BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells?

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Summary
Recent immunohistological and imaging studies emphasize the crucial role of axonal injury in determining the extent of permanent neurological deficits in patients with multiple sclerosis. We have recently shown that human immune cells are capable of producing the neurotrophin brain-derived neurotrophic factor (BDNF), which can prevent axonal and neuronal damage after various pathological insults. BDNF imported into the CNS by immune cells would thus be an attractive candidate for mediating neuroprotective effects in multiple sclerosis. The aim of the present study was to perform a detailed immunohistochemical analysis of the expression of BDNF and its receptor truncated trkB tyrosine kinase receptor (gp145trkB) in a series of multiple sclerosis brain lesions. Our data show that various types of neurones throughout the brain are BDNF immunopositive in multiple sclerosis patients as well as in controls. Furthermore, in multiple sclerosis lesions, BDNF is primarily present in immune cells (T cells, macrophages/microglia) and reactive astrocytes. The number of BDNF immunopositive cells correlates with lesional demyelinating activity. The BDNF receptor gp145trkB is found in neurones in the immediate vicinity of multiple sclerosis plaques as well as in reactive astrocytes within the lesion, but not in immune cells. Our results demonstrate that both BDNF and gp145trkB are expressed in multiple sclerosis lesions. This suggests that BDNF and gp145trkB are involved in immune-mediated neuroprotective interactions in multiple sclerosis, and supports the concept that immune cells produce both damaging and protective factors in multiple sclerosis lesions.

Keywords: autoimmune disease; multiple sclerosis; neuroprotection; neurotrophic factors

Abbreviations: BDNF = brain-derived neurotrophic factor; CLA = common leucocyte antigen; gp95trkB = truncated trkB tyrosine kinase receptor; gp145trkB = full-length trkB tyrosine kinase receptor; NGF = nerve growth factor; NT = neurotrophin; trkB = trkB tyrosine kinase receptor

Introduction
Multiple sclerosis is the major inflammatory demyelinating disease of the CNS that may lead to severe neurological deficits (Lassmann et al., 1998). The mechanisms underlying these chronic deficits are only incompletely understood. However, there is evidence, which is mainly derived from neuroimaging studies, that the extent of permanent disability correlates with the loss of neuronal elements rather than with inflammatory activity (De Stefano et al., 1998; van Waesberghe et al., 1999). Recently, substantial axonal damage with axonal transections and spheroid formation in multiple sclerosis lesions has been described (Ferguson et al., 1997; Trapp et al., 1998). Furthermore, clinical and MRI studies report cortical dysfunction and brain atrophy in multiple sclerosis patients further suggesting secondary
damage to neurones in the course of multiple sclerosis (Davie et al., 1995; Jeffery et al., 2000; Rovaris et al., 2000). In line with these findings, a recent study demonstrates a pronounced loss of retinal ganglion cells in a rat model of optic neuritis (Meyer et al., 2001). However, the majority of neurones and a significant number of axons seem to be preserved. It is unclear so far which factors govern the demise and preservation of neurones and their axons in inflammatory CNS diseases.

Inflammation is considered to be the key feature in multiple sclerosis pathogenesis (Lassmann et al., 1998). The neurotoxic properties of inflammation are well established and thought to be at least partially responsible for the observed axonal damage (Piani et al., 1992; Brosnan and Raine, 1996; Bitsch et al., 2000; Gimsa et al., 2000). However, a number of recent studies have proposed that autoimmune inflammation can have a neuroprotective role in the CNS (Moalem et al., 1999; Hammarberg et al., 2000; Hauben et al., 2000). The factors involved in immune-mediated neuroprotection remain to be clarified. Members of the nerve growth factor (NGF) neurotrophin family such as NGF, brain-derived neurotrophic factor (BDNF), neurotrophin (NT)-3 and NT-4/5 are important regulators of neuronal death and survival (Levin and Barde, 1996). Besides their well-established role in neuronal development, they have the capability to protect neurones against various pathological insults (Cheng and Mattson, 1991; Frim et al., 1994; Lindvall et al., 1994; Holtzman et al., 1996). Notably, BDNF can rescue CNS neurones, e.g. spinal and bulbar motor neurones, after axonal transection, and can promote axonal preservation and regeneration (Weibel et al., 1995; Gravel et al., 1997; Kobayashi et al., 1997).

