Brief Definitive Report

Activated Human T Cells, B Cells, and Monocytes Produce Brain-derived Neurotrophic Factor In Vitro and in Inflammatory Brain Lesions: A Neuroprotective Role of Inflammation?

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Summary

Brain-derived neurotrophic factor (BDNF) has potent effects on neuronal survival and plasticity during development and after injury. In the nervous system, neurons are considered the major cellular source of BDNF. We demonstrate here that in addition, activated human T cells, B cells, and monocytes secrete bioactive BDNF in vitro. Notably, in T helper (Th)1- and Th2-type $CD4^+$ T cell lines specific for myelin autoantigens such as myelin basic protein or myelin oligodendrocyte glycoprotein, BDNF production is increased upon antigen stimulation. The BDNF secreted by immune cells is bioactive, as it supports neuronal survival in vitro. Using anti-BDNF monoclonal antibody and polyclonal antiserum, BDNF immunoreactivity is demonstrable in inflammatory infiltrates in the brain of patients with acute disseminated encephalitis and multiple sclerosis. The results raise the possibility that in the nervous system, inflammatory infiltrates have a neuroprotective effect, which may limit the success of nonselective immunotherapies.

Key words: neurotrophic factors • multiple sclerosis • autoimmunity • immunosuppressive therapy • neurodegeneration

Brain-derived neurotrophic factor (BDNF) was cloned in 1989 (1) as the second member of the neurotrophin (NT) family which includes nerve growth factor (NGF) and NT-3, -4/5, -6, and -7 (2). Since then, the important role of BDNF in regulating the survival and differentiation of various neuronal populations including sensory neurons, cerebellar neurons, and spinal motor neurons has been firmly established (2). Neurons are the major source of BDNF in the nervous system (2, 3). It is thought that BDNF and NT4/5 exert their biological function via the full-length form of *trk*B receptor, called gp145*trk*B (4), expression of which seems to be restricted to neuronal cell populations (5).

Several studies have shown that the therapeutic application of BDNF prevents neuronal degeneration after axotomy and other forms of neuronal injury (6–10). In addition, beneficial effects of BDNF have been reported in animal models of neurodegenerative disease (11). Hence, BDNF is now considered as a potential therapeutic agent for human neurodegenerative disease, e.g., motor neuron disease, although clinical trials have not yet been successful (12). Considering that inflammation is a universal tissue reaction crucial for defense and repair, we asked whether the immune cells accumulating in traumatic, degenerative, ischemic, infectious, and autoimmune lesions of the nervous system might provide a natural imported source of BDNF.

Materials and Methods

Cell Preparations. Ag-specific $CD4^+$ human T cell lines were isolated as described (13). PBMCs were separated into $CD4^+$ T cells, $CD8^+$ T cells, $CD19^+$ B lymphocytes, and $CD14^+$ mono-

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cytes using immunomagnetic beads (Dynal). The purity of the isolated subsets was >98% as determined by flow cytometry for relevant and irrelevant subset markers (not shown).

For proliferation and BDNF production assays, 5×10^5 freshly isolated $CD4^+$ T cells, $CD8^+$ T cells, and B lymphocytes were cultured in RPMI 1640 medium (GIBCO BRL) supplemented with 5% FCS (GIBCO BRL) in 96-well plates (Nunc). After a resting period of 72 h, T lymphocytes were stimulated with 5×10^4 irradiated allogeneic PBMCs and PHA (10 μg/ml; Sigma). B cells were stimulated with heat-inactivated *Staphylococcus aureus* Cowan strain 1 (SAC; Calbiochem) at a final dilution of 1:7,500. Monocytes were stimulated in the course of purification using anti-CD14–coated magnetic beads. Ag-specific T line cells (10^5) were cultured in RPMI 1640 medium supplemented with 5% FCS plus irradiated autologous or HLA-matched PBMCs and the relevant Ag (10–30 μg/ml). Proliferation was assessed by [^{3}H]thymidine uptake. Supernatants were removed at different time intervals after stimulation and analyzed for BDNF protein concentration and bioactivity. BDNF production by APCs was measured in separate wells and subtracted. The cytokine profile of $CD4^+$ T cell lines was assessed by intracellular flow cytometry staining according to the manufacturer's protocol (PharMingen).

