NF-κB Regulates B-Cell-Derived Nerve Growth Factor Expression

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In the mammalian brain, four neurotrophins have been identified: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5). NGF exerts an important role in the development and functions of the central and peripheral nervous system. However, it has recently been documented that several types of immune cells, such as mast cells, lymphocytes, basophils and eosinophils, produce, store and release NGF. Accumulating preclinical and clinical data indicate that dysfunctions of NGF and the other neurotrophins may contribute to impaired immune responses and concentration of NGF frequently correlates with disease severity. Thus, the aim of this study was to elucidate the potential signaling mechanisms of cytokine-neurotrophins interactions contributing to increased NGF levels. Our data show that the transcription factor NF-κB plays a pivotal role in regulating B-cell-derived NGF expression. *Cellular & Molecular Immunology*. 2006;3(1):63-66.

**Key Words:** immune system, inflammation, interleukin, NGF, neurotrophin

**Introduction**

Neurotrophins are a family of growth factors critical for the development and function of the nervous system. Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) are included in this family. Although originally identified as neuronal survival factor, NGF is capable of exerting a far broader spectrum of biological activities (1). It has been documented that NGF is released by several types of immune cells such as mast cells, lymphocytes, basophils and eosinophils. NGF receptors are widely expressed in the immune system, indicating the possibility that the immune system responds to this neurotrophin through an autocrine mechanism. Heightened levels of NGF have been reported in patients with allergies, systemic lupus erythematosus, systemic sclerosis and chronic autoimmune arthritis (2, 3). Elevated levels of NGF found in sera and fluids of patients affected by autoimmune diseases could be explained by an increased production of NGF by immuno-competent B and T cells. Activation of these cells in response to antigens or cytokine stimulation could be the cause of this enhanced production. Data have suggested that NGF might be viewed as a Th2-like cytokine with a regulative role in inflammation and tissue remodeling and NGF might represent a clinical marker of inflammation (3).

Expression of neurotrophins in T cells, however, remains a controversial issue. A recent report indicates that B cells rather than T cells are the predominant if not the only source of leukocyte-derived NGF and BDNF and as such may provide "protective autoimmunity" in repair and regeneration of the injured nervous system (4-6). BDNF plays a pivotal role in B cell development. In BDNF-/- mice, maturation of B lymphocytes is specifically blocked at the pre-BII stage (7). Activated B cells release BDNF (5-7) and express the functional truncated form of the BDNF-receptor. B cells also respond to NGF by increasing their proliferation rate and NGF is essential for the survival of memory B cells (1, 3, 8). Thus, there might be a cross-talk between NGF and BDNF during B cell activation and BDNF may have autocrine functions under certain conditions.

It is known that B cells are able to produce cytokines such as IL-4 and IL-6 in an autocrine fashion. The activation of human B lymphocytes has been worked out in some detail affected by autoimmunity.
and has been shown to be dependent on the synergistic effect of IL-4 and CD40 cross-linking (9).

CD40 is a member of the tumor necrosis factor (TNF) receptor family and the development of the acquired immune response is dependent on bi-directional signaling mediated by CD40 and its ligand CD154 (CD40L), which is primarily expressed on activated CD4+ T cells. Specifically, CD40 signaling is essential for activation of B cells by T cell-dependent antigens, germinal center formation, immunoglobulin (Ig) class switching, affinity maturation of B lymphocytes, and the development of plasma and memory B cells (10). Various analyses have shown that CD40L induces all NF-κB/Rel proteins, whereas LPS activates predominantly p50 and c-Rel (11-13).

In view of the important role played by the CD40-, IL-4-, NGF- and BDNF systems in B cell function and based on our previous reports about NGF activation in other cellular systems in the CNS (14-16), the present study aimed at getting insight into the potential role of BDNF and the transcription factor NF-κB on various B cell activators in regulating B-cell-derived NGF expression.

