(1985); G. Wiederrecht, D. J. Shuey, W. A. Kibbe, C. S. Parker, *ibid.* **48**, 507 (1987).
10. P. Becker and C. Wu, unpublished observations.
11. C. J. Goldenberg *et al.*, *J. Biol. Chem.* **263**, 19734 (1988).
12. C. Wu *et al.*, *Science* **238**, 1247 (1987).
13. P. K. Sorger and H. R. B. Pelham, *EMBO J.* **6**, 3035 (1987).
14. B. K. Jakobsen and H. R. B. Pelham, *Mol. Cell. Biol.* **8**, 5040 (1988); C. Szent-Györgyi, D. B. Finkelstein, W. T. Garrard, *J. Mol. Biol.* **193**, 71 (1987); D. McDaniel *et al.*, *Mol. Cell Biol.* **9**, 4789 (1989).
15. V. Zimarino and C. Wu, *Nature* **327**, 727 (1987).
16. J. S. Larson *et al.*, *Nature* **335**, 372 (1988).
17. D. D. Mosser, P. T. Kotzbauer, K. D. Sarge, R. Morimoto, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 3748 (1990).
18. Temperatures higher than 35°C result in less activation and eventual loss of activity (V. Zimarino, unpublished observations). The discrepancy between in vivo and in vitro maximal activation temperatures could be due to proteolysis, protein denaturation, or to the unphysiological conditions of the extract.
19. V. Zimarino, S. Wilson, C. Wu, unpublished observations.
20. S. I. Taylor and B. Marcus-Samuels, *J. Clin. Endocrinol. Metab.* **58**, 182 (1984); R. A. Roth, B. A. Maddux, D. J. Cassell, I. D. Goldfine, *J. Biol. Chem.* **258**, 12094 (1983).
21. S. J. Bray, B. Burke, N. H. Brown, J. Hirsh, *Genes Dev.* **3**, 1130 (1989).
22. O. Perisic, H. Xiao, J. T. Lis, *Cell* **59**, 797 (1989).
23. H. Ueda, S. Sonoda, J. L. Brown, M. P. Scott, C. Wu, *Genes Dev.* **4**, 624 (1990).
24. The increase is possibly on the order of 100-fold, although it is difficult to reach a quantitative estimate when the activity of the inactive factor is below the limit of detection.
25. P. K. Sorger and H. C. M. Nelson, *Cell* **59**, 807 (1989).
26. If the monomer to trimer transition is required for activation of HSF, anti-HSF may also assist in oligomerization by bridging HSF monomers.
27. A. D. Dignam, P. L. Martin, B. S. Shastry, R. G. Roeder, *Methods Enzymol.* **101**, 582 (1983).
28. We thank R. E. Kingston for sharing a prepublication manuscript (*16*), R. Morimoto for unpublished results on in vitro activation, and C. Tsai and J. T. Westwood for HSF protein and data, respectively. We also thank M. Mage and C. Klee for helpful suggestions and discussion.

10 January 1990; accepted 16 May 1990

Specific Tropism of HIV-1 for Microglial Cells in Primary Human Brain Cultures

BRYNMOR A. WATKINS,* HENRY H. DORN, WALTER B. KELLY, REGINA C. ARMSTRONG, BARBARA J. POTTS,† FRANK MICHAELS, CONRAD V. KUFTA, MONIQUE DUBOIS-DALCQ

Human immunodeficiency virus (HIV) frequently causes neurological dysfunction and is abundantly expressed in the central nervous system (CNS) of acquired immunodeficiency syndrome (AIDS) patients with HIV encephalitis or myelopathy. The virus is found mostly in cells of the monocyte-macrophage lineage within the CNS, but the possibility of infection of other glial cells has been raised. Therefore, the effects of different HIV-1 and HIV-2 strains were studied in primary cultures of adult human brain containing microglial cells, the resident CNS macrophages, and astrocytes. These cultures could be productively infected with macrophage-adapted HIV-1 isolates but not with T lymphocyte–adapted HIV-1 isolates or two HIV-2 isolates. As determined with a triple-label procedure, primary astrocytes did not express HIV *gag* antigens and remained normal throughout the 3-week course of infection. In contrast, virus replicated in neighboring microglial cells, often leading to their cell fusion and death. The death of microglial cells, which normally serve immune functions in the CNS, may be a key factor in the pathogenesis of AIDS encephalitis or myelopathy.

HUMAN IMMUNODEFICIENCY VIRUS not only infects T lymphocytes and cells of the monocyte-macrophage lineage (*1–3*) but also often spreads to the nervous system and causes a variety of neurological dysfunctions (*4*) such as acute and chronic meningitis, inflammatory and sensory neuropathies and myelopathy, as well as encephalitis (*5*). Encephalitis gives rise to a decline in memory and cognitive functions, in addition to sensory and motor disturbances, generally known as AIDS dementia. Pathologically, the CNS diseases caused by HIV-1 are characterized by the presence in several cortical and subcortical regions, as well as in the basal ganglia and spinal cord, of perivascular and infiltrating macrophages and lymphocytes, microglial nodules, and multinucleated giant cells (*5, 6*). These features are accompanied by diffuse gliosis and various degrees of demyelination in the white matter of brain or spinal cord. In postmortem samples of brains from AIDS patients, expression of HIV genes has been detected by in situ hybridization in multi- and mononucleated cells that express monocyte-macrophage lineage markers and, more rarely, in endothelial cells, astrocytes, and oligodendrocytes (*7–9*). In addition, HIV *gag* antigens have been shown by immunohistochemistry to be in individual microglial cells and microglial nodules (*7*). Moreover, macrophages infected with HIV have been identified in cultures derived from the brain of AIDS victims (*1*). These studies indicate that the principal sites of viral replication within the CNS are cells that express markers for the macrophage-monocyte lineage, including microglial cells.