Traditionally, neurones have been considered the major cellular source of BDNF in the CNS (Hofer et al., 1990; Levin and Barde, 1996). Recently, we and others have been able to demonstrate the production of substantial amounts of BDNF in immune cells (Besser and Wank, 1999; Braun et al., 1999; Kerschensteiner et al., 1999). Preliminary observations have shown that immune cell-derived BDNF can be detected in human immune-mediated CNS inflammation. BDNF binds two different types of receptors (Bothwell, 1995): the tyrosine kinase receptor B (trkB), which exists in two isoforms—the full-length receptor (gp145trkB) and the truncated receptor (gp95trkB) lacking the tyrosine kinase domain (Klein et al., 1990, 1991)—and the p75 neurotrophin receptor. Most, if not all, known BDNF functions are transmitted via the full-length gp145trkB receptor (Bothwell, 1995). Furthermore, it is known that gp145trkB is upregulated after brain insults (Frisen et al., 1992; Merlio et al., 1993; Kobayashi et al., 1997). BDNF and gp145trkB, therefore, are attractive candidates for mediating neuroprotective effects in multiple sclerosis.

The focus of the present study was to further characterize the cellular sources of BDNF in multiple sclerosis lesions and to study the expression pattern of its receptor gp145trkB. Our present data show that immune cells are a major source of BDNF in multiple sclerosis lesions. Furthermore, in actively demyelinating lesions, a higher percentage of BDNF-immunoreactive cells is detected compared with inactive lesions. Additionally, neurones in the direct vicinity of active multiple sclerosis lesions, as well as reactive astrocytes in the lesions, express high levels of gp145trkB. These observations underscore the potential importance of BDNF-mediated neuroimmune interactions in multiple sclerosis.

**Material and methods**

**Tissue**

Formalin-fixed and paraffin-embedded autopsy brain tissue from nine multiple sclerosis patients [age 43.9 ± 11.3 years (mean ± standard deviation)] (Table 1) was examined. Five control brains were included in this series from patients without evidence of neurological disease or neuropathological alterations (age 56 ± 13.9 years). Clinical and pathological data of the multiple sclerosis cases are summarized in Table 1. Routine neuropathology (haematoxylin and eosin, luxol fast blue, periodic acid–Schiff-reaction and Bielschowsky silver impregnation) and immunohistochemistry for myelin proteins (myelin basic protein, proteolipid

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age (years)/sex</th>
<th>Disease duration</th>
<th>Disease course</th>
<th>Demyelinating activity of the lesions examined (% of LFB+CD68+ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48/F</td>
<td>n.a.</td>
<td>n.a.</td>
<td>A (99.2)/IA (2.6)</td>
</tr>
<tr>
<td>2</td>
<td>45/M</td>
<td>21 d</td>
<td>Acute</td>
<td>A (94.7)/IA (5.6)</td>
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<td>3</td>
<td>30/M</td>
<td>6 a</td>
<td>SP</td>
<td>A (63.9)/IA (0)</td>
</tr>
<tr>
<td>4</td>
<td>33/F</td>
<td>10 a</td>
<td>SP</td>
<td>IA (0)</td>
</tr>
<tr>
<td>5</td>
<td>53/F</td>
<td>21 a</td>
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<td>IA (0)</td>
</tr>
<tr>
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<td>66/F</td>
<td>n.a.</td>
<td>n.a.</td>
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</tr>
<tr>
<td>7</td>
<td>34/F</td>
<td>13 a</td>
<td>SP</td>
<td>IA (0)</td>
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<tr>
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</tr>
<tr>
<td>9</td>
<td>46/M</td>
<td>3 mo</td>
<td>Acute</td>
<td>A (n.a.)/IA (n.a.)</td>
</tr>
</tbody>
</table>

