Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5162–5166, June 1992
Medical Sciences

Human immunodeficiency virus type 1 infection of neural xenografts

(AIDS/central nervous system/fetal brain/monocyte/macrophage)

THERESE A. CVETKOVICH*, ELIOT LAZAR†‡, BENJAMIN M. BLUMBERG§¶, YOSHIHIRO SAITO§, THOMAS A. ESKIN||, RICHARD REICHMAN**, DAVID A. BARAM††, COCA DEL CERRO†, HOWARD E. GENDELMAN‡‡§§, MANUEL DEL CERRO†‡, AND LEON G. EPSTEIN*§¶

Departments of §Neurology, *Pediatrics, ¶Microbiology and Immunology, ||Pathology, †Neurobiology and Anatomy, ‡Ophthalmology, **Medicine, and ††Obstetrics and Gynecology, University of Rochester, Rochester, NY 14642; and ‡‡The Henry M. Jackson Foundation of the Advancement of Military Medicine and §§The HIV Immunopathogenesis Program, Department of Cellular Immunology, Walter Reed Army Institute of Research, Rockville, MD 20850

Communicated by D. Carleton Gajdusek, February 21, 1992 (received for review November 29, 1991)

ABSTRACT **Human immunodeficiency virus type 1 (HIV-1) infection is highly specific for its human host. To study HIV-1 infection of the human nervous system, we have established a small animal model in which second-trimester (11 to 17.5 weeks) human fetal brain or neural retina is transplanted to the anterior chamber of the eye of immunosuppressed adult rats. The human xenografts vascularized, formed a blood–brain barrier, and differentiated, forming neurons and glia. The xenografts were infected with cell-free HIV-1 or with HIV-1-infected human monocytes. Analysis by polymerase chain reaction revealed HIV-1 sequences in DNA from xenograft tissue exposed to HIV-1 virions, and *in situ* hybridization demonstrated HIV-1 mRNA localized in macrophages and multinucleated giant cells. Pathological damage was observed only in neural xenografts containing HIV-1-infected human monocytes, supporting the hypothesis that these cells mediate neurotoxicity. This small animal model allows the study of direct and indirect effects of HIV-1 infection on developing human fetal neural tissues, and it should prove useful in evaluating antiviral therapies, which must ultimately target HIV-1 infection of the brain.**

In children and adults with human immunodeficiency virus type 1 (HIV-1) infection, clinical neurologic dysfunction is a frequent and prominent manifestation (1, 2). Vertical transmission and HIV-1 isolation from fetal brain are well documented (3, 4); however, details regarding the timing, cellular location, and nature of HIV-1 infection in the developing central nervous system (CNS) remain unknown.

Early neuropathologic studies in children with AIDS revealed that neuroinvasion occurred frequently, but a discrepancy was observed between the number of productively infected macrophages and multinucleated giant cells, and the severity of tissue damage (5). These observations suggested that indirect mechanisms, including the release of cytokines, may be mediators of tissue damage (6, 7). Several *in vitro* studies demonstrated that factors secreted by HIV-1-infected macrophages are toxic to neurons (8, 9), and the soluble HIV-1 glycoprotein gp120 may itself cause neuronal death (10). Moreover, interactions between HIV-1-infected macrophages and glial cells result in the production of neurotoxic factors (ref. 11 and H.E.G., unpublished data). Such factors of host or viral origin, produced systemically or locally in the CNS, could have profound effects on the developing nervous system.

Major biological features common to the lentiviral subfamily of retroviruses include neurotropism and host specificity (12). This necessitates a human tissue target for study of HIV-1 infection. Moreover, the replication activity of lentiviruses *in vitro* is a poor predictor of pathogenic potential *in vivo* (12). The importance of specific virus–cell interactions is emphasized by the varying replication efficiency of lentiviruses in different cell types or at different stages of cellular activation or maturation (13).

The best animal model to date for human AIDS and lentiviral encephalitis is experimental infection of rhesus macaques (*Macaca mulatta*) with the simian immunodeficiency virus (SIV) (14). SIV produces immunodeficiency and neuropathological changes in juvenile macaques virtually indistinguishable from the findings of HIV-1 brain infection in children (15). This model, however, has not proved useful in studies of maternal–fetal transmission (16). Studies of HIV-1 infection of chimpanzees have been restricted by the lack of overt disease and by the expense and protected status of these animals (17). The SCID-hu mouse (18) is an excellent model system for study of HIV-1 infection of the human immune system. However, despite numerous attempts to infect rabbits and rodent species with HIV-1, no satisfactory small animal model exists to study HIV-1 pathogenesis in the nervous system (19, 20).

The chief requirements for an *in vivo* model of HIV-1 infection of neural tissue are vascularized human tissue containing all cellular components (neurons, glia, microglia), capable of differentiation and/or proliferation, and a blood–brain barrier. With the goal of developing such a small animal model, second-trimester fetal human brain or retinal tissue was implanted and grown as a xenograft in the anterior chamber of the eye of immunosuppressed adult rats, where it can serve as a target for HIV-1 infection and as a model for HIV-1 neurotoxicity.