An in vitro system is clearly desirable to study the mechanisms of entry, replication, and assembly of HIV in CNS cells; to examine whether certain strains of HIV have a preferential tropism for specific cell types; and to determine the fate of CNS cells infected by HIV. Although adult CNS neurons have not yet been maintained in culture, all nonneuronal cell types of the brain have been cultured successfully, including astrocytes, oligodendrocytes, and microglial cells (*10, 11*). Adult human brain tissue was resected from temporal lobe as a therapeutic measure in patients with intractable epilepsy (this form of epilepsy is resistant to drug therapy; histopathologically, tissue is normal or shows mild gliosis). Tissue was enzymatically dissociated; cells were seeded onto plastic dishes and cultured as described (legend to Fig. 1). These primary cultures contained significant numbers of microglial cells expressing monocyte-macrophage lineage markers such as LDL-R (low-density lipoprotein receptor) (*12*), Leu M3 and Leu M5 (*13*), EBM11 (*14*), and OX42 (*15*); astrocytes that express GFAP (glial fibrillary acidic protein) (*16*); and flat cells that express cell surface fibronectin (fibroblasts or meningeal cells) (*17*), as seen by fluorescence microscopy (legend to Fig. 2). The survival of microglial cells was optimized by addition of giant cell tumor (GCT) supernatant, a mixture of cytokines (*18*). Higher density cultures of purified microglial cells were obtained from a freshly resected and dissociated malignant glioma by means of a differential adhesion method (legend to Fig. 3). Cultures were infected at 14 to 18 days in vitro with HIV-1 and HIV-2 strains of different origins and cellular tro-

B. A. Watkins, H. H. Dorn, W. B. Kelly, R. C. Armstrong, M. Dubois-Dalcq, Laboratory of Viral and Molecular Pathogenesis, National Institute of Neurological Disorders and Stroke (NINDS), National Institutes of Health (NIH), 36/5D04, 9000 Rockville Pike, Bethesda, MD 20892.
B. J. Potts, Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, NIH, 9000 Rockville Pike, Bethesda, MD 20892.
F. Michaels, Laboratory of Tumor Cell Biology, National Cancer Institute, NIH, 9000 Rockville Pike, Bethesda, MD 20892.
C. V. Kufta, Surgical Neurology Branch, NINDS, NIH, 9000 Rockville Pike, Bethesda, MD 20892.

*To whom correspondence should be addressed.
†Present address: Repligen Corporation, 1 Kendall Square, Building 700, Cambridge, MA 02139.

pisms (*19*). The production of HIV by these cultures was followed by measuring reverse transcriptase (RT) (*20*) and p24 *gag* core antigen levels (*21*) in the tissue culture supernatant.

Using these assays, we found that our glial cell cultures could be productively infected by two macrophage-adapted isolates of HIV-1 (HIV-$1_{AD87(M)}$ and HIV-1_{BaL}) but not by T lymphocyte–adapted HIV-1 strains (HIV-1_{LAV} and HIV-$1_{HTLV\text{-}IIIB}$), a primary HIV-1 isolate (HIV-1_{A1638}), or by two isolates of HIV-2 (HIV-$2_{sbl/isy}$ and HIV-2_{B59}) (Fig. 1). In cultures infected with the two macrophage-adapted strains, peak levels of RT and p24 core antigen production were seen at 12 to 17 days postinoculation (PI); cultures infected with HIV-$1_{AD87(M)}$ produced higher levels of RT and p24 than cultures infected with HIV-1_{BAL} (Fig. 1). The levels of RT and p24 produced by HIV-$1_{AD87(M)}$–infected cultures were proportional to the number of microglial cells present in the cultures (*22*). Cultures infected with other HIV-1 or HIV-2 strains did not produce measurable RT or p24 (*21*).

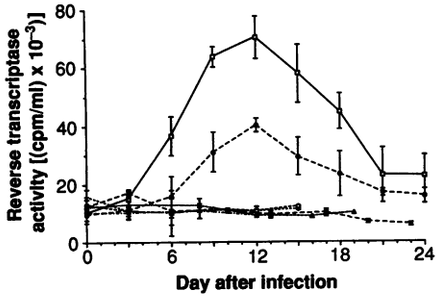

Fig. 1. Only HIV-$1_{AD87(M)}$ and HIV-1_{BaL} gave rise to productive infections in cultures of adult human brain. Peak levels of RT activity were seen at 12 to 17 days PI. These were 7.04×10^4 cpm/ml for HIV-$1_{AD87(M)}$ (□) and 3.6×10^4 cpm/ml for HIV-1_{BaL} (●). All other strains of virus failed to produce RT activity above mock-infected control or background levels ($9.9 \times 10^3 \pm 3.6 \times 10^3$ cpm/ml). (▲) HIV-$1_{LAV\text{-}03}$, (+) HIV-$1_{HTLV\text{-}IIIB}$, (○) HIV-1_{A1638}, (·) HIV-$2_{sbl/isy}$, and (X) HIV-2_{B59}. In addition, p24 core antigen assays (*21*) were performed on these supernatants and showed peak p24 concentrations of 104.9 ng/ml for HIV-$1_{AD87(M)}$ and 40.8 ng/ml for HIV-1_{BaL}. RT levels of supernatants from cultures infected with HIV-$1_{AD87(M)}$ were similar whether the inoculating virus had been grown only in macrophages or grown in PBL after 12 passages in macrophages; the results shown here are all from infection with HIV-$1_{AD87(M)}$ grown in PBL. Cultures infected with HIV-$1_{AD87(M)}$ produced by infected microglia also gave similar RT levels. Tissue obtained from partial temporal lobe resection for intractable epilepsy was disaggregated, as previously described (*11*). Cells were plated (in 100-μl drops for 2 hours) onto poly-D-lysine–coated 35-mm dishes, then refed and maintained in DMEM plus 10% GCT and 5% FBS (*18*). By 14 days in vitro, cultures were clear of myelin debris and contained ~5×10^3 LDL-R^+ (*12*) microglial cells, 1×10^3 $GFAP^+$ (*16*) astrocytes (staining method described in Fig. 2), and 1×10^3 fibronectin-positive flat cells (*17*). No staining for factor VIII–related antigen (*32*), a marker for endothelial cells, was observed. At 14 days in vitro, cultures were inoculated with HIV overnight at 37°C by incubation with viral samples containing 1×10^5 to 2×10^5 cpm/ml of RT activity for all strains except HIV-1_{A1638} (1×10^4 cpm/ml of inoculum). Supernatants were collected at 3- to 4-day intervals, stored at −70°C, and assayed for RT activity with [α-^{32}P]thymidine triphosphate (*20*). Experiments were performed seven times with HIV-$1_{AD87(M)}$, three times with HIV-1_{BaL} and twice with the other strains, each time with cultures from a different biopsy. Values given are the mean of three or more duplicates in a single experiment; error bars are the standard deviation.