SP = secondary progressive multiple sclerosis; A = active lesion; IA = inactive lesion; n.a. = not available; in parentheses: percentage of LFB+ cells per CD68+ macrophages/microglia.
protein, myelin oligodendrocyte glycoprotein, cyclic nucleotide phosphodiesterase and myelin-associated glycoprotein) and immune cells [common leucocyte antigen (CLA): leucocytes; CD68: macrophages/microglia; CD3: T cells; Ig (immunoglobulin): plasma cells] were performed for diagnostic purposes. Lesional demyelinating activity was classified according to the presence of luxol fast blue-positive myelin degradation products in macrophages/microglia indicating ongoing demyelination. The percentage of luxol fast blue-positive macrophages/microglia (CD68+ cells) was calculated for each lesional area (Table 1).

Immunohistochemistry
Immunohistochemistry was performed applying antibodies against BDNF (4.FL.1A1; \(c = 10\, \mu\text{g/mL}\), IgG1; kindly provided by R. Kolbeck and Y.-A. Barde, Martinsried, and I. Bartke, Penzberg, Germany) and trkB [TrkB (794), cat sc-12, rabbit polyclonal, \(c = 0.2\, \mu\text{g/ml}\); Santa Cruz Biotechnology, Santa Cruz, Calif., USA]. The TrkB (794) antibody exclusively recognizes full-length trkB. Briefly, after deparaffinization and blocking with 10% FCS/PBS (foetal calf serum/phosphate-buffered saline), primary antibodies were applied at the concentrations indicated and permitted to bind overnight at 4°C. Control sections were incubated with isotype control antibodies, rabbit serum or without primary antibody. A standard avidin-biotin-peroxidase technique with 3,3′-diaminobenzidine hydrochloride as the chromogenic substrate was used to visualize bound antibody. Alternatively, an alkaline-phosphatase/anti-alkaline-phosphatase-based technique (APAAP; Dako, Glostrup, Denmark) with Fast Red (Sigma, St Louis, Mo., USA) was used. All antibodies were applied after pre-treatment of the sections three times for 5 min in the microwave in citric acid.
buffer, 10 mmol/l, pH 6.0. BDNF-, CD68-, CLA- and CD3-immunoreactive cells were counted in corresponding areas on serial sections. Quantification and photodocumentation were performed on sections stained with the above-mentioned antibodies against BDNF and trkB. The patterns of immunostaining were confirmed applying the anti-BDNF antibody N20 (cat sc-546, 2 μg/ml; Santa Cruz Biotechnology) and the polyclonal anti-trkB T16030-050 (6 μg/ml; Transduction Laboratories, San Diego, Calif., USA), which recognizes full-length and truncated trkB. For multiple sclerosis Cases 1–8, BDNF and trkB immunohistochemistry were performed, whereas from multiple sclerosis Case 9, only sections for trkB immunostaining were available.

**Morphometry**

The number of BDNF-, CLA-, CD68-, CD3- and LFB-positive cells was determined in at least 10 standardized microscopic fields of 10 000 μm² each, defined by an ocular morphometric grid at a total magnification of ×1000. Cells from perivascular inflammatory infiltrates were not included in these counts. Corresponding lesional areas were counted in serial sections. In sections stained for BDNF, cells with astrocytic morphology and neurones were not counted. Cell numbers are given as cells/mm². BDNF-immunoreactive inflammatory cells in perivascular infiltrates were determined separately counting at least 150 perivascular inflammatory cells/case except for Case 6, where only 66 cells were available. Statistical analysis was performed applying the non-parametric Mann–Whitney U-test; a P value of P < 0.05 was considered significant.