MATERIALS AND METHODS

Transplantation of Human Fetal Tissues to the Anterior Chamber of Adult Rat Eyes. *Host animals.* The hosts for this study were 100- to 120-day-old male albino rats, 200–350 g body weight, of specific-pathogen-free Sprague–Dawley strain (Charles River Breeding Laboratories), housed in sterilized microisolator cages. With xenografts (human neural tissue to rat) it was necessary to maintain the host animals on cyclosporin A (Sandoz; 6.0 mg/kg per day).

Donor fetal tissues. Fetal tissues were procured from elective therapeutic abortions in strict accordance with scientific and ethical guidelines of the National Institutes of Health and the University of Rochester. The gestational age

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV-1, human immunodeficiency virus type 1; SIV, simian immunodeficiency virus; CNS, central nervous system; $TCID_{50}$, tissue culture 50% infective dose; PTD, post-transplantation day.

was determined by using heel-to-toe measurement and standardized charts. The retina and brain regions of interest from fetuses of gestational age 11–17.5 weeks were submerged in fresh human plasma at 4°C, dissected under a microscope, and minced into small (<0.5 mm^3) fragments. The brain region used routinely was telencephalon, including both the ventricular and cortical surfaces, thus ensuring the presence in the donor tissue of both neuronal and glial elements. Excess donor fetal tissue was stored at −70°C and screened for HIV-1 infection by using polymerase chain reaction (PCR).

Surgical procedure and periodic examination. Tissue fragments prepared in human plasma at 4°C as above, untreated or mixed with concentrated HIV-1, or HIV-1-infected or uninfected monocytes, were backloaded into a 50-μl Hamilton syringe. The rats were anesthetized with ketamine (60 mg/kg), pentobarbital (20 mg/kg), and topical xylocaine (1%). Ten microliters of tissue suspension was injected via a 27-gauge butterfly needle into the anterior chamber, under direct observation using a dissecting microscope. The growth of the transplant and the status of the eye's anterior segment were easily monitored through the transparent cornea by direct opthalmoscopy or with a stereo microscope. Control animals that received only neural tissue xenografts and sentinel animals with no graft were included in each experiment.

Preparation of HIV-1 Inoculum. Low-speed supernatants from tissue culture-grown HIV-1 (patient samples or standard reference strains) were filtered (0.2-μm pore diameter) and treated with RQ1 RNase-free DNase (Promega) at 2 μg/ml for 20–30 min at room temperature in the presence of 0.01 M $MgCl_2$ to eliminate carryover HIV-1 proviral DNA. Supernatants were placed in 4.0-ml ultracentrifuge tubes, underlayered with a cushion of 0.5 ml of 40% glycerol in 50 mM Hepes at pH 7.8, and centrifuged for 1 hr at 40,000 rpm at 15°C in a Beckman SW60 rotor. The supernatant plus 0.2 ml of the glycerol solution was removed, and the pelleted virus was resuspended in the remaining 0.3 ml by gentle pipetting.

Isolation, Culture, and HIV-1 Infection of Human Monocytes. Monocytes cultured for 7 days in Teflon bottles at a density of 1×10^6 cells per ml were exposed at a multiplicity of infection of 0.01 infectious virus per target cell to monocyte-tropic HIV-1 strain ADA as previously described (21–23). Twenty percent of these monocytes were found to be HIV-1_{ADA}-infected by *in situ* hybridization in control experiments (not shown). All viral stocks were tested and found free of mycoplasma contamination (22, 23) (Gen-Probe II; Gen-Probe, San Diego).

HIV-1 Infection of Human Neural Xenografts. Animals in group I ($n = 16$) were experimentally inoculated by multiple routes with concentrated cell-free HIV-1 [titered to 1×10^3 to 1×10^4 tissue culture 50% infective dose ($TCID_{50}$)/ml] 7–10 days after neural xenografts were placed. Of these animals; group IA ($n = 3$) received 1×10^3 $TCID_{50}$ intravenously (i.v.), 5×10^2 $TCID_{50}$ intramuscularly (i.m.) and intraperitoneally (i.p.), and 7×10^1 $TCID_{50}$ intraocularly (i.o.); group IB ($n = 3$) animals were inoculated similarly i.m., i.p., and i.o. but not i.v.; group IC ($n = 2$) animals were inoculated with 2×10^1 $TCID_{50}$ intracerebrally (i.c.) and 1×10^3 $TCID_{50}$ i.m.; group ID ($n = 4$) animals were inoculated with 1×10^4 $TCID_{50}$ i.v.; and group IE ($n = 4$) animals were inoculated with 5×10^3 $TCID_{50}$ i.v. and i.m..

