Available online at www.sciencedirect.com
SCIENCE DIRECT®
Brain, Behavior, and Immunity 19 (2005) 173–180
BRAIN, BEHAVIOR, and IMMUNITY
www.elsevier.com/locate/ybrbi

Brain-derived neurotrophic factor supports facial motoneuron survival after facial nerve transection in immunodeficient mice

Craig J. Serpe[a,*], Susanna C. Byram[a,b], Virginia M. Sanders[c,1], Kathryn J. Jones[a,b,1]

[a] *Rehabilitation, Research and Development Service, Hines VA Hospital, 5th and Roosevelt Road, Hines, IL 60141, USA*
[b] *Department of Cell Biology, Neurobiology and Anatomy, Loyola University Medical Center, 2160 South First Avenue, Maywood, IL 60153, USA*
[c] *Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Graves Hall, 333 W. 10th Ave, Columbus, OH 43210, USA*

Received 27 April 2004; received in revised form 11 June 2004; accepted 20 July 2004
Available online 17 September 2004

Abstract

Numerous studies have shown that motoneuron survival can be facilitated by neurotrophic factors (NTF) after injury. However, the ability of specific NTF to rescue facial motoneurons (FMN) from axotomy-induced death in immunodeficient mice has not been tested. Therefore, one goal of this study was to determine if brain-derived neurotrophic factor (BDNF), an NTF with a known ability to rescue FMN from axotomy-induced death, supports FMN from axotomy-induced death in recombinase activating gene-2 knockout (RAG-2 KO) mice that lack functional T and B lymphocytes. Nerve growth factor, which has been shown not to play a role in motoneuron survival, was used as a negative control. Brain derived neurotrophic factor treatment restored FMN survival to wild-type (WT) control levels 4 weeks post-operative (wpo) ($80\% \pm 1.9$, $83\% \pm 2.4$, respectively). The second goal of this study was to begin to elucidate if $CD4^+$ T cells produce NTF after facial nerve axotomy. Cervical lymph nodes were collected from WT mice 9 days post-operative, re-activated with anti-CD3 and supernatant collected 24 h later. Immediately after injury, the supernatant was administered to RAG-2 KO mice leading to an increase in FMN survival equivalent to WT controls ($80\% \pm 1.4$, $84\% \pm 2.1$, respectively, 4 wpo). In addition, cervical lymph node supernatant treated with anti-BDNF attenuated FMN rescue in RAG-2 KO mice ($62\% \pm 3.3$) 4 wpo. These data lend support to the hypothesis that $CD4^+$ T cells produce NTF that support motoneuron survival before target reconnection occurs.

Keywords: CD4+ T Lymphocytes; Facial nerve axotomy; Motoneuron survival; Neurotrophic factors; BDNF; Neuroimmune interactions

1. Introduction

The first, and most important, criterion for successful regeneration after peripheral nerve injury is neuronal survival (Fu and Gordon, 1997; Ide, 1996; Lieberman, 1971). A central factor in determining neuronal death or survival in both the peripheral nervous system (PNS) and central nervous system (CNS) is the release of neurotrophic factors (NTF) (Fu and Gordon, 1997). Neurotrophic factors have been shown to be released by non-neuronal cells near both the cell body (within the CNS) and axon (in the periphery) after injury (Barde, 1989; Korsching, 1993; Richardson, 1991, 1994). Since the discovery of the NTF, nerve growth factor (NGF), and the subsequent identification of its role in sympathetic neuronal survival many years ago (Levi-Montalcini, 1987), a significant portion of neuroscience research has been devoted to the search for other NTF and for the determination of their role

* Corresponding author. Fax: +1 708 202 2327.
E-mail addresses: cserpe1@aol.com, craig.serpe@med.va.gov (C.J. Serpe).
[1] These authors contributed equally to this work.

0889-1591/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbi.2004.07.005

in the development and maintenance of the nervous system (Terenghi, 1999).

Evidence to support the role of NTF and their receptors in neuronal survival comes from data demonstrating that using either antibodies against NGF or mice carrying specific mutations within NTF or trk genes leads to a decrease in neuronal survival (Farinas et al., 1994; Levi-Montalcini, 1987; Pinon et al., 1996). Additionally, over the last few years, evidence has mounted that exogenous NTF administration after injury is critical for survival of various neuronal subpopulations during development and into adulthood (Hughes et al., 1993; Li et al., 1994; Sendtner et al., 1990, 1992a,b; Vejsada et al., 1995; Yan et al., 1992). For example, it has previously been shown that, without NTF support, more than 80% of neonatal rat FMN die after facial nerve axotomy (Schmalbruch, 1982; Sendtner et al., 1990). However, exogenous administration of brain derived neurotrophic factor (BDNF) has been shown to rescue neonatal rat FMN from axotomy-induced death (Sendtner et al., 1992a).