**Results**

**BDNF is detected in inflammatory cells in multiple sclerosis lesions**

In the multiple sclerosis patients and control cases examined, BDNF immunoreactivity was detected in various neuronal populations including cortical, hippocampal and brainstem neurones (Fig. 1D and E). No other BDNF-expressing cells were found in control cases. In contrast, in multiple sclerosis plaques a proportion of immune cells was found to contain BDNF immunoreactivity. This was especially pronounced perivascularly, but also found in the lesion parenchyma (Fig. 1A). In Cases 1–3 with actively demyelinating lesions, 48.8 ± 6.6% (mean ± standard deviation) of perivascular inflammatory cells were BDNF⁺, and in Cases 4–8 with inactive lesions 30.6 ± 10.5% were BDNF⁺ (P = 0.07). The immunostaining of serial sections indicated that CD68⁺ macrophages/microglia and CD3⁺ T cells are BDNF⁻ immunoreactive (Fig. 1A–C). Occasional BDNF-immunoreactive plasma cells were seen in the infiltrate. BDNF⁺ inflammatory cells were also detected in other conditions, such as acute disseminated encephalomyelitis and ischaemic brain lesions (C. Stadelmann, M. Kerschensteiner, unpublished results). Reactive astrocytes showed immunoreactivity for BDNF, which was more pronounced in active multiple sclerosis lesions (Fig. 1F). In contrast, no BDNF-immunoreactivity was detected in oligodendrocytes in tissue from multiple sclerosis patients or controls.

**Table 2**

<table>
<thead>
<tr>
<th>Case number</th>
<th>Active area</th>
<th>CLA⁺ cells</th>
<th>% BDNF⁺/CLA⁺</th>
<th>Inactive area</th>
<th>CLA⁺ cells</th>
<th>% BDNF⁺/CLA⁺</th>
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<tr>
<td>1</td>
<td>290</td>
<td>1823</td>
<td>15.9</td>
<td>30</td>
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<tr>
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<td>80</td>
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<td>9.3</td>
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<tr>
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<td>190</td>
<td>1118</td>
<td>17.0</td>
<td>10</td>
<td>425</td>
<td>2.4</td>
</tr>
<tr>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>7</td>
<td>1353</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>20</td>
<td>373</td>
<td>5.4</td>
</tr>
<tr>
<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>60</td>
<td>477</td>
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</tr>
<tr>
<td>7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>9</td>
<td>340</td>
<td>2.6</td>
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<tr>
<td>8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0</td>
<td>935</td>
<td>0</td>
</tr>
</tbody>
</table>

BDNF⁺ cells = BDNF-immunoreactive cells/mm²; CLA⁺ cells = CLA-immunoreactive cells/mm²; % BDNF⁺/CLA⁺ = percentage of BDNF-immunoreactive cells/CLA-immunoreactive cells. Cases 4–8 do not contain active areas; from multiple sclerosis Case 9 only trkB immunostaining was available.

**BDNF expression in inflammatory cells correlates with lesional demyelinating activity**

BDNF-immunoreactive immune cells were detected in lesions with ongoing demyelination as well as in inactive lesions (Table 2). However, comparisons between the numbers of BDNF⁺ cells in actively demyelinating areas and inactive areas in the same lesion (Cases 1–3) reveal that 3.1 ± 1.0% (mean ± standard deviation) of the total number of CLA⁺ immune cells are BDNF⁺, whereas this rises to 14.1 ± 4.2% in actively demyelinating areas (Table 2 and Fig. 2). Analysing the total number of inactive multiple sclerosis lesions (Cases 1–8), 3.8 ± 4.0% of CLA-expressing cells show BDNF-immunoreactivity (two-tailed P = 0.02, Mann–Whitney U-test). However, a single multiple sclerosis
case with an inactive, chronic lesion containing relatively high numbers of BDNF+ cells was observed (Case 6).