In group II ($n = 8$), concentrated HIV-1 (1×10^3 $TCID_{50}$) was mixed with the neural tissue explant at the time of engraftment in the anterior chamber. In Group III ($n = 25$), animals were inoculated with HIV-1_{ADA}-infected human monocytes (10^4 cells in 5 μl) mixed with 5 μl of fetal brain tissue fragments. In this experiment, 7 animals received neural tissue only bilaterally, 7 animals received neural tissue plus uninfected human monocytes, and 11 animals received neural tissue plus HIV-1_{ADA}-infected monocytes bilaterally. Representative animals were euthanized on post-transplantation days (PTDs) 7–14. Grafted tissue was recovered by enucleation and immediately frozen or prepared for histologic or ultrastructural studies. Grafts survived *in oculo* for 30 days or more; however, to assess infectibility with HIV-1, the animals were euthanized prior to this point.

***In Situ* Hybridization for HIV-1 mRNAs.** The pBenn-6 6.5-kilobase (kb) *Hin*dIII fragment recloned in the RNA transcription vector pSP64 (Promega) was used to prepare HIV-1-specific sense and antisense RNA transcripts (24, 25). These were synthesized by using SP6 RNA polymerase and tritium-labeled UTP and CTP to a specific activity of 1.13×10^8 dpm/μg. *In situ* hybridization was performed as previously described (25, 26).

PCR. DNA was extracted from tissue fragments (10–20 mg) by the method of Boom *et al.* (27). To identify HIV-1 in xenografts, we used nested *pol* (JA17, JA18, JA19, JA20), and *env* (JA9, JA10, JA11, JA12) primer sets that recognize diverse HIV-1 field isolates (28). We have previously described the use of efficient primers and conditions for PCR amplification and cloning of the V3 region of HIV-1 (29). We also used a nested primer set for the HIV-1 *nef* gene (outer set, starting at nucleotide 8796, 5′-TTCGCCACATACCTAGAAGAATAAGA-3′ and, starting at nucleotide 9532, 5′-CCGCCCAGGCCACGCCTCCCT-3′; inner set, starting at nucleotide 8840, 5′-TTGCTATAAGATGGGTGGCAAGTG-3′ and, starting at nucleotide 9498, 5′-CGGAAAGTCCCTTGTAGCAAGCTC-3′; positions are relative to the HXB2 clone of HIV). To distinguish rat from human fetal DNA, we designed a set of nested primers based on the sequences of the human and rat D_1 dopamine receptor genes recently published by Zhou *et al.* (30). These were D_1 human outer 5′-GTGTTTGTGTGGTTTGGGTGG-3′, inner 5′-ATAACAATGGGGCCGCGATGT-3′, and reverse 5′-CAGGTTGGGTGCTGACCGTTT-3′; and D_1 rat outer 5′-TGGTTTGGGTGGGCGAATTCT-3′, inner 5′-CATTAACAACAATGGGGCTGT-3′, and reverse 5′-ATGCTGTCCACTGTGTGTGACA-3′. In addition, we used the β-globin primer set (GH20/GH21 outer, PC03/PC04 inner) of Saiki *et al.* (31) to test the efficiency of extraction; this set identified both human and rat DNA. Results were scored as positive only if specific bands were observed with at least two primer sets for HIV-1 and one for host DNA.

RESULTS

Description of Anterior Chamber Human Neural Xenografts. The overall success rate in establishing viable xenografts in rat eyes was 85%. Preliminary experiments established optimal conditions for the xenograft procedure using second-trimester human fetal neural tissue; neuronal, glial, and microglial cells were identified within these grafts (32).

Biomicroscopical observations of intact brain and retinal grafts in the anterior chamber showed that these grafts became vascularized and grew into spherical or oblong masses with well-defined limits and a characteristic opalescent glow on their smooth surface (Fig. 1). Light microscopical observations on recovered xenograft tissue demonstrated that neuronal cells maintained normal cellular morphology and differentiation within these grafts. Ultrastructural analysis of neural graft tissues revealed well-defined axonal growth cones (not shown) and synaptic endings with synaptic vesicles (Fig. 2); this confirmed that neuronal differentiation could occur within these grafts. In addition, ultrastructural studies demonstrated that these vascularized xenografts excluded the protein marker horseradish peroxidase, indicating that they developed an intact blood–brain (blood–graft) barrier (32).

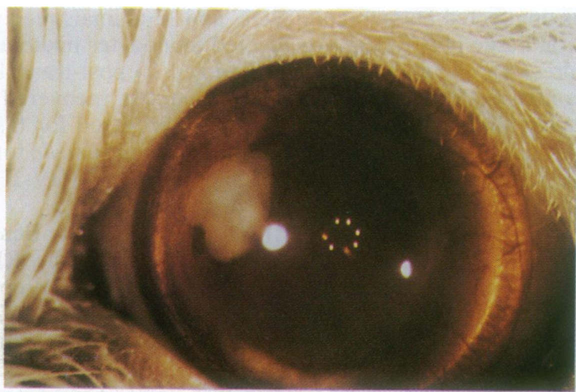

FIG. 1. Biomicroscopy of a rat eye, anterior chamber, containing xenograft of telencephalon from a 15-week gestation human fetus, PTD 7.