To determine which of the different cell types present in these cultures were expressing viral antigens, we used a triple-color labeling procedure at different time points after infection. This permitted the identification of (i) microglial cells by labeling with Dil-LDL (1,1′-dioctadecyl-1-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate–conjugated low-density lipoprotein) (*12*) (red fluorescence, Fig. 2); (ii) astrocytes by labeling with antibodies to GFAP (anti-GFAP) (*16*) (blue fluorescence, Fig. 2); and (iii) intracellular HIV-1 antigens by labeling with antibodies to p17 *gag* antigens (*23*) (green fluorescence, Fig. 2A). The HIV *gag* gene products p17 and p24 could both be identified in these cultures by immunostaining; anti-p17 was used routinely to detect HIV-1 expression. None of the cells in our cultures expressed *gag* antigens after inoculation with strains of HIV-1 other than HIV-$1_{AD87(M)}$ and HIV-1_{BaL}. Triple staining on parallel control cultures clearly shows the absence of green fluorescence for p17 and the presence of higher numbers of LDL-R^+ microglial cells in the absence of viral infection (Fig. 2B). All $p17^+$ cells were also LDL-R^+, identifying them as microglial cells (Fig. 2A); in no case did we find staining for p17 in $GFAP^+$ astrocytes in primary glial cell cultures infected with HIV-1 (Fig. 2A) or HIV-2 (*21*). We concluded from these experiments that microglial cells were the only cells in these cultures synthesizing significant levels of viral proteins.

The progression of infection of microglial cells in cultures inoculated with either HIV-$1_{AD87(M)}$ or HIV-1_{BaL} was examined in detail. Microglial cells in mock-infected cultures, in which they often resemble activated microglial cells (*24*), stayed scattered or formed small groups (two to five cells) during the experiment. In contrast, microglial cells in infected cultures formed progressively larger clusters (up to 20 cells) from 1 week PI and fused to form multinucleated cells. Such microglial cell clustering and

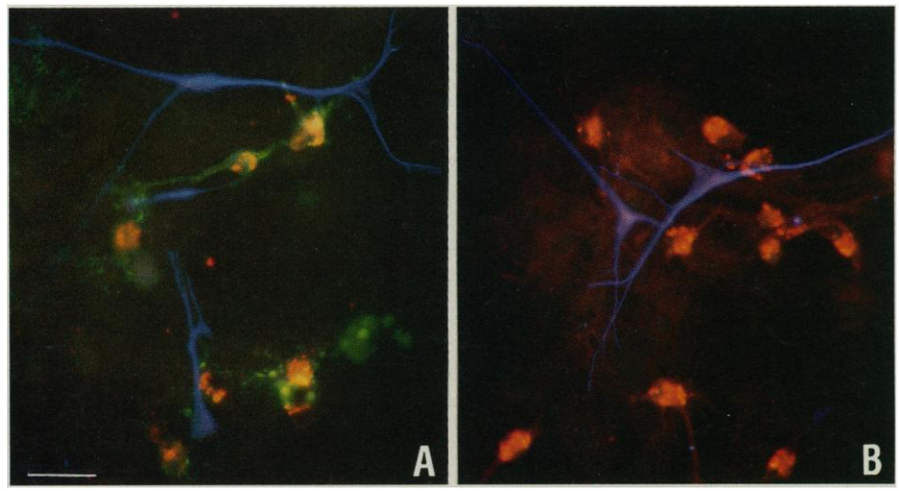

Fig. 2. Triple immunofluorescence labeling of adult human brain cultures for LDL-R (red), GFAP (blue), and HIV-1 p17 (green); triple exposure: (**A**) HIV-$1_{AD87(M)}$–infected culture at 10 days PI; only LDL-R^+ microglial cells were labeled with anti-p17, which stained cytoplasmic aggregates, while $GFAP^+$ astrocytes were not labeled. Infected cultures contained fewer LDL-R^+ microglial cells than mock-infected cultures. (**B**) Mock-infected culture at 10 days PI; no labeling with anti-p17 was seen in any cell. Scale bar, 50 μm. Cultures of adult human brain were grown and infected as described in Fig. 1. Live cells were labeled with Dil-LDL, which binds to the LDL-R (*12*) on microglial cells, fixed with 2% paraformaldehyde, and permeabilized with Triton X-100 (0.05%). Cultures were then stained with rabbit antiserum to GFAP (to identify astrocytes), and visualized with donkey antiserum to rabbit immunoglobulins (Igs) conjugated to biotin followed by streptavidin-AMCA (*16*). HIV antigens were identified by staining with mouse monoclonal anti–HIV-1 p17 antibody (*23*), and visualized with goal antiserum to mouse IgG conjugated to fluorescein. Preparations were examined and photographed on a Zeiss Axiophot fluorescence microscope equipped with appropriate fluorescence filters. This staining was performed on more than 50 HIV-$1_{AD87(M)}$–infected cultures from seven different biopsies at different times PI; the same pattern of staining was seen in cells infected with HIV-1_{BaL}.

