JOURNAL OF VIROLOGY, Apr. 1987, p. 1244–1247
0022-538X/87/041244-04$02.00/0

Vol. 61, No. 4

Infection of Brain-Derived Cells with the Human Immunodeficiency Virus

F. CHIODI,[1]* S. FUERSTENBERG,[2] M. GIDLUND,[2] B. ÅSJÖ,[1] AND E. M. FENYÖ[1]

Department of Virology,[1] and Department of Immunology,[2] Karolinska Institutet, Stockholm, Sweden

Received 9 September 1986/Accepted 2 December 1986

A malignant glioma cell line was infected with the human T-lymphotropic virus type IIIB isolate of the human immunodeficiency virus. Infection appeared to be latent rather than productive. Through contact with monocytic or lymphoid cells, the virus present in the glioma cells could be transmitted and gave rise to a fully productive infection.

The human immunodeficiency virus (HIV) (2) is morphologically and genomically related to lentiviruses (6), a subfamily of retroviruses. Like lentiviruses, it has a pronounced cytopathic effect in vitro and generates persistent infection in vivo. The prototype lentivirus visnavirus is responsible for a chronic central nervous system disease in sheep (7, 9), whereas the caprine arthritis encephalitis virus induces chronic, progressive leukoencephalomyelitis in young goats (3). In humans, HIV is etiologically linked to the acquired immunodeficiency syndrome (AIDS). AIDS is frequently accompanied by the AIDS dementia complex (10), which is characterized by paraparesis, ataxia, and progressive dementia in adults and loss of milestones in children (10, 11, 14, 17).

The deterioration of superior cognitive functions observed in patients with AIDS strongly suggests that the virus has a direct action on brain cells. To study the target cell of HIV in the brain, we attempted to infect three human malignant glioma (MG) lines that were originally derived from adult males. Cell lines 373-MG and 489-MG (19) were derived from temporal astrocytic gliomas that were histologically classified as grade IV. The cell line 138-MG (16) was derived from a frontal astrocytic glioma and contained a large number of oligodendroglia cells. The tumor was classified histologically as grade III. Figure 1 shows the morphology of culture lines. Cell lines 373-MG (Fig. 1, lane a) and 138-MG (Fig. 1, lane c) had the basic astrocytoid shape, showed nuclear polymorphism, and were occasionally multinuclear (16, 19). Cells in the 489-MG cultures had the basic bipolar shape and varied in amount of cytoplasm (19). None of the glioma lines expressed the T4 antigen when tested by indirect membrane immunofluorescence with the Fab fragment (13) of an anti-T4 monoclonal antibody (received from Ellis Reinherz) and fluorescein isothiocyanate-labeled rabbit anti-mouse immunoglobulin G (IgG; Dakopatts, Glostrup, Denmark).

Because differences in the biological properties of several HIV isolates have been observed (1), the three glioma lines were inoculated with two different virus strains (kindly provided by R. C. Gallo, National Institutes of Health, Bethesda, Md.): human T-lymphotropic virus type IIIB (HTLV-IIIB), obtained from two patients, one with AIDS and one with AIDS-related complex, and RF-I, derived from a patient with AIDS. Infection was carried out both with free virus (Table 1) and by cocultivation of glioma cells with virus-infected peripheral blood mononuclear cells (PBMC; data not shown). In the cell-free transmission experiment, 1 ml of HTLV-IIIB or RF-I (reverse transcriptase activity, 96×10^3 and 140×10^3 cpm/ml, respectively) was added directly to the monolayer of glioma cells and incubated for 1 h at 37°C. Virus-infected cultures did not show any cytopathic changes during the 6-month observation period, although the infected cells initially grew more slowly than did the uninfected cells. Table 1 shows that during the course of the entire experiment, none of the infected glioma cultures released a detectable amount of virus into the supernatant fluid.

In the cell-free transmission experiment, the HTLV-IIIB-infected 138-MG line exhibited, transiently, bright eccentric fluorescence in 20% of the cells when stained with mouse monoclonal antibodies directed against the viral core proteins p15 and p24 (gift of R. C. Gallo; Table 1). Uninfected cells and cells from the PBMC cocultivation experiment were negative. This suggests that the 138-MG line became infected with HTLV-IIIB. The infection may have been latent or productive in a few cells only and, thereby, escaped detection.