In line with previous observations, the composition and cell numbers of infiltrating immune cells were comparable between active and inactive multiple sclerosis lesions. However, actively demyelinating lesions were characterized by a very high percentage of CD68+ macrophages/microglia containing LFB+ myelin degradation products (Table 1). In
all cases, CD68+ macrophages/microglia were the predominant inflammatory cell population in the lesions. In active areas, $1160 \pm 594$ (mean $\pm$ standard deviation) CD68+ cells/mm$^2$ were observed compared with $758.4 \pm 359$ CD68+ cells/mm$^2$ in inactive lesions ($P = 0.38$). In contrast, numbers of CD3+ cells were low in the lesional areas examined, ranging from $31.6 \pm 19.5$ T-lymphocytes/mm$^2$ in inactive areas to $39.2 \pm 30$ T-lymphocytes/mm$^2$ in actively demyelinating areas ($P = 0.8$). Thus, macrophages/microglia are most likely to constitute the predominant BDNF-containing inflammatory cells in the lesions.

**gp145trkB is detected in neurones in the immediate vicinity of multiple sclerosis plaques**

Immunohistochemistry for full-length trkB showed abundant immunoreactivity in various neuronal populations including cortical, hippocampal and brainstem neurones in multiple sclerosis lesions. Immunostaining for full-length trkB showed abundant immunoreactivity in various neuronal populations including cortical, hippocampal and brainstem neurones in multiple sclerosis lesions. Immunostaining for full-length trkB showed abundant immunoreactivity in various neuronal populations including cortical, hippocampal and brainstem neurones in multiple sclerosis lesions.
sclerosis patients as well as in controls (Fig. 3A and B). In multiple sclerosis lesions, reactive astrocytes showed strong, distinct staining for full-length trkB (Fig. 3D). Only rare astrocytic staining was observed in control brains. However, immune cells in the lesions, as well as perivascularly, were consistently negative independent of lesional activity (Fig. 3C). In addition, no immunoreactivity for full-length and truncated trkB was observed in oligodendrocytes in multiple sclerosis cases and controls. In some multiple sclerosis cases, staining for trkB was more abundant in neurones in the immediate vicinity than far away from the lesion, although the irregular distribution of neurones in and close to multiple sclerosis lesions precluded a quantitative evaluation. Immunohistochemistry applying both antibodies against both full-length and truncated trkB revealed a similar staining pattern. Again, immune cells were negative. In some multiple sclerosis lesions, immunoreactivity for trkB was found in neurones immediately adjacent to BDNF+ inflammatory cells.

Discussion

In the present study, we extend our previous observation that immune cells can be a potent source of the neuroprotective factor BDNF in neuroinflammatory disease (Kerschensteiner et al., 1999). We find BDNF-immunoreactivity in T cells and macrophages/microglia in active and inactive multiple sclerosis lesions. Lesions with ongoing demyelination show higher ratios of BDNF+ immune cells. Furthermore, BDNF is found in neurones and astrocytes. The BDNF receptor trkB is present equally in neurones and astrocytes, but not in inflammatory cells or oligodendroglial cells. Our results demonstrate the presence of BDNF and trkB in multiple sclerosis lesions, and suggest possible neurotrophic interactions between infiltrating immune cells and resident cells of the CNS.

