GLIA

GLIA 59:603–614 (2011)

Expression and Detrimental Role of Hematopoietic Prostaglandin D Synthase in Spinal Cord Contusion Injury

ADRIANA REDENSEK,[1] KHIZR I. RATHORE,[1] JENNIFER L. BERARD,[1] RUBÈN LÓPEZ-VALES,[1] LEIGH ANNE SWAYNE,[2] STEFFANY A.L. BENNETT,[2] IKUKO MOHRI,[3] MASAKO TANIIKE,[3] YOSHIHIRO URADE,[3] AND SAMUEL DAVID[1]*

[1]*Center for Research in Neuroscience, Research Institute of the McGill University Health Center, Montreal, Quebec, Canada H3G 1A4*

[2]*Neural Regeneration Laboratory, Department. of Biochemistry, Microbiology, and Immunology, University of Ottawa, ON, Canada*

[3]*Department of Molecular Behavioral Biology, Osaka Bioscience Institute, Suita, Osaka 565-0874, Japan*

KEY WORDS
spinal cord injury; inflammation; prostaglandins

ABSTRACT
Prostaglandin D_2 (PGD_2) is a potent inflammatory mediator, which is implicated in both the initiation and resolution of inflammation in peripheral non-neural tissues. Its role in the central nervous system has not been fully elucidated. Spinal cord injury (SCI) is associated with an acute inflammatory response, which contributes to secondary tissue damage that worsens functional loss. We show here, with the use of hematopoietic prostaglandin D synthase (HPGDS) deficient mice and a HPGDS selective inhibitor (HQL-79), that PGD_2 plays a detrimental role after SCI. We also show that HPGDS is expressed in macrophages in the injured mouse spinal cord and contributes to the increase in PGD_2 in the contused spinal cord. *HPGDS*$^{-/-}$ mice also show reduced secondary tissue damage and reduced expression of the proinflammatory chemokine CXCL10 as well as an increase in IL-6 and TGFβ-1 expression in the injured spinal cord. This was accompanied by a reduction in the expression of the microglia/macrophage activation marker Mac-2 and an increase in the antioxidant metallothionein III. Importantly, HPGDS deficient mice exhibit significantly better locomotor recovery after spinal cord contusion injury than wild-type (Wt) mice. In addition, systemically administered HPGDS inhibitor (HQL-79) also enhanced locomotor recovery after SCI in Wt mice. These data suggest that PGD_2 generated via HPGDS has detrimental effects after SCI and that blocking the activity of this enzyme can be beneficial.

INTRODUCTION

Prostaglandin D_2 (PGD_2) is a potent inflammatory mediator produced by two synthases, hematopoietic prostaglandin D synthase (HPGDS), and lipocalin-type prostaglandin D synthase (L-PGDS). Arachidonic acid associated with membrane phospholipids is cleaved by phospholipase A_2 and converted to a prostanoid precursor by the cycloxygenases-1/2 (COX-1/2). The prostanoid precursor, prostaglandin H_2, can then be converted by HPGDS or L-PGDS to PGD_2. In the central nervous system (CNS), L-PGDS is expressed by oligodendrocytes, meningeal cells, and the choroid plexus (Beuckmann et al., 2000; Urade et al., 1993). In contrast, HPGDS is expressed by certain immune cells of hematopoietic origin (Lewis et al., 1982; Mohri et al., 2003; Ujihara et al., 1988; Urade et al., 1989, 1990). PGD_2 can induce the production of IL-2, IL-4, IL-5, and IL-13 in Th2 cells (Tanaka et al., 2004) and act as a chemoattractant for Th2 cells, eosinophils, and basophils (Hirai et al., 2001), which has led to studies on the role of PGD_2 and HPGDS in allergic immune responses (Kostenis and Ulven, 2006). Various studies have demonstrated that PGD_2 can also promote the resolution of inflammation (Angeli et al., 2004; Matsuoka et al., 2000; Rajakariar et al., 2007; Spik et al., 2005). As PGD_2 can act through a variety of mechanisms, its role as either a pro or anti-inflammatory mediator remains to be fully elucidated. PGD_2 is very abundant in the cerebrospinal fluid, likely derived from the choroid plexus and meninges; and in the normal CNS plays a role in the regulation of the sleep-wake cycle (Hayaishi and Urade, 2002). Recent studies suggest that PGD_2 generated via HPGDS plays a role in CNS inflammatory responses. HPGDS is expressed in activated microglia surrounding senile plaques in Alzheimer's brains (Mohri et al., 2007) and is detrimental in the mouse model of human globoid cell leukodystrophy (Mohri et al., 2006). PGD_2 also mediates neuronal damage *in vitro* via microglia (Bate et al., 2006). However, PGD_2 may also be beneficial in models of CNS ischemia (Liu et al., 2009; Saleem et al., 2007; Taniguchi et al., 2007).

Inflammation after spinal cord injury (SCI) contributes to secondary tissue damage and functional loss

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: Wings for Life Spinal Cord Research Foundation (Austria); Grant sponsor: Canadian Institutes of Health Research (CIHR); Grant number: MOP-89999.

*Correspondence to: Samuel David, Center for Research in Neuroscience, McGill University Health Center Research Institute, Livingston Hall, Room L7-210, 1650 Cedar Ave., Montreal, Québec, Canada H3G 1A4. E-mail: sam.david@mcgill.ca

Received 22 July 2010; Accepted 30 November 2010

DOI 10.1002/glia.21128

Published online 3 February 2011 in Wiley Online Library (wileyonlinelibrary.com).

(Donnelly and Popovich, 2008; Dumont et al., 2001; Kwon et al., 2004). Inhibition of COX-2, the enzyme immediately upstream of HPGDS, results in some improvement in functional recovery, reduced lesion size, and an increase in viable tissue in mild forms of SCI (Faden et al., 1988; Hains et al., 2001; Lopez-Vales et al., 2006; O'Banion et al., 2002; Resnick et al., 1998). As COX enzymes give rise to both pro- and anti-inflammatory mediators, maximal therapeutic benefits would be expected to arise from targeting only the proinflammatory mediators generated downstream of COX. We therefore assessed the potential role of PGD_2 produced by HPGDS in inflammation-induced secondary damage triggered after spinal cord contusion injury in mice. We show here that PGD_2 produced from HPGDS after SCI is proinflammatory and contributes to secondary damage and greater locomotor deficits and may be a possible target for therapeutic intervention for the treatment of acute SCI.

MATERIALS AND METHODS

Spinal Cord Contusion Injury

The *HPGDS*$^{-/-}$ and *HPGDS*$^{+/+}$ mice (littermate controls) on a C57BL/6 background were generated as previously described (Mohri et al., 2006). Adult female mice (18–22 g body weight) were anesthetized with ketamine/xylazine/acepromazine (50/5/1 mg/kg). A partial laminectomy was done at the 11th thoracic level to expose the spinal cord, and a contusion injury was performed as described previously (Ghasemlou et al., 2005). Briefly, adjacent vertebrae to the laminectomy were immobilized with modified serrated Adson forceps (Fine Science Tools) and the spinal cord contused with the Infinite Horizons spinal cord impactor (Precision Scientific Instrumentation). Moderate type of contusion injuries were made as we have described before (Ghasemlou et al., 2005) with displacements of the spinal cord tissue at the time of impact ranging between 400 and 600 μm ($n = 6$ for each group). Because of the difficulty in obtaining sufficient numbers of knockout mice born at the same time, these experiments were done in two separate small groups and the data pooled. Locomotor analysis was performed using the Basso Mouse Scale (BMS) (Basso et al., 2006), which is a nine-point scale that was designed for evaluating locomotor control after contusion injuries in mice. For this analysis, mice were scored by two observers trained at the Basso laboratory at Ohio State University and the consensus score taken. The BMS scoring as well as the subsequent histological analysis were all performed blind. C57BL/6 mice (Charles River Canada) showed no differences in locomotor recovery after SCI when compared to HPGDS$^{+/+}$ littermate controls.

In another set of experiments, female C57BL/6 mice (18–22 g body weight) were treated daily with subcutaneous injections with 4-benzhydryloxy-(1) {3-(1H-tetrazol-5-yl)-propyl}piperidine (HQL-79; Cayman Chemicals), a selective small molecule inhibitor of HPGDS (Aritake et al., 2006). Five mice were used in each group but one from the HQL-79 group died a few days after surgery ($n = 4$ HQL-79 and $n = 5$ for vehicle controls). HQL-79 was suspended in 0.5% methylcellulose and the treatments started 1 h after SCI for 28 days at a dose of 50 mg/kg body weight. This dose and subcutaneous route of administration was previously reported to be effective in a model of CNS demyelination (Mohri et al., 2006). Control mice were treated similarly with vehicle. HQL-79 has also been shown to have protective effects in transient cerebral ischemia (Liu et al., 2009).

All procedures were approved by the McGill University Animal Care Committee and followed the guidelines of the Canadian Council on Animal Care.