The HTLV-IIIB-infected 138-MG line was therefore cocultivated with cells susceptible for infection with HIV. We used PBMC and a monocytic cell line U_{937} clone 16. The U_{937} cell line was derived from a patient with generalized histiocytic lymphoma (18). It grows as a suspension culture, and its monocytoid properties have been documented (18). One of the clonal lines, clone 16, used in these cocultivation experiments was positive (95% of the cells) for the OKT_4 antigen in indirect membrane immunofluorescence and was particularly sensitive to HTLV-IIIB infection (B. Åsjö, I. Ivhed, M. Gidlund, S. Fuerstenberg, E. M. Fenyö, K. Nilsson, and H. Wigzeu, Virology, in press). Cocultivation was initiated 2, 3, and 4 months after HTLV-IIIB infection of glioma cell lines. In three experiments (of four) virus could be demonstrated in the HTLV-IIIB-infected 138-MG lines.

The results of one such experiment are shown in Fig. 2. Among all the infected MG lines, only the cocultures of the two HTLV-IIIB-infected 138-MG lines became virus positive, as determined by reverse transcriptase activity and immunofluorescence. The results indicate that the HTLV-IIIB-infected 138-MG lines transmit viral infection to sensitive target cells. It is conceivable that reactivation of HTLV-

* Corresponding author.

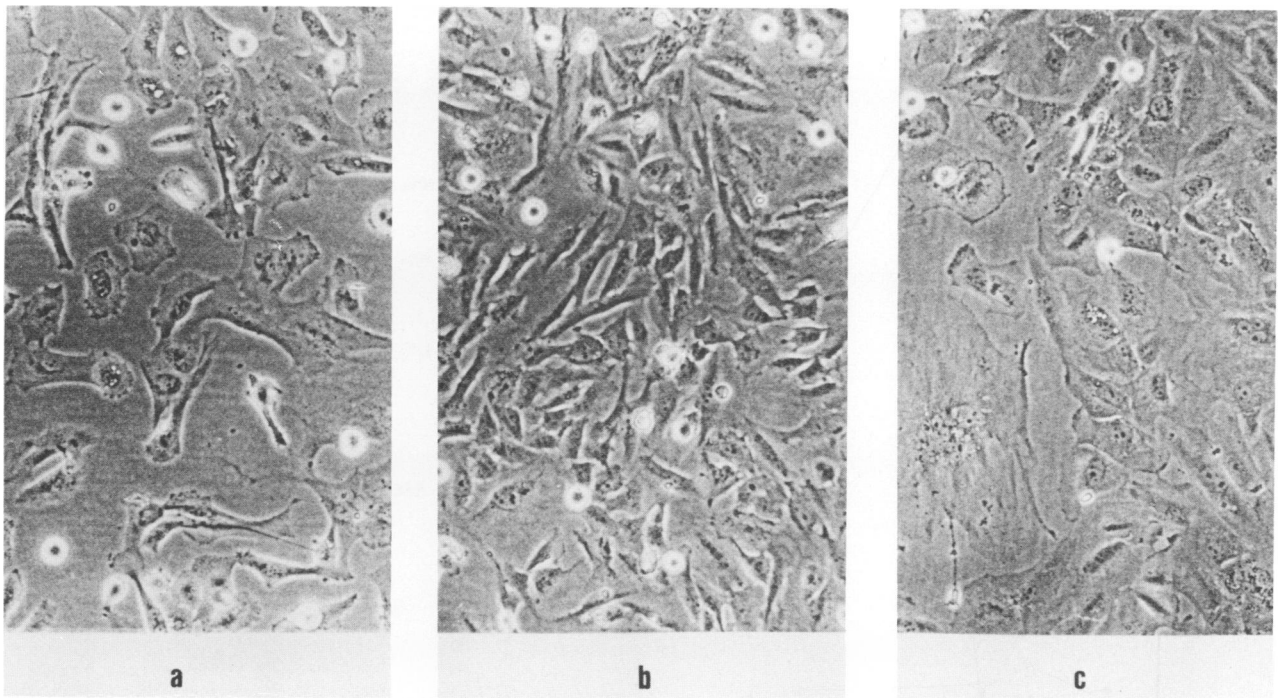

FIG. 1. Photomicrographs of cell lines 138-MG (lane c), 373-MG (lane a), 489-MG (lane b). The MG lines (kindly provided by J. Pontén, Department of Pathology, University of Uppsala, Uppsala, Sweden) were grown in Eagle minimum essential medium with 10% fetal calf serum. The cultures were trypsinized and split 1:3 or 1:5 twice a week.