The notion that infiltrating immune cells could provide BDNF to support neuronal survival has only emerged recently (Kerschensteiner et al., 1999), and since then has been confirmed by a number of studies (Batchelor et al., 1999; Besser and Wank, 1999; Braun et al., 1999; Hammarberg et al., 2000; Kipnis et al., 2000; Barouch et al., 2001). In this study, we detect a significant number of immune cells containing BDNF, mainly macrophages/microglia and T-lymphocytes in active and inactive multiple sclerosis lesions. We have shown previously that these cells are capable of releasing bioactive BDNF in vitro (Kerschensteiner et al., 1999). A relevant question is whether BDNF is induced by immune cell activation in vivo. In vitro data show an increase of BDNF mRNA and protein after activation of immune cells (Braun et al., 1999; Kerschensteiner et al., 1999; Barouch et al., 2001). In vivoto, an upregulation of BDNF mRNA expression is demonstrated in mouse alveolar macrophages after repeated allergenic challenge (Braun et al., 1999). In the present study, we show that a higher percentage of inflammatory cells in actively demyelinating areas of multiple sclerosis lesions contains BDNF compared with areas without ongoing myelin breakdown. Thus, the observations presented here confirm that in vivo activation also upregulates BDNF in immune cells. In addition, a high percentage of BDNF+ macrophages and lymphocytes is found in perivascular infiltrates. Taken together, these observations indicate that relatively high numbers of BDNF-containing immune cells are present at early stages of lesion development. In general, in chronic inactive lesions, only very few BDNF+ cells are encountered. However, exceptional multiple sclerosis cases contain relatively high numbers of BDNF+ inflammatory cells in inactive lesions. Astrocytes, which have been shown to store and release exogenous BDNF (Rubio, 1997), also show increased BDNF-immunoreactivity in actively demyelinating areas. All these factors result in the highest number of BDNF+ cells being found in the actively demyelinating edge early in the development of a multiple sclerosis lesion, just in the vicinity of axons that are not directly subject to the inflammatory insult, but still at high risk of inflammation-induced bystander damage.

Outside the lesion area neurones are the major sources of BDNF in multiple sclerosis brains as well as in controls. It is well established that BDNF can be transported anterogradely and released by neurones (von Bartheld et al., 1996; Zhou and Rush, 1996; Canals et al., 2001). Notably this anterograde transport of BDNF is upregulated after axotomy (Tonra et al., 1998). Autocrine and paracrine interactions between neurones releasing BDNF and bearing gp145trkB receptors have been reported (Kokaia et al., 1993; Acheson et al., 1995; Marini et al., 1998) suggesting that neuronal BDNF may further enhance the endogenous neurotrophic support in multiple sclerosis lesions. However, we did not find BDNF in dystrophic axonal spheroids of multiple sclerosis lesions. Thus, expression levels in dystrophic axons are possibly below the detection limit of our immunohistochemical methods. This observation further supports the view that inflammatory cells are the major source of BDNF in multiple sclerosis lesions.

In addition to BDNF itself, we characterized the expression pattern of the major BDNF receptor, trkB. Two major species of trkB protein have been described: the full-length gp145trkB, capable of transducing BDNF signalling, and a truncated variant, pg95trkB, which is devoid of an active tyrosine kinase domain (Klein et al., 1990, 1991; Bothwell, 1995). In addition, BDNF, like the other neurotrophins of the NGF family, binds to the p75 neurotrophin receptor. Recent studies have demonstrated that the p75 neurotrophin receptor is primarily expressed in oligodendroctes and oligodendrocyte precursors, as well as macrophages/microglia in multiple sclerosis lesions (Dowling et al., 1999; Chang et al., 2000). As most, if not all, known BDNF functions are transmitted via the the full-length gp145trkB receptor, we have focused on the expression of gp145trkB.

We find a robust expression of gp145trkB in neurones in the immediate vicinity of multiple sclerosis plaques. Single neurones with clearly pronounced trkB-immunoreactivity
close to multiple sclerosis lesions are observed, suggesting an upregulation of trkB in a proportion of damaged neurones. Additionally, we find full-length trkB immunoreactivity in reactive astrocytes within the lesions, as has previously been described for reactive astrocytes in a rodent chronic brain injury model (McKean et al., 1997). Thus far, no relevant function of BDNF signalling in astrocytes has been identified, and so the significance of our observation of full-length trkB expression in astrocytes in multiple sclerosis lesions remains elusive. In contrast to the expression of full-length trkB in neural cells, we were unable to detect full-length trkB in immune cells within multiple sclerosis lesions. This contrasts with in vitro observations that demonstrate full-length trkB mRNA expression in antigen-specific T-cell lines from human donors (Besser and Wank, 1999), an observation that we were able to confirm in individual human T-cell lines. However, until now, we have been unable to find full-length trkB protein in isolated human peripheral immune cells (M. Kerschensteiner, unpublished data). Whether immune cells can or cannot express full-length trkB currently remains an open question, nevertheless, our data clearly show that in neuroinflammatory lesions, autocrine or paracrine BDNF signalling to immune cells via trkB is unlikely to occur. The restriction of trkB expression to neural cell types in multiple sclerosis underscores the possibility that there may be BDNF signalling from infiltrating cells to neurones in neuroinflammatory lesions, as would be necessary for immune cells to support neuronal survival or to provide axonal protection.