Reverse Transcriptase Polymerase Chain Reaction

Spinal cord contusion injuries were made in adult C57BL/6 mice as described above. A 4 mm length of spinal cord centered on the lesion was collected on days 1, 3, 7, 14, 21, and 28 after injury ($n = 3$ for each time point). This tissue was homogenized in QIAzol reagent (Qiagen) and total RNA extracted using the RNeasy Lipid Mini Tissue Kit (Qiagen). The RNA concentrations were determined by spectrophotometry, and 1 μg of RNA was converted to cDNA using the Omniscript RT Kit (Qiagen) according to the manufacturer's protocol. Semiquantitative PCR was performed using HSTaq Master Mix (Qiagen). Primers and conditions for HPGDS and L-PGDS were the same as that used in a previous publication (Mohri et al., 2006). PCR products were separated on a 2% agarose gel, visualized by ethidium bromide staining, and densitometric analysis carried out using ImageQuant 5.0 (Molecular Dynamics). Each time point was compared to naïve uninjured spinal cord and normalized to peptidylprolyl isomerase A (PPIA).

Quantitative Real-Time PCR

Contused spinal cord tissue from *HPGDS*$^{-/-}$ and wild type (Wt) controls were collected as stated above on days 1, 3, 14, and 28 after injury ($n = 3$ for each time point). Total RNA was extracted in a similar manner as for RT-PCR. Following this, 0.5 μg of RNA was converted to cDNA using the Stratascript RT set (Stratagene) according to the manufacturers' protocol. Quantitative real-time PCRs were performed using the Brilliant SYBR Green QPCR Master Mix and MX4000 (Stratagene). Gene-specific primers were designed using PrimerQuest (Integrated DNA Technology). The sequence-specific primers used were as follows:

- TGF β1 forward, 5′-TGGAGCTGGTGAAACGGAAG-3′;
- TGF β1 reverse, 5′-ACAGGATCTGGCCACGGAT-3′;
- Mac-2 forward, 5′-TGTGTGCCTTAGGAGTGGGAAA CT-3′;
- Mac-2 reverse, 5′-AGAACACTTGCCTAGCAGTCACGA-3′;
- Metallothionein III forward, 5′-TGTGAGAAGTGTG-CAAGGACTGT-3′
- Metallothionein III reverse, 5′-TTTACATAGGCTGTGT GGGAGGG-3′

- TNF-α forward, 5′-AGACCCTCACACTCAGATCATC TTC-3′
- TNF-α reverse, 5′-CCTCCACTTGGTGGTTTGCT-3′
- IL-1β forward, 5′-GCTTCAGGCAGGCAGTATCACT-3′
- IL-1β reverse, 5′-CACGGGAAAGACACAGGTAGCT-3′
- Glyceraldehydes 3-phosphate dehydrogenase (GAPDH) forward, 5′-TCAACAGCAACTCCCACTCTTCCA-3′;
- GAPDH reverse, 5′-ACCCTGTTGCTGTAGCCGTATT-CA-3′.

Annealing temperature was 60°C for all primer sets. Each time point was compared to uninjured controls and normalized to GAPDH.

Immunofluorescence, Immunohistochemical, and Histological Staining

Under deep anesthesia (ketamine/xylazine/acepromazine [50/5/1 mg/kg]), mice were perfused transcardially with 0.1 M phosphate buffer (PB) followed by 4% paraformaldehyde in 0.1 M PB. A 1 cm length of spinal cord centered on the lesion was removed and postfixed in the same fixative and processed for cryostat sectioning (12 μm). Tissue sections were incubated with 1% bovine serum albumin and 0.1% Triton-x-100 in PBS to block nonspecific binding. Sections were subsequently washed and incubated overnight at 4°C with the following primary antibodies: rabbit anti-HPGDS (1:500; Cayman Chemical) or rabbit anti-L-PGDS (1:2,000; Cayman Chemical). Differential cell types were identified with the following antibodies: rat Mac-1 antibody (1:200; Serotec, for macrophages/microglia), rat Mac-2 antibody (1:2; supernatant from Mac-2 producing hybridoma), rat anti-CD3 (1:100; BD Bioscience, for T cells), rat anti-B220 (1:100; BD Bioscience, for B cells), mouse anti-APC (1:50; Calbiochem, for oligodendrocytes), mouse anti-NeuN (1:50; Chemicon, for neurons), and rabbit anti-GFAP (1:500, Dako, for astrocytes). Serotonergic innervation was assessed using rabbit anti 5-HT (1:5,000; Sigma Aldrich). Tissue sections were subsequently washed and incubated for 1 h at room temperature with the following secondary antibodies: Alexa Fluor 488 goat anti-rabbit IgG (1:400 for GFAP staining, 1:600 for all other incubations; Invitrogen) and either Alexa Fluor 594 donkey anti-rat IgG (1:200 for Mac-1, T cell, and B cell staining, 1:600 for all other incubations; Invitrogen) or rhodamine conjugated goat anti-mouse IgG (1:500; Jackson ImmunoResearch). Myelin was visualized by staining with Luxol Fast Blue (LFB; Fisher) as described previously (Ghasemlou et al., 2005). For neuronal counts, tissue sections were stained with cresyl violet (Sigma-Aldrich) for 10 min at room temperature followed by dehydration through ascending alcohols and Hemo-De (Thermo Fisher Scientific).

Quantification of Histological Results

Histological quantification was performed from spinal cord cross-sections obtained from mice 28 days after SCI. All images were captured with a QImaging Retiga 1300C camera and viewed using a Zeiss Axioskop2 Plus microscope and quantification done using BioQuant Nova Prime image analysis system (BioQuant Image Analysis Corp.). Tissue sparing was calculated by manually outlining the GFAP stained area in cross-sections. Myelin sparing was assessed by calculating the LFB-stained area in the dorsal column. Neuronal survival was assessed by counting neuronal profiles in the ventral horn below the level of the central canal in tissue sections stained with cresyl violet. Serotonergic innervation was quantified by calculating the area occupied by serotonergic axons in a fixed area of the ventral horn at a distance of 1,000 μm caudal to the lesion site. All analysis was carried out blind.

Cytokine Protein Expression

Contusion injuries were done in adult HPGDS$^{-/-}$ and Wt mice ($n = 4$) and a 4 mm length of spinal cord centered on the lesion collected at 12 h after surgery and snap-frozen. The tissue was homogenized in Tissue Extraction Reagent I (Invitrogen), and protein concentration was determined using the DC Protein Assay (Bio-Rad). Samples were concentrated using MicroCon centrifugation filters (Millipore) and the protein concentration re-determined. All samples were diluted to 3.7 μg/μL to ensure equal amounts of protein. The protein levels of 20 cytokines and chemokines (FGF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p40/p70, IL-13, IL-17, IP-10, CXCL1/KC, CCL2/MCP-1, MIG, CCL3/MIP-1α, TNF-α, and VEGF) were then analyzed using the BioSource Mouse Cytokine 20-PlexMultiplex Bead Immunoassay (Invitrogen) on a Luminex-100LS system (Luminex Corp.) as per manufacturers' protocol. Results were analyzed using Beadview multiplex data analysis software (UpState Biotechnology).

PGD_2 Enzyme Immunoassay

Contusion injuries were done in *HPGDS*$^{-/-}$ and Wt mice ($n = 4$) and 4 mm length of spinal cord centered on the lesion collected on 1, 5, and 14 days after injury and snap-frozen. Total lipids were extracted using a modified Bligh and Dyer method (Bonin et al. 2004) and prostaglandin D_2 (PGD_2) levels analyzed using a PGD_2 Enzyme Immunoassay (EIA) Kit (Cayman Chemical) as per manufacturer's protocol with the following modifications: to control for extraction efficiency, tissue was spiked with a synthetic internal standard [187.5 ng C13:0 lysophosphatidylcholine (LPC)] added at the time of lipid extraction. Concentrations of C13:0 LPC were determined by high-performance liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS). Variation in extraction efficiency between samples was less than 2%. For PGD_2 quantification, samples were analyzed in triplicate and concentrations assessed in comparison with a standard curve at three different dilutions to ensure a

linear response. Assays were conducted in replicate for a total of 18 measurements per time point averaged to give one data point per animal ($n = 4$/condition/time point). Data are expressed as pg/milligram of tissue wet weight at time of extraction.

Statistical Analyses

The data are shown as mean ± SEM. The RT-PCR was analyzed by one-way ANOVA with *post hoc* Dunnett's test. The EIA data were analyzed by two-way ANOVA with *post hoc* Bonferroni test. The BMS data and histological assessments were performed by using two-way repeated measures ANOVA with *post hoc* Tukey's test for multiple comparisons. Differences were considered significant at $P < 0.05$.

