Brief Definitive Report

THE CELLULAR RECEPTOR (CD4) OF THE HUMAN IMMUNODEFICIENCY VIRUS IS EXPRESSED ON NEURONS AND GLIAL CELLS IN HUMAN BRAIN

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The peculiar tropism of the human immunodeficiency virus (HIV) for T helper lymphocytes can be explained by a specific interaction between the virus and the CD4 molecule on these cells (1, 2). The tropism for T lymphocytes, however, can hardly account for the early brain infection observed in some AIDS (acquired immune deficiency syndrome) patients (3, 4). Since CD4 is also expressed on virus-susceptible non-T cell lines we wondered whether an additional expression site of CD4 could be demonstrated in neural tissue (5). To this end, CD4 expression in brain was analyzed with several different anti-CD4 mAbs, and using a CD4-specific cDNA probe in Northern blot analyses. $CD4^+$ cells and CD4-specific mRNA were found in the cerebellum, thalamus, and pons. The reactive cells could be identified as neurons as well as glial cells.

Material and Methods

Tissues. Specimens of normal brain tissues obtained 4–8 h postmortem were generously provided by Dr. K. Biese (Institut für Neuropathologie, Munich). Tissue samples were snap frozen in isopentane precooled with liquid nitrogen and stored at −80°C.

Monoclonal Antibodies. As anti-CD4 antibodies, the three different mAbs MT 151 (IgG2A); MT 321 (IgG1), recognizing the OKT4 epitope; and MT 310 (IgG1), recognizing the OKT4 A determinant were used (6, 7). The antimonocyte antibodies M-M 522 (IgG1), and M-M 42 (IgG1), and anti-T cell mAbs MT 910 (CD2) and MT 811 (CD8) were described previously (6, 8, 9). The anti-GFAP antiserum was obtained from Dakopatts, Copenhagen, Denmark. Control stainings were performed with the mouse myeloma IgGs MOPC 21 (IgG1) and UPC 10 (IgG2A) from Sigma Chemical Co., St. Louis, MO.

Staining Procedures. Immunoperoxidase staining was carried out on 20-μm cryosections using appropriate dilutions of the above listed mAbs as described elsewhere (10). Controls were performed with the peroxidase-conjugated second antibody alone (P260, P217; Dakopatts) or with isotype-matched mAbs of the same protein concentration. The sections were counterstained with either cresyl violet or methylene blue for Nissl substance.

RNA Isolation and Northern Blot Analyses. Total RNA was isolated by the guanidinium isothiocyanate method (11). Solid-frozen tissues were homogenized in liquid nitrogen before RNA isolation. Blotting was carried out according to standard protocols with a 1.7 kb CD4-specific cDNA probe containing part of the CD4 coding and the 3′ untranslated region of the message (to be published elsewhere). Control hybridization with T cell receptor–specific probes was done with cDNA probes for the α, β, and γ chains. The probes were a kind gift of Dr. Tak Mak, Toronto, Canada.

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TABLE I
CD4 Expression on Cells of Different Cerebral Subareas

Cerebral subarea	Staining with Anti-CD4 antibodies*		Northern blot‡
	Glial cells	Neurons	
Cerebellum	−	+	+
Cortex (temporal)	−	−	−
Cortex (parietal)	−	−	−
White matter (parietal)	−	−	−
Thalamus	+	+	+
Basal ganglia	−	−	−
Corpus amygdaloideum	−	−	−
Hippocampus	ND	ND	+
Pons	+	+	+

* mAbs MT 151, MT 321, and MT 310 were used in the indirect immunoperoxidase staining technique on 20-μm cryosections. Sections were counterstained with cresyl violet for Nissl substance.
‡ Northern blot analyses were performed with a 1.7 kb CD4-specific cDNA probe as described.