Materials and Methods

Animals

All experiments were carried out according to the guidelines of the Japanese Community’s Council for Animal Experiments and conformed to international guidelines on the ethical use of animals. All animal protocols were approved by the Institutional Animal Care and Use Committee in compliance with the Public Health Service policy on human care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Isolation of B cells

Twelve-week-old 129SW or three-week-old BDNF-/- mice (kindly provided by Dr. Hatanaka (Osaka, Japan)) and wildtype littermates were sacrificed, genotyped (17) and spleens were collected. Resting splenic B cells were isolated by depletion of CD43+ cells with magnetic beads by spleens were collected. Resting splenic B cells were isolated (kindly provided by Dr. Hatanaka (Osaka, Japan)) and expressed on activated CD4+ T cells. Specifically, CD40 and its ligand CD154 (CD40L), which is primarily response is dependent on bi-directional signaling mediated

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Though the NGF receptor p75NTR (also a member of the TNFR superfamily) and CD40 are expressed in a co-ordinate fashion on B cells, they may differ in their activities at different stages of B cell differentiation as a result of their interactions with other cytokine- or growth factor-systems (19). It remains unclear whether p75NTR expression is essential for B cell functioning. However, it is of special interest as to whether p75NTR mediates apoptosis upon activation by proNGF (20). Recently, important differences in cytokine production and expression of surface-bound growth factors by CD40 L-activated B cells, compared to LPS-activated B cells, have been described. The findings indicate that the means by which B cells are activated and not simply the enhanced expression of co-stimulatory molecules underpin the ability of B cells to respond to the stimuli and to activate, for instance, naïve CD8+ T cells (10-13). Although continuous studies have revealed CD40 signaling pathways that ultimately result in B cell activation, it still remains to be explored how these signaling events are initiated after receptor ligation and how the CD40- and NGF-receptor systems interact with each other.

To determine the mechanism involved in the enhanced release of NGF protein upon cytokine stimulation of B cells, we studied different NF-κB inhibitors. Using ELISA and RT-PCR techniques, the effects of the antioxidants PDTC (known to prevent cellular reactive oxygen intermediate (ROI) production and cytokine-induced NF-κB activation) and N-acetyl-L-cystein (NAC) were investigated (12, 16). B cells incubated with PDTC (30 μM), which alone does not influence basal NGF mRNA/protein expression levels (data not shown) (16), revealed the inhibitory effect of PDTC on LPS-/cytokine-induced NGF protein release 24 h after stimulation (Figure 1). Moreover, the LPS-/cytokine-induced NGF protein release was abolished by NAC (2 mM) (Figure 1) suggesting that reactive radical intermediates are indeed involved in LPS-/cytokine-induced B cell NGF synthesis. It is interesting to note that both inhibitors, PDTC and NAC, blocked the effect on cytokine-induced NGF release (Figure 1). PDTC and NAC also reduced the LPS-/cytokine-activated NGF mRNA expression at maximal inhibitory concentrations (data not shown). Thus, our present study suggests that LPS-, IL-4- and CD40-stimulated NF-κB activation is a key regulatory event for B-cell-derived NGF production. The investigation on BDNF-deficient mice points to a crucial cross-talk between these two neurotrophins during B cell activation (12, 13) (Figures 1, 2). However, the biological significance of the interaction between the CD40- and the neurotrophin-systems and its pivotal impact on specific B cell functions remain to be elucidated.

An inflammation-induced increase of NGF might cause this factor to act like a cytokine that modifies functions of mast cells, macrophages and B cells. Instead of working singly, neurotrophins and cytokines appear to follow a concerted path to achieve an array of responses necessary to preserve homeostatic integrity. Acute or chronic dysfunction of this cross-talk can upset the delicate equilibrium that

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**Figure 1.** Induction of NGF protein release from purified splenic B cells by various B cell activators or NF-κB inhibitors. (A) LPS (1 ng/ml), anti-CD40 (5 μg/ml), IL-4 (5 ng/ml) and IL-6 (5 ng/ml) were used (*p < 0.05 compared to unstimulated controls, #p < 0.05 compared to LPS-stimulated cells, †p < 0.05 compared to anti-CD40 or IL-4 alone, ‡p < 0.05 compared to BDNF+/- mice). (B) B cell activators used were: LPS (1 ng/ml), anti-CD40 (5 μg/ml), IL-4 (5 ng/ml), IL-6 (5 ng/ml); inhibitors: PDTC (30 μM) and NAC (2 mM) (*p < 0.05 compared to unstimulated controls, †p < 0.05 compared to stimulated cells in the absence of NF-κB inhibitors).

**Figure 2.** Schematic illustration of the signaling mechanisms underlying the coupling of CD40 to NGF expression in B cells.
regulates intersystemic communication, hence may potentially provoke or exacerbate immune-mediated pathological processes, such as multiple sclerosis. A clearer understanding of the specific roles played by these neurotrophins in this context will lead to new opportunities for pharmacological modulation of neuro-immune dysfunctions.

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References