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Susceptibility of human glial cells to infection with human immunodeficiency virus (HIV)

Stephen Dewhurst, Joel Bresser+, Mario Stevenson, Koji Sakai, Mary Jean Evinger-Hodges° and David J. Volsky

Molecular Biology Laboratory, Department of Pathology and Microbiology, University of Nebraska Medical Center, 42nd & Dewey Avenue, Omaha, NE 68105, +Clinical Immunology and Biological Therapy and *°Department of Hematology, M.D. Anderson Hospital and Tumor Institute, Texas Medical Center, 6723 Bertner Avenue, Houston, TX 77030, USA*

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Three human brain-derived cell lines (including two of astrocytic origin) were exposed in vitro to the human immunodeficiency virus (HIV), the etiologic agent of immunodeficiency in AIDS. In all three lines, HIV transcripts were detected by in situ hybridisation in 20–30% of cells 48 h after infection. Synthesis of virus *gag* gene products p24 and p55 was demonstrated by immunoblotting. No cytopathic effects typical of HIV-infected human T lymphocytes were observed. Our data indicate that HIV is neurotropic, and support the hypothesis that this virus may infect astrocytes in the brain.

AIDS; Central nervous system; HIV receptor; Glial fibrillary acidic protein; Human immunodeficiency virus; Astrocyte

1. INTRODUCTION

One of the characteristic features of AIDS is the high prevalence of CNS disease [1]. This typically manifests itself as a dementia with subacute encephalopathy that occurs in the absence of recognised opportunistic CNS pathogens [2]. HIV, the etiologic agent of the immune dysfunction in AIDS (reviews [3,4]), has also been implicated in the AIDS-associated neurological disorders, on the basis of the detection of HIV genome in the brains of neurosymptomatic AIDS patients [5] and isolation of the virus from the brain tissue [6]. Although these data suggest that HIV could be neurotropic, proof that HIV can directly infect nervous system (NS)-derived cells, such as astrocytes, has been lacking.

We have recently demonstrated that several cell lines originating from human brain tumors express gene transcripts encoding the helper T lymphocyte-specific T4 glycoprotein [7], which also serves as a cellular receptor for HIV [8,9]. Furthermore, two such cell lines exhibited detectable levels of the HIV receptors on cell surfaces [7], suggesting that they may be susceptible to the virus. Here we present data showing that T4/T4+-positive human astrocytes can be infected by HIV in vitro. These results provide evidence for HIV neurotropism and suggest the possibility for a direct involvement of HIV in AIDS-related nervous system disorders.

Correspondence address: D.J. Volsky, Molecular Biology Laboratory, Dept of Pathology and Microbiology, University of Nebraska Medical Center, 42nd & Dewey Avenue, Omaha, NE 68105, USA

Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immune deficiency syndrome; GFAP, glial fibrillary acidic protein; CNS, central nervous system

2. MATERIALS AND METHODS

2.1. *Cell lines*

Human brain tumor-derived cell lines U-251MG [10,11], U-373MG [11] and Te671 [12] were ob-

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tained from D.D. Bigner (U-251MG) and American Type Culture Collection (ATCC, Rockville, MD). The $T4^+$ cell line of human T cell leukemia origin, CEM [13], was obtained from L. Montagnier.

2.2. *Preparation of subgenomic HIV RNA probes*

Restriction endonuclease map of HIV molecular clones N1G-B and N1G-G, and the respective positions of subcloned subgenomic fragments used for the generation of RNA probes are illustrated in fig.1F. Molecular clones N1G-B and N1G-G were derived from CEM cells infected with N1G isolate of HIV [14]. The pGAP probe was derived by subcloning the 2.6-kb *Bgl*II-*Eco*RI fragment of HIV clone N1G-B in forward orientation into the *Bam*HI-*Eco*RI sites of pGEM3 (Promega Biotec). The pGgag and pGenv were made by subcloning the 1.4-kb *Sac*I-*Bgl*II or the 2.1-kb *Kpn*I-*Bam*HI fragment from HIV clone N1G-G into the *Sac*I-*Bam*HI sites or the *Kpn*I-*Bam*HI sites of pGEM-4 (Promega Biotec), respectively. A ^{32}P-labelled antisense RNA probe was transcribed from pGEM-3 and pGEM-4 vectors using the T7 promoter of the *Hin*dIII-linear transcription plasmid template.