Results

To demonstrate expression of CD4 in human brain, two independent methods were chosen: immunoperoxidase stainings with three different mAbs were performed on serial sections of various brain subareas and, in parallel, Northern blot analysis was carried out with RNA extracted from these regions, as demonstrated in Table I. Discrete neurons staining with anti-CD4 antibodies were found in the cerebellum, thalamus, and pons. Spurious epitope sharing of unrelated molecules could be ruled out because staining with mAbs MT 151, MT 321, and MT 310, recognizing different epitopes of CD4, resulted in virtually identical staining patterns. It is noteworthy that no positive staining was obtained with other anti–T cell reagents (anti-CD2 or anti-CD8), which were tested on various sections. CD4-expressing monocytes could be excluded by control staining with antimonocyte antibodies (M-M 522, M-M 42). According to morphological criteria and staining patterns with anti-GFAP, and by the Nissl stain, the positive cells could be identified on serial sections either as glial cells or as neurons. In the cerebellum of both examined brains, Purkinje cells gave a variable reaction ranging from few strongly positive cells to numerous faintly stained cells. A most impressive reaction was found on sections of both thalami in an area close to the plexus choroideus, where clustering neurons gave a strong reaction with anti-CD4 antibodies (Fig. 1). In the pons area, only rare dispersed neurons stained with the anti-CD4 antibodies. As indicated in Table I, a distinct reaction of glial cells with anti-CD4 antibodies was seen in the thalamus and pons. Here, dispersed cells exhibiting the morphology of glial cells and reacting also with the astrocyte-specific marker GFAP could be identified on serial sections as $CD4^+$ cells.

Northern blot analyses with a CD4-specific cDNA probe revealed a 3 kb mRNA in RNA preparations of the above-described subareas and in the hippocampus, which was not examined in the immunoperoxidase staining (Fig. 2). This RNA, specifically hybridizing with the CD4 cDNA probe, was seen only in those subareas where anti-CD4–reactive cells had been detected by peroxidase staining. In one of the two cerebellum specimens, an additional 1.7 kb CD4-specific mRNA was found. In contrast to cerebellum, thalamus, pons, and hippocampus, no hybridization signal was obtained on RNA extracts of basal

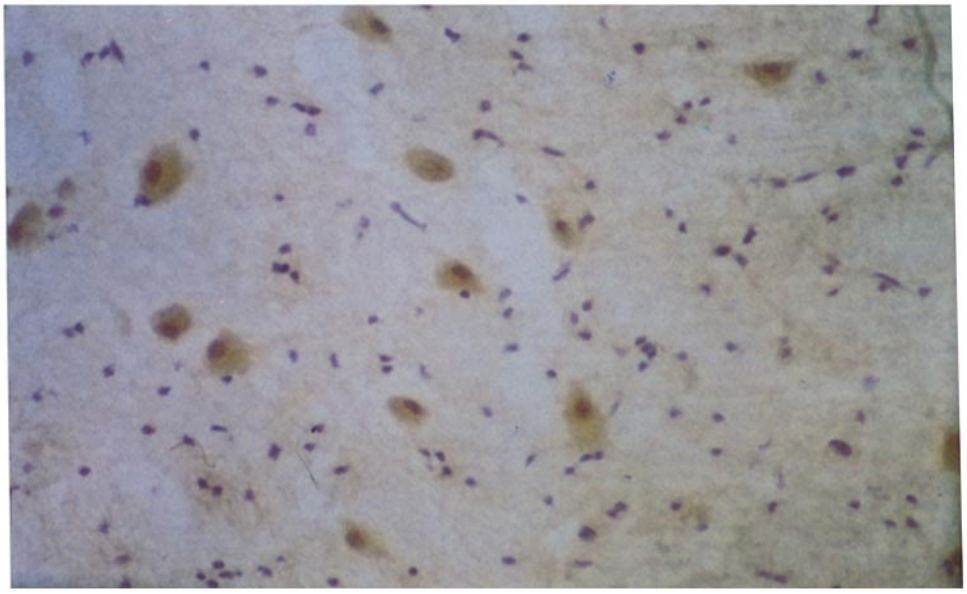

FIGURE 1. 20-μm cryosection originating from an area of the thalamus close to the plexus choroideus stained with the anti-CD4 mAb MT 321 using the indirect immunoperoxidase technique (counterstained with cresyl violet; magnification × 25).

