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Absence of brain-derived neurotrophic factor and trkB receptor immunoreactivity in glia of Alzheimer's disease

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Abstract Alterations in the neuronal expression of some neurotrophins have been shown in various neurodegenerative processes, particularly Alzheimer's disease (AD). Glia may up-regulate neurotrophins and their high-affinity tyrosine kinase (trk) receptors in response to neural injury. In human immunodeficiency virus type 1 (HIV-1) encephalitis, activated microglia were shown to express brain-derived neurotrophic factor (BDNF), while reactive astrocytes expressed trkB receptor. This observation has suggested the existence of local neurotrophic regulation between different glial populations. To characterize the glial cellular distribution of BDNF and trkB receptor proteins in AD, we studied selected regions of postmortem brains from four AD and three age-matched control patients by double-immunofluorescence confocal microscopy. In both groups, BDNF immunoreactivity was distributed in neuronal perikarya and neuritic processes in the neocortex and hippocampus. No BDNF immunoreactivity was observed in microglia or astrocytes within and between senile plaques of AD. Catalytic trkB receptor immunoreactivity was present in neuronal perikarya in the neocortex and hippocampus. Reactive astrocytes and microglia were not immunoreactive for catalytic trkB. The absence of BDNF and trkB proteins in glia in AD patients is in contrast to the finding in patients with HIV-1 encephalitis. This difference suggests that glial expression of BDNF and trkB proteins may be characteristic of particular disease processes, rather than merely representing a stereotyped response to any type of neural injury.

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Introduction

The role of neurotrophic factors in the pathogenesis of various neurodegenerative disorders has been investigated in recent years [9]. In Alzheimer's disease (AD), most studies had examined the integrity of the cholinergic basal forebrain neurons and their projections to the neocortex and hippocampus. Their findings have supported the notion that retrograde transport of nerve growth factor (NGF) from the cerebral cortex to the basal forebrain is diminished in AD due to defective expression of its high-affinity tyrosine kinase receptor, trkA [1, 13]. Decreased levels of the expression of brain-derived neurotrophic factor (BDNF) mRNA were shown in AD hippocampi [14, 15].

Chronic activation of glial cells may promote progressive neurodegeneration in AD via both autocrine and paracrine effects of inflammatory mediators such as interleukin-1, interleukin-6, tumor necrosis factor-α and S100β [4, 8]. Activated microglia are physically associated with dense amyloid cores in senile plaques of AD, and are likely involved in phagocytic activity. Reactive astrocytes are usually located at the periphery of senile plaques and extend their processes into the plaque cores [4]. The microglial phagocytosis of senile plaque cores may be regulated by astrocytes [3]. Expression of NGF, BDNF, neurotrophin-3, and neurotrophin-4/5 mRNA was shown in microglial cultures [5, 10]. As BDNF and neurotrophin-3 were shown to promote microglial proliferation in vitro [5], we hypothesized that in response to neural injury in AD, glial cells up-regulate neurotrophins and their receptors, which may locally modulate the complex interaction between glial cells.

In the present study, we applied double-immunofluorescence (IF) confocal microscopy to evaluate the glial cellular distribution of BDNF and its high-affinity trkB receptor proteins in postmortem brain tissues from AD patients.

Materials and methods

We studied four brains from AD patients (aged 67, 80, 82 and 90 years). Three brains from patients without neurological diseases (aged 70, 72 and 77 years) were used as age-matched controls (two-tailed $P = 0.3$, unpaired Student's *t*-test). The postmortem intervals ranged from 3 to 6 h for AD, and 12 to 19 h for controls. Clinico-neuropathological studies of both AD and control brains followed the protocol of the Alzheimer's Disease Research Center at the University of Pittsburgh [16]. All AD brains (mean fresh weight 1098 ± 121 g) were diagnosed with "definite AD" according to the Consortium to Establish a Registry for Alzheimer's Disease criteria [12] and showed a high frequency of neurofibrillary tangles with the Braak and Braak stage VI [2]. All control brains (mean fresh weight 1370 ± 70 g) showed age-related astrocytosis, without evidence of AD pathological features. Paraffin sections of the mid-frontal gyrus and hippocampus (at the level of the lateral geniculate body) were used for IF staining.