2.3. *Detection of HIV expression by hybridisation in situ*

Cells were washed and harvested at 48 h post-infection. CEM/LAV-N1T [14] producer cells were included as positive controls. Smears of fixed cells were taken through a modified prehybridisation procedure [15] prior to overnight incubation with a Photobiotin™ [16]-labelled antisense single-stranded RNA probe. The probe consisted of pGAP, pGgag and pGenv combined in equal amounts in hybridisation cocktail as described [16]. Following hybridisation, slides were treated with RNase and washed. Hybrids were detected by incubation of the slides with streptavidin-conjugated Texas red according to the manufacturer's instructions (BRL). Photographs were taken on a Nikon microscope using Kodak Ektachrome PS 800/1600 film push processed at 1600 ASA. All slides were photographed with the same exposure time so as to allow direct comparison of the relative levels of fluorescence.

2.4. *Immunocytochemical staining of cells for GFAP*

Acetone fixed cell smears were reacted with GFAP-specific monoclonal antibodies (G-A-5, obtained from Boehringer-Mannheim and used at a concentration of 5–10 μg/ml). Detection of bound antibody was achieved by the avidin-biotin-peroxidase method (Vector Laboratories).

2.5. *Immunoprecipitation-enriched immunoblot analysis*

Cell extracts were immunoprecipitated using monoclonal antibodies to virus *gag* gene products p55 and p24 (Chemicon International), prior to electrophoresis and transfer to nitrocellulose [17]. Filters were incubated with antibody at 1:1000 dilution at 37°C overnight prior to detection using gold-conjugated anti-mouse immunoglobulin (Boehringer Mannheim) and silver enhancement (IntenSE, Boehringer Mannheim).

3. RESULTS

The principal characteristics of the three NS-derived cell lines used in the present study are summarized in table 1. None of the cell lines expressed epithelial cell markers (EMA, V-72-3, keratin), nor did they bind the monoclonal antibodies OKM-1 and OKM-3, indicating that they were not of the monocyte/macrophage series. All the cell lines were also free of T-lymphocytic characteristics, as assessed by Northern blot analysis with probes recognizing the constant region of the T-cell receptor. We may thus conclude that the cell lines tested were free of contamination by known HIV-host cells, i.e., monocyte/macrophages and T lymphocytes. Two of the cell lines expressed the astrocyte-specific marker, GFAP, as detected by immunocytochemistry and molecular hybridisation with a GFAP cDNA probe (fig.2E). As reported previously, all three cell lines expressed elevated levels of mRNA encoding the T4 receptor for HIV (table 1 and [7]).

For infection studies, glial cell cultures were exposed overnight to HIV-containing culture supernatants from HIV-producer cells CEM/HIV [13], followed by washing and analysis for the expression of viral products at various time points. Virus genome transcription was demonstrated by hybridisation in situ 48 h after infection (fig.1).

Table 1

Characterisation of human NS-derived cell lines and their response to HIV infection

Cell line	Origin	Characteristics of uninfected cells				Parameters of HIV infection 7 days after exposure to the virus			
		Monocyte/ macrophage markers[a]	T-lympho-cytic character-istics[b]	T4 mRNA	GFAP[c]	HIV transcripts[d]	HIV antigens		Cytopathic effects[e]
							IF	IP/WB	
U-251MG	glioma	–	–	+	+	+	–	+	–
U-373MG	glioblastoma	–	–	+	+	+	–	+	–
Te671	medullo-blastoma	–	–	+	–	+	–	+	–
CEM	T cell leukemia	–	+	+	–	+	+	+	+

[a] OKM1, OKM3
[b] T cell receptor gene rearrangement, CT gene expression
[c] By molecular hybridisation, immunocytochemistry
[d] By in situ hybridisation (fig.1) and Northern blotting (not shown)
[e] Cell lysis, formation of syncytia

IF, indirect immunofluorescence on smears of acetone-fixed cells; IP/WB, immunoprecipitation-enriched Western blotting (see also fig.2)

Virally encoded messsages were detected using subgenomic antisense HIV RNA probes (fig.1F) in approx. 30% of virus producing CEM/HIV cells (fig.1A) and 20% of infected brain-derived cells (fig.1B,D). In contrast, uninfected cells did not hybridise with these probes (fig.1C), and control hybridisations using RNA probes of opposite orientation (i.e., sense) also showed no reaction. A comparison of fig.1D and E clearly reveals that the GFAP-positive and virus infectable U-373MG cell populations (90 and 20%, respectively) must overlap, thereby demonstrating that cells of astrocytic origin are, indeed, infectable by HIV. The detection of viral RNA transcripts by in situ hybridisation was confirmed by RNA blot analysis 2 and 7 days after infection (not shown).