RESULTS

HPGDS Is Upregulated in Microglia/Macrophages After SCI

We first assessed the changes in expression and localization of the two PGD_2 synthases [L-PGDS and hematopoietic prostaglandin D synthase (HPGDS)] in the uninjured and injured spinal cord of adult C57BL/6 mice at several time-points after contusion injury. HPGDS mRNA levels begin to show an increase at 3 days and are significantly increased between seven and eightfold at 7 and 14 days after SCI and decrease at later times (Fig. 1A). In contrast, the mRNA levels of L-PGDS did not change after SCI (Fig. 1A). Double immunofluorescence labeling showed that HPGDS is expressed in $Mac1^+$ cells (Fig. 1B–G), a marker that is highly expressed by activated microglia and macrophages of hematogenous origin. Activated microglia/macrophages have been shown to reach their peak numbers at 7 days after contusion injury (Sroga et al., 2003). Double immunofluorescence labeling of L-PGDS in naïve and injured spinal cord showed L-PGDS was localized to oligodendrocytes as previously reported (Urade et al., 1985a, 1985b) with no discernible differences in staining before and after SCI (data not shown).

HPGDS Is Responsible for the Increase in PGD_2 After SCI

We next examined the changes in the levels of prostaglandin D_2 (PGD_2) after SCI and, in particular, assessed the contribution of hematopoietic prostaglandin D synthase (HPGDS) to the increase in PGD_2 after SCI. This was done by quantifying PGD_2 in spinal cord tissue at 1, 5, and 14 days after injury in Wt and $HPGDS^{-/-}$ mice using a competitive EIA approach ($n = 4$ for each group). Comparisons of the differences in the PGD_2 levels in Wt and $HPGDS^{-/-}$ mice would also reveal the relative contributions of HPGDS and L-PGDS to PGD_2 production. The EIA results showed a striking 3-fold increase in PGD_2 levels at 14 days after contusion injury (Fig. 2A). Interestingly, this increase in PGD_2 was completely abrogated in the $HPGDS^{-/-}$ mice. These results therefore suggest that the increase in PGD_2 in the injured spinal cord is produced via HPGDS and not L-PGDS. It is possible that the assay is not sensitive enough to detect small changes in PGD_2 that is likely to occur earlier than day 14 after injury, as PGD_2 is very labile. This may account for why changes in locomotor recovery and cytokine expression are detected at earlier time points (see below).

HPGDS Mediated Production of PGD_2 After SCI Is Detrimental

The experiments described earlier indicate that the increased level of PGD_2 in the spinal cord after contusion injury is mainly attributable to hematopoietic prostaglandin D synthase (HPGDS), which is expressed by microglia/macrophages located in the lesion epicenter. To determine what role PGD_2 plays after SCI, spinal cord contusion injuries were done in $HPGDS^{+/+}$ and $HPGDS^{-/-}$ mice, and locomotor recovery assessed using the BMS analysis. Significant improvement in locomotor control was observed in $HPGDS^{-/-}$ mice when compared to Wt controls, starting from 5 days after injury and continuing until day 28. The $HPGDS^{-/-}$ mice reached an average maximal BMS score of 4.5, which indicates stepping with both hind limbs, while $HPGDS^{+/+}$ mice reached an average score of three, which indicates that the mice can only place their hind limb paws in the correct placement, with or without the ability to bear weight (Fig. 2B). The significant improvement seen in the $HPGDS^{-/-}$ indicates a detrimental role for PGD_2 after SCI. These results were further confirmed in Wt mice treated with a selective inhibitor of HPGDS (HQL-79) after SCI (Fig. 2C). C57BL/6 mice that were given daily subcutaneous injections of HQL-79 starting 1 h after SCI for 28 days showed a significant improvement in locomotor recovery as judged by the BMS analysis when compared to the vehicle treated controls. The earliest time point at which statistically significant differences in locomotor control were observed between HQL-79 and vehicle-treated mice occurred at day 10 after SCI. This improvement is 5 days later than that seen in $HPGDS^{-/-}$ mice. This difference between HQL-79 and $HPGDS^{-/-}$ mice may have to do with the concentration and frequency of the inhibitor used. Additional experiments will need to be done to optimize the conditions of the inhibitor treatment. Similar to the $HPGDS^{-/-}$ mice, Wt mice given HQL-79 reached an average score of 5 by 28 days after injury, which indicates frequent stepping with both hind limbs. Wt mice given vehicle performed similar to $HPGDS^{+/+}$ mice and were only able to place both hind limbs but unable to step frequently by day 28. These additional experiments with the HPGDS inhibitor confirmed that PGD_2 produced via HPGDS after SCI is detrimental.

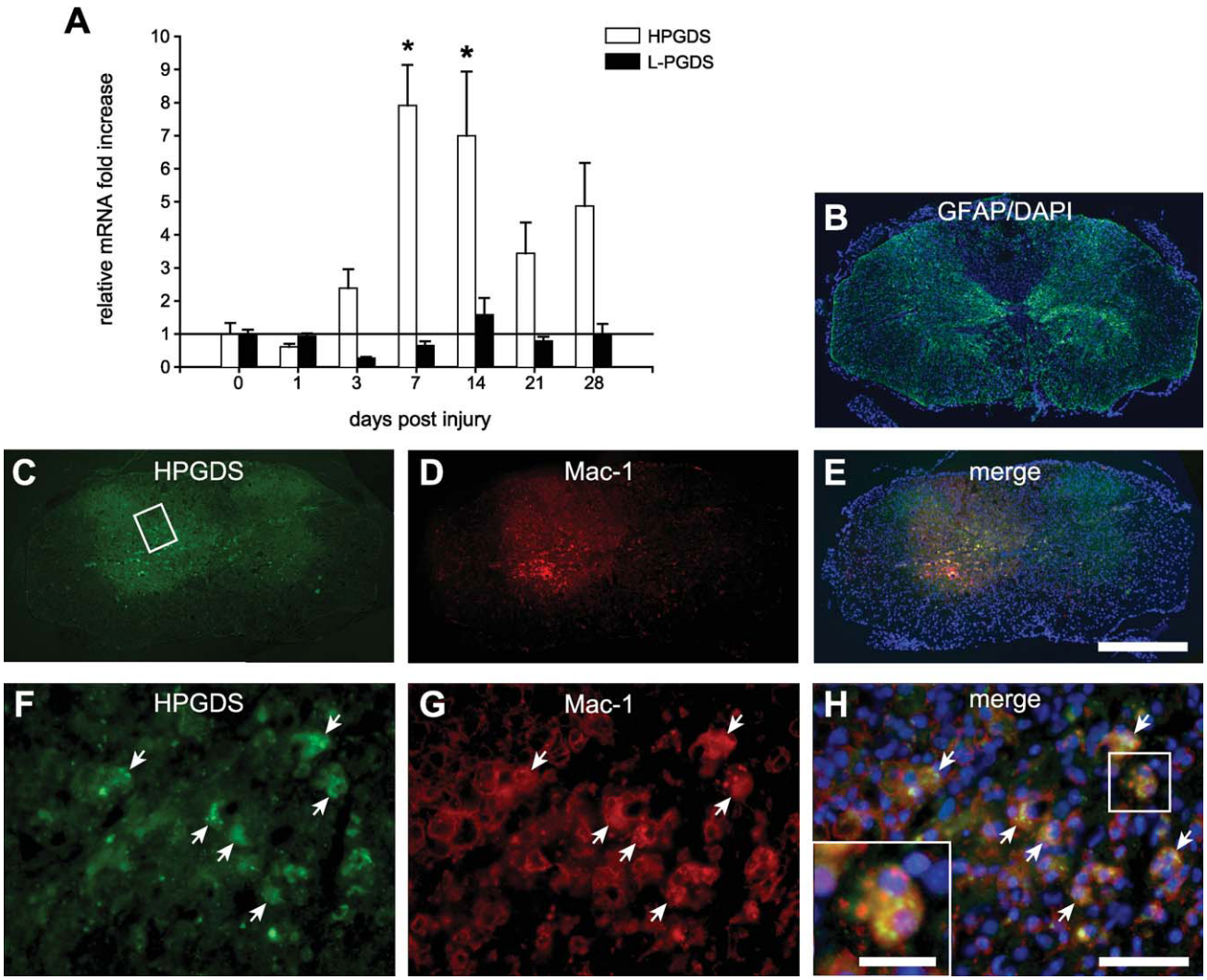

Fig. 1. HPGDS expression is increased after spinal cord contusion injury. The mRNA expression levels of the two PGD_2 synthases (HPGDS and L-PGDS) in the spinal cord were analyzed at several time points after contusion injury. **A:** Quantification of relative mRNA fold increases over levels in uninjured spinal cord tissue; values normalized to PPIA. HPGDS expression peaks at 7 and 14 days after injury and remains high for up to 28 days. L-PGDS mRNA levels do not change after SCI. **B:** Low magnification image of a spinal cord cross-section stained for GFAP at 7 days postinjury to show the lesion architecture. This section is at a distance of 100 μm caudal to the lesion epicenter. Nuclei are labeled with DAPI. **C–H:** Double immunofluorescence labeling of HPGDS and Mac-1, 7 days after SCI of a section adjacent to that shown in B. C–E: Low magnification image of the entire cross-section of the spinal cord showing labeling for HPGDS (C), Mac-1 (D), and the merged image showing HPGDS, Mac-1, and nuclear staining with DAPI (E). F–H: Higher magnification of the area outlined in the dashed line in panel C, showing HPGDS labeling (F), Mac-1 (G), and merged image showing HPGDS, Mac-1, and DAPI staining (H). Note that HPGDS is expressed in Mac-1^+ macrophages/microglia (arrows). Scale bars: E = 500 μm, H = 50 μm (inset = 20 μm). n = 3 for all analyses. Values represent mean ± SEM; $^*P < 0.05$.