IIIB in the glioma cells may occur spontaneously or through the contact with monocytic or T-lymphoid cells. Alternatively, the small amount of virus produced by a few glioma cells spreads in cultures of sensitive target cells.

The virus obtained from the cocultivation experiment was designated 138-MG/HTLV-III-B and was compared with the original infecting HTLV-IIIB type virus in a radioimmunoprecipitation assay. U_{937} clone 16 cells were infected with the cell-free supernatant fluid obtained from cocultures at day 20 (Fig. 2), and HTLV-IIIB was used as a control. Cells were metabolically labeled with [^{35}S]cysteine 7 days later, and the antigen lysate was immunoprecipitated with the serum of a patient with lymphadenopathy which was known to react with several viral proteins. Figure 3 shows that 138-MG/HTLV-IIIB and HTLV-IIIB give identical bands (cf. lanes d and e), suggesting that passage through glioma cells did not alter the protein pattern of the infectious agent, as measured by the radioimmunoprecipitation assay.

The T4 marker, mostly present on the T-helper cells or a region closely associated with this surface structure, has been described to act as a receptor for HIV (4, 8). The

TABLE 1. Virus expression in glioma lines infected with two strains of HIV

Cells	Virus strain	Virus expression on the following days after infection[a]				
		8 (RT activity [cpm][b])	12		26	
			RT activity (cpm)	Antigen-positive cells (%)[c]	RT activity (cpm)	Antigen-positive cells (%)
138-MG	HTLV-IIIB	–[d]	–	2	–	20
	RF-I	–	–	–	–	–
489-MG	HTLV-IIIB	–	–	–	–	–
	RF-I	–	–	–	–	–
373-MG	HTLV-IIIB	–	–	–	–	–
	RF-I	–	–	–	–	–
Peripheral blood mononuclear cells	HTLV-IIIB	26×10^3	72×10^3			
	RF-I	21×10^3	24×10^3			

[a] On days 55 and 110 after infection there was no reverse transcriptase activity and there were no antigen-positive cells.

[b] RT, Reverse transcriptase. The enzyme activity was measured in 0.3% Triton X-100-disrupted, high-speed pellets of supernatant culture fluids, as described previously (1).

[c] Indirect immunofluorescence on fixed cells (acetone-methanol; 1:1) with monoclonal antibodies against the viral core proteins p24 and p15 and fluorescein isothiocyanate-labeled rabbit anti-mouse IgG (Dakopatts).

[d] –, None.

