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Expression of the T4 molecule (AIDS virus receptor) by human brain-derived cells

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Three human cell lines of astrocytic origin were evaluated for expression of a human T-lymphocyte surface glycoprotein, T4, which also serves as a cellular receptor for the human immunodeficiency virus (AIDS virus, HIV). T4 antigen was detected on the cell surface of 2 of these cell lines using monoclonal OKT-4 antibody and flow cytometry. Gene transcripts encoding the T4 molecule were detected by a ribonuclease protection assay in surface T4-positive and -negative cells. Our results suggest that astrocytes may serve as targets for HIV infection in the brain.

Human immunodeficiency virus (HIV); AIDS; HIV receptor; Astrocyte; T4 antigen; Glial fibrillary acidic protein

1. INTRODUCTION

Human immunodeficiency virus, the etiologic agent of AIDS (reviews [1,2]), is the first animal retrovirus for which a cellular receptor has been identified. This receptor is a 62 kDa polypeptide which is expressed on human helper/inducer T lymphocytes and is known as the T4, or CD4/Leu3, antigen [3–5]. The cytopathic interaction of HIV with $T4^+$ lymphocytes is thought to be responsible for the depletion of helper/inducer T cells observed in AIDS patients [6], which is theorised to lead to the immune defects that are associated with this disease. The T4 antigen is also expressed by cells of the mononuclear phagocyte series [7], but has not been reported to be present on non-hematopoietic cells.

Whereas severe immunodeficiency is the most dramatic feature of AIDS [1,2,6], up to 75% of AIDS patients also develop unexplained progressive neurological disorders [8]. HIV has been isolated from cerebrospinal fluid of AIDS patients [9] and detected in brain tissue by hybridisation in situ [10], indicating that this virus infects the central nervous system (CNS). Indeed, HIV has been demonstrated in macrophages in the brain [11] as well as in endothelial cells [12] and possibly also astrocytes [13]. This raises the possibility that human brain cells, other than invading hematopoietic cells, may display the HIV receptor (T4 molecule), thereby providing a basis for HIV neurotropism. Interestingly, both T4 and its murine homolog, L3T4, have been recently reported to be expressed at the RNA level in human and mouse brain, respectively [14,15]. However, neither report demonstrated the production of the corresponding proteins, nor were the cell type(s) encoding these mRNAs resolved.

Here, we demonstrate the cell surface expression

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

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of the T4 molecule and describe the detection of an RNA species encoding T4 in well-characterised human brain-derived cell lines of astrocytic lineage.

2. MATERIALS AND METHODS

2.1. *Cell lines*

Human brain tumor-derived cell lines U-251MG [16,17], U-343aMG and U-373MG [15] were obtained from D.D. Bigner, B. Westermark and American Type Culture Collection (ATCC, Rockville, MD), respectively. The $T4^+$ cell line of human T cell leukemia origin, CEM [18], was obtained from L. Montagnier.

2.2. *Immunocytochemical detection of GFAP*

GFAP was identified immunocytochemically by the avidin-biotin-peroxidase complex (ABC) method (Vector Laboratories) using monoclonal antibodies. Reagents were obtained from Boehringer Mannheim (GFAP-specific monoclonal antibody G-A-5) and D.D. Bigner (GFAP-specific monoclonal antibody 1B4), and were used at 5–10 μg/ml.

2.3. *Ribonuclease protection assay for T4 gene expression*

Levels of T4 receptor RNA were determined by ribonuclease protection using the T4 RNA probe as illustrated in fig.1B. The T4 RNA probe was derived by subcloning, in reverse orientation, a 600 bp *Eco*RI-*Sac*I fragment of the T4 receptor cDNA [19] into the transcription plasmid pGEM3 (Promega Biotec, Inc.). ^{32}P-labelled single-stranded antisense probe was generated using the Sp6 promoter of the *Eco*RI linearised template in the presence of 10 μM [^{32}P]CTP (600 μCi/mM) and excess (1 mM) cold ATP, GTP and UTP. 10^6 cpm of probe was hybridised for 12 h at 42°C with 10 μg of total cellular RNA (extracted by the guanidinium isothiocyanate method [20]) or 20 μg tRNA in 30 μl of a solution containing 80% formamide, 400 mM NaCl, 20 mM Tris-HCl (pH 7.5) and 1 mM EDTA at 452°C for 12 h. The hybridisation was terminated by the addition of 300 μl of 300 mM NaCl, 10 mM Tris, 1 mM EDTA containing 5 μg/ml RNase T1 and 20 μg/ml RNase A (Boehringer Mannheim). Digestion of unhybridised probe proceeded at 22°C for 1 h, after which 20 μl of 10% SDS and 15 μl of proteinase K (10 mg/ml) were added for 15 min at 37°C. The samples were extracted once with phenol, once with chloroform, and the RNA/RNA hybrids were precipitated with ethanol in the presence of 20 μg of carrier tRNA. The precipitated sample pellets were dissolved in 10 μl of formamide loading buffer (95% formamide, 10 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol). The samples were electrophoresed on denaturing 7 M urea/5% polyacrylamide gels, dried and subjected to autoradiography. End-labelled *Msp*I-digested pBR322 DNA served as molecular mass markers.