Primary antibodies

The anti-BDNF (clone N-20, 1:200 dilution) and anti-trkB (clone 794, 1:100) antibodies were affinity-purified rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, Calif.) as previously described [18]. Antibodies to cell-specific markers included mouse anti-HLA-DR IgG2b (LN-3, ICN Biomedicals, Aurora, Ohio; neat), mouse anti-human glial fibrillary acidic protein (GFAP) IgG1 (6F2, Dako, Carpinteria, Calif.; 1:100), and affinity-purified biotinylated lectin for *Ricinus communis* agglutinin I (RCA-1, Vector, Burlingame, Calif.; 1:2000).

Immunocytochemistry

Double IF staining was performed with the following pairs of primary antibodies: BDNF/RCA-1; BDNF/HLA-DR; BDNF/GFAP; trkB/RCA-1; trkB/HLA-DR; trkB/GFAP. For antigen retrieval, sections were immersed in preheated target retrieval solution (Dako) at 95°–99 °C for 1 h (for HLA-DR staining) or treated with pepsin (0.4%; Dako) at 37 °C for 30 min (for all other staining). As previously described [18], for BDNF and trkB the direct tyramide signal amplification method was used first (FITC-conjugated tyramide, DuPont NEN, Boston, Mass.), followed by cell-specific marker labeling using the conventional IF staining method (Cy5-conjugated streptavidin or Cy5-conjugated goat anti-mouse IgG serum). Negative controls (replacing primary antibodies with normal rabbit serum) showed no immunoreactivity (IR). Double IF sections were analyzed by confocal laser-scanning microscopy (Molecular Dynamics, Sunnyvale, Calif.), as previously described [17].

Results

BDNF

In the frontal gyrus of both AD and control brains, BDNF IR was present in subpopulations of neuronal perikarya of the neocortical layers 2–6, and in fiber and punctate profiles throughout the neuropil (Fig. 1 A). In the white matter, BDNF IR was uniformly distributed and followed the axonal orientation. In the hippocampus, BDNF IR was observed in most granule cells of the dentate gyrus (Fig. 1 B) and most pyramidal neurons of CA1–CA4 (Fig. 1 C). In the subiculum, pyramidal neurons were less often immunoreactive for BDNF. There was no consistent difference in neuronal BDNF IR patterns observed in individual brain regions examined between AD and control brains, except for lesser neuronal density in AD brains.

In AD brains, microglia labeled with either RCA-1 or HLA-DR predominantly clustered within senile plaque cores (Fig. 2), while GFAP-labeled astrocytes gathered around the plaque core (Fig. 3). Within and in between senile plaques, microglia (Fig. 2) and astrocytes (Fig. 3) did not show BDNF IR, nor did those cells in control brains. Plump BDNF-immunoreactive structures were observed within senile plaques, probably representing dystrophic neurites (Fig. 3).

TrkB

In both AD and control brains, trkB IR was present in subpopulations of neuronal perikarya. The neocortical

Fig. 1 A–C Immunofluorescence staining for BDNF protein *(green)*. **A** The frontal cortical layer 5 from a non-AD control brain shows BDNF immunoreactivity in subpopulations of neuronal perikarya *(arrows)* and in fiber and punctate profiles in the neuropil *(arrowheads)*. **B** Most granule cells of the dentate gyrus from an AD brain are immunoreactive for BDNF. **C** The hippocampal CA1 region from an AD brain shows BDNF immunoreactivity in most pyramidal neurons and in fiber and punctate profiles in the neuropil (*AD* Alzheimer's disease, *BDNF* brain-derived neurotrophic factor) *Bars* **A–C** = 50 μm

