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A Chimpanzee-Passaged Human Immunodeficiency Virus Isolate Is Cytopathic for Chimpanzee Cells but Does Not Induce Disease

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The human immunodeficiency virus type 1 (HIV-1) readily infects both humans and chimpanzees, but the pathologic outcomes of infection in these two species differ greatly. In attempts to identify virus-cell interactions that might account for this differential pathogenicity, chimpanzee peripheral blood lymphocytes and bone marrow macrophages were assessed in vitro for their ability to support the replication of several HIV-1 isolates. Although the IIIb, RF, and MN isolates did not readily infect chimpanzee peripheral blood lymphocytes, an isolate of HIV-1 passaged in vivo in chimpanzees not only replicated well in both chimpanzee peripheral blood lymphocytes and bone marrow macrophages but also was cytopathic for chimpanzee CD4$^+$ lymphocytes. Because no evidence of HIV-induced disease has been observed in chimpanzees infected with this isolate, in vitro replication to high titers with concomitant loss of CD4$^+$ cells is not, in this instance, a correlate of pathogenicity. These observations, therefore, indicate that caution must be used when making extrapolations from in vitro data to in vivo pathogenesis.

Great apes are the only nonhuman species readily infected with the human immunodeficiency virus type 1 (HIV-1) (1, 3, 7, 9). Chimpanzees (*Pan troglodytes*), therefore, have become extremely important in animal experimentation related to AIDS. It is of interest, however, that HIV-1-infected chimpanzees do not develop an AIDS-like disease (7, 18). This has led to the suggestion that studying differences in the biology of HIV-1 infection in chimpanzees and humans may facilitate the identification of factors that lead to disease progression. Since the pathogenic consequences of HIV-1 infection of humans may result, at least in part, from infection of CD4$^+$ lymphocytes and monocyte/macrophages with the subsequent loss of these cells (2), we sought to determine whether there is an inherent difference in the ability of cells of human and chimpanzee origin to support the replication of HIV-1.

Initially, we assessed the susceptibility of peripheral blood lymphocytes (PBLs) from chimpanzees to HIV-1 infection in vitro. The viruses used in these studies included HIV-1 IIIb, RF, and MN isolates. Stocks of cell-free virus-containing supernatants were prepared by using concanavalin A-stimulated, interleukin-2-expanded human PBLs. A fourth HIV-1 isolate, LAV-1b, was also studied. This strain was derived from the original LAV-1_{BRU} isolate (obtained from L. Montagnier, J.-C. Chermann, and F. Barre-Sinoussi) following inoculation into a naive chimpanzee (C-459) and subsequent passage into a second chimpanzee 5 months later by transfusion of 10 ml of blood from C-459 (7). Virus was reisolated from the second chimpanzee 2 weeks after the transfusion by cocultivation of chimpanzee PBLs with phytohemagglutinin-stimulated PBLs from a normal human donor; the cell-free culture supernatant was filtered and designated LAV-$1b_0$ (passage 0). The original stock was then expanded for use in in vitro assays by two successive passages in normal human PBLs.

HIV-1 isolates IIIb, RF, and MN infected and replicated to high titers in phytohemagglutinin-stimulated, interleukin-2-expanded human PBLs, but infection of chimpanzee PBLs by these isolates resulted in little or no production of virus (Fig. 1). In repeated experiments, while these three laboratory isolates of HIV-1 did on occasion replicate in chimpanzee PBLs, they never replicated as efficiently as in human cells (13, 15). Interestingly, the LAV-1b isolate readily infected both human and chimpanzee PBLs and produced comparable levels of virus under the culture conditions used (Fig. 1). Even though the chimpanzee PBLs supported efficient replication of the LAV-1b strain, it was possible that the virus was not cytopathic for chimpanzee CD4$^+$ lymphocytes. Although previous studies indicated that LAV-1b is cytopathic for chimpanzee CD4$^+$ cells (7), we repeated the experiments not only with unfractionated lymphocytes but also with a CD4-enriched lymphocyte population obtained by the technique of panning to remove CD8$^+$ cells. The results confirmed those of the earlier study and demonstrated the unequivocal loss of CD4$^+$ cells concomitant with virus production (Table 1). The decrease in viability and loss of cell numbers ruled out the possibility that the observed decreases in CD4/CD8 ratios in the infected cells were due to downmodulation of the CD4 molecule.