2.4. *Detection of cell surface T4 antigen expression*

The OKT4 surface marker was detected using a monoclonal antibody purchased from Ortho Diagnostic Systems, using an Ortho System 50H cytofluorograph (FACS).

3. RESULTS

For the purpose of this study, it was important to confirm that the three cell lines used, all originally derived from brain tumors [16,17], still expressed characteristics indicative of their nervous system origin and that they were free of any monocyte or T-lymphocyte contamination. Immunocytochemical staining for the astrocyte-specific antigen, GFAP [21], revealed that approx. 99% of the U-251MG and U-343aMG cells ex-

Fig.1. Immunocytochemical staining for GFAP using monoclonal antibody 1B4 on the U-373MG cell line. Magnification, × 3200

pressed this protein. Typical GFAP staining is illustrated in fig.1. Thus, the overwhelming majority of these cells were astrocytic in origin. The proportion of the U-373MG cells that stained for GFAP was lower, about 90%. However, the cells neither bound OKM1, OKM3 antibodies (as detected by flow cytometry), nor did they exhibit T cell receptor gene rearrangement or CTβ gene expression (not shown), indicating a lack of contamination by known HIV-host cells.

We next analysed the cells for surface T4 receptor expression using monoclonal OKT4 antibody and flow cytometry. As shown in fig.2, the U-343aMG and U-373MG, but not U-251MG cells, clearly exhibited detectable levels of OKT4 binding. The proportion of glial cells showing T4-specific fluorescence and the mean fluorescence intensity (fig.2B,D) were lower than those observed with control $T4^+$ CEM cells (fig.2F), indicating a lower density of the T4 receptor on astrocytes as compared to a T-lymphocytic cell line.

Since flow cytometric analysis of surface receptors in cells grown in monolayer cultures is often

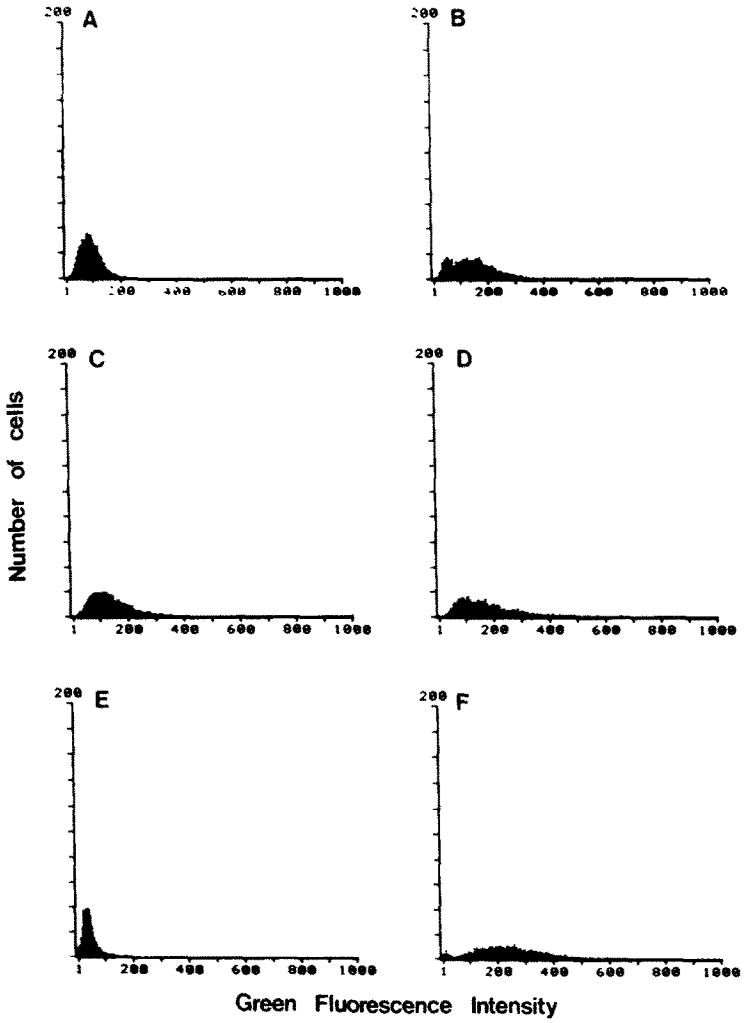

Fig.2. Flow cytometric analysis of cell surface T4 expression by: (B) U-373MG (33.7%); (D) U-343aMG (11.1%); (E) U-251MG (0.6%); (F) CEM (94.1%) cells. Numbers in parentheses represent the proportion of cells exhibiting T4-specific fluorescence, which was calculated by subtraction of background fluorescence from that observed with anti-T4 antibodies. Control staining with FITC alone is illustrated for (A) U-373MG and (C) U-343aMG cells.