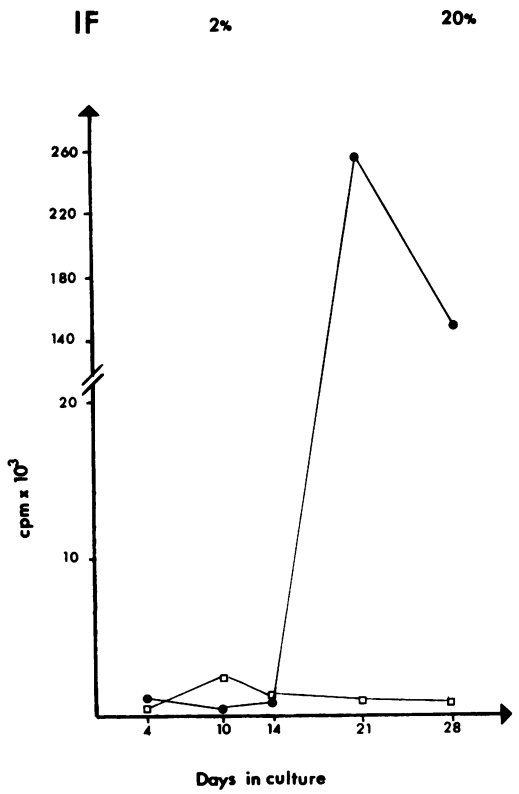

FIG. 2. Reverse transcriptase activity in cocultures of HTLV-IIIB-infected 138-MG (●) and 373-MG (□) lines. A total of 1×10^6 glioma cells that adhered to the bottom of tissue culture bottles were cocultivated with 3×10^6 U_{937} clone 16 cells in RPMI 1640 medium with 10% fetal calf serum and 2 μg of polybrene per ml. The U_{937} cells were subcultivated at a ratio of 1:8 twice a week in the medium described above. IF, Immunofluorescence (see Table 1, footnote *c*).

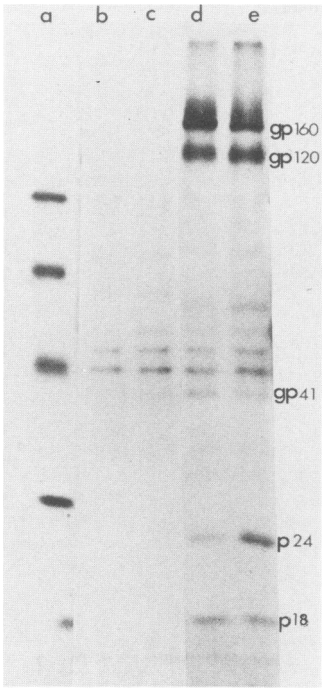

FIG. 3. Comparison of HTLV-IIIB and 138-MG/HTLV-IIIB antigen radioimmunoprecipitation with serum from a patient with persistent generalized lymphadenopathy. U_{937} clone 16 cells were infected with virus and were labeled with [^{35}S]cysteine 7 days later, as described previously (F. Chiodi, U. Bredberg-Råden, G. Biberfeld, B. Böttiger, J. Albert, B. Åsjö, E. M. Fenyö, and E. Norrby, submitted for publication). Fractions of 10 μl of clarified cell lysates were precipitated overnight with 5 μl of serum, and the immunocomplexes were bound to protein A-Sepharose beads and analyzed by polyacrylamide gel electrophoresis on 9 to 16% acrylamide gradient gels. Viral proteins gp160, gp120, gp41, p24, and p18 were immunoprecipitated from HTLV-IIIB and 138-MG/HTLV-IIIB cells. Lane b, HTLV-IIIB antigen precipitated with normal serum; lane c, 138-MG/HTLV-IIIB antigen precipitated with normal serum; lane d, HTLV-IIIB antigen precipitated with the serum of a patient with persistent generalized lymphadenopathy; lane e, 138-MG/HTLV-IIIB antigen precipitated with the serum of a patient with persistent generalized lymphadenopathy; lane a, ^{14}C-labeled molecular weight markers (phosphorylase *b*, 92,000; bovine serum albumin, 69,000; ovalbumin, 46,000; carbonic anhydrase, 30,000; lactoglobulin A, 18,367).

remarkable observation is that the 138-MG line, which apparently lacks the T4 marker, could be infected. Brain cells of mice have been described to contain cell surface structures that are similar to those of thymus cells. In fact, antisera reacting with thymus cells could be produced by immunization of mice with brain cells (12). Similarly, human glioma cells may contain at least a partial structure of the T4 molecule that is present on T-helper lymphocytes (8). This structure may not be detected by the particular monoclonal antibody used in this study. Alternatively, the virus may use different surface structures as its receptor.

In summary, it seems that HTLV-IIIB can latently infect brain-derived cells. Similar to other latent virus infections of the brain (9, 15), the presence of virus can be demonstrated by cocultivation with sensitive target cells. This may also explain the presence of HIV particles in multinucleated giant cells resembling macrophages in brain sections of patients with AIDS encephalopathy (5). No detectable viral expression occurs in the brain cells themselves; but through contact with monocytic or lymphoid cells, the virus may be transmitted and give rise to productive infection.

The technical assistance of Robert Fredriksson, Birgitta Lind, and Agneta von Gegerfelt is gratefully acknowledged. We also thank E. Norrby and J. Pontén for helpful discussion.

