Brain, Behavior, and Immunity xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Impact of peripheral immune status on central molecular responses to facial nerve axotomy

D.O. Setter [a,b,*], E.M. Runge [a,b], N.D. Schartz [a], F.M. Kennedy [a,b], B.L. Brown [a], K.P. McMillan [a,b], W.M. Miller [a,b], K.M. Shah [a], M.M. Haulcomb [a,b], V.M. Sanders [c], K.J. Jones [a,b]

[a] Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN, United States
[b] Research and Development Service, Richard L. Roudebush VAMC, Indianapolis, IN, United States
[c] Molecular Virology, Immunology, and Medical Genetics, The Ohio State University, Columbus, OH, United States

ARTICLE INFO

Article history:
Received 26 June 2017
Received in revised form 3 October 2017
Accepted 3 October 2017
Available online xxxx

Keywords:
Motoneuron
Nerve injury
Axotomy
T cells
Amyotrophic lateral sclerosis

ABSTRACT

When facial nerve axotomy (FNA) is performed on immunodeficient recombinase activating gene-2 knockout (RAG-2$^{-/-}$) mice, there is greater facial motoneuron (FMN) death relative to wild type (WT) mice. Reconstituting RAG-2$^{-/-}$ mice with whole splenocytes rescues FMN survival after FNA, and CD4+ T cells specifically drive immune-mediated neuroprotection. Evidence suggests that immunodysregulation may contribute to motoneuron death in amyotrophic lateral sclerosis (ALS). Immunoreconstitution of RAG-2$^{-/-}$ mice with lymphocytes from the mutant superoxide dismutase (mSOD1) mouse model of ALS revealed that the mSOD1 whole splenocyte environment suppresses mSOD1 CD4+ T cell-mediated neuroprotection after FNA. The objective of the current study was to characterize the effect of CD4+ T cells on the central molecular response to FNA and then identify if mSOD1 whole splenocytes blocked these regulatory pathways.

Gene expression profiles of the axotomized facial motor nucleus were assessed from RAG-2$^{-/-}$ mice immunoreconstituted with either CD4+ T cells or whole splenocytes from WT or mSOD1 donors. The findings indicate that immunodeficient mice have suppressed glial activation after axotomy, and cell transfer of WT CD4+ T cells rescues microenvironment responses. Additionally, mSOD1 whole splenocyte recipients exhibit an increased astrocyte activation response to FNA. In RAG-2$^{-/-}$ + mSOD1 whole splenocyte mice, an elevation of motoneuron-specific Fas cell death pathways is also observed. Altogether, these findings suggest that mSOD1 whole splenocytes do not suppress mSOD1 CD4+ T cell regulation of the microenvironment, and instead, mSOD1 whole splenocytes may promote motoneuron death by either promoting a neurotoxic astrocyte phenotype or inducing Fas-mediated cell death pathways. This study demonstrates that peripheral immune status significantly affects central responses to nerve injury. Future studies will elucidate the mechanisms by which mSOD1 whole splenocytes promote cell death and if inhibiting this mechanism can preserve motoneuron survival in injury and disease.

1. Introduction

A growing body of literature indicates that peripheral immune status is an important factor in central nervous system responses to injury or disease (Beers et al., 2008; DeFrancesco-Lisowitz et al., 2015; Ip et al., 2015; Spani et al., 2015). Using the facial nerve axotomy (FNA) model of peripheral nerve injury, our laboratory discovered that immunodeficient mice lacking functional B and T cells have significantly more facial motoneuron (FMN) death within the central nervous system (CNS) after FNA relative to wild type (WT) mice (Serpe et al., 1999; Serpe et al., 2000; Serpe et al., 2003). Immunoreconstitution of immunodeficient mice with WT whole splenocytes prior to FNA rescues FMN survival, and the CD4+ T cell population alone is specifically responsible for mediating neuroprotection after axotomy (Serpe et al., 2003). Key cellular elements within the facial motor nucleus that interact with peripheral neuroprotective CD4+ T cells include both astrocytes and microglia (Byram et al., 2004; Wainwright et al., 2009b; Wainwright et al., 2009c).