Reverse-transcription PCR Analysis of BDNF Transcription. Total cellular RNA was extracted using two different RNA extraction systems (QIAGEN, and WAK Chemie). The RNA (0.5–2 μg) was transcribed with random hexamer primers (Boehringer-Mannheim) or oligo(dt) primers (GIBCO BRL), dNTP (MBI Fermentas), and Superscript™ Reverse Transcriptase (GIBCO BRL). All reactions were carried out in a total volume of 25 μl containing 1 U *Taq* polymerase (QIAGEN), 200 μM of each dNTP, cDNA, and 15 pmol of each primer for 35 or 40 PCR cycles with annealing at 60°C. The correct size of the bands was determined by comparison with a DNA mass standard (pUC MIX 8; MBI Fermentas). RNA samples transcribed in the absence of reverse transcriptase were used as negative controls to exclude genomic contamination. All PCR products were sequenced (Medigene). The primer sequences were as follows: β-actin forward: 5′-CGAGCGGGAAATCGTGCGTGA-3′ (position 622–642); β-actin reverse: 5′-CAGCGAGGCCAGGATGGAGCC-3′ (position 1057–1037); BDNF forward: 5′-AGCGTGAATGGGCCCAAGGCA-3′ (position 208–228); BDNF reverse 5′-TGTGACCGTCCCGCCCGACA-3′ (position 570–551).

Quantification of BDNF Protein Secretion. BDNF protein concentration was determined with a sensitive sandwich ELISA. In brief, 96-well flat-bottomed plates (Immulon 4; Dynatech) were coated with a chicken anti–human BDNF Ab (Promega). Recombinant human BDNF (used as standard; Research Diagnostics, Inc.) and cell supernatants (all made up in RPMI, 5% FCS plus 0.05% Tween 20 [Sigma]) were used in serial dilutions. Bound BDNF was detected by incubating the plates with a mouse anti–human BDNF Ab (Research Diagnostics, Inc.) followed by peroxidase-labeled goat anti–mouse IgG (Dianova). The plates were developed using a liquid substrate system (Sigma), and the OD was determined at 450 nm. For enhanced detection of BDNF in biological samples, the cell supernatants were acid-treated for 15 min before the assays. This treatment did not change detection of the standard. Specificity of the BDNF ELISA was established using recombinant BDNF and control neurotrophic factors. The sensitivity of this ELISA ranges from 20 to 40 pg/ml. Quantification of BDNF was confirmed by a second highly specific ELISA.

Assay for BDNF Bioactivity. Biological assays were performed using dissociated sensory neurons prepared from nodose ganglia of 8-d-old chick embryos (14). Neurons were plated in laminin/polyornithine-coated 48-well plastic dishes. Exogenous BDNF (1 ng/ml) and immune cell supernatants were added in the presence or absence of the anti-BDNF mAb 4.D3.3A3, which neutralizes the biological activity of BDNF. After 24 h, the surviving neurons were counted using light microscopy. For each experiment, duplicate wells were evaluated.

Immunohistochemical Analysis of Brain Sections. Immunohistochemistry was performed on formalin-fixed, frozen, and paraffin-embedded human brain tissue using a mouse IgG1 mAb against BDNF (4.FL.1A1; 10 μg/ml) and a polyclonal anti-BDNF antiserum (N-20, used at 1:500 dilution; Santa Cruz Biotechnology) (15). Two actively demyelinating cases of multiple sclerosis, one case of acute disseminated (postinfectious) leukoencephalitis, and two controls without neurological disease were examined. Control sections were incubated without primary Ab or with control IgG1 Ab. Serial sections were labeled for CD3 (1:300; Serotec), CD68 (1:100; Dako), or human IgG (1:200; Amersham Pharmacia Biotech), using an avidin-biotin peroxidase method.