fusion was characteristic of the progression of HIV-1 infection in normal brain cultures and was especially striking in cultures enriched in microglial cells derived from a malignant glioma (Fig. 3, A to L). To correlate the cytopathic changes seen in microglial cells with viral antigen expression, we examined preparations that had been double-labeled with Dil-LDL and anti-p17 at 3, 9, 15, and 21 days PI. At 3 days PI, only a few LDL-R^+ cells had a dotted pattern of p17 cytoplasmic immunofluorescence (Fig. 3, B and C). The percentage of cells expressing p17 increased during the next week (15 to 20% at 6 to 9 days PI to 60 to 70% at 15 days PI) and was similar for both HIV-$1_{AD87(M)}$ and HIV-1_{BaL}; p17 protein became more diffuse in the cytoplasm and was also associated with the plasma membrane in clusters of microglial cells, multi-nucleated giant cells, and individual mononucleated LDL-R^+ cells (Fig. 3, D to L). At 15 to 21 days PI, the multinucleated giant cells lysed, and the majority (60 to 70%) of remaining microglial cells were labeled with anti-p17 (Fig. 3, K and L). Electron microscopy (EM) showed virions budding only from the cell surface in HIV-$1_{AD87(M)}$–infected cells as observed in infected cells in AIDS patients' brains (*9*), and clusters of mature virions in the extracellular space (Fig. 3M). The cells with budding virions were identified as cells of the monocyte-macrophage lineage by EM immunogold techniques with monoclonal antibodies Leu M3 and Leu M5 (*13*).

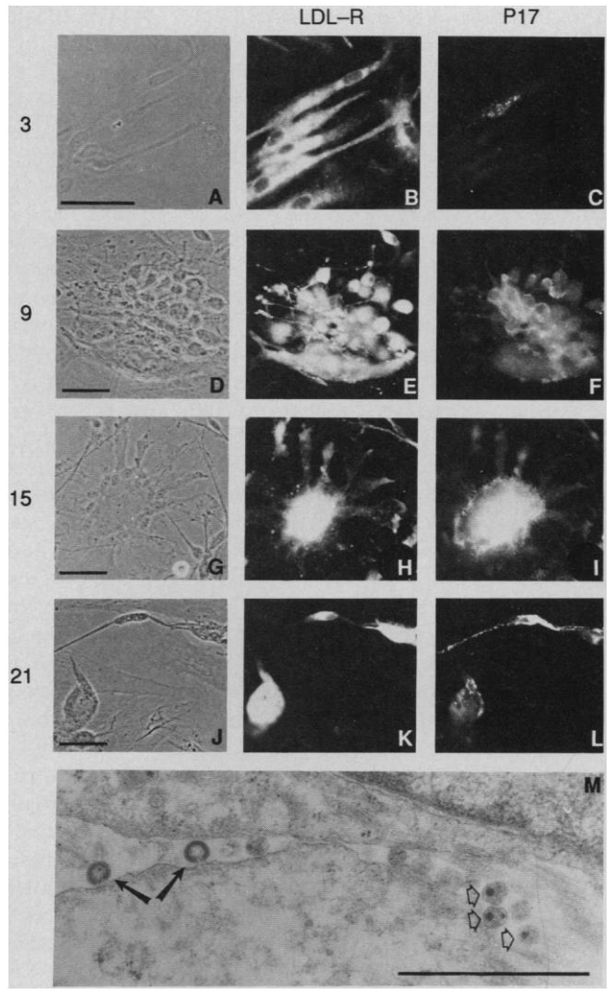

Fig. 3. Sequential analysis of cytopathic changes, viral expression, and budding in enriched microglial cell cultures infected with HIV-$1_{AD87(M)}$. (A, D, G, and J) Phase contrast; scale bars, 50 μm; (B, E, H, and K) LDL-R labeling; (C, F, I, and L) anti–HIV-1 p17*gag* (P17) immunostaining; (M) electron micrograph; scale bar, 1.0 μm. (**A** to **C**) Three days PI; culture was composed of evenly distributed LDL-R^+ microglial cells; <1% of these cells were labeled with antibodies to p17. (**D** to **F**) Nine days PI; microglial cells have formed clusters as well as some multinucleated giant cells; 16.7% of the microglial cells were labeled with antibodies to p17. (**G** to **I**) Fifteen days PI; LDL-R^+ multinucleated giant cells (H) and clusters of microglial cells were more evident than at 9 days PI; 66.0% of LDL-R^+ microglial cells were labeled with anti-p17 (I). (**J** to **L**) Twenty-one days PI; many fewer microglial cells were present in infected cultures than in mock-infected control cultures; 71.3% of LDL-R^+ microglial cells were also $p17^+$. (**M**) Electron micrograph of HIV-$1_{AD87(M)}$–infected microglial cell; immature particles are seen budding from the cell's plasma membrane (closed arrows); mature particles, seen in the extracellular space, have characteristic asymmetric cylindrical cores (open arrows). Glioma biopsies were disaggregated with collagenase (Sigma type 1A, 1%, 1 to 1.5 hours), followed by trypsin (0.25% for 20 min), then trypsin inhibitor (0.1%) and deoxyribonuclease (DNase) (0.05%) for 5 min. Glioma cultures were enriched for microglia by plating cells on plastic dishes for 1 hour at 37°C, and then washed to remove nonadherent cells. These cultures were inoculated with HIV-$1_{AD87(M)}$ after 7 days in vitro (legend to Fig. 1). At four time points PI, cultures were stained with Dil-LDL and anti-p17 and examined as described in Fig. 2. No labeling of mock-infected cells with anti-p17 was seen at any time point. Two other sequential analysis of microglial cells–enriched cultures from the same glioma infected with HIV-$1_{AD87(M)}$ gave similar results. For EM, cells were fixed at 19 days PI, first in a mixture of 1.25% glutaraldehyde and 1% paraformaldehyde in 0.08 M cacodylate buffer, and then 60 min in 4% glutaraldehyde in the same buffer. Cells were scraped from dishes, pelleted, fixed in osmium tetroxide, uranyl-stained, and embedded in epon before thin sectioning and photographing on a Phillips EM 400-T.