CD4+ T cells are implicated in amyotrophic lateral sclerosis (ALS), a neurodegenerative disease affecting motoneurons. CD4+ T cells are decreased in ALS patients relative to age-matched

* Corresponding author at: 320 W 15th St. NB211C, Indianapolis, IN 46202, United States.
E-mail address: dolmstea@iupui.edu (D.O. Setter).

https://doi.org/10.1016/j.bbi.2017.10.005
0889-1591/

controls (Chen et al., 2014), and patients with ALS exhibit increased peripheral immune activation and circulating cytokines (Chen et al., 2014; Lu et al., 2016). The mutant superoxide dismutase (mSOD1) mouse model of ALS also demonstrates significant immunodysregulation, including lymphopenia and a failure of T cells to generate a proliferation or immunization response to stimuli (Kuzmenok et al., 2006; Banerjee et al., 2008). CD4+ T cell deficiency in mSOD1 mice results in accelerated disease progression, and adoptive cell transfer of WT CD4+ T cells into mSOD1 mice modestly improves survival (Banerjee et al., 2008; Beers et al., 2008). Thus, the immune system may be a new therapeutic target for ALS.

When FNA is superimposed on the mSOD1 mouse in the presymptomatic stages, FMN death after FNA mirrors that of immunodeficient mice, corroborating evidence for immunodysregulation in mSOD1 mice (Mariotti et al., 2002; Mesnard et al., 2011). These intriguing findings led us to explore the molecular changes that occur in the facial motor nucleus of both FMN and the surrounding neuropil in WT and mSOD1 mice (Mesnard et al., 2011; Haulcomb et al., 2014). Genes associated with motoneuron regeneration, glial activation, inflammation, and cell death were examined. While the FMN regenerative phenotype in mSOD1 mice was unaffected, astrocyte and microglia responses were found to be dysregulated after peripheral axotomy in mSOD1 mice. Furthermore, mSOD1 mice also exhibited increased expression of motoneuron-specific Fas cell death pathway components, indicating a disease-induced prevalence of Fas-mediated cell death (Haulcomb et al., 2014).

We next explored the neuroprotective capacity of the mSOD1 peripheral immune system in immunodeficient mice. When whole splenocytes from mSOD1 mice, including CD4+ T cells, were transferred into RAG-$2^{-/-}$ mice, significant FMN death still occurred after FNA. However, when isolated mSOD1 CD4+ T cells were transferred into RAG-$2^{-/-}$ mice, we observed that FMN survival was rescued to WT levels (Mesnard-Hoaglin et al., 2014). These findings suggest that an inhibitory factor present in the mSOD1 whole splenocyte milieu blocks mSOD1 CD4+ T cell-mediated neuroprotection.

While we now have an understanding of the cellular elements involved in CD4+ T cell-mediated promotion of FMN survival after target disconnection, the accompanying key molecular changes that occur in the CNS, and how they may factor in ALS, are unknown. The well-characterized nerve cell body response to injury in the facial motor nucleus provides the opportunity to exploit the advantages inherent in laser microdissection and qPCR in order to identify a CNS molecular signature modifiable by the peripheral immune system after injury (Lieberman, 1971; Grafstein, 1975; Moran and Graeber, 2004). This approach is both powerful and efficient because it captures large amounts of gene expression data reflecting different functional states and multiple cell types using a single method of tissue processing from individual animals.

The results indicate that the CNS astrocytic response to peripheral nerve injury appears to be differentially affected by peripheral immune status. Furthermore, mSOD1 immune cells induce Fas expression in the facial motor nucleus, which may contribute to motoneuron death. Collectively, these data suggest that cell-cell interactions between the CNS glia and adaptive immune are potential therapeutic targets in neurodegenerative diseases such as ALS.