associated with a significant background fluorescence (cf. fig.2A,C), we wished to confirm whether the positive fluorescence observed with anti-T4 antibodies indeed represented expression of the T4 gene. RNAs isolated from surface $T4^+$ and $T4^-$ U-373MG and U-251MG cells, respectively, were therefore analysed for the presence of T4 gene transcripts using human T4 cDNA probe [19] and RNase protection assay. The results of this analysis are presented in fig.3. The two human astrocytic cell lines tested clearly contained mRNAs specific for the T4 gene. The presence of T4 mRNA in the surface $T4^-$ line U-251MG is particularly noteworthy because it indicates the limitation of flow cytometry as a method of detecting low-density surface antigens. As expected, the $T4^+$ lymphocytic cell line, CEM [18] expressed high levels of T4 gene transcripts. The relative levels of T4-specific mRNA in glial cells were much lower (20–30 ×) than in T-lymphoid cell lines. At this stage, it is unclear whether that reflected a lower expression of T4 gene per cell, or the fact that only a subpopulation of cells expressed the gene.

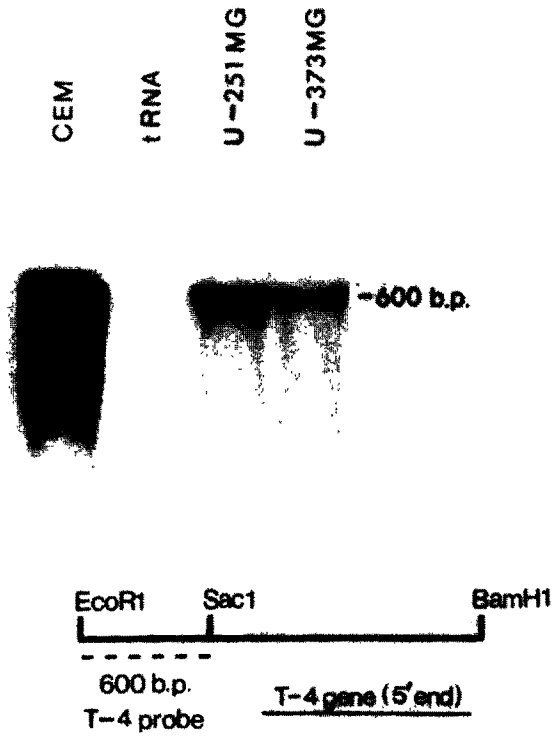

Fig.3. Detection of T4 gene transcripts in the indicated cell lines by an RNase protection assay.

4. DISCUSSION

This is the first demonstration that well characterised cell lines derived from human brain, in this case GFAP-positive and therefore astrocytic in origin, also express the gene encoding the T4 molecule – an antigen normally expected to be found on helper/inducer T lymphocytes. This observation is not entirely surprising. Since the original report of a shared antigen on murine lymphocytes and brain cells [22], several investigators have reported the existence of shared brain-lymphoid antigens, including Thy-1 [23] and OX-2 [24]. Furthermore, during the preparation of this work, Maddon et al. reported the detection of T4 mRNA in human cerebral cortex, although the brain cell type(s) that express the T4 gene was not specified [14]. Our results clearly indicate that brain-derived cells, such as astrocytes, rather than rare invading hematopoietic cells, may be responsible for the observed expression of the T4 molecule in human brain. Moreover, we demonstrate that the T4 gene is not only expressed at the mRNA level, but is also present on the astrocyte surface as an antigenically-recognisable T4 molecule (fig.2). This finding may have implications with regard to the possible functional significance of T4 antigens in human brain, particularly in view of the reported interaction between Class II HLA molecules and the T4 molecule [25]. Such an interaction may, for example, participate in the transduction of a signal required for B cell activation [25], leading to possible autoimmune events.

The observation that the T4 gene is expressed in cells of astrocytic origin, and perhaps other non-hematopoietic cells in the brain, may have relevance to the pathogenesis of AIDS-related brain disease. Although HIV can undoubtedly infect the human brain [9,10], the HIV-susceptible cell type(s) in this organ is unknown. HIV has been convincingly demonstrated in macrophages in the brain [11], but these cells could become infected in the peripheral circulatory system [26,27] and migrate across the brain-blood barrier. Our demonstration of the presence of the T4 molecule (HIV receptor) on astrocytes may thus provide an explanation for the presumed neurotropism of HIV and encourage studies on the infectivity of brain cells by this virus. These experiments are in progress in our laboratory.

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