Histologic examination of xenografts from group I animals (infected with cell-free HIV-1 by systemic inoculation) and group II animals (infected with cell-free HIV-1 inoculated *in oculo*) did not reveal any histologic differences compared with the appearance of grafts from control animals with neural tissue not exposed to HIV-1. In group III, grafts were compared between animals receiving only fetal brain tissue and animals receiving brain plus uninfected human monocytes, or brain plus HIV-1_{ADA}-infected monocytes, at 7 and 14 days after inoculation. Grafts composed of neural tissue only, or neural tissue and uninfected monocytes, at both time points, exhibited normal-appearing neuronal and glial precursors with distinct nuclei and occasional mitotic forms (Fig. 3 *A* and *B*). In sharp contrast, the xenografts containing HIV-1_{ADA}-infected monocytes showed significant degeneration at both time points (Fig. 3 *C* and *D*). In these grafts, the HIV-1-infected cells had the appearance of activated macrophages with vacuolated cytoplasm. Neuroblasts in close proximity to the infected macrophages were small and dark, with poorly defined nuclei indicating cell death (Fig. 3*D*). Notably absent were lymphocytes and other inflammatory cells in and around the graft, as is usually seen with transplant rejection. Thus, HIV-1-infected macrophages were specifically associated with tissue pathology.

HIV-1 Gene Sequences in Xenograft and Rat Tissues. DNA prepared from the xenografts and from selected rat and human tissues was analyzed by PCR using primers specific for HIV-1 and host gene sequences. Grafts from one eye of

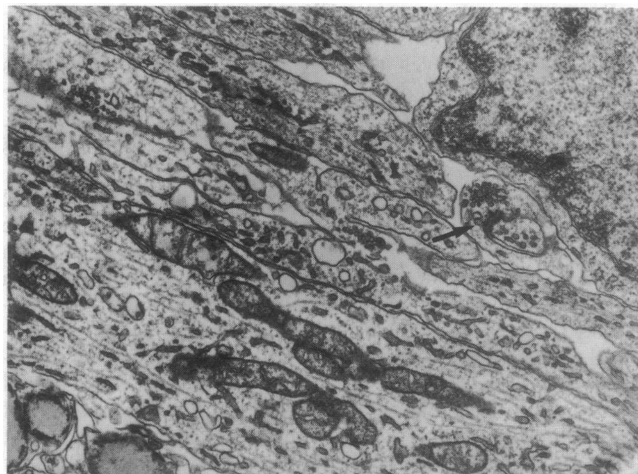

FIG. 2. Electron micrograph of human fetal brain xenograft, demonstrating a cluster of synaptic vesicles adjacent to a synaptic cleft (arrow). (×10,400.)