This study was supported by grants from the Swedish Cancer Society and Swedish Medical Research Council (project no. K86-16H-7737-01A).

LITERATURE CITED

1. **Åsjö, B., L. Morfeldt-Månson, J. Albert, G. Biberfeld, A. Karlsson, K. Lidman, and E. M. Fenyö.** 1986. Differences in the replicative capacity of AIDS-virus isolated from patients with varying clinical conditions. Lancet **ii**:660–662.
2. **Coffin, J., A. Haase, J. A. Levy, L. Montagnier, S. Oroszlam, N. Teich, H. Temin, K. Toyoshima, H. Varmus, P. Vogt, and R. A. Weiss.** 1986. Human immunodeficiency viruses. Science **232**:697.
3. **Dahlberg, J. E., J. H. Gaskin, and K. Perk.** 1981. Morphological and immunological comparison of caprine arthritis encephalitis and ovine progressive pneumonia viruses. J. Virol. **39**:914–919.
4. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312**:763–767.
5. **Epstein, L. G., L. R. Sharer, E.-S. Cho, and M. Myenhofer.** 1984/1985. HTLV-III/LAV-like retrovirus particles in the brains of patients with AIDS encephalopathy. AIDS Res. **1**:447–453.
6. **Gonda, M. A., F. Wong-Staal, R. C. Gallo, J. E. Clements, O.**

Narayan, and R. V. Gilden. 1985. Sequence homology and morphologic similarity of HTLV-III and visna virus, a pathogenic lentivirus. Science **227**:173–177.
7. **Haase, A. T.** 1975. The slow infection caused by visna virus. Curr. Top. Microbiol. Immunol. **72**:101–156.
8. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J.-C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus, LAV. Nature (London) **312**:767–768.
9. **Nathanson, N., G. Georgsson, P. A. Palsson, J. A. Najjar, R. Lutley, and G. Petursson.** 1985. Experimental visna in Icelandic sheep: the prototype lentivirus infection. Reviews of Infectious Diseases 7(1), 75–82.
10. **Navia, B. A., E.-S. Cho, C. K. Petito, and R. W. Price.** 1986. The AIDS dementia complex. II. Nueropathology. Ann. Neurol. **19**:525–535.
11. **Navia, B. A., B. D. Jordan, and R. W. Price.** 1986. The AIDS dementia complex. I. Clinique features. Ann. Neurol. **19**:517–524.
12. **Oger, J., S. Szuchet, J. Antel, and B. G. W. Arnason.** 1982. A monoclonal antibody against human T suppressor lymphocytes binds specifically to the surface of cultured oligodendrocytes. Nature (London) **295**:66–68.
13. **Parham, P. J.** 1983. On the fragmentation of monoclonal IgG1, IgG2a and IgG2b from Balb/c mice. Immunology **131**:2895–2902.
14. **Petito, C. K., B. A. Navia, E.-S. Cho, B. D. Jordan, D. C. George, and R. W. Price.** 1985. Vacuolar myelopathy pathologically resembling subacute combined degeneration in patients with the acquired immune deficiency syndrome. N. Engl. J. Med. **312**:874–879.
15. **Petursson, G., N. Nathanson, G. Georgsson, H. Panitch, and P. A. Palsson.** 1976. Pathogenesis of visna I. Sequential virologic, serologic, and pathologic studies. Lab. Invest **35**:402–412.
16. **Pontén, J., and E. M. Macintyre.** 1968. Long term culture of normal and neoplastic human glia. Acta Pathol. Microbiol. Scand. **74**:465–486.
17. **Snider, W. B., D. M. Simpson, S. Kielsen, J. W. M. Gold, C. E. Metroka, and J. B. Posner.** 1983. Neurological complications of acquired immune deficiency syndrome: analysis of 50 patients. Ann. Neurol. **14**:403–418.
18. **Sundström, C., and K. Nilsson.** 1976. Establishment and characterization of a human histiocytic lymphoma cell line (u-937). Int. J. Cancer **17**:565–577.
19. **Westermark, B., J. Pontén, and R. Hugosson.** 1973. Determinants for the establishment of permanent tissue culture lines from human gliomas. Acta Pathol. Microbiol. Scand. Sect. A **81**:791–805.