The translation of viral gene transcripts was demonstrated by immunoprecipitation-enriched immunoblot analysis using monoclonal antibodies to HIV *gag* gene products (fig.2). Viral polypeptides p55 and p24 were identified in glial cell extracts 48 h after infection, albeit at reduced levels in comparison to HIV-infected $T4^+$ lymphocytic cells CR-10/HIV [18] (fig.2). No virus-specific antigens could be detected by indirect immunofluorescence staining (table 1), confirming the low-level production of viral structural proteins in these cells. The production of mature progeny virus by HIV-infected NS-derived cells was demonstrated by cocultivation assays using PHA/IL-2 stimulated peripheral blood lymphocytes. Reverse transcriptase assays performed on supernatants from such cultures indicated that low levels of virus replication occurred in brain cell lines up to 7 days after infection with HIV. Infectious progeny virus was also obtained after transfection of glial cells with biologically active recombinant clones of HIV (not shown), confirming that these cells are permissive to HIV infection and replication.

In contrast to HIV-infected T lymphocytes, no cytopathic effects (cell lysis, formation of syncytia, growth retardation) were observed during short- or long-term culture of HIV-infected glial cells (table 1). However, the long-term infected cultures were found to contain viral gene transcripts and express the tat^{HIV} gene-encoded transactivation function [19] as detected by the chloramphenicol acetyl transferase (CAT) assay, but did not release infectious virus particles (not shown).