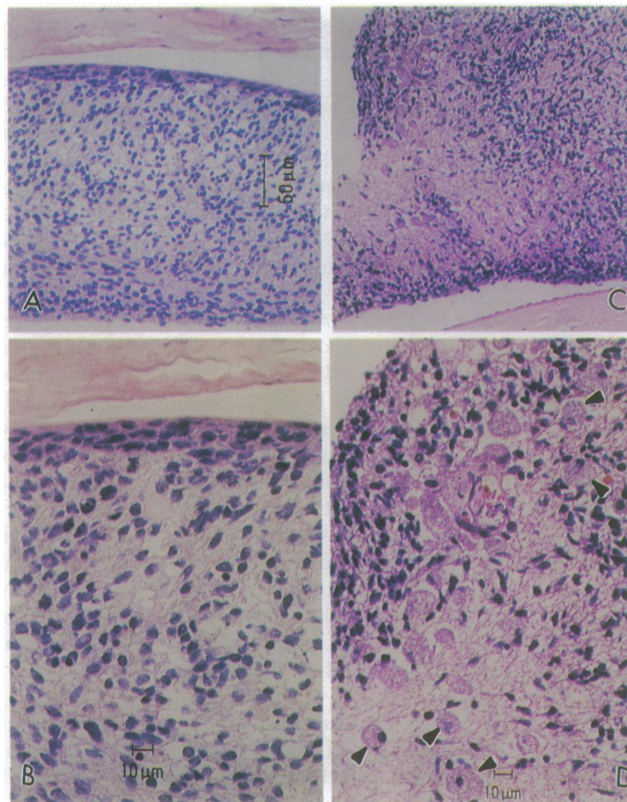

FIG. 3. (*A*) Low-power photomicrograph of anterior chamber xenograft from an animal euthanized on PTD 7 after placement of 14-week gestation human fetal brain and uninfected human monocytes. A healthy graft with uniformly distributed nuclei is demonstrated. (Hematoxylin and eosin, ×130.) (*B*) High-power field of graft shown in *A*. The presence of healthy-looking immature neural tissue separated by a distinct neuropil can be appreciated. Proliferating neuroblasts undergoing mitosis are present. (Hematoxylin and eosin, ×270.) (*C*) Low-power field of anterior chamber xenograft from another animal euthanized on PTD 7 after placement of 14-week gestation human fetal brain and HIV-1_{ADA}-infected human monocytes. Note patchy areas with less dense cell population distributed throughout the graft, corresponding to clusters of macrophages and cell debris. The media are free of inflammatory cells and notably absent are surveillant rat macrophages, which characteristically invade the graft from the surface during rejection. (Hematoxylin and eosin, ×110.) (*D*) High-power field of graft shown in *C*. Numerous activated macrophages (arrowheads) are seen in close proximity to and in direct contact with neuroblasts undergoing degeneration. In contrast to the graft shown in *B* the neuroblasts are less densely packed, and their nuclei are small and pyknotic. (Hematoxylin and eosin, ×250.)

each of the 24 rats from groups I and II were subjected to PCR analysis individually and compared with other tissues from the same rat as well as with positive and no-DNA controls (Fig. 4).

A series of similar experiments (not shown) demonstrated that in 3 of 16 animals in groups IA–IE, inoculated after engraftment with concentrated cell-free HIV-1 by multiple routes, HIV-1-specific bands were observed in recovered xenograft tissue. None of the animals from groups IA or IB had infected grafts, while one graft each from groups IC, ID, and IE gave HIV-1-specific bands. Two of the three infected grafts contained fetal brain tissue, and one had retinal tissue. In group II animals, in which concentrated HIV-1 was engrafted simultaneously with the neural tissue in the anterior chamber, five of eight xenografts examined by PCR were positive for HIV-1 sequences. Three of these HIV-1-infected grafts were fetal brain tissue and two were fetal retinal tissue. As care was taken to eliminate HIV-1 proviral DNA from the

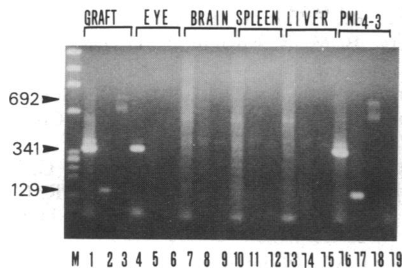

FIG. 4. PCR analysis demonstrates HIV-1 DNA in xenograft but not in rat tissues. Human fetal brain fragments mixed with DNase-treated HIV-1 were grafted into the anterior chamber of the rat eye and grown *in situ* for 9 days. PCR was performed with nested primers for the *env*, *pol*, and *nef* genes of HIV-1. The PCR products were analyzed on a 1.2% agarose gel with ethidium bromide staining. *env* primers were used for lanes 1, 4, 7, 10, 13, and 16 [expected product length 341 base pairs (bp)]; *pol* primers for lanes 2, 5, 8, 11, 14, and 17 (product, 129 bp); and *nef* primers for lanes 3, 6, 9, 12, 15, and 18 (product, 692 bp). The appearance of bands at the expected lengths (arrowheads on the left, in bp) indicates HIV-1 proviral or unintegrated DNA. The signal in lane 4, weak by comparison with that of lanes 1 and 16, suggests that a small amount of graft tissue sloughed off into the eye compartment. Positive HIV-1 control was plasmid pNL4-3. *env* primers but no DNA was used in negative-control lane 19. Lane M contained *Hae* III-digested marker DNA from phage ϕX174.

inocula, the finding of HIV-1 DNA in these experiments is proof of viral replication in xenograft tissue.

***In Situ* Hybridization.** Animals in group III (engrafted with HIV-1_{ADA}-infected monocytes) could not be assessed by PCR due to the presence of HIV-1 proviral DNA in the monocytes. We therefore studied xenografts from these animals by using *in situ* hybridization. Two grafts from group III and two grafts from group II (inoculated with cell-free HIV-1) that were HIV-1 positive by PCR were selected for *in situ* hybridization, and serial 5-μm paraffin sections were prepared. Grafts with neural tissue only and grafts with neural tissue plus uninfected monocytes served as controls. Each graft from HIV-1-inoculated animals contained a small number of macrophages that hybridized to the HIV-1-specific riboprobe. Fig. 5 demonstrates the presence of a strong cytoplasmic hybridization signal over macrophages within the graft. Hybridization occurred with the HIV-1 antisense probe, but not with the sense probe, indicating the presence of HIV-1 RNAs. (HIV-1 DNA would not be expected to hybridize under these nondenaturing conditions.) No hybridization signal was observed in the grafts containing only neural tissue or neural tissue plus uninfected monocytes. In Fig. 5 *D* and *E* the signal was localized over a multinucleated giant cell, similar in appearance to syncytial cells characteristic of HIV-1 encephalitis (2, 5, 33).