Secondary Tissue Damage After SCI Is Reduced in HPGDS$^{-/-}$ Mice

Because HPGDS$^{-/-}$ mice showed improved locomotor recovery, we next examined whether the lack of HPGDS also had an impact on secondary tissue damage. To assess this, the extent of myelin loss after SCI was examined by staining cross sections of the spinal cord with LFB. *HPGDS*$^{-/-}$ mice showed significantly greater sparing of myelin when compared to Wt mice (Fig. 3A,B). *HPGDS*$^{-/-}$ mice showed greater myelin sparing at the epicenter and at distances of up to 1 mm rostral and caudal to the injury (Fig. 3C). Images of spinal cord cross sections stained for GFAP at different distances from the lesion epicenter are shown in Supporting Information Fig. 1 and display the extent of the moderate injury. Mice treated with the HPGDS inhibitor also showed greater myelin sparing at some regions of the injured spinal cord with a trend to an increase in other regions (see Supp. Info. Fig. 2). We also assessed the number of surviving neurons in the ventral gray matter of the spinal cord at varying distances from the epicenter of the lesion. *HPGDS*$^{-/-}$ mice had significantly more neurons caudal to the epicenter when compared to *HPGDS*$^{+/+}$ mice (Fig. 3D–F). Serotonergic axons that descend from the raphe nuclei in the brainstem and innervate the ventral horn are required for locomotor control. We therefore assessed the serotonergic innervation in the ventral gray matter

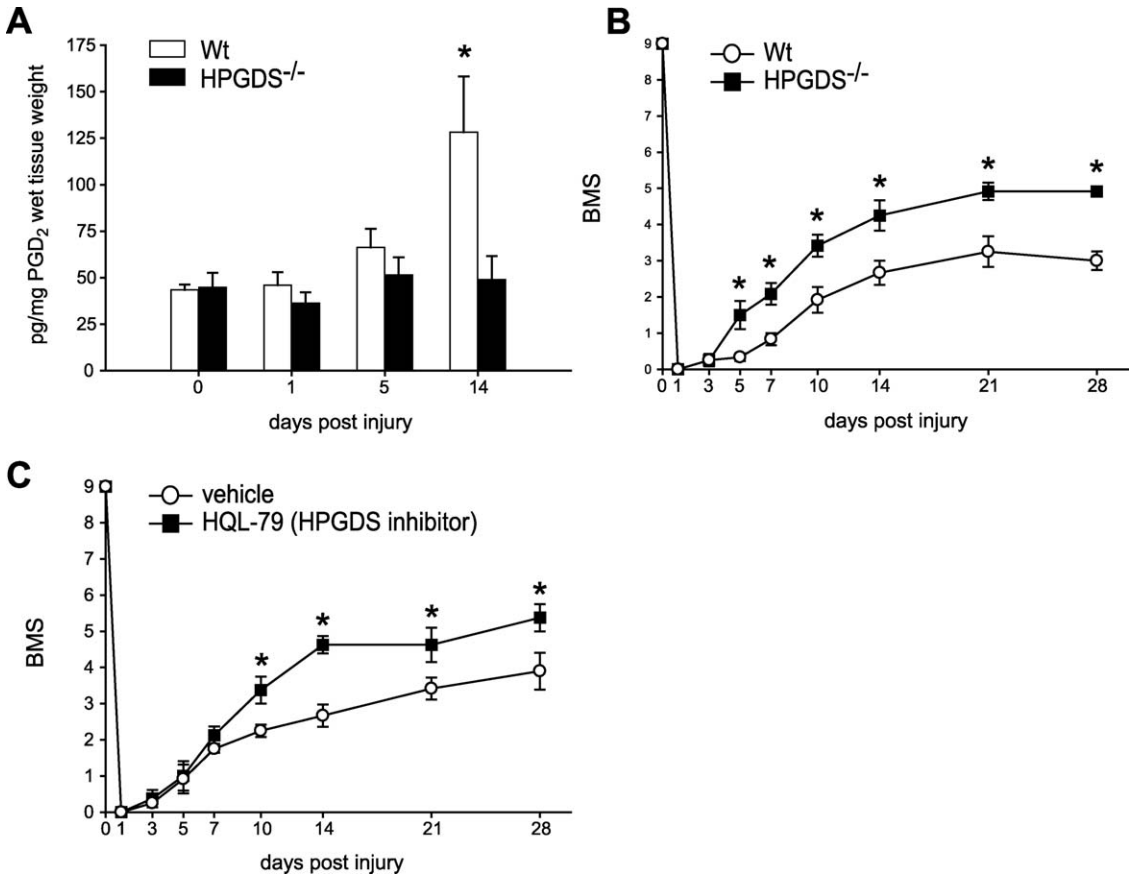

Fig. 2. Increased PGD_2 after contusion injury is synthesized by HPGDS and is detrimental after SCI. **A:** The level of PGD_2 in spinal cord tissue from uninjured and contused spinal cord at 1, 5, and 14 days after SCI from wild type (Wt) and *HPGDS*$^{-/-}$ mice were analyzed by EIA. In Wt mice, PGD_2 levels begin to rise at 5 days postinjury and is significantly greater than *HPGDS*$^{-/-}$ mice at 14 days after injury (n = 4 per group; ANOVA, *post hoc* Bonferroni). This increase is completely abrogated in the HPGDS$^{-/-}$ mice. **B:** HPGDS$^{-/-}$ mice show significant improvement in locomotor recovery after SCI when compared to Wt controls assessed using the BMS analysis. Improvement in locomotor control is seen beginning on day 5 after SCI and sustained for the entire 28-day period (n = 6 for both HPGDS$^{-/-}$ and Wt). **C:** Wt C57BL/6 mice treated daily with the HPGDS inhibitor, HQL-79, starting 1 h after SCI show significant improvement in locomotor recovery when compared to vehicle-treated controls. The improvement in locomotor scores seen in HQL-79 treated mice is similar to that seen with HPGDS$^{-/-}$ mice [n = 4 (HQL-79); n = 5 (vehicle)]. Values are represented as mean ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$.

1,000-μm caudal to the epicenter at 28 days after SCI. The *HPGDS*$^{-/-}$ mice had a greater than twofold increase in sparing of serotonergic innervation of the ventral horn when compared to the injured Wt controls (Fig. 3G–I). Interestingly, the extent of macrophage/microglial infiltration into the injury site based on Mac-1 staining appears to be similar in Wt and *HPGDS*$^{-/-}$ mice 28 days after SCI (Fig. 4A–C). This suggests that the metabolic products of HPGDS are likely to contribute to secondary damage not via mediating the infiltration of macrophages into the injured cord but likely by modulating the responses of macrophages/microglia or other neighboring cell types.

The cross sections of the spinal cord taken 28 days after contusion were also examined for changes in T-cell and B-cell infiltration. T cells were only detected at the epicenter of the lesion. Their numbers in the *HPGDS*$^{-/-}$ mice were increased when compared to Wt mice, although the total numbers of these cells appear to be very small (WT = 3.3 ± 1.9; KO = 24 ± 3.2; $P < 0.01$, n = 3). Their significance, if any, to the pathology remains unclear. The numbers of B cells were not significantly different between the groups (WT = 64 ± 44.3; KO = 40 ± 19.6, n = 3).

Differences in Chemokine/Cytokine Expression in the Injured Spinal Cord in HPGDS$^{-/-}$ Mice

As PGD_2 is a potent inflammatory mediator in the periphery, we examined the expression of several inflammatory chemokines and cytokines after SCI. As chemokine and cytokine levels in the spinal cord are increased early after injury (Bartholdi and Schwab, 1997; Pineau and Lacroix, 2007; Yang et al., 2004), we assessed changes in chemokine/cytokine levels in the spinal cord 12 h after SCI in Wt and hematopoietic prostaglandin D synthase (*HPGDS*)$^{-/-}$ mice using a Cytokine 20-Plex Multiplex Bead Immunoassay. At this time, both Wt and *HPGDS*$^{-/-}$ mice showed a marked increase in CXCL1/KC, CCL2/MCP-1, CCL3/MIP-1α, CXCL10/IP-10, IL-1α, IL-5, and IL-6, when compared to uninjured spinal cord in which these cytokines were not detected (Fig. 5A,B).