Results

T Cells, B Cells, and Monocytes Transcribe BDNF mRNA and Secrete BDNF Protein. BDNF was detected in $CD4^+$ T cells, $CD8^+$ T cells, B cells, and monocytes (Fig. 1, a and b). $CD4^+$ T cells and $CD8^+$ T cells were stimulated with PHA and B cells with SAC. Monocytes were stimulated in the course of purification with anti-CD14–coated magnetic beads. Fig. 1 b shows the BDNF concentrations in supernatants of stimulated and nonstimulated cells after 72 h. Constitutive BDNF production was observed in T cells and B cells (white bars in Fig. 1 b). All cell types pro-

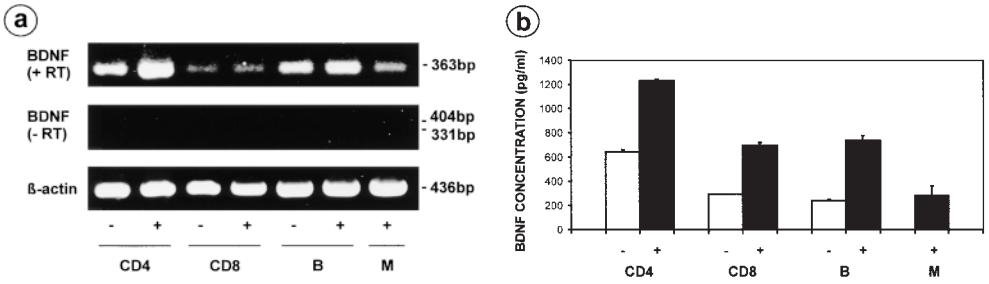

Figure 1. BDNF transcription (a) and protein secretion (b) by stimulated (+) and nonstimulated (−) human immune cells. (a) RT-PCR analysis of BDNF transcripts. RNA was extracted with RNAzol B (WAK Chemie) after 24 h of culture and reverse-transcribed with oligo(dt) primers. The middle lane represents control without reverse transcriptase (−RT). (b) BDNF protein secretion measured in supernatants after 72 h of culture. White bars (−), nonstimulated cells; black bars (+), stimulated cells (see Materials and Methods). Bars indicate mean of duplicates; vertical lines indicate individual measurements. Lymphocyte proliferation was measured in parallel (not shown). Stimulation indices, measured by [^{3}H]thymidine incorporation, were >5 in all samples. B, B cells; M, monocytes.

Table I. *BDNF Production by Ag-specific CD4⁺ T Cell Lines*

TCL	Antigen	IFN⁺	IFN⁺/IL-4⁺	IL-4⁺	BDNF, −Ag	BDNF, +Ag	SI
		%	%	%	pg/ml	pg/ml	
CABP1	MBP	98	1	1	11	621	13
HKBP	MBP	100	0	0	239*	1,320*	ND
TNMOG6	MOG	99	1	0	8*	960*	ND
CLBP3	MBP	61	8	31	220	876	172
HWβ70	rTCR	59	12	29	0*	370*	ND
HWPPD	PPD	24	36	40	658	1,096	131
HWTT	TT	31	38	31	1,364	1,003	6
MKTT11	TT	7	9	84	0	100	5
MKB6	rPhl	3	20	77	0	356	33
MKB7	rPhl	2	14	84	0	88	687
CLBP7	MBP	2	2	96	482	1,154	471
CLBP14	MBP	0	2	98	158	920	892
CLBP16	MBP	2	4	94	62	977	393

Columns 1 and 2 list T cell line (TCL) identification code and Ag specificity (MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; rTCR, recombinant T cell receptor β chain; TT, tetanus toxoid; PPD, purified protein derivate of *Mycobacterium tuberculosis*; rPhl, recombinant *Phleum pratense*). Columns 3–5 indicate the cytokine profile as determined by intracellular double-staining for IFN-γ (IFN) and IL-4 and FACS® analysis (percentage of stained cells). Columns 6 and 7 show BDNF concentrations as measured by ELISA in supernatants of nonstimulated (−AG) and Ag-stimulated (+AG) T cell lines after 72 h (*after 24 h). Column 8 lists Ag-induced stimulation measured by [³H]thymidine incorporation between 48 and 72 h (SI, stimulation index).

duced enhanced levels of BDNF after stimulation (black bars in Fig. 1 b). Reverse-transcription (RT)-PCR analysis showed BDNF transcripts in T cells, B cells, and monocytes (Fig. 1 a).