DISCUSSION

Our studies indicate that second-trimester human fetal brain or retinal tissue can be established as xenografts in the anterior chamber of the rat eye and can be productively infected with cell-free or monocyte-associated HIV-1. Further, these xenografts vascularize, differentiate, and reconstitute a blood–brain (blood–graft) barrier that excludes blood-borne proteins such as the marker horseradish peroxidase (32). The anterior chamber of the rabbit eye has been used as an immune-privileged transplantation site since the last century, preceding the development of cell cultures (34). Clearly, this model still offers some advantages over *in vitro* systems due to the ability to study functionally intact neural tissue (32, 35).

Macrophages and multinucleated (syncytial) giant cells (MGC) were recognized early as the major cell types har-

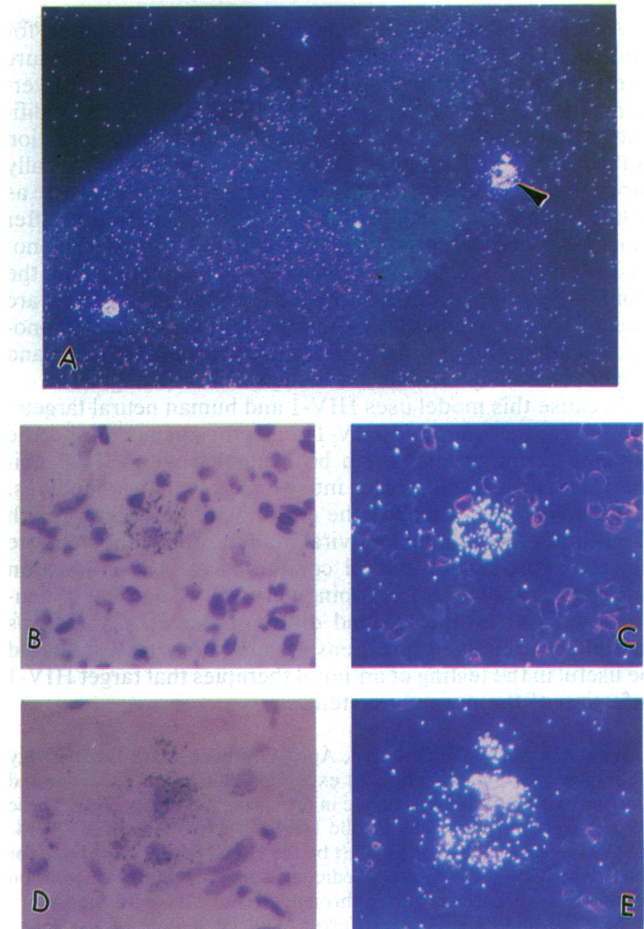

FIG. 5. *In situ* hybridization of an intraocular human fetal brain xenograft tissue coinoculated with HIV-1_{ADA}-infected human monocytes (PTD 8). Tritium-labeled antisense strand HIV-1 riboprobe hybridizes with HIV-1 RNAs, giving a strong signal indicating productive infection localized to cells identified morphologically as macrophages. (*A*) Panoramic dark-field view of a graft section. (×75.) The two positive cells seen in the picture are shown at higher magnification (×300) in photomicrographs *B*–*E*. (*B* and *C*) Light-field (*B*) dark-field (*C*) photographic pair showing the positive cell seen on the lower left of *A*. (*D* and *E*) Light-field (*D*) dark-field (*E*) photographic pair showing the multinucleated giant cell seen on the upper right of *A* (arrowhead).

boring HIV-1 infection in the CNS (5, 33, 36, 37). Macrophage tropism may be essential for HIV-1 (and SIV) neuroinvasiveness (38, 39). On this basis we sought to determine the pathologic consequences of introducing HIV-1-infected human monocytes into human neural xenografts. The finding of HIV-1 in macrophages and MGC suggests that HIV-1 infection in this model reproduces the chief finding of AIDS encephalitis (5, 33, 36, 37). The comparison of neural xenografts with HIV-1-infected versus uninfected monocytes revealed pathologic changes, including degeneration and death of neural cells in contact with macrophages, while grafts with uninfected monocytes continued to thrive and differentiate. These data strongly support hypotheses that neurotoxic effects are mediated by HIV-1-infected mononuclear cells (8, 9, 40). Similar pathology was not observed in grafts infected with cell-free HIV-1, suggesting that direct cell-to-cell contact of HIV-1-infected macrophages with neural cells or, alternatively, the local release of cytokines or other neurotoxic factors may be required for neuronal damage. More rigorous testing of these hypotheses is possible by employing the model herein described but will require additional studies with larger numbers of animals.