We have recently discovered that FMN survival after injury is significantly reduced in immunodeficient mice lacking both functional T and B lymphocytes (Serpe et al., 1999, 2000). Reconstitution of immunodeficient mice with whole splenocytes, obtained from wild-type mice, restores FMN survival after injury to wild-type controls (Serpe et al., 1999, 2000). Subsequently, we identified the $CD4^+$ T cell as the immune cell type responsible for mediating FMN survival after facial nerve injury (Serpe et al., 2003). Additionally, we examined the kinetics of FMN survival by extending the post-axotomy time from 4 weeks to 10 weeks. While FMN survival in immunodeficient mice remained the same as at 4 weeks post-operative (wpo), FMN survival in both the wild-type and reconstituted mice continued to significantly decline through 10 wpo (Serpe et al., 2000). These results may be due, in part, to the loss of target-derived NTF, with permanent target disconnection. Since $CD4^+$ T cells produce NTF, e.g., BDNF (Besser and Wank, 1999; Kerschensteiner et al., 1999), it is hypothesized that, following facial nerve transection in wild-type controls, NTF released from activated $CD4^+$ T cells initially support neuronal survival until target reconnection occurs.

The ability of specific NTF to rescue FMN from axotomy-induced death in immunodeficient mice is unknown. Therefore, the ability of BDNF to rescue FMN from axotomy-induced death was examined in recombinase activating gene-2 knockout (RAG-2 KO) mice that lack functional T and B lymphocytes. Nerve growth factor has been implicated in sensory neuron survival in rats and mice (Levi-Montalcini, 1987). However, NGF has not been found to play a role in motoneuron survival after peripheral nerve injury (Sendtner et al., 1996). Therefore, both phosphate saline buffer (PBS) and NGF were used as negative controls in the present study. The results indicate that BDNF, but not NGF, has the capacity to rescue FMN from axotomy-induced death after injury in immunodeficient mice. In agreement with the literature, NGF lacks the capability to rescue motoneurons from axotomy-induced death after injury (Sendtner et al., 1996). Our next goal was to determine if cervical lymph node cells, obtained from wild-type (WT) mice that received a facial nerve axotomy 9 days post-axotomy, could support FMN survival in RAG-2 KO mice 4 wpo. The results indicate that supernatant collected from re-activated cervical lymph node cells, from WT mice, protects FMN in RAG-2 KO mice from axotomy-induced death 4 wpo. In addition, RT-PCR shows that cervical lymph node cells from WT mice express BDNF. Therefore, supernatant from cervical lymph node cells was treated with anti-BDNF 24 h before experimental use to determine if the BDNF protein was released and functional. Anti-BDNF treated supernatant failed to restore FMN survival, to WT numbers, in RAG-2 KO mice 4 wpo.

2. Materials and methods

2.1. Animals and surgical procedures

All mice used in the present study were on a C57BL/6 background. Seven-week old female wild-type mice, and recombinase activating gene-2 knockout (RAG-2 KO) mice, which lack functional T and B lymphocytes only, were obtained from Taconic labs (Germantown, New York, NY). All mice were provided autoclaved pellets and water ad libitum. Mice were permitted 1 week to acclimate to their environment before manipulation and used at 8 weeks of age in all experiments. All mice were housed under a 12 h light/dark cycle in microisolater cages contained within a laminar flow system to maintain a pathogen-free environment. All experimental manipulations were performed approximately 4 h into the light cycle under aseptic conditions. All surgical procedures were completed in accordance with National Institutes of Health guidelines on the care and use of laboratory animals for research purposes. Mice were anesthetized with 3% halothane for all surgical procedures. Using aseptic techniques, the right facial nerve of each animal was exposed at its exit from the stylomastoid foramen (SMF) and completely transected (Serpe et al., 1999). The distal nerve stump was pushed away from the proximal nerve stump, thereby preventing reconnection of the facial nerve. The left facial nerve was exposed, but not transected, and served as a sham-operated internal control. The present study contains seven experimental groups (for information on either NTF, supernatant, or anti-BDNF treatment, see descriptions below): (1) wild-type mice, (2) PBS-treated, (3) BDNF-treated, (4) NGF-treated, (5) supernatant-treated (right