The appeal of neurotrophin-mediated neuroprotection in multiple sclerosis lies in the pleiotropy inherent to neurotrophin actions (Levi-Montalcini et al., 1996; Lewin and Barde, 1996). It is well established that BDNF can prevent neuronal cell death after various pathological insults including experimental transection of axons in the spinal cord (Gravel et al., 1997; Kobayashi et al., 1997; Liu et al., 1999). Moreover, BDNF can also protect axons against elimination during development, as well as against degeneration after axotomy or in experimental neurodegenerative diseases (Mitsumoto et al., 1994; Ikeda et al., 1995; Weibel et al., 1995; Sagot et al., 1998). Furthermore, the preservation of axons provides the basis for neuroregenerative attempts including axonal regeneration and sprouting, which are directly supported by BDNF (Kobayashi et al., 1997; Gallo and Letourneau, 1998; Mamounas et al., 2000). Apart from influencing survival and regeneration of neuronal elements, BDNF supports remyelination after both peripheral and CNS injury (McTigue et al., 1998). Finally, BDNF has been shown to downregulate the expression of MHC (major histocompatibility complex) molecules in hippocampal slices, and may thus also act as an immunomodulator (Torcia et al., 1996; Neumann et al., 1998).

The neuroprotective capacity of immune cells is not restricted to BDNF: a panel of other potentially neuroprotective and neurotrophic mediators is released by immune cells, e.g. NGF, NT-3, transforming growth factor β and platelet-derived growth factor (Ehrhard et al., 1993; Blotnick et al., 1994; Diemel et al., 1998; Letterio and Roberts, 1998; Hammarberg et al., 2000; Kipnis et al., 2000). Moreover, immune-derived cytokines have been demonstrated to exert neuroprotective effects (Cheng et al., 1994; Bruce et al., 1996). This is relevant in the context of observations showing that neuroinflammation directed to sites of traumatic injuries to the optic nerve (Moalem et al., 1999) or the spinal cord (Hammarberg et al., 2000; Hauben et al., 2000) can protect neurones from secondary degeneration. Moreover, immune cells infiltrating spontaneously into sites of neural injury seem to protect brainstem neurones after experimental trauma (Serpe et al., 1999) and could potentially do so in neurodegenerative diseases (Batchelor et al., 1999), which show substantial immune activation in foci of pathological change (McGeer and McGeer, 1995).

In summary, we demonstrate here that immune cells, reactive astrocytes and neurones contain BDNF, a potent neuroprotective agent, in multiple sclerosis. Within the multiple sclerosis lesion immune cells seems to be the major source of BDNF. They are likely to release this substance in the immediate vicinity of nerve cell processes, which—according to the trkB expression that we observed—are likely to be responsive to the neuroprotective effects of BDNF. This neurotrophin-mediated neuroimmune signalling network could be a major factor that helps to preserve axons in a microenvironment that is clearly capable of exerting significant neurotoxicity. Thus, it should be considered as a beneficial aspect of neuroinflammation that could be worth preserving therapeutically, or even reinforcing using tailored immunomodulatory treatment strategies (Hohlfeld, 1997; Hohlfeld et al., 2000).

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