Because human macrophages support the replication of HIV-1 (2, 10–12, 14), we also assessed the ability of the four HIV-1 isolates to replicate in chimpanzee bone marrow-derived adherent cells. Heparinized bone marrow samples were obtained from chimpanzees by posterior iliac crest aspiration, and adherent cells were isolated and then incubated for 2 h with supernatants containing the various HIV-1 isolates. As in the studies with PBLs, the IIIb, RF, and MN isolates apparently did not infect the chimpanzee bone marrow macrophages, since very little p24 antigen was detected in culture supernatants. However, the LAV-1b isolate readily infected the chimpanzee bone marrow-derived adherent cells, as evidenced by p24 antigen accumulation in cultures (Fig. 2A).

Replication of the LAV-1b isolate in the adherent bone marrow-derived cells clearly reflected infection of a macro-

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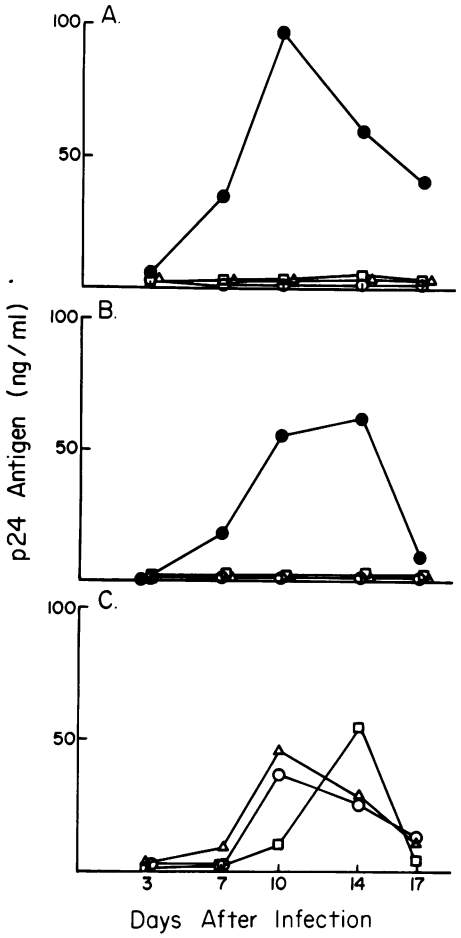

FIG. 1. HIV-1 isolate LAV-1b (●), but not IIIb (○), MN (□), and RF (△), efficiently infects chimpanzee PBLs. PBLs of normal, seronegative chimpanzees (A and B) and a normal human volunteer (C) were isolated by Ficoll-diatrizoate density gradient centrifugation, activated with 0.1% phytohemagglutinin for 3 days, and then expanded in interleukin-2 (20 U/ml; Hoffmann LaRoche)-containing medium. These PBLs were then incubated for 2 h with supernatants containing 32 infectious doses of the various HIV-1 isolates. One infectious dose is defined as the minimum dilution of this stock virus-containing supernatant which infects human PBLs under these conditions. After an 18-h incubation at 37°C, cells were washed and resuspended in medium. The medium was changed regularly, and supernatants were assessed for HIV p24 antigen as determined by enzyme-linked immunosorbent assay (Coulter p24 antigen assay).