ganglia, corpus amygdaloideum, and the parietal and temporal cortex containing grey and white matter (Table I and Fig. 2). Confirming the immunohistochemical findings with the anti–T cell antibodies (anti-CD2 and anti-CD8), the Northern blot rehybridized with probes specific for the T cell receptor α, β, and γ chains turned out to be negative in all lanes containing brain RNA.

Discussion

The presented data unequivocally demonstrate that CD4 is expressed on genuine neural cells because contaminating T cells or monocytes also displaying CD4 could be excluded by the negative staining results with anti–T cell and antimonocyte antibodies. Moreover, hybridization with T cell receptor–specific cDNA probes failed to detect mRNA coding for the various chains indicative of T cells.

On the whole, a picture of remarkable concordance emerged, in so far as CD4-expressing cells were found only in those regions where a CD4-specific mRNA was detected. In view of the discrete distribution of $CD4^+$ neurons and glial cells in the brain, the finding of an additional 1.7 kb mRNA in one of the two examined cerebella deserves further comment. The discrepant hybridization patterns may be due to different localizations of the examined cerebellar specimens, thus also underlining the discontinuous distribution of CD4 in brain. Because the CD4 mRNA contains a 3′ untranslated region representing half of its total transcribed length, the difference in mRNA length may be due to an alternative polyadenylation or splicing site in the 3′ untranslated region (12). A second, smaller mRNA has recently been reported for human brain, and a 2.7 kb L3T4-specific message has been found in mouse brain (13, 14). The broad