A dramatic decrease in the number of microglial cells was seen in the 3 weeks after infection (compare Fig. 3B with 3K), whereas microglial cells survived in control (mock-infected) cultures. In cultures infected with HIV-$1_{AD87(M)}$ or HIV-1_{BaL}, 80% of LDL-R^+ microglial cells died 15 days after infection (Fig. 4), while only a minimal loss of LDL-R^+ microglial cells occurred in mock-infected cultures (3% loss at 19 days PI, 33% loss at 24 days PI). The most pronounced loss of infected microglial cells occurred between 6 and 12 days PI, as supernatant RT levels increased to their maximum (Fig. 1). The rate and degree of the microglial cell loss was approximately the same with both HIV-$1_{AD87(M)}$ and HIV-1_{BaL}, even though cultures infected with HIV-$1_{AD87(M)}$ produced twice the level of RT activity in the cell-free supernatant (Fig. 1). Parallel experiments on macrophages derived from blood monocytes showed no loss of cells in cultures infected by HIV-$1_{AD87(M)}$ or HIV-1_{BaL} (*25*), suggesting that the cytopathic effect of the virus is specific for microglia among cells of the monocyte-macrophage lineage. The remaining 10 to 15% of LDL-R^+ microglial cells present at 3 weeks PI seemed to be resistant to the cytopathic effects of the virus, and most of them expressed p17 (Fig. 3L). These cells may be persistently infected with HIV-1.

Thus, microglial cells isolated from adult human brain are especially susceptible to macrophage-adapted strains of HIV-1, which concurs with the observation that most brain isolates of HIV-1 replicate best in monocyte-macrophages in vitro (*1, 26*). In contrast, both HIV-2 strains, which are able to grow in monocyte-macrophages (*19*), did not replicate significantly in human glial cell cultures, suggesting that macrophage-tropic HIV-2 strains may less readily infect brain microglial cells than macrophage-tropic HIV-1 strains. Two HIV-1 (HIV-$1_{HTLV-IIIB}$ and HIV-1_{LAV}) strains that did not replicate in microglial cells have, however, been adapted to transformed T lymphocyte cell lines and may have therefore been selected to grow preferentially in T lymphocytes. Since passage history may influence the success of the infection of brain microglial cells, we inoculated human brain cultures with two primary isolates from asymptomatic seropositive patients (HIV-1_{A1638} and HIV-2_{B59}), each of which had been passaged only once in either peripheral blood lymphocytes (PBLs) or monocyte-macrophages. None of these viruses replicat-

ed to detectable levels in our glial cell cultures. In the case of HIV-1_{A1638}, this could have been due to the lower RT activity of the viral inoculum (legend to Fig. 1); however, productive infection was observed with an inoculum of HIV-$1_{AD87(M)}$ with the same RT activity. These observations suggest that HIV isolates from the PBLs of asymptomatic patients may require several cycles of replication in monocyte-macrophages before they can productively infect microglial cells. It is possible that the strains of HIV that did not actively replicate in human glial cell cultures may have infected the cells and been reverse-transcribed, but that viral expression and replication did not occur. Similarly, astrocytes or other glial cell types could be latently infected in vitro by some strains of HIV as has been described in some glioma cell lines and in fetal nerve cells (*27*).

Microglial cells are derived from bone marrow precursors that have migrated into the brain during development, where they differentiate and remain thereafter undergoing slow turnover (*15*). These bone marrow precursors also give rise to circulating monocytes and tissue macrophages, but despite their common origins, microglial cells and monocyte-macrophages react differently

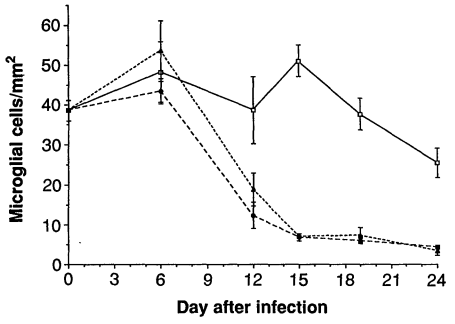

Fig. 4. Loss of microglial cells in HIV-$1_{AD87(M)}$– and HIV-1_{BaL}–infected cultures. The number of microglial cells present in HIV-$1_{AD87(M)}$ (●), HIV-1_{BaL} (▲), and mock-infected (□) cultures showed no significant change during the first 6 days PI. A highly significant decrease occurred between 6 and 12 days PI in cultures infected with HIV-$1_{AD87(M)}$ and HIV-1_{BaL}, but not in mock-infected control cultures or in cultures infected with other strains of HIV-1 and HIV-2. The number of LDL-R^+ microglial cells present in these cultures showed a small further decrease after 15 days PI. No significant change in the number of $GFAP^+$ astrocytes was observed in any of these cultures. Cultures of human brain cells were prepared and infected as described in Fig. 1. At five time points after infection (6, 12, 15, 19, and 24 days PI), cultures were triple-stained with anti-GFAP, Dil-LDL, and anti–HIV-1 p17 antibodies (legend to Fig. 2). The number of LDL-R^+ microglia present in each culture was counted using a Zeiss Axiophot fluorescence microscope. Values are the mean of three dishes, error bars are the standard deviation. Similar results were obtained in four experiments with cultures from different biopsies.

to HIV. In infections of microglial cells with HIV-$1_{AD87(M)}$ or HIV-1_{BaL}, virus production and cell death proceed with a pace and severity resembling the acute lytic infection seen in T4 lymphocytes with RT activity accumulating in the cell-free supernatant peaks as microglial cell death increases (at 12 days PI) and declining afterwards. In contrast, infections of monocytes and macrophages with HIV-$1_{AD87(M)}$ or HIV-1_{BaL} progress more slowly and occur in the absence of substntial cell death (*1, 25*). Moreover, microglial cells show virus budding and release at the cell surface, whereas the site of virus production in macrophage appears restricted to intracellular vacuoles (*28*). Both monocyte-macrophages and brain microglial cells have the intriguing ability to sustain HIV replication and expression in the absence of cell mitosis.