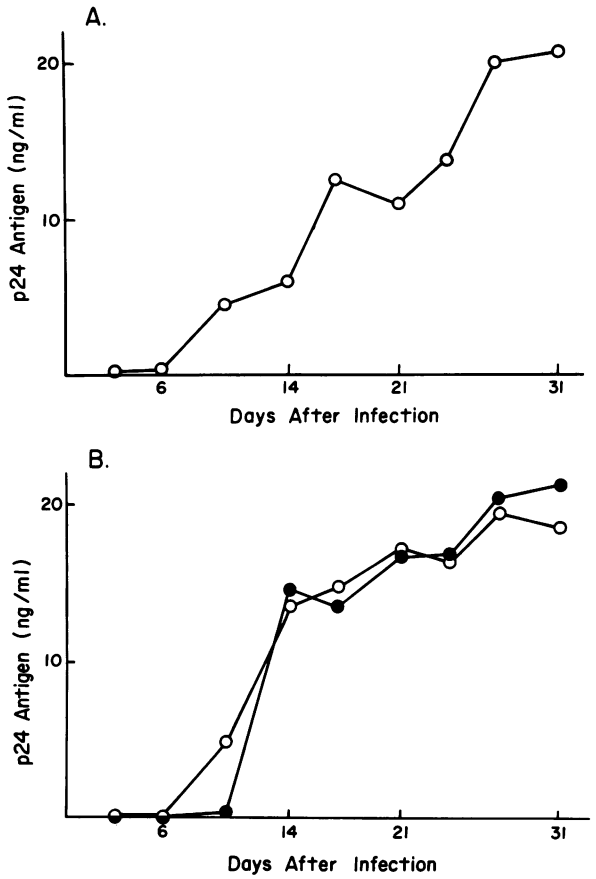

FIG. 2. (A) HIV-1 isolate LAV-1b readily infects chimpanzee bone marrow-derived adherent cells in vitro. Heparinized bone marrow samples were obtained by posterior iliac crest aspiration from ketamine-anesthetized chimpanzees. Cells were isolated by density gradient centrifugation, washed, and placed in culture in two-chamber Lab-Tek tissue culture slides (Nunc) in Iscove modified Dulbecco minimum essential medium supplemented with 12.5% fetal bovine serum and 12.5% horse serum without lectin stimulation or cytokines (20, 21). After 7 days of culture, nonadherent cells were removed and the adherent cells were washed with medium two times. Adherent cells were then incubated for 2 h with a supernatant containing 64 infectious doses of LAV-1b. The medium was changed regularly, and supernatants were assessed for HIV p24 antigen. (B) LAV-1b infects T-lymphocyte-depleted, bone marrow-derived, adherent cells. Bone marrow mononuclear cells were depleted of T lymphocytes by using magnetic beads (19). Cells were incubated with an anti-CD2 monoclonal antibody (3PT2H9; S. Schlossman, Dana-Farber Cancer Institute) (●) or an irrelevant control monoclonal antibody (5E5C) (○) in ascites form at a dilution of 1:500 for 30 min at room temperature. Cells were washed three times and then incubated with Dynabeads H-450 coated with goat anti-mouse immunoglobulin G (Dynal) (bead/cell ratio, 8:1) for 1 h at 37°C with continuous gentle stirring. Magnetic separation of cells was carried out with a magnetic particle concentrator. The T-lymphocyte-depleted bone marrow cells were then washed twice and used in the HIV-1 infection experiment, as described above.

phage population. T-lymphocyte-depleted adherent bone marrow-derived cells supported virus replication (Fig. 2B). Moreover, immunohistochemical studies demonstrated that viral antigen was expressed by cultured cells which not only resembled macrophages morphologically (Fig. 3B) but also stained with a monoclonal antibody that recognizes CD68, a 110-kDa macrophage-specific cytoplasmic and surface molecule (Fig. 3A). Finally, electron microscopic analysis demonstrated lentivirus particles budding from cultured cells with a macrophagelike morphology (Fig. 4).

If only those studies with the IIIb, RF, and MN isolates of HIV-1 were considered, there might appear to be a fundamental difference between human and chimpanzee cells in their ability to support HIV-1 replication. However, when assessed in the context of the studies of the LAV-1b isolate, these experiments suggest that the apparent differences in biologic properties of cells of the two species may simply reflect characteristics of the particular virus isolate being evaluated. The present studies clearly indicate that chimpanzee PBLs and macrophages can support HIV-1 replication and that HIV-1 infection of chimpanzee PBLs is associated

TABLE 1. Cytopathicity of HIV-1 strain LAV-1b for chimpanzee CD4$^+$ lymphocytes[a]

PBLs	Days in culture	Reverse transcriptase (cpm, 10^3/ml)		CD4/CD8 ratio		% Viability of cells		Total cells (10^{-6})	
		−LAV-1b	+LAV-1b	−LAV-1b	+LAV-1b	−LAV-1b	+LAV-1b	−LAV-1b	+LAV-1b
Unfractionated	5	0.2	1.3	0.6	0.6				
	6	0.4	8.9	0.5	0.5				
	7	0.2	11.0	0.4	0.3				
	10	0.8	108.0	0.3	<0.1				
	11	0.4	34.0	0.2	<0.1				
CD4 enriched	5	0.7	4.6			46.0	57.0	9.5	14.0
	10	0.6	114.0			42.0	19.0	15.0	5.5
	14	0.8	116.0			46.0	9.0	19.0	3.0
	18	1.1	53.2			40.0	4.0	21.0	1.0

[a] The CD4-enriched PBLs were obtained by first incubating chimpanzee PBLs on plastic dishes to remove adherent cells, followed by overnight incubation in tissue culture dishes coated with a monoclonal anti-CD8 antibody (OKT8; Ortho). The CD4$^+$ cells were removed by gentle pipetting, washed, and then used for infectivity studies. Dual fluorescence flow cytometric analysis with anti-CD4 (OKT4; Ortho) and anti-CD8 monoclonal antibodies demonstrated that the recovered cells were essentially 100% CD4$^+$.

