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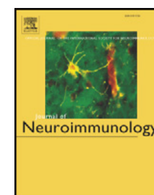

Short communication

Myeloid Kruppel-like factor 2 deficiency exacerbates neurological dysfunction and neuroinflammation in a murine model of multiple sclerosis

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ABSTRACT

Cells of the innate immune system are important mediators of multiple sclerosis (MS). We have previously identified Kruppel-like factor 2 (KLF2) as a critical negative regulator of myeloid activation in the setting of bacterial infection and sepsis, but the role of myeloid KLF2 in MS has not been investigated. In this study, myeloid KLF2 deficient mice exhibited more severe neurological dysfunction and increased spinal cord demyelination and neuroinflammation in experimental autoimmune encephalomyelitis. This study represents the first description of a significant role of myeloid KLF2 in neuroinflammation, identifying KLF2 as a potential target for further investigation in patients with MS.

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1. Introduction

Multiple sclerosis (MS) is a chronic and often debilitating immune mediated inflammatory disease that affects the central nervous system (CNS) causing significant neurological disability (Noseworthy et al., 2000). In MS the normally immunologically privileged brain and spinal cord are invaded by multiple leukocyte cell types that initiate an inflammatory process resulting in demyelination and axonal degeneration (Shechter and Schwartz, 2013). In addition to T lymphocytes, cells of the innate immune system, in particular peripheral blood macrophages and microglial cells, have also been demonstrated to play important roles in mediating MS. Multiple loss of function studies using the experimental autoimmune encephalomyelitis (EAE) murine model have been conducted that demonstrate the detrimental role of these cells in MS (Rawji and Yong, 2013).

Kruppel-like factor 2 (KLF2) is a member of the Kruppel-like family of zinc-finger transcription factors that are critically involved in regulating cellular development and function (McConnell and Yang, 2010). Recent studies from our laboratory identified KLF2 as a critical negative regulator of myeloid pro-inflammatory activation and alterations in KLF2 levels serve as a key determinant of the innate immune response in vivo (Das et al., 2006; Mahabeleshwar et al., 2011). Further, a reduction in myeloid KLF2 levels occurs in patients with acute and chronic inflammatory disease states such as sepsis (Mahabeleshwar et al., 2011) and atherosclerosis (Das et al., 2006). While these seminal studies were the first to identify an in vivo role for myeloid KLF2, the importance of this regulatory pathway in CNS disease has not been investigated.

The aim of the current study is to evaluate the role of myeloid KLF2 in MS. Our study demonstrates that myeloid KLF2 is an important mediator of murine EAE via regulation of neuroinflammation.

2. Materials and methods

2.1. Animals and EAE murine model

All animal experiments were performed in accordance with guidelines of and approved by the Institutional Animal Care and Use Committee, Case Western Reserve University. Myeloid specific KLF2 deficient mice (LysM$^{Cre/Cre}$:KLF2$^{fl/fl}$, designated MY-K2-KO) were generated as previously described (Mahabeleshwar et al., 2011). In MY-K2-KO mice

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greater than 95% deletion of KLF2 is observed in myeloid cells (Mahabeleshwar et al., 2011) with no significant change in KLF4 and KLF6 expression (two KLF family members demonstrated to have important roles in myeloid cells) (Liao et al., 2011; Date et al., 2014)(Supplemental Fig. S1). In addition, no substantial effect was seen on other hematopoietic lineages, tissues, or monocytic subsets (Mahabeleshwar et al., 2011). EAE was induced in 8 week old female MY-K2-KO mice and age matched control mice ($LysM^{Cre/Cre}$) (n = 20–25 mice per group) by subcutaneous immunization with myelin oligodendrocyte glycoprotein $(MOG)_{35–55}$ peptide (300 μg/mouse, Cleveland Clinic Molecular Biotechnology Core Laboratory) emulsified in Freund's complete adjuvant (Sigma). On day 0 and day 2 after immunization mice received an intraperitoneal injection of pertussis toxin (200 ng/mouse, Sigma). Mice were weighed and observed daily for 28 days after immunization for neurological deficits. Mean daily clinical neurological scores were determined based on the observed neurological deficit of each group: 0 = healthy, 1 = limp tail, 2 = limp tail and hind leg weakness or impaired righting reflex or paresis of one limb, 3 = limp tail and complete paralysis of hind legs or limp tail with paralysis of one front and one hind leg, 4 = limp tail, complete hind leg and partial front leg paralysis, and 5 = complete hind and complete front leg paralysis, no movement around cage.

2.2. Histology and immunofluorescence

Lumbar spinal cord cryosections (10 μm) from three evenly spaced levels between T12 and L2 of each spinal cord (n = 5–7 spinal cords per group) were stained with Luxol fast blue (LFB) and hematoxylin and eosin (H&E), or fixed in 4% paraformaldehyde for 10 min, washed and blocked for 30 min with 5% BSA in PBS-T (0.1 M PBS containing 0.2% Tween 20) and incubated overnight at 4 °C with antibodies to myelin basic protein (MBP; Millipore), CD3 (Dako), chemokine C-C motif ligand 2 (CCL2; Santa Cruz), inducible nitric oxide synthase (iNOS), CD45, CD68 (BD Biosciences) as indicated, followed by Alexa-488 or 594 conjugated secondary antibodies (Invitrogen). All imaging was performed using a Leica video imaging system. To quantify immunostaining results identical light intensity and exposure times were applied to all photographs from each experimental set. Images were acquired separately from the bilateral dorsal, ventral, and lateral white matter columns from three levels of the spinal cord for each mouse. All images were converted to grayscale and then analyzed by density measurement with ImageJ software. A fixed threshold range of 0–160 was chosen to highlight the staining signals in normal spinal cord sections, and the total area within this range was measured, averaged, and compared.