The progressive clustering and fusion of infected microglial cells observed in our cultures mimics the two pathological hallmarks of AIDS infection in the brain: the microglial nodules and the multinucleated giant cell formation. Such events may be a prelude to microglial cell death in vivo as well as in vitro. In addition, a fraction of the brain microglial cells may become persistently infected and constitute a reservoir of HIV infection. Acute and chronic HIV infection of microglial cells may have various consequences for brain function, since these cells normally assume immune functions within the CNS (*24, 29*). Microglial cells can express class II major histocompatibility complex (MHC) molecules, in response to γ-interferon or other stimuli, and present antigen to T lymphocytes in an MHC-restricted fashion (*29*). Therefore, their destruction may well result in altered immune-mediated reaction to HIV-1 and other pathogens in AIDS patients' brains. In addition, microglial cells infected with HIV-1 may release a number of compounds, which could have deleterious effects on nerve cells. Among these are tumor necrosis factor, which has been shown to cause oligodendrocyte cell death and demyelination in vitro (*30*), and could cause demyelination in vivo. Death of microglial cells may release reactive oxygen intermediates (for example, free radicals) in the CNS parenchyma, which could impair nerve cell functions such as synaptic transmission (*31*). Moreover, a nonproductive infection of astrocytes (*27*) could interfere with their multiple functions in the CNS without the virus entering a full replicative cycle. Thus HIV, a lymphotropic, monocyte-tropic, and neurotropic human retrovirus, may have more than one way to interfere with nervous system functions, just as it has with immune functions, and it is now possible to study the basis of HIV neurotropism with well-characterized in vitro systems of differentiated human CNS cells.

REFERENCES AND NOTES

1. S. Gartner *et al.*, *Science* **233**, 215 (1986); S. Gartner, P. Markovits, D. M. Markovitz, R. F. Betts, M. Popovic, *J. Am. Med. Assoc.* **256**, 2365 (1986); Y. Koyanagi *et al.*, *Science* **241**, 1673 (1988).
2. H. E. Gendelman *et al.*, *J. Exp. Med.* **167**, 1428 (1988); B. J. Potts, W. Maury, M. A. Martin, *Virology* **175**, 465 (1990).
3. D. H. Ho, T. R. Rota, M. S. Hirsch, *J. Clin. Invest.* **77**, 1712 (1986); C. D. Pauza, *Cell. Immunol.* **112**, 414 (1988); S. Roy and M. A. Wainberg, *J. Leukocyte Biol.* **43**, 91 (1988).
4. T. Tucker, *J. Neurol. Sci.* **89**, 119 (1989); R. T. Johnson, J. C. McArthur, O. Narayan, *FASEB J.* **2**, 2970 (1988).
5. C. K. Petito, E.-S. Cho, W. Lemann, B. A. Navia, R. W. Price, *J. Neuropathol. Exp. Neurol.* **45**, 635 (1986); B. A. Navia, E.-S. Cho, K. Petito, R. W. Price, *Ann. Neurol.* **19**, 525 (1986); S. M. de la Monte, D. D. Ho, M. S. Hirsch, E. P. Richardson, *Neurology* **37**, 562 (1987).
6. H. Budka, *Acta Neuropathol.* **69**, 253 (1986); D. J. Eilbott *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 3337 (1989).
7. R. Vazeux *et al.*, *Am. J. Pathol.* **126**, 403 (1987).
8. C. A. Wiley, R. D. Schrier, J. A. Nelson, P. W. Lampert, M. B. Oldstone, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7089 (1986); M. H. Stoler, T. A. Eskin, S. Benn, R. C. Angerer, L. M. Angerer, *J. Am. Med. Assoc.* **256**, 2360 (1986).
9. S. Koenig *et al.*, *Science* **233**, 1089 (1986).
10. S. U. Kim, G. Moretto, D. H. Shin, V. M. Lee, *J. Neurol. Sci.* **69**, 81 (1985); G. M. Hayes, M. N. Woodroofe, M. L. Cuzner, *J. Neuroimmunol.* **19**, 177 (1988); Y. Grenier, T. C. G. Ruijs, Y. Robitaille, A. Olivier, J. P. Antel, *ibid.* **21**, 103 (1989).
11. H. Dorn, R. Armstrong, C. V. Kufta, M. Dubois-Dalcq, *Ann. N.Y. Acad. Sci.*, in press. Preliminary experiments have shown no evidence of productive infection of human oligodendrocyte cultures by HIV-1.
12. Dil-LDL (1,1′-dioctadecyl-1-3,3,3′,3′-tetramethylindocarbocyanine perchlorate–conjugated LDL) was purchased from Biomedical Technologies Inc., Stoughton, MA [J. L. Goldstein, Y. K. Ho, S. K. Basu, M. S. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 333 (1979); R. E. Pitas, T. L. Innerarity, J. N. Weinstein, R. W. Mahley, *Arteriosclerosis* **1**, 177 (1981)].
13. Leu M3: A. Dimitriu-Bona, G. R. Burmester, S. J. Waters, R. J. Winchester, *J. Immunol.* **130**, 145 (1983). Leu M5: L. L. Lanier, M. A. Arnaout, R. Schwating, N. L. Warner, G. D. Ross, *Eur. J. Immunol.* **15**, 713 (1985). Only Leu $M3^+$ or Leu $M5^+$ cells were seen budding virus in immunogold-stained EM preparations.
14. M. E. Esiri and J. O. McGee, *J. Clin. Pathol.* **39**, 615 (1986).
15. V. H. Perry and S. Gordon, *Trends Neurosci.* **11**, 273 (1988).
16. Antibody to human GFAP purchased from Chemicon International, El Segundo, CA [M. Noppe, R. Crols, D. Andries, A. Lowenthal, *Clin. Chim. Acta* **155**, 143 (1986)]. Streptavidin conjugated to AMCA (7-amino-4-methylcoumarin-3-acetate) was purchased from Molecular Probes, Junction City, OR [N. Appel, M. Wessendorf, R. Elde, *Soc. Neurosci. Abstr.* **13**, 776 (1987)].
17. H. M. Geller and M. Dubois-Dalcq, *J. Cell Biol.* **107**, 1977 (1988).
18. GCT supernatant contains granulocyte-macrophage–colony-stimulating factor (GM-CSF), macrophage-CSF (M-CSF), and granulocyte-CSF (G-CSF) [C. L. Erickson-Miller, C. N. Abboud, J. K. Brennan, *Exp. Hematol.* **16**, 184 (1988)] and was a gift of N. Sarver at National Institute of Allergy and Infectious Diseases (NIAID), NIH. We determined optimal growth and survival conditions for microglial cells in various culture conditions. After 1 to 2