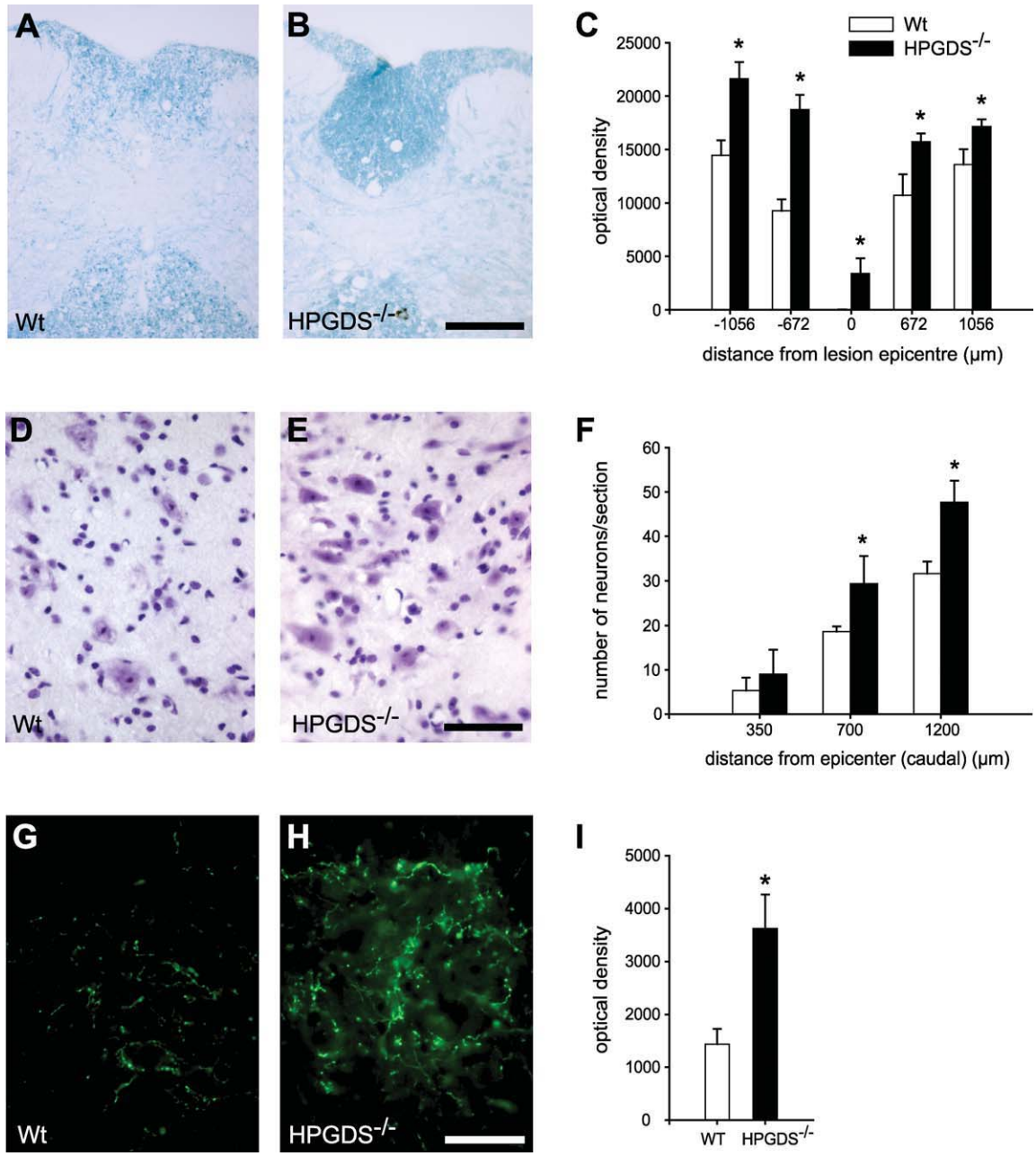

Fig. 3. HPGDS$^{-/-}$ mice have reduced secondary damage after SCI. **A, B:** Micrographs showing LFB staining of the dorsal white matter in wild-type (Wt) and HPGDS$^{-/-}$ mice at 28 days after SCI, taken from a distance of 627-μm rostral to the epicenter of the lesion. Note that there is greater sparing of myelin in the dorsal columns in the HPGDS$^{-/-}$ mice (B) when compared to the Wt mice (A). **C:** Graph showing that there is significantly greater myelin sparing at all distances 1 mm rostral and caudal to the lesion epicenter. Micrographs of cresyl violet-stained sections of the ventral horn of the spinal cord Wt (**D**) and HPGDS$^{-/-}$ (**E**) mice taken from ~ 1,000 μm caudal to epicenter. Note the greater sparing of neurons in the HPGDS$^{-/-}$ mouse (E). **F:** Graph showing that there is significantly greater sparing of neurons in the ventral horn of HPGDS$^{-/-}$ mice when compared to Wt controls caudal to the lesion epicenter at 28 days after SCI. **G, H:** Micrographs of 5-HT staining of the ventral gray matter of Wt (G) and HPGDS$^{-/-}$ (H) mice 1,000 μm caudal to lesion epicenter 28 days after SCI. **I:** Graph shows that there is significantly greater sparing of 5-HT fibers in the ventral horn of HPGDS$^{-/-}$ mice when compared to Wt controls 28 days after SCI. $n = 3$; values represent means ± SEM; $^*P < 0.05$. Scale bars: B = 200 μm and E and H = 50 μm.

Of these, *HPGDS*$^{-/-}$ showed a significant decrease in CLCL10 and a significant increase in IL-6 when compared to Wt mice. Interferon-γ was not detected in any of the groups. Unexpectedly, neither TNF-α nor IL-1β levels were detected in either genotype after injury, which was likely due to technical problems as both these cytokines have been reported to be expressed at this time point after SCI (Pineau and Lacroix, 2007). We therefore carried out quantitative real-time-PCR analysis of the mRNA levels of IL-1β, TNF-α, and TGF-β1 in the uninjured spinal cord and at 1 and 14 days after injury. The later time point was chosen as some cytokines have

also been reported to have a second peak at 2 weeks after SCI (Pineau and Lacroix, 2007). The mRNA levels of IL-1β showed a ~ 30-fold increase at 14 days after SCI, but no differences were seen between the genotypes (Fig. 6A). TNF-α mRNA showed an early increase at 1 day (20-fold) and a more substantial increase (~ 80-fold) at 14 days after SCI (Fig. 6B). However, there was no difference in these levels between *HPGDS*$^{-/-}$ and *HPGDS*$^{+/+}$ mice (Fig. 6B). TGF-β1 mRNA showed ~ sevenfold increase in Wt mice at 1 day and a 19-fold increase at 14 days postinjury (Fig. 6C). Although there was no difference in TGF-β1 mRNA levels between Wt and HPGDS$^{-/-}$ at 1 day after SCI, the expression level was almost twofold greater in HPGDS$^{-/-}$ mice at 14 days (Fig 6C). As these cytokines and chemokines can influence the infiltration and activation of peripheral immune cells, we assessed by quantitative real-time-PCR the mRNA expression of Mac-2, a galectin expressed by activated macrophages and microglia (Liu et al., 1995; Rotshenker, 2009). There was a significant decrease in Mac-2 expression in *HPGDS*$^{-/-}$ that was statistically significant at 14 days after SCI (Fig. 7A). Collectively, these data suggest that PGD_2 produced via HPGDS after SCI may mediate inflammatory responses in the spinal cord. As IL-6 mRNA levels were significantly higher in the injured spinal cord in HPGDS$^{-/-}$, we assessed the expression of metallothionein I, II, and III, which are potent anti-oxidants that can be regulated by IL-6 (West et al., 2008). Metallothionein III mRNA was found to be significantly higher in the injured spinal cord of HPGDS$^{-/-}$ mice early after injury when compared to Wt mice (Fig. 7B). No differences between genotypes were found in the expression of metallothionein I and II (data not shown).

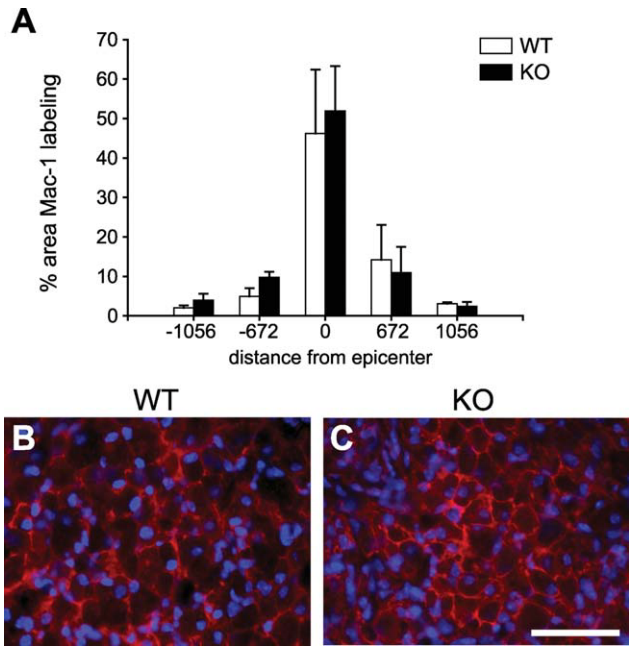

Fig. 4. **A:** Graph showing the percentage of the dorsal column area labeled with Mac-1 in cross sections of the spinal cord at 28 days after contusion injury. Measurements were made from sections taken at different regions rostral and caudal to the lesion epicenter (0). No differences were seen between *HPGDS*$^{-/-}$ and wild-type (Wt) mice. **B, C:** Micrographs showing similar areas from the epicenter of the lesion taken from Wt (B) and *HPGDS*$^{-/-}$ (C) mice. Note the similarity in the staining. Scale bar in *C* = 50 μm.