A major limitation of the human neural xenograft model for studying pathogenesis is the absence of the selective pressure exerted by the human immune system. This may be overcome by placing the xenografts in SCID-hu mice, a modification that should be technically feasible. A second limitation is the relative inefficiency of HIV-1 infection by systemically inoculated cell-free virus (19, 20), even with human tissue as a target. Infection was established more consistently after placement of concentrated HIV-1 or HIV-1-infected monocytes directly into the neural tissue, thus circumventing the "natural" route of neuroinvasion. Additional studies are needed to test whether HIV-1-infected primary human monocytes can traffic through the rodent vascular system and target previously established human neural xenografts.

Because this model uses HIV-1 and human neural targets, the *in vivo* relevance of HIV-1 regulatory genes or specific envelope configurations can be evaluated with HIV-1 chimeric constructs transfected into primary human monocytes. This *in vivo* model avoids the extrapolation necessary with the use of other animal lentiviral models, and it should more closely approximate natural conditions of HIV-1 infection and neurotoxicity in developing nervous tissue, where neurons and glia proliferate and differentiate. Finally, as this model uses relatively inexpensive laboratory rats, it should be useful in the testing of antiviral therapies that target HIV-1 infection of the nervous system.

We thank Mary Morgenstern, April Whitbeck, Kirk Dzenko, Ky Dehlinger, and Robert White for exceptional technical expertise and Corrinne Gartland for assistance in preparing the manuscript. These studies were supported by Public Health Service Grants NS28754, EY09217, and AI32305 and in part by the Henry M. Jackson Fund for the Advancement of Military Medicine. Some of the instrumentation used in this work was acquired through private gifts to the laboratory of M.d.C., and H.E.G. is a Carter-Wallace Fellow of the Johns Hopkins University School of Public Health and Hygiene, Department of Immunology and Infectious Diseases.

