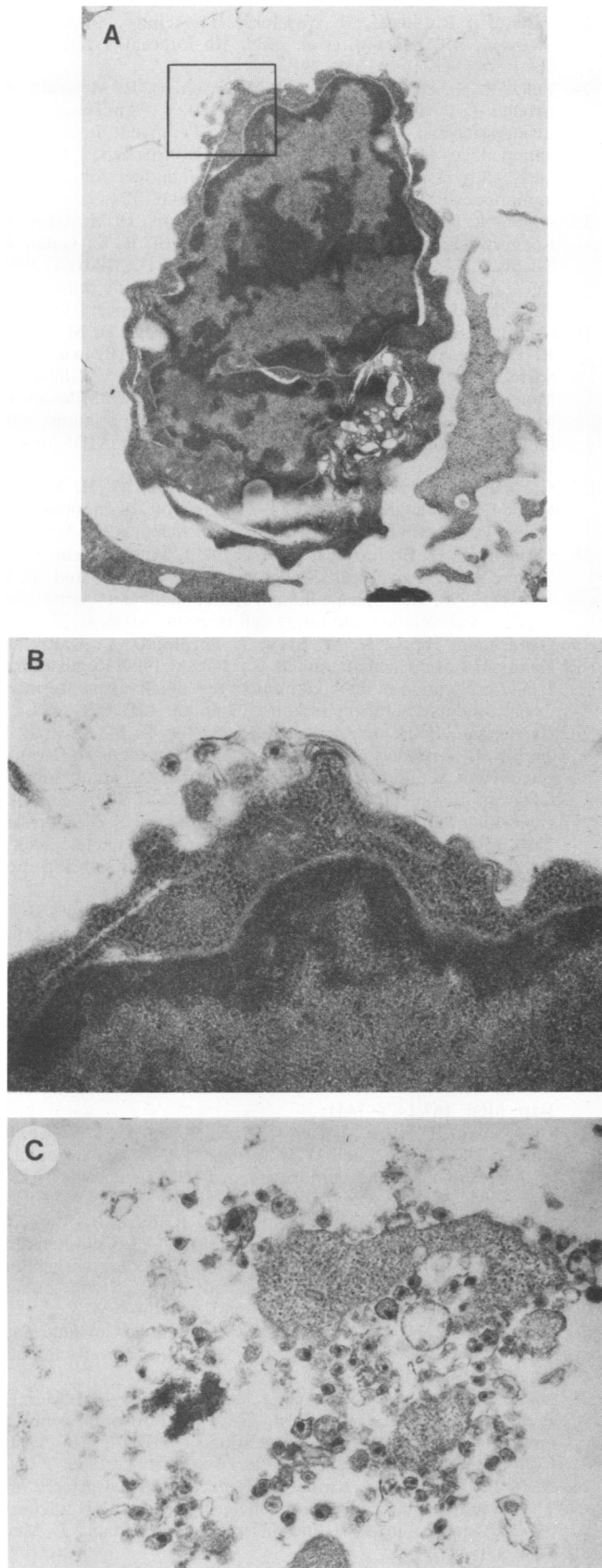
FIG. 7. Comparative electron micrographs of HIV-1-infected chimpanzee and human PHA/IL-2-stimulated PBMCs. Human and chimpanzee PBMCs previously cultured for 3 days in medium with PHA and IL-2 were exposed to HIV-1 ADA at a MOI of 0.01. (A and B) Focal budding of HIV-1 particles from the surface of a chimpanzee lymphocyte. Note the thickened cell/virus-associated membranes involved in virus budding in panel B. (C) Cytolytic response of human PBMCs to HIV-1. Note the large numbers of virus particles associated with cellular debris. Magnification, ×96,000.

resistance of these cells in chimpanzees comes on the realization that after experimental inoculation of large amounts of virus or virus-producing cells into animals, a full range of virus-specific immunity develops (7) without detectable infection in macrophages (27, 28). This immunity requires efficient Ag processing of the virus-infected cells in mononuclear phagocytes, leading to an abortive infection, and thus further supports the findings presented here. Macrophages also have protective functions through their abilities to ingest and kill invading microorganisms and therefore provide an important element for host defense against disease (32). Paradoxically these same cells are targets for a number of microbial pathogens including HIV-1. It is of particular interest that the target tissues in which large numbers of HIV-1-infected macrophages reside constitute the identical tissues involved in disease (31). In humans, the lung, brain, and spinal cord are all primary sites of replication for HIV-1 in the macrophage and all demonstrate significant and persistent levels of viral replication which correlate with histopathological alterations and clinical disease (24). HIV-1-related interstitial pneumonitis, subacute encephalopathy, and spinal cord myelopathy are features associated with HIV-1 infection of macrophages (24). Furthermore, the numbers of infected cells correlate with the observed histopathology in many cases. Not all tissue macrophages are equally permissive for lentiviral infections. For example, in horses infected with equine infectious anemia virus, Kupffer cells present in the liver have been demonstrated to be a major site of virus replication (33). In HIV-1-infected humans we studied, liver or Kupffer cell infection and associated pathology is less frequent (26, 34). In a 8-month-old chimpanzee chronically infected with HIV-1 IIIB, liver infection was not demonstrated (27). We previously demonstrated that monocytes cultured from peripheral blood of HIV-1 IIB-infected chimpanzees did not contain HIV-specific gene products or release progeny virions (27, 28). The lack of susceptibility of liver, connective tissue, bone, and kidney tissues to pathological processes may be traced to the lack of permissiveness of their respective tissue macrophages in supporting productive HIV-1 infection.

The lack of apparent HIV-1 infection in chimpanzee monocytes coupled with the previously reported continuous but noncytopathic infection of lymphocytes demonstrate two aspects of controlled virus replication in the host and further defines the animal models' application to AIDS research. Further investigations into the unique aspects of these virus-host cell interactions should further elucidate the necessary mechanisms that correlate with and precipitate disease in humans. This will likely require the use of an HIV-1 isolate or strain which efficiently infects chimpanzee macrophages in vivo. Indeed, one such virus strain was found. Ultimately, understanding the mechanisms of virus adaptation to its natural host cells should provide insights into the pathogenesis of HIV-1 in its human host.

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