BDNF was also produced by Ag-specific CD4⁺ T cell lines of different Ag specificity and cytokine profile (Table I). Ag-induced BDNF secretion was observed both in Th1- and in Th2-type cells (Table I).

Immune Cell–derived BDNF Supports Neuronal Survival In Vitro. Bioactivity of the BDNF produced by human immunocompetent cells was assessed by measuring the ability to rescue cultured sensory neurons from cell death (14). CD4⁺ T cells, CD8⁺ T cells, B cells, and monocytes were stimulated with PHA, SAC, or anti-CD14–coated magnetic beads, respectively. After 96 h, the supernatants were collected and added to chicken embryonic nodose ganglia neurons to determine the neuronal survival rate (Fig. 2). Neuronal survival was supported by externally supplied recombinant BDNF and by supernatants of activated CD4⁺ T cells, CD8⁺ T cells, B cells, and monocytes (black bars in Fig. 2). The promotion of neuronal survival by immune cell supernatant was due to biologically active BDNF as demonstrated by inhibition with anti-BDNF mAb 4.D3.3A3, which blocks the biological activity of BDNF (white bars in Fig. 2).

BDNF Is Expressed by Inflammatory Cells in Acute Disseminated Encephalitis and Multiple Sclerosis. Strong BDNF immunoreactivity was observed in inflammatory cells forming perivascular infiltrates in cases of disseminated (postinfectious) leukoencephalitis and multiple sclerosis (Fig. 3). Using serial sections, BDNF⁺ cells were found to correspond to infiltrating mononuclear cells (Fig. 3). In multiple sclerosis, BDNF⁺ lymphocytes and macrophages were not restricted to the perivascular localization but were found throughout the lesion (Fig. 3 g). Lesional areas with high numbers of macrophages actively involved in demyelination showed enhanced BDNF immunoreactivity. In

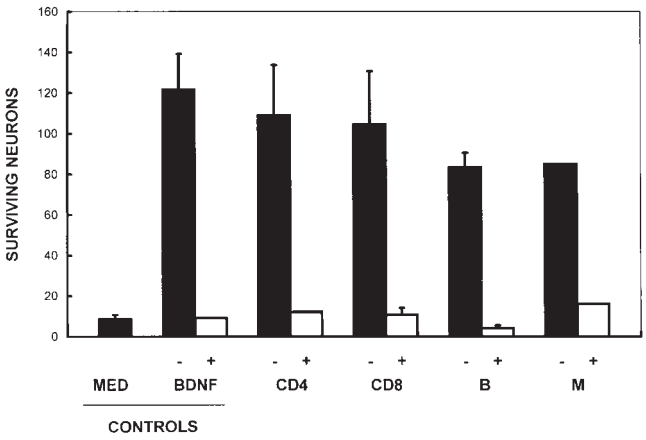

Figure 2. Bioactivity of immune cell–derived BDNF. Chicken nodose ganglia neurons were cultured in the presence of supernatants (diluted 1:2–1:16) of activated CD4⁺ and CD8⁺ T cells, B cells (B), and monocytes (M). Results are shown only for the 1:2 dilution. The neuronal cell assays were done in the presence (white bars, +) or absence (black bars, −) of the neutralizing anti-BDNF mAb 4.D3.3A3 (4 μg/ml). Medium (Med) alone served as the negative control and exogenous BDNF (1 ng/ml) as the positive control. Bars indicate mean of duplicates; vertical lines indicate individual measurements.