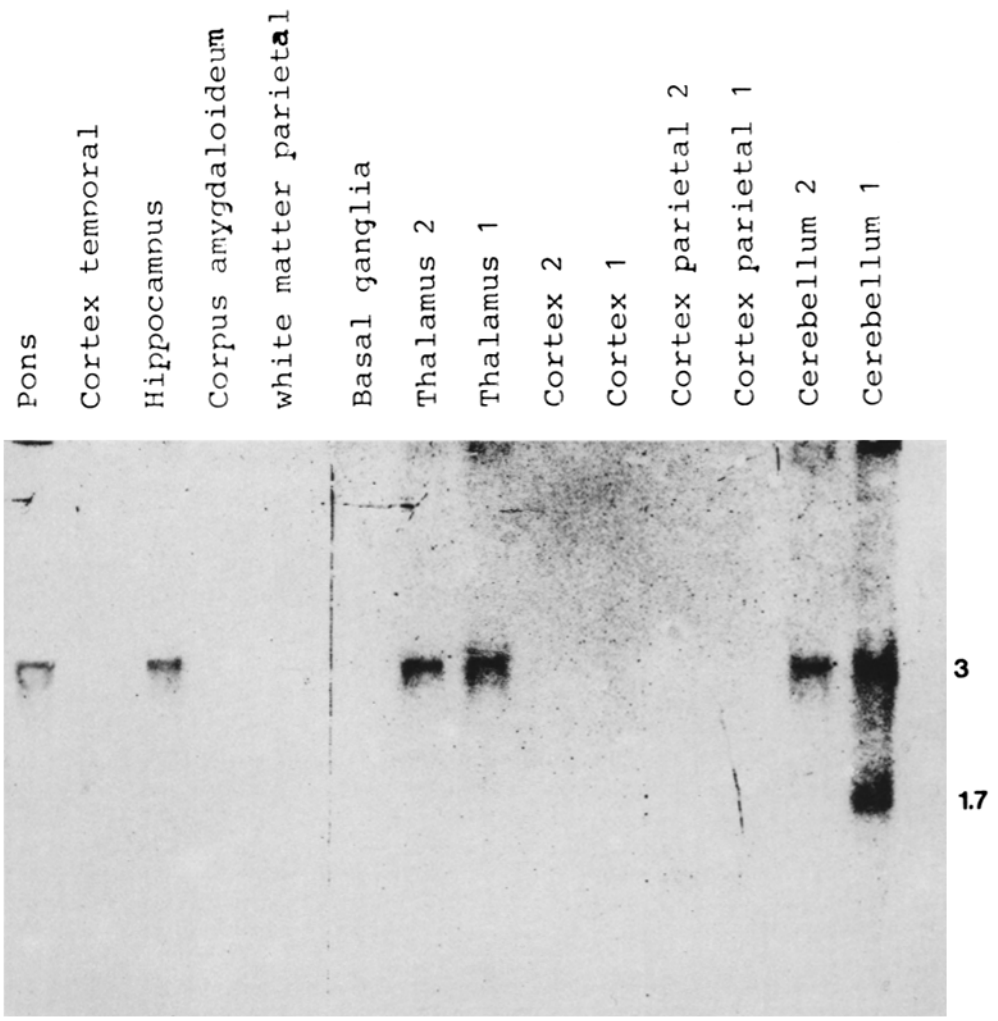

FIGURE 2. Northern blot analysis of RNA from cerebral subareas. 25 μg of total RNA were electrophoresed through an 1.2% agarose gel containing formaldehyde. After transfer to Hybond-N membrane RNA was hybridized to the nick-translated CD4-specific cDNA probe, followed by high-stringency washes down to 0.1 × SSC. Exposure time was 7 d at −80°C with intensifying screen. Fragment size markers (kb) are at right.

spectrum of neurological symptoms of AIDS patients, ranging from subacute subcortical dementia, spasticity, and gait disturbance to paraparesis may well be explained by a differential involvement of those regions where $CD4^+$ cells are located. Based on the immunohistochemical findings, it cannot be determined precisely whether the CD4-reactive molecules are located only on the cell surface or also in the cytoplasm. We propose that these $CD4^+$ cells are a direct target for HIV.

This virus as a typical member of the lentivirus group, exhibits not only the special affinity for cells of the central nervous system, but also a peculiar penetration capacity, length of incubation time, antigen shedding, and a high mutation rate of envelope glycoproteins (15). Because the $CD4^+$ cells present in

discrete subareas of the brain are slowly proliferating or nonproliferating cells, they most likely serve as an ideal reservoir for this retrovirus. Although the pathway of HIV invading the brain cannot be exactly traced at present, the intracerebral spreading of the virus may occur via syncytium formation involving CD4 of noninfected cells (16). Whether this process is initiated by virus-infected macrophages cannot be decided at present (17). Furthermore, the intimate contact of glial cells and neurons might facilitate virus dissemination by this CD4-dependent cell fusion process.

There may be a connection between the early appearance of the virus in the cerebrospinal fluid and the location of $CD4^+$ neurons in close proximity to the plexus choroideus (18). More defined neuroanatomical studies are required to draw a complete map of CD4-expressing cells in the brain and peripheral nervous system. Besides Thy-1 and OX-2, CD4 is another member of the immunoglobulin supergene family shown to be expressed both on lymphocytes and brain tissues (19–21).

Summary

The expression of the CD4 antigen in normal human brain was investigated in parallel by immunohistochemical and Northern blot analyses. With anti-CD4 antibodies detecting different epitopes of the molecule, $CD4^+$ neurons were defined in the cerebellum, thalamus, and pons. $CD4^+$ glial cells were identified in the thalamus and pons. CD4-specific mRNA was detected in all three subareas and in the hippocampus, while other subareas were negative. The $CD4^+$ cells were negative with anti–T cell antibodies (anti-CD2 and anti-CD8), as well as with antimonocyte antibodies (M-M 522 and M-M 42).

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