2.3. Real time quantitative PCR analysis

Lumbar spinal cords from MY-K2-KO and Control mice at peak stage (i.e., days 16–19 after immunization, n = 3–4 mice per group) were harvested and total RNA was extracted from the spinal cord tissue with TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. 2 μg of total RNA was reverse transcribed with M-MuLV reverse transcriptase (New England BioLabs Inc.) and oligo-dT primers. Real-time PCR was performed with Universal SYBR Green PCR Master Mix (Roche) on a LightCycler 480 System (Roche) with gene specific primers for iNOS, CCL2, TNFα, IL1β, CD16, CD86, MMP-2, and MMP-9.

2.4. Statistics

Data are expressed as mean ± SEM. Differences between statistical groups were evaluated for statistical significance using the Student's *t*-test for unpaired data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Myeloid KLF2 deficiency exacerbates neurological dysfunction and spinal cord demyelination in EAE

To assess the role of myeloid KLF2 in MS, EAE was induced in MY-K2-KO and control mice. MY-K2-KO mice exhibited a more rapid onset of neurological dysfunction and higher clinical neurological scores when compared to control mice (Fig. 1A). Myelin staining of lumbar spinal cords using LFB (Fig. 1B and C) or immunostaining for MBP (Fig. 1D) reveals less staining in MY-K2-KO mice compared to control mice, indicating greater demyelination of the CNS. These data demonstrating that

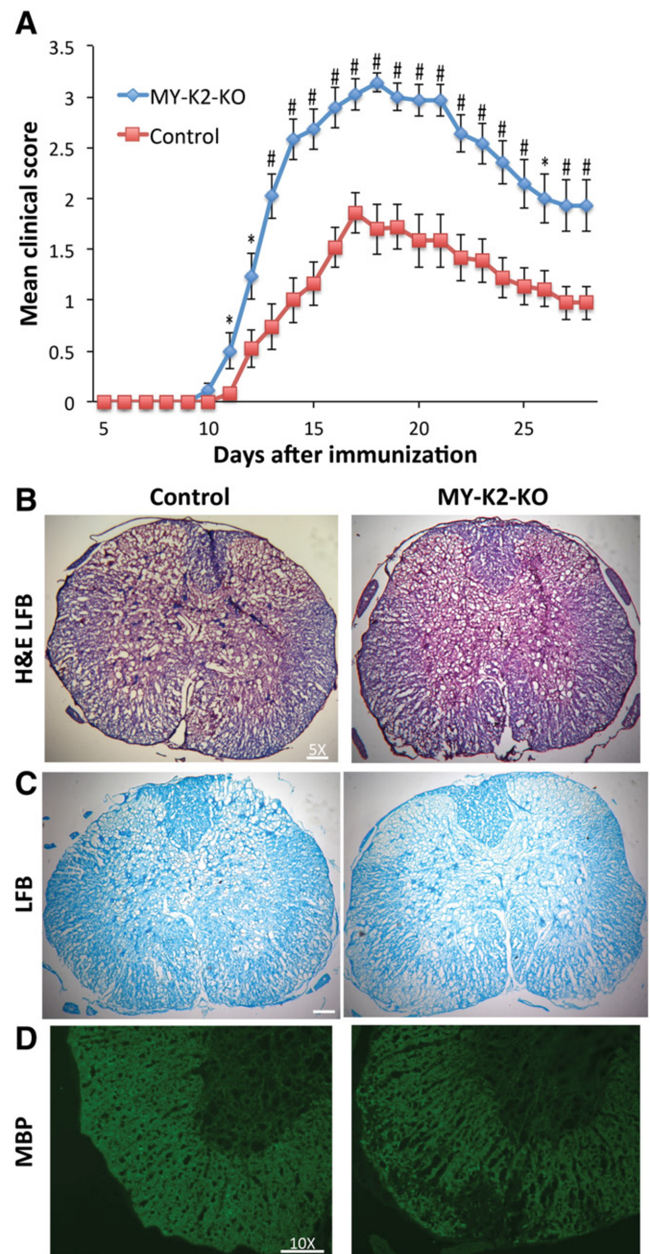

Fig. 1. Myeloid KLF2 deficient mice have more severe neurological deficits and spinal cord demyelination in EAE. (A) Clinical neurological scores from 8 week old female MY-K2-KO and control mice after immunization with $MOG_{35–55}$ peptide (n = 20–25 per group). *$P < 0.05$, #$P < 0.005$. (B–D) Lumbar spinal cords from MY-K2-KO and control mice at peak stage (i.e., days 16–19 after immunization) stained with: (B) hematoxylin, eosin, and Luxol fast blue (H&E LFB), (C) Luxol fast blue alone (LFB), and (D) myelin basic protein (MBP) antibody. Representative results are shown (n = 5–7 per group).

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