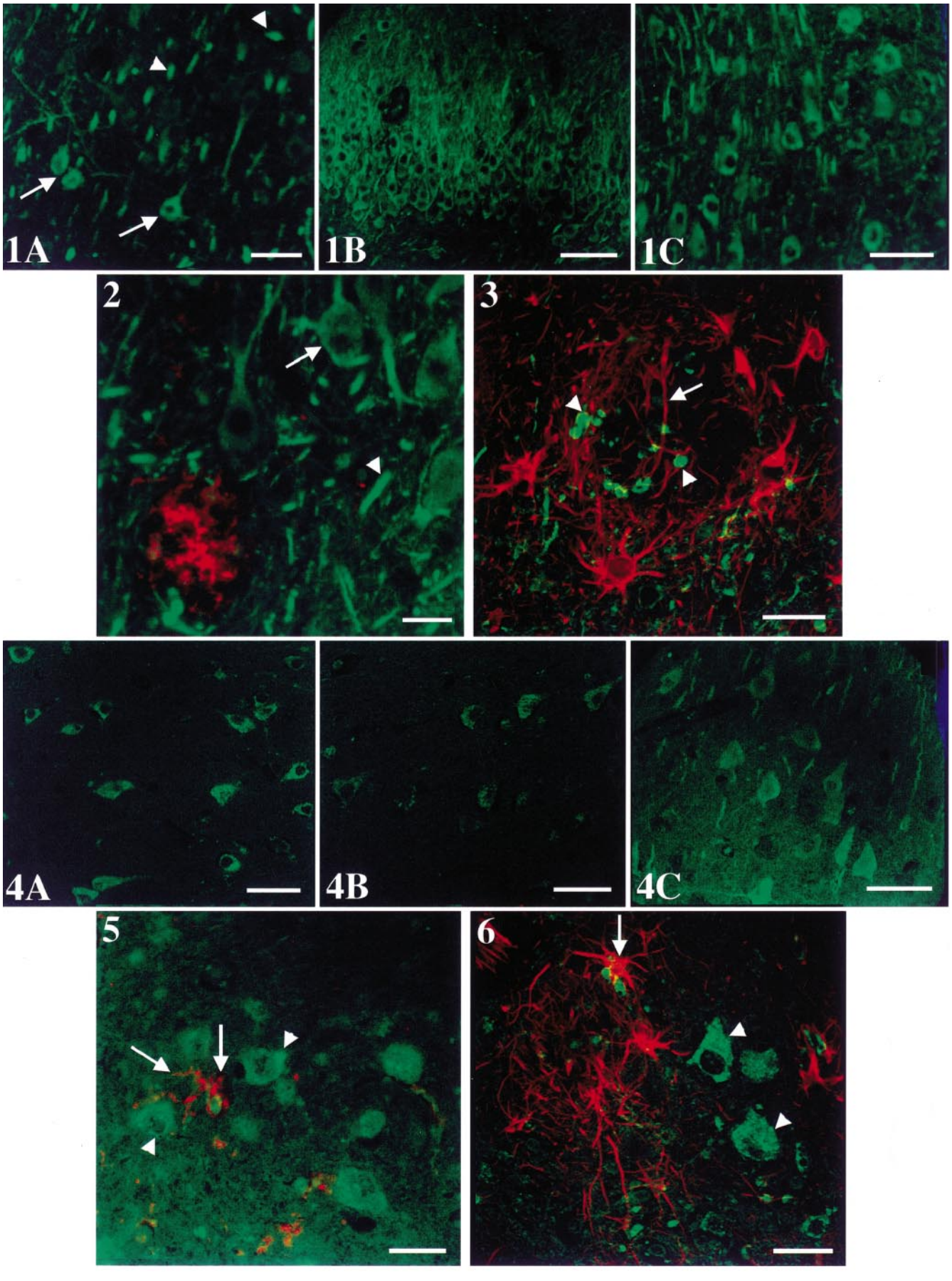
Fig. 2 Double-immunofluorescence staining for BDNF *(green)* and HLA-DR *(red)* of the hippocampal CA1 region from an AD brain. HLA-DR-labeled microglia predominantly cluster within a senile plaque core. Confocal microscopy shows that microglia are not immunoreactive for BDNF. Note BDNF immunoreactivity in pyramidal neurons *(arrow)* and in fiber profiles *(arrowhead)*. *Bar* = 20 μm