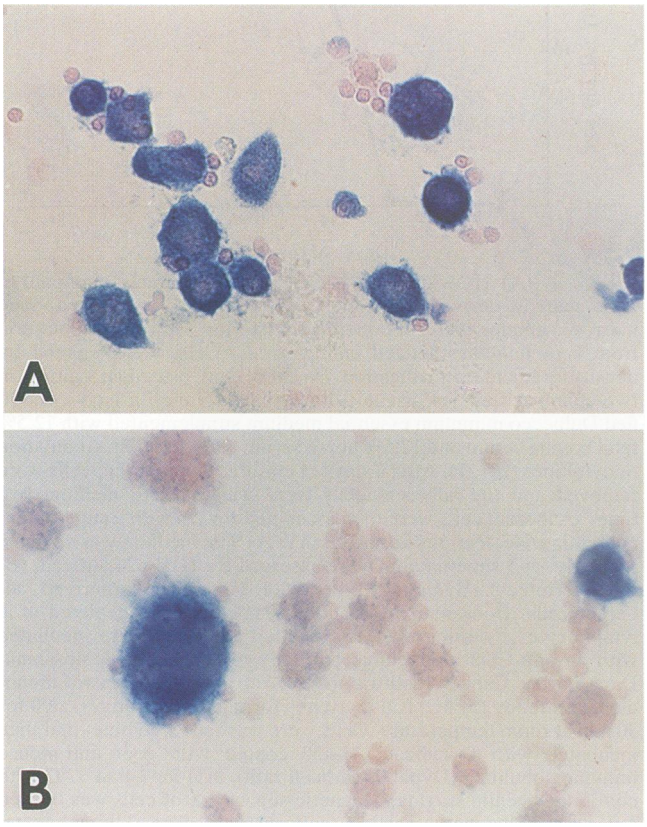

FIG. 3. Photomicrographs of chimpanzee bone marrow adherent cells infected in vitro with LAV-1b. A three-layer alkaline phosphatase–anti-alkaline phosphatase technique, performed as described previously (21), was used with monoclonal antibody EBM11 (Dakopatts), a CD68-specific reagent, and RIC7, a reagent specific for HIV-1 p24 protein (17). RIC7 was kindly provided by M. Popovic and A. Minassian, National Cancer Institute. Antigen was visualized by using a blue alkaline phosphatase substrate kit (Vector), and the slides were counterstained with nuclear fast red. (A) Adherent cells express the macrophage-specific CD68 antigen (blue). Magnification, ×285. (B) Cells with a macrophagelike morphology express HIV-1 core antigens (p24). Magnification, ×360.

with cytopathicity and loss of CD4$^+$ cells. The HIV-1 isolates IIIb, RF, and MN were selected for their in vitro replicative properties in human cells; thus, the results obtained in the present study may simply reflect their preference for replication in human rather than chimpanzee cells. Substantial data exist which show that the in vitro propagation of HIV-1 preferentially selects for a subset of viruses present in the population of viruses which can replicate most efficiently in the selecting cell type (10, 11, 16). It follows, therefore, that an in vivo passage of LAV-1_{BRU} in chimpanzees may have selected for a strain that not only infects and replicates to high titers in chimpanzee CD4$^+$ cells but also is cytopathic for those cells. This is supported by experiments in which replication in chimpanzee PBLs by LAV-1b was compared with that of an independent stock of LAV-1_{BRU} obtained from another investigator. The non-chimpanzee-passaged LAV-1 replicated more poorly in chimpanzee PBLs than the chimpanzee-passaged LAV-1, as evidenced by both maximum production of reverse transcriptase activity and titers of the viruses in culture supernatants (4).