DISCUSSION

A number of factors have been shown to be implicated in triggering the inflammatory response after SCI that contributes to secondary damage and functional loss (Donnelly and Popovich, 2008; Kwon et al., 2004). Despite the considerable work done in this area of SCI, the potential role of prostaglandins in modulating the inflammatory response after SCI has not been examined thus far. We have examined the expression and role of PGD_2 in spinal cord contusion injury. We show that (i) expression of only one of the two synthetic enzymes that produce PGD_2, namely, HPGDS but not L-PGDS is increased after SCI; (ii) the expression of HPGDS in SCI is largely confined to microglia/macrophages at the lesion site; (iii) mice lacking HPGDS show improved locomotor recovery when compared to Wt mice; (iv)

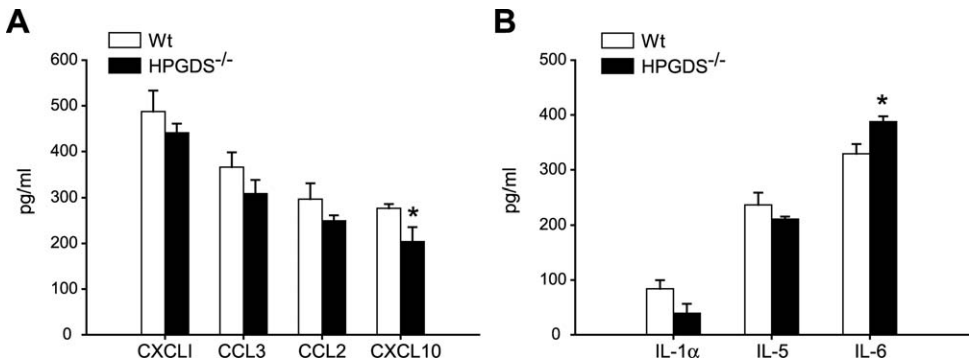

Fig. 5. Changes in chemokines and cytokines in the spinal cord after SCI. The protein levels of several chemokines (**A**) and cytokines (**B**) were analyzed at 12 h after SCI in wild type (Wt) and HPGDS$^{-/-}$ mice. A: The level of CXCL10 protein was significantly reduced in HPGDS$^{-/-}$ mice when compared to Wt controls. Although the levels of other chemokines were elevated after SCI, there were no differences between the two genotypes. B: The level of IL-6 protein was significantly increased in the spinal cord of HPGDS$^{-/-}$ mice when compared to Wt controls. Values represent means ± SEM; *$P < 0.05$; $n = 4$ for each group. Protein levels were assayed using the BioSource Mouse Cytokine 20-Plex Multiplex Bead Immunoassay.

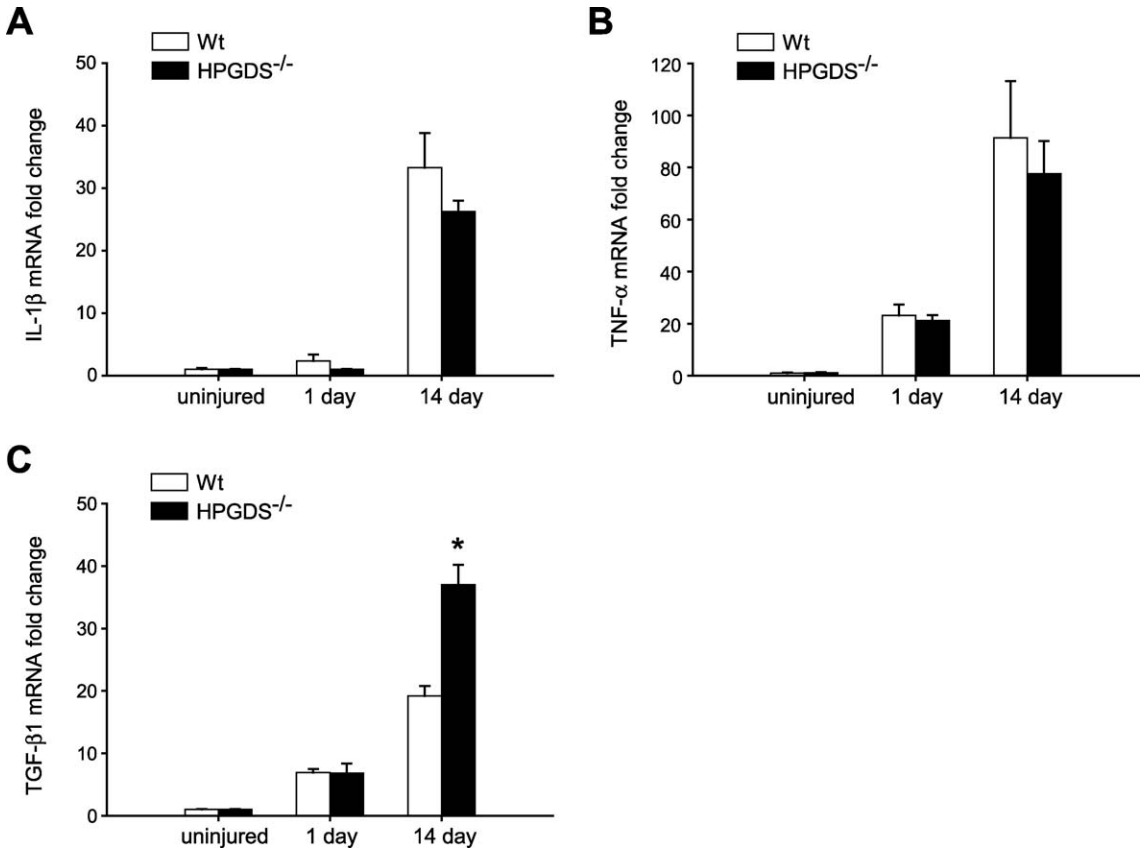

Fig. 6. The mRNA expression by quantitative real-time-PCR analysis of IL-1β (**A**), TNF-α (**B**), and TGF-β1 (**C**) was examined in uninjured spinal cord and spinal cord tissue at 1 and 14 days after SCI. Note that although there is a delayed increase in IL-1β (A) and TNF-α (B) in both strains of mice at 14 days, there are no differences between the two genotypes. C: In contrast, there was a significant increase in the expression of TGF-β1 in HPGDS$^{-/-}$ mice at 14 days post-SCI. Values represent means ± SEM; *$P < 0.05$; $n = 3$ for each group.

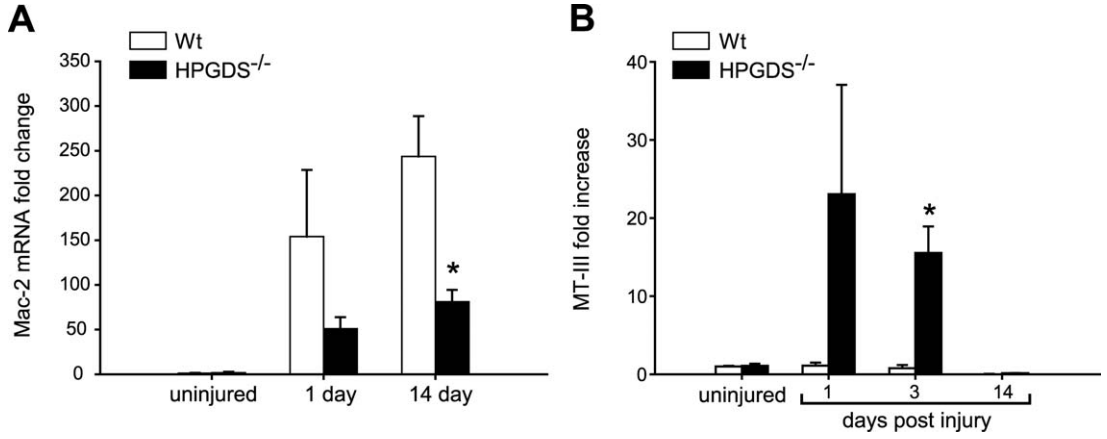

Fig. 7. **A:** The mRNA level of Mac-2 (a galectin expressed on activated microglia/macrophages) is significantly decreased at 14 days after SCI in *HPGDS*$^{-/-}$ mice when compared to wild-type (Wt) mice, suggesting a reduction in macrophage/microglial activation. **B:** There is increased mRNA expression of the free radical scavenger and potent antioxidant, metallothionin-III, in HPGDS$^{-/-}$ mice when compared to Wt controls. Values represent means ± SEM; *$P < 0.05$; $n = 3$ for each group.

improved locomotor recovery is also seen in Wt mice treated with a small molecule inhibitor of HPGDS (HQL-79); (v) HPGDS$^{-/-}$ mice show reduced secondary damage in the spinal cord after SCI as reflected in increased sparing of myelin, neurons, and serotonergic innervation of the ventral horn; (vi) HPGDS$^{-/-}$ mice also show reduced expression of CXCL10 and an increase in IL-6 and TGF-β1 after SCI; (vii) HPGDS$^{-/-}$ mice also showed increased expression of metallothionein (MT)-III, which has antioxidant activity; (viii) the macrophage/microglial activation marker Mac-2 is also reduced after SCI in HPGDS$^{-/-}$ mice. These data sug-

gest that PGD_2 produced by macrophages after SCI has detrimental effects, which can be blocked by treatment with a small molecule HPGDS inhibitor.