Fig. 3 Double immunofluorescence staining for BDNF *(green)* and GFAP *(red)* of the frontal cortex from an AD brain. GFAP-labeled astrocytes gather around a senile plaque core and extend their processes into the plaque core *(arrow)*. Confocal microscopy shows that astrocytes are not immunoreactive for BDNF. Plump BDNF-immunoreactive structures in the plaque probably represent abnormal neurites *(arrowhead)*. Note that thin lines of yellow fluorescence represent superimposed signals, not co-localization (distinguishable by optical sectioning) (*GFAP* glial fibrillary acidic protein). *Bar* = 20 μm

Fig. 4 A–C Immunofluorescence staining for catalytic trkB receptor protein *(green)*. **A, B** The frontal cortex from a non-AD control brain shows that trkB immunoreactivity in subpopulations of neuronal perikarya in the cortical layer 5 (**A**) is more intense than in the layer 3 (**B**). **C** Most pyramidal cells of the hippocampal CA4 region from a control brain are immunoreactive for trkB (*trkB* tyrosine kinase B). *Bars* **A–C** = 50 μm

Fig. 5 Double-immunofluorescence staining for trkB *(green)* and HLA-DR *(red)* of the frontal cortex from AD brain. Confocal microscopy shows that HLA-DR-labeled microglia are not immunoreactive for trkB *(arrows)*. Note trkB immunoreactivity in pyramidal neurons *(arrowheads)*. *Bar* = 20 μm

Fig. 6 Double-immunofluorescence staining for trkB *(green)* and GFAP *(red)* of the frontal cortex from an AD brain. Confocal microscopy shows that GFAP-labeled astrocytes associated with a senile plaque *(arrow)* are not immunoreactive for trkB. Pyramidal neurons show trkB immunoreactivity *(arrowheads)*. Note that thin lines of yellow fluorescence *(arrow)* represent superimposed signals, not co-localization (distinguishable by optical sectioning). *Bar* = 20 μm


1A
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layers 5–6 (Fig. 4 A) generally showed more intense trkB IR than did the neocortical layers 2–3 (Fig. 4 B). In the hippocampus, trkB IR was observed in most granule cells of the dentate gyrus and most pyramidal cells of CA2–CA4 (Fig. 4 C). Pyramidal cells of the CA1 and subiculum were weakly and less often immunoreactive for trkB. There was no consistent difference in neuronal trkB IR patterns observed in individual brain regions examined between AD and control brains, except for less neuronal density in AD brains.

In AD brains within and in between senile plaques, microglia labeled with either RCA-1 or HLA-DR (Fig. 5) and GFAP-labeled astrocytes (Fig. 6) did not show trkB IR, nor did those cells in control brains.

Discussion

Microglia in vitro can both express and respond to neurotrophins [5, 10]. Reactive astrocytes can express catalytic trkB receptors following chronic neural injury [7, 11]. In our recent study of HIV-1 encephalitic brains, activated microglia were shown to express BDNF protein, while reactive astrocytes expressed trkB receptor protein [18]. This observation has suggested the existence of local neurotrophic regulation between different glial populations in the vicinity of neural injury.

In AD, microglia and astrocytes are intimately associated with senile plaques [4]. It is possible that these glial cells express neurotrophins and their receptors, which may locally affect the complex interplay between glial cells. Recent studies of AD showed increased expression of hepatocyte growth factor protein in glia [6, 19]. In the present study of brains with advanced-stage AD (Braak and Braak stage VI) using double IF confocal microscopy, we did not find evidence of BDNF or trkB protein expression in microglia or astrocytes within and in between senile plaques. The difference in glial expression of BDNF and trk B proteins between AD and HIV-1 encephalitis suggests this aberrant expression may be characteristic of particular disease processes, rather than merely representing a stereotyped response to any type of neural injury. As the present study was limited to end-stage AD, it would be interesting to delineate the glial expression of neurotrophins and their receptors in various stages of AD development.

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