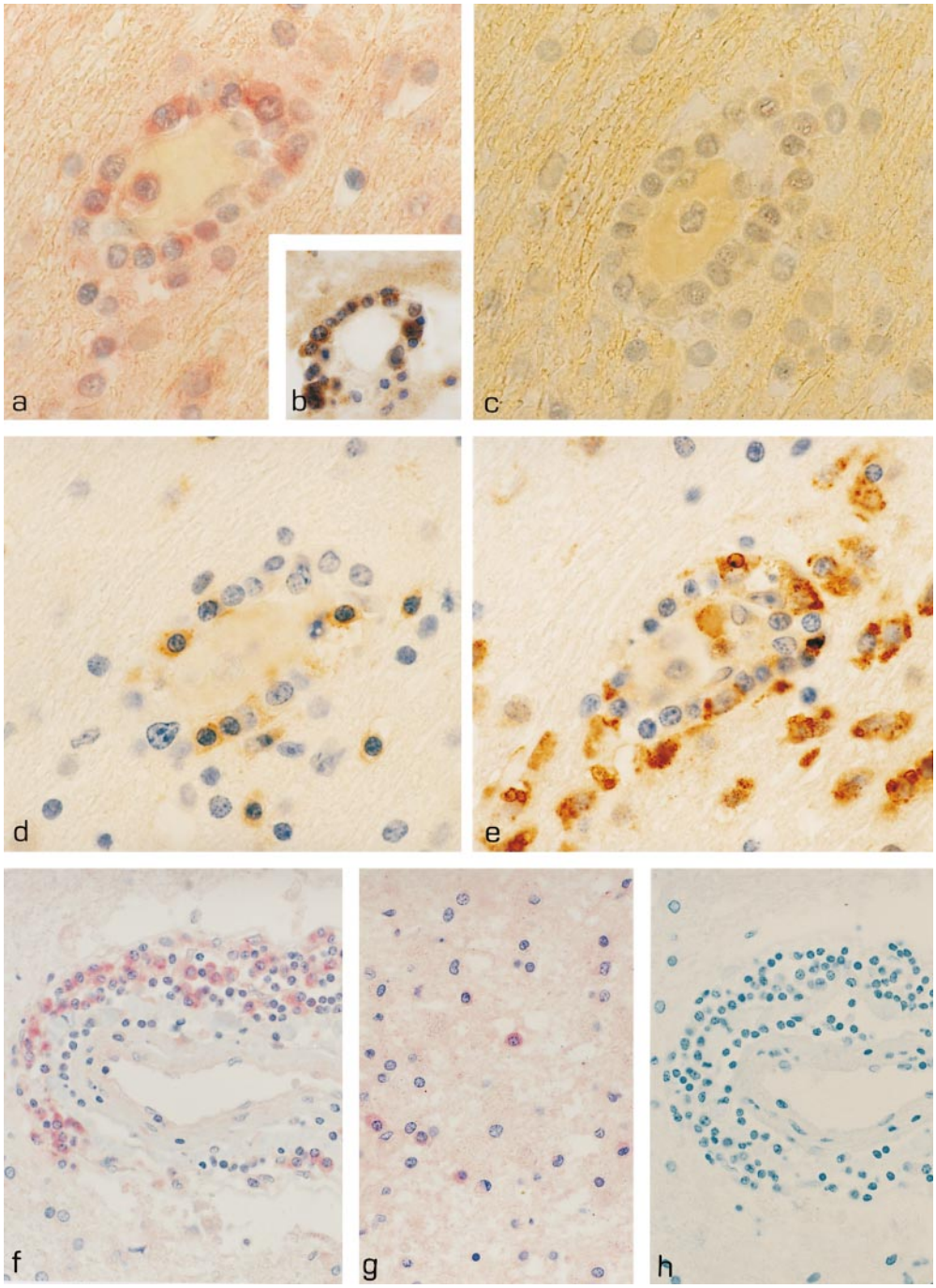

Figure 3. Expression of BDNF in inflammatory cells in acute disseminated (postinfectious) leukoencephalitis and multiple sclerosis. (a, c–e) Serial sections of a perivascular inflammatory infiltrate in postinfectious leukoencephalitis (original magnification: ×1,000): (a) immunolabeling of BDNF (mAb); (c) negative control without primary Ab; (d) CD3-labeled T cells; (e) $CD68^+$ macrophages; (b) inflammatory cells in a cryostat section from the same patient stained with a polyclonal anti-BDNF antiserum (original magnification: ×500). (f–h) BDNF immunoreactivity in multiple sclerosis (original magnification: ×450): (f) in inflammatory cells forming a perivascular infiltrate, and (g) in inflammatory cells invading the plaque area; (h) negative control.