2. Materials and methods

2.1. Animals and surgical procedure

For this study, the following mouse strains were purchased from The Jackson Laboratory (Bar Harbor, ME) where they were bred and maintained in separate lines: C57BL/6J (WT, RRID: IMSR_JAX:000664), B6(Cg)-Rag2tm1.1Cgn/J (RAG-$2^{-/-}$, RRID: IMSR_JAX:008449), and B6.Cg-Tg(SOD1G93A)1Gur/J (mSOD1, RRID:IMSR_JAX:004435). The Jackson Laboratory maintains WT and RAG-$2^{-/-}$ strains as homozygous inbred colonies, and the mSOD1 strain is generated by breeding a C57BL/6J female with a male hemizygous for the SOD1G93A mutation. All mice were obtained at 6 or 7 weeks of age and allowed to acclimate for 1 week prior to any manipulation. Female mice were exclusively used, which is a limitation of this study. Male mice were excluded because their propensity for aggression after surgery requires individual housing to prevent trauma and infection of the surgical site (Edwards, 1968; Van Loo et al., 2003; Lockworth et al., 2015). Individual housing significantly stresses the animal, resulting in adverse effects on both immune physiology and animal welfare (Olsson and Westlund, 2007; Kamakura et al., 2016; Weber et al., 2017).

All animal procedures complied with National Institutes of Health guidelines on the care and use of laboratory animals and were approved by Indiana University School of Medicine's Institutional Animal Care and Use Committee. Mice were housed in sterilized microisolator cages with a 12 h light/dark cycle and fed autoclaved food pellets and drinking water *ad libitum*. The animal facility uses a laminar flow system to maintain a pathogen-free environment.

Aseptic procedures were followed during the FNA following National Institutes of Health guidelines. FNA was performed on 8 week old mice following previously established methods (Olmstead et al., 2015). Briefly, mice were anesthetized with 2.5% isoflurane in 0.9 L/min oxygen, and the facial nerve was exposed and transected at its exit from the stylomastoid foramen. The remaining nerve stumps were separated and resected to prevent reconnection. No operation was performed on the left facial nerve, allowing for the left facial motor nucleus to serve as a paired internal control. Prior to euthanasia, behavioral observations of ipsilateral facial paralysis verified that no functional recovery occurred.

2.2. Isolation and adoptive transfer of whole splenocytes and CD4+ T cells

Adoptive cell transfers were performed on recipient mice at 7 weeks of age, 1 week prior to FNA following a protocol modified from Serpe et al., 2003. Donor mice (1:1 donor:recipient ratio) were euthanized with CO_2 inhalation followed by cervical dislocation. The spleen was dissected out and a single-cell suspension of whole splenocytes was generated following the Miltenyi Biotec gentleMACS protocol.

For preparation of whole splenocytes, red blood cell lysis was performed on the cell suspension with ACK Lysing Buffer (Thermo Fisher Scientific; A1049201) for 4 min at RT, and cells were washed and resuspended in PBS. 50×10^6 whole splenocytes in 100 µl of PBS were injected into the recipient mouse tail vein.

For preparation of CD4+ T cells, red blood cell lysis was not performed to maximize CD4+ T cell yield. The whole splenocyte cell pellet was incubated with CD4 (L3T4) MicroBeads (Miltenyi Biotec, 130-049-201) per manufacturer protocol, and magnetic separation was performed with the Possel_d2 program on an autoMACS™ Pro Separator. Cells were washed and resuspended in PBS, and 5×10^6 CD4+ T cells in 100 µl of PBS were injected into the recipient mouse tail vein. This cell number was selected based on data indicating that approximately 10% of whole splenocytes are CD4+ T cells in the C57BL/6J mouse strain (JAX Phenome Database, RRID: SCR_003212, MPD:Jaxpheno6).

CD4+ T cell fraction purity was measured using flow cytometry with FITC rat anti-mouse CD4 antibody (BD Biosciences Cat# 557307 RRID:AB_396633), and average purity levels were greater

Please cite this article in press as: Setter, D.O., et al. Impact of peripheral immune status on central molecular responses to facial nerve axotomy. Brain Behav. Immun. (2017), https://doi.org/10.1016/j.bbi.2017.10.005