The reduction of secondary damage and the locomotor improvement after SCI in *HPGDS*$^{-/-}$ mice indicates a potential role for PGD_2 in the proinflammatory response following SCI. In support of this, we observed a significant decrease in the expression of the macrophage marker Mac-2 in the *HPGDS*$^{-/-}$ mice after SCI. PGD_2 has previously been shown to mediate microglial activation in the *twitcher* mouse mutant (Mohri et al., 2006), a model of human Krabbes disease. In those experiments, the double *twitcher*/*HPGDS*$^{-/-}$ mice showed reduced microglial activation and signs of reduced inflammation in the CNS as well as a reduction in iNOS expression (Mohri et al., 2006). Although we found a reduction in the macrophage activation marker Mac-2, we did not see differences in iNOS mRNA expression on days 1, 3, or 14 after SCI in *HPGDS*$^{-/-}$ and Wt mice (data not shown). Furthermore, the *twitcher*/*HPGDS*$^{-/-}$ mice showed no differences in the level of IL-6, in contrast to the increase we have seen in *HPGDS*$^{-/-}$ mice early after SCI. Although both models highlight PGD_2 as a proinflammatory mediator in the CNS, the mechanisms of action appear to be different in these two models. In addition to the changes in Mac-2, the expression of the chemokine CXCL10 was also significantly reduced in the *HPGDS*$^{-/-}$ mice after spinal cord contusion injury. Despite the reduction in this cytokine, T cells, which were found only at the epicenter of the lesion, appeared to be increased in the *HPGDS*$^{-/-}$ mice, although the total number of these cells remained very low. The reason for this is not clear at present. However, other studies have shown that CXCL10 can mediate effects independent of its T-cell chemoattractant properties. For instance, the inhibition of CXCL10 using a function-blocking antibody in a dorsal spinal cord hemisection lesioning model in rats was found not only to reduce macrophage numbers but also increase angiogenesis (Glaser et al., 2004) and neuroprotection (Glaser et al., 2006). The decrease in CXCL10 in *HPGDS*$^{-/-}$ seen in our work may therefore mediate beneficial effects on angiogenesis after SCI.

Interestingly, IL-6 and TGF-β1 were significantly increased in *HPGDS*$^{-/-}$ mice after SCI. Some studies have suggested that IL-6 has proinflammatory effects after SCI (Okada et al., 2004), while a number of other studies indicate that it may have anti-inflammatory and/or protective effects after SCI, for example, IL-6 plays an important role in peripheral nerve regeneration and is required for regenerating dorsal column axons (Cafferty et al., 2004) as well as promoting axonal regeneration in the presence of myelin inhibitors in SCI (Cao et al., 2006; Hannila and Filbin, 2008). Increased levels of IL-6 have also been found after spinal cord contusion injury in transgenic mice lacking active NF-κB (Brambilla et al., 2005), as well as in Wt mice injected with 15d-PGJ2, a metabolite of PGD_2 (Kerr et al., 2008) in which secondary damage was reduced and accompanied by improvement in locomotor recovery. IL-6 has also been found to be neuroprotective when administered to rodents, which are exposed to excitotoxic insults or ischemia (Allan and Rothwell, 2001). IL-6 has also been linked to the expression of MT, free radical scavengers, and antioxidant proteins (West et al., 2008). We found increased expression of MT-III in *HPGDS*$^{-/-}$ mice after SCI, which could also contribute to reducing secondary damage. The increase in IL-6 found in the *HPGDS*$^{-/-}$ mice may therefore have a positive impact on tissue protection after spinal cord contusion injury and could potentially impact either indirectly or directly on the increased serotonergic innervation after SCI in the *HPGDS*$^{-/-}$ mice. We also found that TGF-β1 expression was increased significantly in *HPGDS*$^{-/-}$ when compared to Wt mice at 14 days after SCI. TGF-β1 is a pleiotropic cytokine, which is expressed in the injured spinal cord (McTigue et al., 2000). We did not find differences in expression of GFAP or laminin by qRT-PCR or chondroitin sulfate proteoglycan by immunostaining in the injured spinal cord of *HPGDS*$^{-/-}$ and Wt mice (data not shown). However, this cytokine exhibits a variety of anti-inflammatory properties (Lefer et al., 1990; Perrella et al., 1994) that could contribute to the reduction in secondary damage seen after SCI.

Our laboratory has previously reported that daily injections of low doses of 15d-PGJ2, a dehydration product of PGD_2, has beneficial effects after SCI, while higher doses are detrimental (Kerr et al., 2008). Similar beneficial and detrimental effects of 15d-PGJ2 were also seen in experimental autoimmune encephalomyelitis (Diab et al., 2002). In the present study, we show that reducing PGD_2 produced by HPGDS in hematogenous macrophages in *HPGDS*$^{-/-}$ mice after SCI results in beneficial effects in terms of histopathology and locomotor recovery. In these *HPGDS*$^{-/-}$ mice, the unaffected L-PGDS may produce low basal levels of PGD2 that can get nonenzymatically converted to the lower protective concentrations of 15d-PGD_2 that would add to the beneficial effects seen. Our results also suggest that PGD_2 produced via HPGDS in macrophages either directly or indirectly mediate inflammatory responses that contribute to secondary tissue damage after SCI. Future work needs to focus on delineating the downstream receptor mechanisms, as well as other possible beneficial mechanisms, which might result from blocking HPGDS. As the inhibition of PGD_2 using the small molecule inhibitor (HQL-79) was also effective in improving locomotor function after SCI, HPGDS could be a target for therapeutic intervention in the treatment of acute SCI.

ACKNOWLEDGMENTS

AR was a recipient of a studentship from the CIHR Training Program in Neuroinflammation. LAS was a recipient of a Vision 2010/Ontario Ministry of Research Innovation post-doctoral fellowship. The authors thank Hiba Kazak, Ourania Tsatas, and Claude Lachance for technical help, Ashleigh McLean for critical reading of this manuscript, and Margaret Attiwell for help with the illustrations.

REFERENCES

Allan SM, Rothwell NJ. 2001. Cytokines and acute neurodegeneration. Nat Rev Neurosci 2:734–744.

Angeli V, Staumont D, Charbonnier AS, Hammad H, Gosset P, Pichavant M, Lambrecht BN, Capron M, Dombrowicz D, Trottein F. 2004. Activation of the D prostanoid receptor 1 regulates immune and skin allergic responses. J Immunol 172:3822–3829.

Aritake K, Kado Y, Inoue T, Miyano M, Urade Y. 2006. Structural and functional characterization of HQL-79, an orally selective inhibitor of human hematopoietic prostaglandin D synthase. J Biol Chem 281: 15277–15286.

Bartholdi D, Schwab ME. 1997. Expression of pro-inflammatory cytokine and chemokine mRNA upon experimental spinal cord injury in mouse: An in situ hybridization study. Eur J Neurosci 9:1422–1438.

Basso DM, Fisher LC, Anderson AJ, Jakeman LB, McTigue DM, Popovich PG. 2006. Basso Mouse Scale for locomotion detects differences in recovery after spinal cord injury in five common mouse strains. J Neurotrauma 23:635–659.

Bate C, Kempster S, Williams A. 2006. Prostaglandin D2 mediates neuronal damage by amyloid-β or prions which activates microglial cells. Neuropharmacology 50:229–237.

Beuckmann CT, Lazarus M, Gerashchenko D, Mizoguchi A, Nomura S, Mohri I, Uesugi A, Kaneko T, Mizuno N, Hayaishi O, Urade Y. 2000. Cellular localization of lipocalin-type prostaglandin D synthase (β-trace) in the central nervous system of the adult rat. J Comp Neurol 428:62–78.