weeks, numbers were highest when Dulbecco's modified Eagle's medium (DMEM) was supplemented with 5% adult human serum, but long-term survival was better with 5% fetal bovine serum (FBS). We tested the effects of interleukin-3 and GM-CSF (AMGEN) and of highly purified recombinant human M-CSF, long and short clones [M. B. Ladner *et al.*, *EMBO J.* **6**, 2693 (1987); a gift of Cetus Corp., Emeryville, CA], all factors known to enhance the growth and survival of cells of the monocyte-macrophage lineage or microglial cells (D. Giulian and J. E. Ingeman, *J. Neurosci.* **8**, 4707 (1988)]. Addition of 500 to 1000 units of growth factor per milliliter of defined medium [P. A. Eccleston and D. H. Silberberg, *Dev. Brain Res.* **16**, 1 (1984)] or medium with 5% FBS was compared to addition of 10% GCT. None of these factors or GCT induced microglial cell division, since LDL-R^+ cells were not labeled by tritiated thymidine after a 24-hour labeling period, as determined by whole-cell autoradiography. Numbers of surviving microglial cells after 2 weeks were highest in medium containing 10% GCT and 5% FBS compared with all other factors tested either singly or in combinations of two.

19. Viral strains. (i) HIV-$1_{AD87(M)}$: HIV-1_{Ada} passaged 12 times in macrophages (*2*); stock was grown in PBLs. RT level of stock was 7×10^5 cpm/ml. (ii) HIV-1_{BaL}: Routinely passaged only in macrophages. A gift from S. Gartner in R. Gallo's laboratory, National Cancer Institute (NCI). RT level of stock was 8×10^5 cpm/ml. (iii) HIV-$1_{HTLV-IIIB}$: Original isolate [M. G. Sarngadharan, M. Popovic, L. Bruch, J. Schüpbach, R. C. Gallo, *Science* **224**, 506 (1984)], passaged in PBLs and H9 T cell line. RT level of stock was 8.0×10^5 cpm/ml. Infectivity was checked in H9 T cell line. (iv) HIV-1_{LAV}: Original isolate [F. Barré-Sinoussi *et al.*, *ibid.* **220**, 868 (1983)], passaged in PBLs and A-301 T cell line. RT level of stock was 1.5×10^6 cpm/ml. Infectivity checked in HeLa T4 cells, H9 T cell line, and PBLs. (v) HIV-1_{A1638}: Primary isolate from PBLs of an asymptomatic seropositive patient passaged once in macrophages or PBLs. A gift from A. Valentin and B. Asjo of Karolinska Institute, Stockholm. RT level of stock was 1.5×10^4 cpm/ml. (vi) HIV-2_{B59}: Primary isolate from PBL of an asymptomatic patient passaged once in macrophages or PBLs. A gift from A. Valentin and B. Asjo of Karolinska Institute, Stockholm. RT levels of stocks were 1×10^5 to 2×10^5 cpm/ml. (vii) HIV-$2_{sbl/isy}$: Molecularly cloned virus grown in Hut 78 cells; a gift from G. Franchini, NCI [G. Franchini *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2437 (1989); N. Hattori *et al.*, *ibid.*, in press].
20. R. L. Willey *et al.*, *J. Virol.* **62**, 139 (1988).
21. HIV-1 p24 assay kit was purchased from Du Pont Biotechnology Systems and used as described [Y. Koyanagi *et al.*, *Science* **236**, 819 (1987)]. Expression of *gag* antigens in cultures infected with HIV-2 was assessed by indirect immunofluorescence with antibodies for simian immunodeficiency virus (SIV-1) p24 cross-reacting with HIV-2 p24 (a gift from G. Franchini, NCI). Generally, no immunofluorescence staining was seen in HIV-2–infected cultures; however, some staining was seen in cytoplasmic inclusions of LDL^+ cells in one set of cultures infected with HIV-$2_{sbl/isy}$, but this was not accompanied by an increase in supernatant RT levels.
22. Estimated peak HIV production per microglial cell: Peak RT activity production in microglial cells infected with HIV-$1_{AD87(M)}$ varies from 16 to 54 cpm per cell in different experiments. When infected under similar conditions with HIV-$1_{AD87(M)}$, blood-derived monocyte-macrophages produced ~36 cpm RT activity per cell. Lower production of RT activity per cell (7 cpm) was observed in PBL cultures infected with HIV-$1_{AD87(M)}$, whereas HIV-1_{LAV} infection of these cells resulted in the production of ~41 cpm of RT activity per cell. The level of HIV production by microglial cells is similar to the levels produced by macrophages infected with similar strains in our experiments, as well as those in previous publications (*1, 2*).
23. Anti–HIV-1 p17 antibody was a gift from F. Veronese, NCI, and Bionetics Research, Inc., Rockville, MD [F. D. Veronese, T. D. Copeland, S. Oroszlan, R. C. Gallo, M. G. Sarngadharan, *J. Virol.* **62**, 795 (1988)].
24. J. Streit *et al.*, *Glia* **1**, 301 (1988).
25. No significant cell loss associated with HIV infection was observed in monocyte-macrophage cultures inoculated and maintained as described in Fig. 1. At 21 days PI, mock-infected macrophage cultures contained 26 ± 11 macrophages per square millimeter; HIV-$1_{AD87(M)}$–infected cultures contained 27 ± 10 macrophages per square millimeter; and HIV-1_{BaL}–infected cultures contained 28 ± 8 macrophages square millimeter (compare with Fig. 4).
26. M. Popovic, W. Mellert, V. Erfle, S. Gartner, *Ann. Neurol.* **23**, S74 (1988); C. Cheng-Mayer, C. Weiss, D. Seto, J. A. Levy, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 8575 (1989).
27. F. Chiodi, S. Fuerstenberg, M. Gidlund, B. Asjo, E. M. Fenyo, *J. Virol.* **61**, 1244 (1987); C. Kunsch and B. Wigdahl, *Virology* **173**, 715 (1989).
28. J. M. Orenstein, M. S. Meltzer, T. Phipps, H. E. Gendelman, *J. Virol.* **62**, 2578 (1988).
29. K. Frei, C. Siepl, P. Groscurth, S. Bodmer, A. Fontana, *Ann. N.Y. Acad. Sci.* **540**, 218 (1988).
30. D. S. Robbins *et al.*, *J. Immunol.* **139**, 2593 (1987); K. W. Selmaj and C. S. Raine, *Ann. Neurol.* **23**, 339 (1988).
31. C. Colton and D. Gilbert, *FEBS Lett.* **223**, 284 (1987); C. A. Colton, J. S. Colton, D. L. Gilbert, *J. Free Radicals Biol. Med.* **2**, 141 (1986).
32. R. D. McComb, T. R. Jones, S. V. Pizzo, D. D. Bigner, *J. Neuropathol. Exp. Neurol.* **41**, 479 (1982).
33. We thank M. Martin, NIAID, for advice; G. Franchini and F. Di Veronese in R. Gallo's laboratory, NCI; B. Asjo and A. Valentin in E. Norrby's laboratory, Karolinska Institute, Stockholm; N. Sarver, Division of AIDS, NIAID; and C. Jordan, Laboratory of Viral and Molecular Pathogenesis, National Institute of Neurological Disorders and Stroke, for providing viruses, cells, reagents, and advice throughout this study. We would also like to thank R. Rusten, Jr., for his photographic work and ultramicrotomy and P. Ballew for help with the manuscript.

