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Epstein-Barr Virus Transformation Induces Expression of *trk b* mRNA in Human B Lymphocytes

■ Neurotrophins may play a role in immune function modulation. We have shown that Epstein-Barr virus (EBV)–transformed B lymphocytes express functional Trk B, the high-affinity transmembrane tyrosine kinase receptor for brain-derived neurotrophic factor and neurotrophin-4/5. In this study, we have used reverse transcriptase polymerase chain reaction to examine the expression of *trk b* mRNA in normal human and EBV-transformed B lymphocytes. We demonstrated that *trk b* mRNA was undetectable in normal B lymphocytes but was expressed following EBV transformation. Additionally, normal B lymphocytes up-regulated *trk b* transcription after stimulation with a combination of B cell–specific polyclonal activating agents. ■

Keywords: CD40, RT-PCR, BDNF, NT-4/5

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Neurotrophins are soluble extracellular macromolecules that promote growth, survival, and differentiation of neurons and exert their effects via cell-surface receptors. The neurotrophins include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5) (Korsching, 1993). Trk B, a member of the neurotrophin receptor family, is a transmembrane tyrosine kinase (trk) that binds with high affinity to BDNF and NT-4/5 (Meakin Shooter, 1992; Barbacid, 1994).

It has been proposed that neurotrophins play a role in modulating the immune response (Otten et al, 1989; Ehrhard et al, 1993). In a previous report, we demonstrated the presence of Trk B receptors on Epstein-Barr virus (EBV)–transformed human B lymphocytes and the subsequent tyrosine phosphorylation of Trk B on these cells following treatment with physiologic concentrations of BDNF (Schenone et al, 1996). Given this, it was of interest to determine whether Trk B is present on normal resting B lymphocytes or whether its expression is induced on transformation or stimulation. We used reverse transcriptase polymerase chain reaction (RT-PCR) to examine four samples of highly purified human B lymphocytes, isolated from peripheral blood, before and after EBV transformation for the presence of *trk b* mRNA transcript. Our results showed that in all samples, *trk b* mRNA was undetectable in resting B lymphocytes but was expressed following EBV transformation.

MATERIALS AND METHODS

Cell preparation and culture

One unit of heparinized venous blood was obtained from each of four healthy adult volunteers. From each sample, 10 mL were diluted 1:1 in RPMI 1640 medium (GIBCO BRL, Gaithersburg, MD) and centrifuged over Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ) gradient. Permanent EBV transformation of unfractionated mononuclear leukocytes was performed as previously described (Neitzel, 1986).

Peripheral blood mononuclear cells (PBMCs) were obtained from the remainder of the volunteer samples by centrifugation over sodium diatrizoate/Ficoll gradients (Isolymph; Gallard-Schlesinger Corp., Carle Place, NY). Cells were washed three times in sterile saline prior to additional separation. Unless noted, cells were suspended in complete media, which consisted of RPMI 1640 medium (GIBCO), supplemented with gentamicin (50 μg/mL), L-glutamine (0.3 mg/mL), penicillin G (50 U/mL), streptomycin (50 μg/mL), and 10% fetal bovine serum (Intergen Company, Purchase, NY). PBMCs were separated into B cell–enriched fractions, as previously described (Rosenberg Lipsky, 1979). Briefly, PBMCs were depleted of monocytes and natural killer cells by incubating with 5 mM L-leucine methyl ester (Sigma, St. Louis, MO) in

serum-free medium (Thiele et al, 1983). After washing, B cells were depleted of T cells by incubating with neuraminidase-treated sheep red blood cells (N-SRBCs) (Galili Schlesinger, 1974). The rosetting and nonrosetting populations were separated by centrifugation on diatrizoate/Ficoll gradients. The nonrosetting cells obtained from the interface were again rosetted with N-SRBCs and centrifuged on diatrizoate/Ficoll gradients to remove residual T cells. B cells prepared in this fashion are more than 90% CD20-positive, as has been previously established (Jelinek Lipsky, 1985). Human dorsal root ganglia (DRGs, collected within 12 hours of death, immediately frozen in liquid nitrogen, and stored at –70°C until used) were used as controls.

Reverse Transcriptase Polymerase Chain Reaction

Total RNA was isolated from volunteers' resting B lymphocytes and corresponding EBV-transformed B lymphocytes by a single-step method (RNA STAT-60, Tel-Test "B" Inc., Friendswood, TX). RNA was quantitated by spectrophotometry at 260 nm, and its integrity was tested by formaldehyde/agarose gel electrophoresis. First-strand cDNA was synthesized from 2 μg of total RNA in a 20 μL RT reaction containing 5 mM $MgCl_2$, 1 × PCR buffer, 2.5 μm random hexamers (Perkin-Elmer Co., Norwalk, CT), 1 U/μL RNase inhibitor (Boehringer Mannheim Co., Indianapolis, IN), 1 mM dNTPs, and 2.5 U/μL Moloney Murine Leukemia Virus RT (GIBCO). Polymerase chain reaction was performed in a 50-μL reaction containing 10 μL of the RT reaction, 1 × PCR buffer, 2.5 mM dNTPs, 0.15 μM each 5' and 3' primers, and 1.25 U/50 μL of Taq DNA polymerase (Boehringer Mannheim). Amplification was performed for 35 cycles consisting of 1 minute of denaturation at 95°C, 2 minutes of annealing at 54°C, and 3 minutes of extension at 72°C, with a final extension time of 7 minutes at 72°C.