Perhaps of greater significance is the apparent lack of correlation between the in vitro and in vivo properties of LAV-1b. The infectivity and cytopathicity of the LAV-1b isolate for chimpanzee lymphocytes and macrophages do not appear to correlate with virulence in vivo. In the past 5 years, the original passage 0 stock of LAV-1b was used to infect 14 chimpanzees housed at four different institutions. All of the chimpanzees appear clinically well, although two animals infected for more than 5 years have had persistent thrombocytopenia and low numbers of circulating CD4$^+$ lymphocytes (8). This contrasts with observations made concerning the in vitro cytopathicity and in vivo pathogenicity of the simian immunodeficiency virus isolate from sooty mangabeys (SIVsmm) (5). Although isolates from animals naturally infected with SIVsmm replicate efficiently in PBLs of the sooty mangabey, the virus is not cytopathic for CD4$^+$ cells and does not elicit disease in its natural host. The acquisition of cytopathicity for mangabey CD4$^+$ lymphocytes by a mutant of SIVsmm, termed SIVsmmPBj14, resulted in its concomitant acquisition of pathogenicity for sooty mangabeys (6). Despite this apparent correlation in the simian immunodeficiency virus system between in vitro and in vivo properties, the results of the present chimpanzee study emphasize the lack of universality in extrapolating from in vitro observations to in vivo pathogenesis.

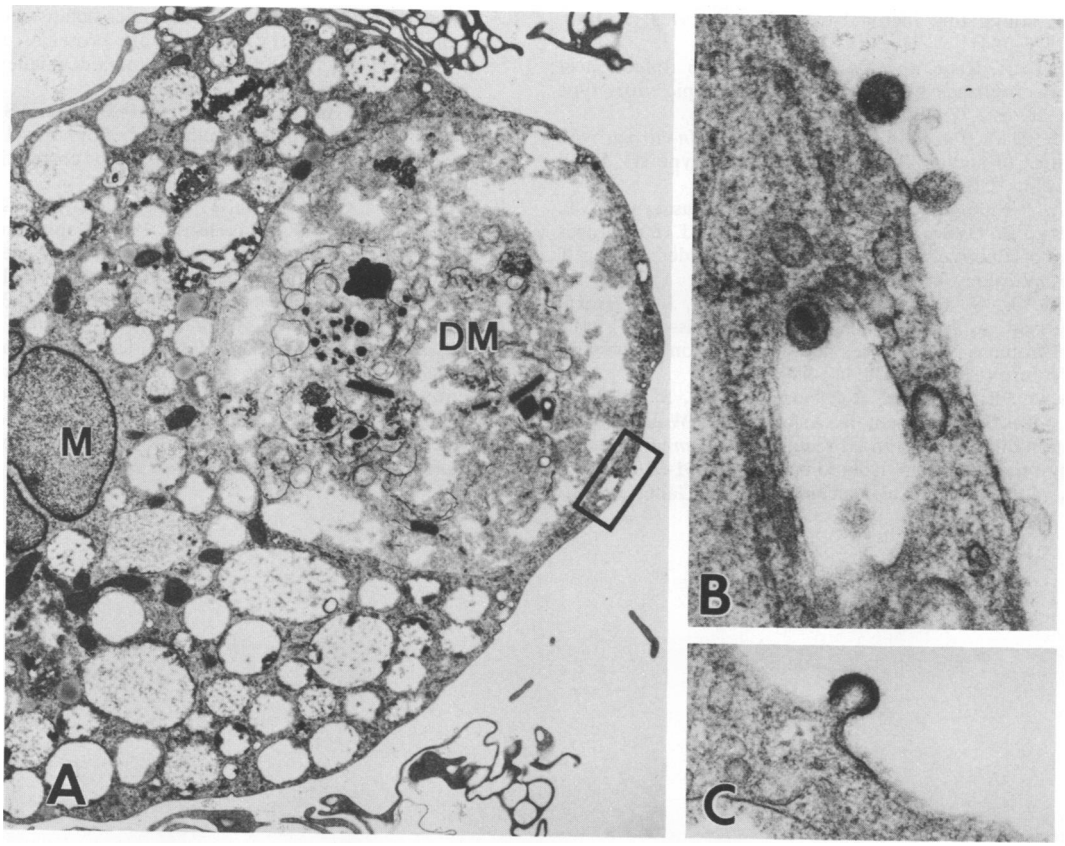

FIG. 4. Transmission electron photomicrographs of bone marrow adherent cells infected in vitro with LAV-1b. Adherent bone marrow cells were scraped from slides and prepared by standard techniques, as described previously (21). (A) A bone marrow macrophage (M) contains cytoplasmic lipid droplets, electron-dense granules, and phagocytic vacuoles, one of which is filled with a degenerative macrophage (DM). Mature lentivirus particles are found adjacent to the plasma membrane (enclosed; enlarged in panel B). (C) In other bone marrow macrophages, budding lentivirus particles can be found at the plasma membrane. Uranyl acetate and lead citrate. Magnifications: panel A, ×3,600; panel B, ×62,500; panel C, ×75,000.

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