1 February 1990; accepted 25 April 1990

Protein Tyrosine Phosphatase Activity of an Essential Virulence Determinant in *Yersinia*

KUNLIANG GUAN AND JACK E. DIXON*

***Yersinia* is the genus of bacteria that is the causative agent in plague or the black death, and on several occasions this organism has killed a significant portion of the world's population. An essential virulence determinant of *Yersinia* was shown to be a protein tyrosine phosphatase. The recombinant 50-kilodalton *Yersinia* phosphatase had a specificity for removal of phosphate from Tyr-containing as opposed to Ser/Thr-containing phosphopeptides and proteins. Site-directed mutagenesis was used to show that the *Yersinia* phosphatase possesses an essential Cys residue required for catalysis. Amino acids surrounding an essential Cys residue are highly conserved, as are other amino acids in the *Yersinia* and mammalian protein tyrosine phosphatases, suggesting that they use a common catalytic mechanism.**

PROTEIN TYROSINE PHOSPHORYLATION is an early event in the signal transduction pathway used by several receptors involved in mediating cellular proliferation and regulation. Tyrosine phosphorylation can also lead to unrestrained cell growth, and a number of tyrosine kinases appear to function as oncogenes (*1*). Tyrosine phosphorylation has been shown to be a key factor in the regulation of the cell cycle (*2*). The levels of protein tyrosine phosphorylation appear to be modulated within the cell by both tyrosine kinases and protein tyrosine phosphatases (PTPases) (*3*). Evidence indicates that PTPases constitute a family of enzymes that most likely function in regulating the extent and duration of tyrosine phosphorylation within the cell (*3*). Tonks *et al.* (*4*) reported the purification of a 35-kD protein tyrosine phosphatase (PTPase 1B) from human placenta, and Charbonneau *et al.* (*5*) showed that this phosphatase shared significant amino acid sequence homology with the cytoplasmic domain of the leukocyte cell surface glycoprotein, CD45. CD45 was subsequently shown to have tyrosine phosphatase activity (*6*). Streuli *et al.* (*7*) have cloned another member of the family called LAR (leukocyte antigen–related protein), which is a tyrosine phosphatase structurally related to CD45.

Work from our laboratory suggests that there are additional members of this family of PTPases that resemble CD45 and LAR (*8*). In our initial screening of a rat brain cDNA library, we isolated several PTPase clones. We have found the cloning, expression, and localization of a PTPase cDNA from rat brain that encodes a protein having 97% identity with the protein sequence reported by Charbonneau *et al.* (*8, 9*). We have referred to the protein as PTP 1. Cool *et al.* (*10*) have cloned a structurally related PTPase from a human T cell cDNA library. Another clone from our cDNA library has been designated as PTP 18 (*11*). The longest PTP 18 cDNA, which is approximately 5.6 kb in length, encodes an enzyme having an

Department of Biochemistry, and Walther Cancer Institute, Purdue University, West Lafayette, IN 47907.

*To whom correspondence should be addressed.