inflammatory central nervous system disease and healthy controls, BDNF was also detected in various types of neurons, ependymal cells, and weakly in astrocytes. No immunoreactivity was observed in oligodendrocytes or ramified microglial cells. Similar staining patterns were observed with the monoclonal and polyclonal Abs.

Discussion

We demonstrate that human immune cells express the neurotrophic factor BDNF both in vitro and in inflammatory brain lesions. BDNF is one of the most potent factors supporting neuronal survival and regulating neurotransmitter release and dendritic growth (2, 16, 17). Several studies have shown that the administration of BDNF protein or the BDNF gene can rescue injured or degenerating neurons and induce axonal outgrowth and regeneration (6–10). Furthermore, BDNF had beneficial effects in several animal models of neurodegenerative diseases (11). Difficulties in delivering sufficient amounts of BDNF to the site of central nervous system lesions have so far hampered the successful application of BDNF for treatment of human diseases (12).

One promising strategy for the delivery of neuroprotective factors relies on retroviral transduction of neurotrophic factors into T cell lines specific for Ag expressed in the nervous system (18). Our present results indicate that this experimental strategy has a natural counterpart: T cells and other immune cells homing to degenerative, infectious, or autoimmune lesions express a potent neurotrophic factor, BDNF, that may help to minimize neuronal damage. Consistent with this hypothesis, it has recently been demonstrated that the implantation of activated macrophages into experimental spinal cord lesions leads to partial functional recovery of paraplegic rats (19). In addition to its neurotrophic effect, locally produced BDNF could have an immunomodulatory function, e.g., by indirectly downregulating MHC class II expression on microglia (20).

NT-mediated effects on immune cells have previously been demonstrated for nerve growth factor (21). Thus far, however, we found no evidence that BDNF acts on peripheral human immune cells. This could be explained by the fact that immune cells express only the truncated form of the *trk*B BDNF receptor (our unpublished observations; consistent with previous reports that the expression of the full-length, signal-transducing form gp145*trk*B is restricted to neuronal cells, whereas the truncated gp95*trk*B form is widely expressed in nonneuronal tissues [5]). Further, we did not detect significant effects of BDNF on the proliferation, cytokine production, or apoptosis of human lymphocytes (our unpublished data). Clearly, these negative observations by no means rule out that BDNF has a role in the immune system, and the question of whether BDNF can affect elements of the immune system deserves more detailed study.

BDNF production by immune cells provides a novel example of the bidirectional interaction between the nervous system and the immune system (22, 23). It is noteworthy that upon stimulation with Ag a large proportion of immune cells, including $CD4^+$ T cell lines specific for brain autoantigens, produce BDNF. Thus, in some circumstances, the immune infiltrates commonly found in inflammatory, ischemic, degenerative, and traumatic lesions of the nervous system may have a protective rather than destructive role. This could help to explain the presence of large numbers of autoreactive T lymphocytes in the healthy immune repertoire, a phenomenon invoked by Cohen's concept of the "immune homunculus" (24).

In conclusion, our demonstration that activated T cells, B cells, and monocytes produce BDNF in vitro and in inflammatory lesions raises the intriguing possibility that neuroinflammatory reactions have a neuroprotective (side) effect. This has obvious therapeutic implications for multiple sclerosis and other inflammatory diseases of the nervous system (25). Production of both neurotoxic and neuroprotective factors emphasizes the complex role of immune cells in inflammatory, degenerative, and regenerative processes of the nervous system.

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