1. Epstein, L. G., Sharer, L. R., Oleske, J. M., Connor, E. M., Goudsmit, J., Robert-Guroff, M. & Koenigsberger, M. R. (1986) *Pediatrics* **78**, 678–687.
2. Michaels, J., Sharer, L. R. & Epstein, L. G. (1988) *Immunodeficiency Rev.* **1**, 42–77.
3. Jovaisis, E., Koch, M. A., Schafer, A., Stauber, M. & Lowenthal, D. (1985) *Lancet* **ii**, 1129.
4. Sprecher, S., Soumenkoff, G., Puissant, F. & Degueldre, M. (1986) *Lancet* **ii**, 288–289.
5. Sharer, L. R., Epstein, L. G., Cho, E.-S., Joshi, V. V., Meyenhofer, M. F., Rankin, L. F. & Petito, C. K. (1986) *Hum. Pathol.* **17**, 271–284.
6. Merrill, J. E. & Chen, I. S. (1991) *FASEB J.* **5**, 2391–2397.
7. Mintz, M., Rapaport, R., Oleske, J. M., Connor, E. M., Koenigsberger, M. R., Denny, T. & Epstein, L. G. (1989) *Am. J. Dis. Child.* **143**, 771–774.
8. Giulian, D., Vaca, K. & Noonan, C. A. (1990) *Science* **250**, 1593–1596.
9. Pulliam, L., Herndier, B. G., Tang, N. M. & McGrath, M. S. (1991) *J. Clin. Invest.* **87**, 503–512.
10. Dreyer, E. B., Kaiser, P. K., Offermann, J. T. & Lipton, S. A. (1990) *Science* **248**, 364–367.
11. Burton, E. W., Bryant, H. U., DeCoster, M., Orenstein, J. M., Meltzer, M. S. & Gendelman, H. E. (1992) *AIDS Res. Hum. Retro.*, in press.
12. Narayan, O. & Clements, J. E. (1989) *J. Gen. Virol.* **70**, 1617–1639.
13. Gendelman, H. E., Narayan, O., Kennedy-Stoskopf, S., Kennedy, P. G. E., Ghotbi, Z., Clements, J. E., Stanley, J. & Pezeshkpour, G. (1985) *J. Virol.* **58**, 67–74.
14. Letvin, N. L., Daniel, M. D., Sehgal, P. K., Desrosiers, D. C., Hunt, R. D., Waldron, L. M., Mackey, K. J., Schmide, D. K., Chalifoux, L. V. & King, N. W. (1985) *Science* **230**, 71–73.
15. Sharer, L. R., Baskin, G. B., Cho, E.-S., Murphey-Corb, M., Blumberg, B. M. & Epstein, L. G. (1988) *Ann. Neurol.* **23** (Suppl), 108–112.
16. Davison-Fairburn, B., Blanchard, J., Fu-Sen, H., Martin, L., Harrison, R., Ratterree, M. & Murphey-Corb, M. (1990) *J. Med. Primatol.* **19**, 381–393.
17. Gajdusek, D. C., Amyx, H. L., Gibbs, C. J., Jr., Asher, D. M., Epstein, L. G., Sarin, P. S., Gallo, R. C., Maluish, A., Arthur, L. O., Montagnier, L. & Mildvan, D. (1985) *Lancet* **i**, 55–56.
18. McCune, J. M., Namikawa, R., Kaneshima, H., Schultz, L. D., Lieberman, M. & Weissman, I. L. (1988) *Science* **241**, 1632–1639.
19. Kindt, T. J., Gordon, M. R., Truckenmiller, M. E., Recker, D. P., Kuta, E. A. & Dickerson, D. R. (1990) *J. Cell Biochem. Suppl.* **14D**, 49 (abstr.).
20. Morrow, W. J. W., Wharton, M., Lau, D. & Levy, J. A. (1987) *J. Gen. Virol.* **68**, 2253–2257.
21. Gendelman, H. E., Orenstein, J. M., Martin, M. A., Ferrua, C., Mitra, R., Phipps, T., Wahl, T. A., Lane, H. C., Fauci, A. S., Burke, D. S., Skillman, D. & Meltzer, M. S. (1988) *J. Exp. Med.* **167**, 1428–1441.
22. Gendelman, H. E., Baca, L., Husayni, H., Orenstein, J. M., Kalter, D. C., Turpin, J. A., Skillman, D., Hoover, D. L. & Meltzer, M. S. (1990) *AIDS* **4**, 221–228.
23. Gendelman, H. E., Orenstein, J. M., Baca, L. M., Weiser, B., Burger, H., Kalter, D. C. & Meltzer, M. S. (1989) *AIDS* **3**, 475–495.
24. Benn, S., Rutledge, R., Folks, T., Gold, J., Baker, L., McCormick, J., Feorino, P., Piot, P., Quinn, T. & Martin, M. (1985) *Science* **230**, 949–951.
25. Stoler, M. H., Eskin, T. A., Benn, S., Angerer, R. C. & Angerer, L. M. (1986) *J. Am. Med. Assoc.* **256**, 2360–2364.
26. Sharer, L. R., Dowling, P. C., Michaels, J., Cook, S. D., Menonna, J., Blumberg, B. M. & Epstein, L. G. (1990) *Neuropathol. Appl. Neurobiol.* **16**, 317–331.
27. Boom, R., Sol, C. J. A., Salimans, M. M. M., Jansen, C. L., Wertheim-van Dillen, P. M. E. & van der Noordaa, J. (1990) *J. Clin. Microbiol.* **28**, 495–503.
28. Albert, J. & Fenyo, E. M. (1990) *J. Clin. Microbiol.* **28**, 1560–1564.
29. Epstein, L. G., Kuiken, C., Blumberg, B. M., Hartman, S., Sharer, L. R., Clement, M. & Goudsmit, J. (1991) *Virology* **180**, 583–590.
30. Zhou, Q.-Y., Grandy, D. K., Thanmbi, L., Kushner, J. A., Van Tol, H. M. H., Cone, R., Pribnow, D., Salon, J., Bunzow, J. R. & Civelli, O. (1990) *Nature (London)* **347**, 76–80.
31. Saiki, R. K., Bugawan, T. L., Horn, G. T., Mullis, K. B. & Erlich, H. A. (1986) *Nature (London)* **386**, 163–166.
32. Epstein, L. G., Cvetkovich, T. A., Lazar, E., Dehlinger, K., Dzenko, K., del Cerro, C. & del Cerro, M. (1992) *J. Neurotransplant. Plast.*, in press.
33. Sharer, L. R., Cho, E.-S. & Epstein, L. G. (1985) *Hum. Pathol.* **16**, 760.
34. van Dooremaal, J. C. (1873) *Onderz. Physiol. Lab. Rijksuniv. Utrechsche Loogeschool* **3**, 277–290.
35. del Cerro, M., Gash, D. M., Rao, G. N., Notter, M. F., Wiegand, S. J., Sathi, S. & del Cerro, C. (1987) *Neuroscience* **21**, 707–724.
36. Epstein, L. G., Sharer, L. R., Cho, E.-S., Meyenhofer, M. F., Navia, B. A. & Price, R. W. (1985) *AIDS Res.* **1**, 447–454.
37. Koenig, S., Gendelman, H. E., Orenstein, J. M., Dal Canto, M. C., Pezeshkpour, G. H., Yungbluth, M., Janotta, M., Aksamit, A., Martin, M. A. & Fauci, A. S. (1986) *Science* **233**, 1089–1093.
38. Desrosiers, R. C., Hansen-Moosa, A., Mori, K., Bouvier, D. P., King, N. W., Daniel, M. D. & Ringler, D. J. (1991) *Am. J. Pathol.* **139**, 29–35.
39. Westervelt, P., Trowbridge, D. B., Epstein, L. G., Blumberg, B. M., Li, Y., Hahn, B., Shaw, G. M., Price, R. W. & Ratner, L. (1992) *J. Virol.* **66**, 2577–2582.
40. Gendelman, H. E. & Gendelman, S. (1992) in *Neurotropic Viruses and Immunity*, eds. Spector, S., Bendinetti, M. & Friedman, H. (Plenum, New York), in press.