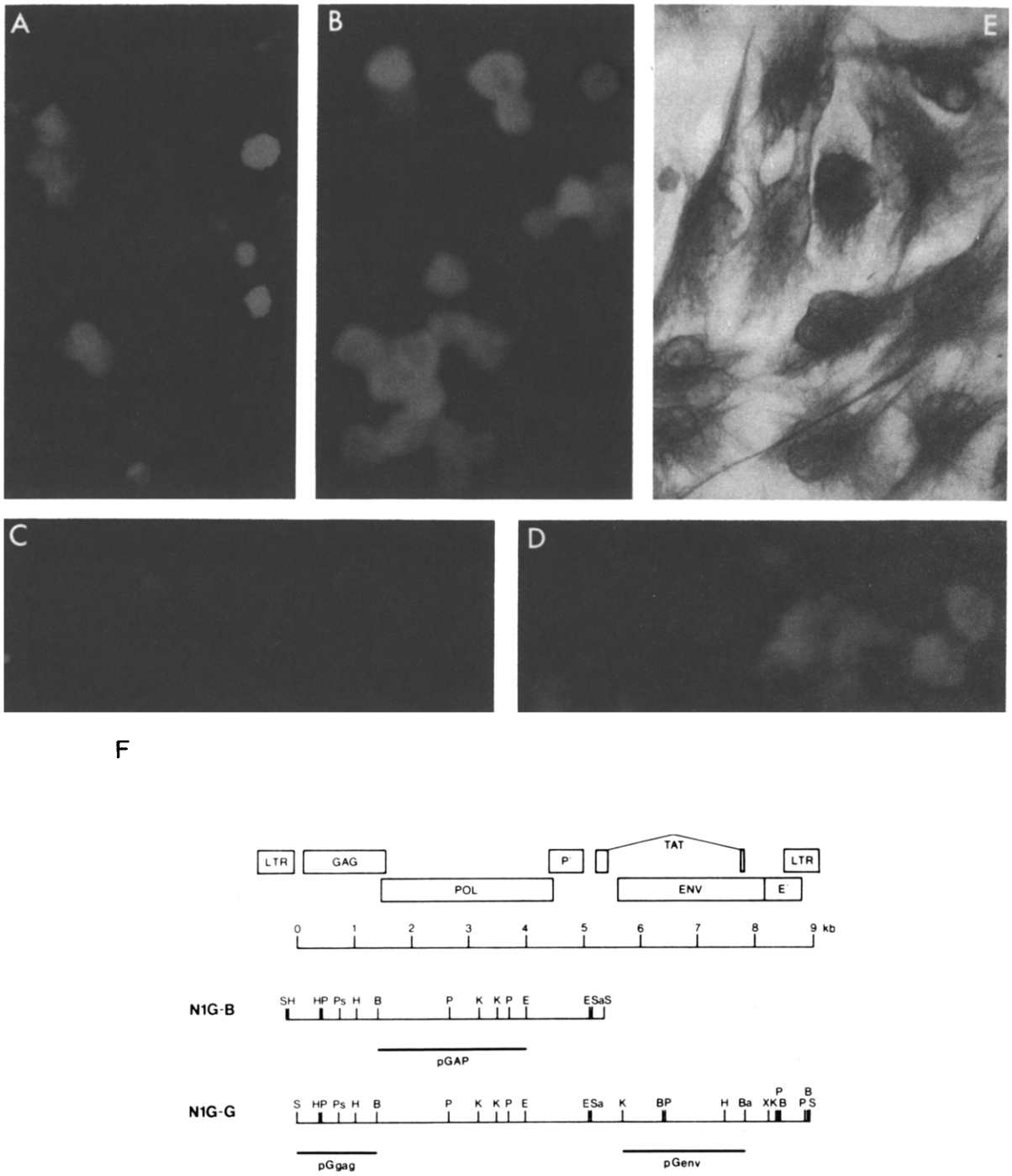

Fig.1. A–D: In situ hybridisation of fixed cell smears from HIV-infected cell cultures (A, CEM/HIV producer cells; B, Te671 cells; D, U-373MG cells) or control uninfected cells (C, U-373MG) with HIV-specific RNA probes. Magnification × 455. E: Immunocytochemical staining of U-373MG cells for GFAP. Magnification × 525. F: Restriction endonuclease map of HIV clones with areas covered by RNA probes indicated. Enzyme cleavage sites: Ba, *Bam*HI; B, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; K, *Kpn*I; Ps, *Pst*I; P, *Pvu*II; S, *Sac*I; Sa, *Sal*I; X, *Xho*I.

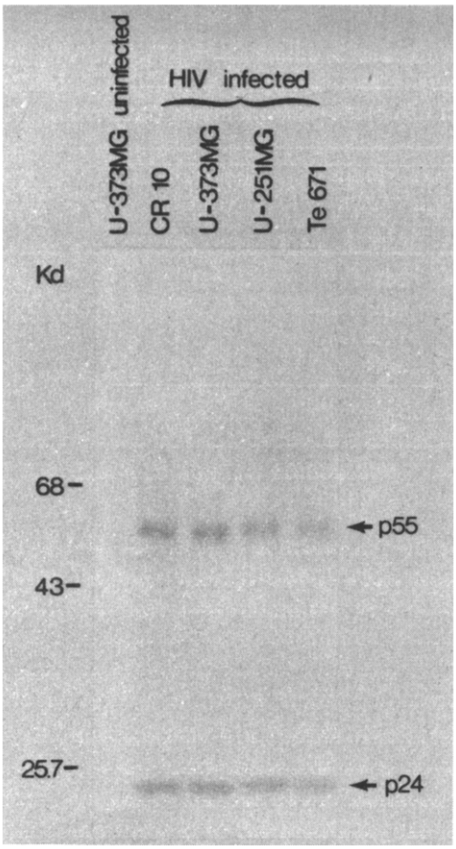

Fig.2. Immunoblot analysis of HIV-specific protein synthesis in infected cell lines using monoclonal antibodies to *gag* gene products p55 and p24. CR-10/HIV [18] lane represents an extract of less than 2×10^6 cells, whereas all other lanes represent over 20×10^6 cells. Numbers on the left refer to molecular mass markers.

4. CONCLUSIONS

The results presented here demonstrate that human astrocytes can be directly infected by the AIDS virus in vitro. This finding is consistent with our recent report showing the expression of the HIV receptor (T4 molecule) by these cells [7]. Taken together with previous reports on the probable localisation of HIV genome in astrocytes in brain autopsy materials from AIDS patients [20,21], our data strongly suggest that astrocytes, and possibly other NS-derived cells, may serve as the cellular targets for HIV neurotropism within the CNS.

It is of interest that virus activity in HIV-infected NS-derived cells was reduced in comparison to T-lymphocytic cells at both transcriptional and translational levels (figs 1 and 2). Furthermore, viral replication in these cells appeared to decline rapidly during culture (being undetectable 14 days after infection). Although the observed decline could be the result of a transient infection, it may also be due to the establishment of a state of viral latency. The latter hypothesis is supported by our preliminary experiments showing the persistence of certain viral transcripts and regulatory functions, such as the transcriptional transactivation, in long-term cultures of HIV-infected glial cells. The observation that HIV did not induce cytopathic effects in glial cells at any time after virus exposure (table 1) can be interpreted as another indication that the pathway of HIV infection in NS-derived cells differs markedly from the highly replicative and cytopathic interaction of the virus with human T lymphocytes.

While HIV can frequently be detected in macrophages in the brains of AIDS patients afflicted with neurological disorders [22,23], the localisation of the virus to astrocytes and other NS-derived cells in vivo has been achieved only rarely, and then, usually in patients at a severe stage of the disease [20,21]. Since our data indicate that in vitro infected NS cells express only low levels of HIV gene products, it is conceivable that the convincing proof for HIV neurotropism in vivo has to await more sensitive techniques for the detection of latent HIV infections in clinical materials.

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