The primers specific for *trk b* have been previously described, and sequence homology of PCR products with published sequences have been verified (Schenone et al, 1996). The 518-bp amplified products were analyzed on ethidium bromide-stained 2.0% agarose gels. Primers specific for ß-actin (Forward-GACTTCGAGCAAGAGATGGCCAC; Reverse-CAATGCCAGGGTACATGGTGGTG) were used as a positive control to ensure that equivalent amounts of cDNA were loaded into each PCR.

Results

Reverse transcriptase polymerase chain reaction on human DRGs with primers specific for *trk b* gave the expected size, 518 bp (data not shown). Purified resting B lymphocytes from each of the four volunteers did not express mRNA for *trk b* (Fig. 1: lanes 2, 5, 8, and 11). On EBV transformation, parallel samples demonstrated PCR products of the expected size for *trk b* (see Fig. 1: lanes 3, 6, 9, and 12). ß-actin mRNA expression was used to ensure that equivalent amounts of cDNA were loaded in the PCR reactions (see Fig. 1). Stimulation of untransformed B lymphocytes with a combination of CD40 ligand (CD40L), interleukin 2 (IL2), and interleukin 4 (IL4) also induced low-level *trk b* expression.

Discussion

This study demonstrates that resting human B lymphocytes isolated from peripheral blood do not express detectable levels of *trk b* mRNA; EBV transformation of parallel same donor samples, however, is sufficient to induce *trk b* mRNA expression. We have shown previously that this mRNA induction translates into a functional Trk B receptor that is tyrosine phosphorylated on exposure to BDNF (Schenone et al, 1996). Because EBV infection acts as a mitogenic stimulus in B lymphocytes (Klein, 1994), our results are consistent with the notion that *trk b* up-regulation

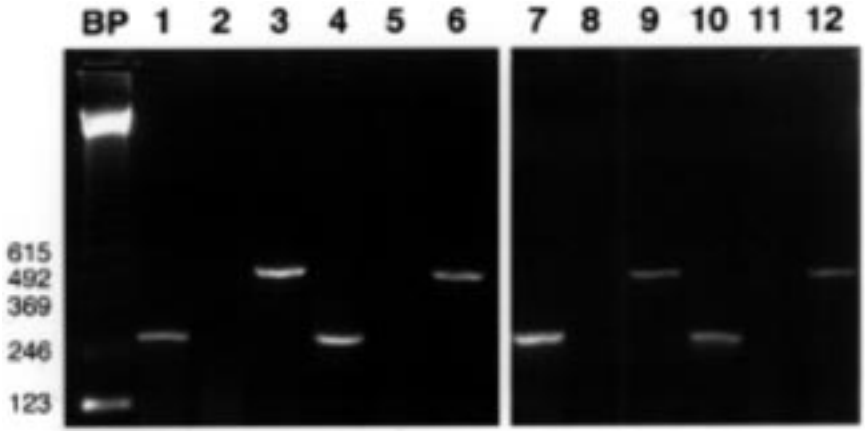

Figure 1. RT-PCR amplification of *trk b* receptor mRNA before and after EBV transformation. None of four samples of purified normal B lymphocytes expressed detectable *trk b* mRNA (lanes 2, 5, 8, and 11). After EBV transformation, parallel samples from the same donor demonstrated PCR products of the expected size for *trk b* (lanes 3, 6, 9, and 12). ß-actin mRNA expression was used to verify that equivalent amounts of normal B lymphocyte cDNA had been loaded into the PCR reaction (lanes 1, 4, 7, and 10).

is a result of cellular activation. Induction of *trk b* expression by stimulation with the combination of polyclonal activating agents CD40L, IL2, and IL4 also suggests that expression is a result of activation rather than transformation. In support of this hypothesis, other investigators have shown that $p75^{NGFR}$ is induced on cultured human melanocytes (Peacocke et al, 1988), and Trk A is similarly induced on KLH-activated T-cell clones (Ehrhard et al, 1994).

The function of mediating "cross-talk" between neuronal and immune cells has been proposed for NGF (Thorpe Perez-Polo, 1987; Ehrhard et al, 1993). This current study, demonstrating inducibility of *trk b* expression in B lymphocytes, suggests a specific role for BDNF and/or NT-4/5 in modulating the immune response within the nervous system. Because BDNF is only expressed locally within the central nervous system (CNS), this provides a potential mechanism by which the function of activated B lymphocytes might be modulated after entry into the CNS. Alternatively, up-regulation of expression of a growth factor receptor after transformation might provide a basis for B lymphocyte mitogenesis in CNS lymphomas. Finally, therapeutic trials have been initiated in which BDNF is used to treat motor neuron disease. Lymphocytes (in circulation, bone marrow, and spleen) are being exposed to high levels of the growth factor. It has been assumed that specific neuronal growth factors will not have stimulatory or mitogenic effects on non-neuronal cells. It is possible thqt this assumption may not be correct and patients in therapeutic trials should be monitored for potential adverse effects resulting from unexpected stimulation of B lymphocytes.

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