Bonin F, Ryan SD, Migahed L, Mo F, Lallier J, Franks DJ, Arai H, Bennett SA. 2004. Anti-apoptotic actions of the platelet-activating factor acetylhydrolase I alpha2 catalytic subunit. J Biol Chem 279: 52425–52436.

Brambilla R, Bracchi-Ricard V, Hu WH, Frydel B, Bramwell A, Karmally S, Green EJ, Bethea JR. 2005. Inhibition of astroglial nuclear factor κB reduces inflammation and improves functional recovery after spinal cord injury. J Exp Med 202:145–156.

Cafferty WB, Gardiner NJ, Das P, Qiu J, McMahon SB, Thompson SW. 2004. Conditioning injury-induced spinal axon regeneration fails in interleukin-6 knock-out mice. J Neurosci 24:4432–4443.

Cao Z, Gao Y, Bryson JB, Hou J, Chaudhry N, Siddiq M, Martinez J, Spencer T, Carmel J, Hart RB, Filbin MT. 2006. The cytokine interleukin-6 is sufficient but not necessary to mimic the peripheral conditioning lesion effect on axonal growth. J Neurosci 26:5565–5573.

Diab A, Deng C, Smith JD, Hussain RZ, Phanavanh B, Lovett-Racke AE, Drew PD, Racke MK. 2002. Peroxisome proliferator-activated receptor-gamma agonist 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 ameliorates experimental autoimmune encephalomyelitis. J Immunol 168:2508–2515.

Donnelly DJ, Popovich PG. 2008. Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. Exp Neurol 209:378–388.

Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, Dumont AS. 2001. Acute spinal cord injury, Part 1: Pathophysiologic mechanisms. Clin Neuropharmacol 24:254–264.

Faden AI, Lemke M, Demediuk P. 1988. Effects of BW755C, a mixed cyclo-oxygenase-lipoxygenase inhibitor, following traumatic spinal cord injury in rats. Brain Res 463:63–68.

Ghasemlou N, Kerr BJ, David S. 2005. Tissue displacement and impact force are important contributors to outcome after spinal cord contusion injury. Exp Neurol 196:9–17.

Glaser J, Gonzalez R, Perreau VM, Cotman CW, Keirstead HS. 2004. Neutralization of the chemokine CXCL10 enhances tissue sparing and angiogenesis following spinal cord injury. J Neurosci Res 77:701–708.

Glaser J, Gonzalez R, Sadr E, Keirstead HS. 2006. Neutralization of the chemokine CXCL10 reduces apoptosis and increases axon sprouting after spinal cord injury. J Neurosci Res 84:724–734.

Hains BC, Yucra JA, Hulsebosch CE. 2001. Reduction of pathological and behavioral deficits following spinal cord contusion injury with the selective cyclooxygenase-2 inhibitor NS-398. J Neurotrauma 18:409–423.

Hannila SS, Filbin MT. 2008. The role of cyclic AMP signaling in promoting axonal regeneration after spinal cord injury. Exp Neurol 209:321–332.

Hayaishi O, Urade Y. 2002. Prostaglandin D2 in sleep-wake regulation: Recent progress and perspectives. Neuroscientist 8:12–15.

Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, Ichimasa M, Sugamura K, Nakamura M, Takano S, Nagata K. 2001. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. J Exp Med 193:255–261.

Kerr BJ, Girolami EI, Ghasemlou N, Jeong SY, David S. 2008. The protective effects of 15-deoxy-delta-(12,14)-prostaglandin J2 in spinal cord injury. Glia 56:436–448.

Kostenis E, Ulven T. 2006. Emerging roles of DP, CRTH2 in allergic inflammation. Trends Mol Med 12:148–158.

Kwon BK, Tetzlaff W, Grauer JN, Beiner J, Vaccaro AR. 2004. Pathophysiology and pharmacologic treatment of acute spinal cord injury. Spine J 4:451–464.

Lefer AM, Tsao P, Aoki N, Palladino MA Jr. 1990. Mediation of cardioprotection by transforming growth factor-β. Science 249:61–64.

Lewis RA, Soter NA, Diamond PT, Austen KF, Oates JA, Roberts LJ II. 1982. Prostaglandin D2 generation after activation of rat and human mast cells with anti-IgE. J Immunol 129:1627–1631.

Liu FT, Hsu DK, Zuberi RI, Kuwabara I, Chi EY, Henderson WR Jr. 1995. Expression and function of galectin-3, a β-galactoside-binding lectin, in human monocytes and macrophages. Am J Pathol 147:1016–1028.

Liu M, Eguchi N, Yamasaki Y, Urade Y, Hattori N, Urabe T. 2009. Protective role of hematopoietic prostaglandin D synthase in transient focal cerebral ischemia in mice. Neuroscience 163:296–307.

Lopez-Vales R, Garcia-Alias G, Guzman-Lenis MS, Fores J, Casas C, Navarro X, Verdu E. 2006. Effects of COX-2 and iNOS inhibitors alone or in combination with olfactory ensheathing cell grafts after spinal cord injury. Spine 31:1100–1106.

Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kabashima K, Sugimoto Y, Kobayashi T, Ushikubi F, Aze Y, Eguchi N, Urade Y, Yoshida N, Kimura K, Mizoguchi A, Honda Y, Nagai H, Narumiya S. 2000. Prostaglandin D2 as a mediator of allergic asthma. Science 287:2013–2017.

McTigue DM, Popovich PG, Morgan TE, Stokes BT. 2000. Localization of transforming growth factor-beta1 and receptor mRNA after experimental spinal cord injury. Exp Neurol 163:220–230.

Mohri I, Eguchi N, Suzuki K, Urade Y, Taniike M. 2003. Hematopoietic prostaglandin D synthase is expressed in microglia in the developing postnatal mouse brain. Glia 42:263–274.

Mohri I, Kadoyama K, Kanekiyo T, Sato Y, Kagitani-Shimono K, Saito Y, Suzuki K, Kudo T, Takeda M, Urade Y, Murayama S, Tanike M. 2007. Hematopoietic prostaglandin D synthase and DP1 receptor are selectively upregulated in microglia and astrocytes within senile plaques from human patients and in a mouse model of Alzheimer disease. J Neuropathol Exp Neurol 66:469–480.

Mohri I, Taniike M, Taniguchi H, Kanekiyo T, Aritake K, Inui T, Fukumoto N, Eguchi N, Kushi A, Sasai H, Kanaoka Y, Ozono K, Narumiya S, Suzuki K, Urade Y. 2006. Prostaglandin D2-mediated microglia/astrocyte interaction enhances astrogliosis and demyelination in twitcher. J Neurosci 26:4383–4393.

O'Banion MK, Kyrkanides S, Olschowka JA. 2002. Selective inhibition of cyclooxygenase-2 attenuates expression of inflammation-related genes in CNS injury. Adv Exp Med Biol 507:155–160.

Okada S, Nakamura M, Mikami Y, Shimazaki T, Mihara M, Ohsugi Y, Iwamoto Y, Yoshizaki K, Kishimoto T, Toyama Y Okano H. 2004. Blockade of interleukin-6 receptor suppresses reactive astrogliosis and ameliorates functional recovery in experimental spinal cord injury. J Neurosci Res 76:265–276.

Perrella MA, Yoshizumi M, Fen Z, Tsai JC, Hsieh CM, Kourembanas S, Lee ME. 1994. Transforming growth factor-β 1, but not dexamethasone, down-regulates nitric-oxide synthase mRNA after its induction by interleukin-1 beta in rat smooth muscle cells. J Biol Chem 269:14595–14600.

Pineau I, Lacroix S. 2007. Proinflammatory cytokine synthesis in the injured mouse spinal cord: Multiphasic expression pattern and identification of the cell types involved. J Comp Neurol 500:267–285.

Rajakariar R, Hilliard M, Lawrence T, Trivedi S, Colville-Nash P, Bellingan G, Fitzgerald D, Yaqoob MM, Gilroy DW. 2007. Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxyΔ^{12-14} PGJ2. Proc Natl Acad Sci USA 104:20979–20984.

Resnick DK, Graham SH, Dixon CE, Marion DW. 1998. Role of cyclooxygenase 2 in acute spinal cord injury. J Neurotrauma 15:1005–1013.

Rotshenker S. 2009. The Role of Galectin-3/MAC-2 in the Activation of the Innate-Immune Function of Phagocytosis in Microglia in Injury and Disease. J Mol Neurosci.

Saleem S, Zhuang H, de Brum-Fernandes AJ, Maruyama T, Narumiya S, Dore S. 2007. PGD(2) DP1 receptor protects brain from ischemia-reperfusion injury. Eur J Neurosci 26:73–78.

Spik I, Brenuchon C, Angeli V, Staumont D, Fleury S, Capron M, Trottein F, Dombrowicz D. 2005. Activation of the prostaglandin D2 receptor DP2/CRTH2 increases allergic inflammation in mouse. J Immunol 174:3703–3708.

Sroga JM, Jones TB, Kigerl KA, McGaughy VM, Popovich PG. 2003. Rats and mice exhibit distinct inflammatory reactions after spinal cord injury. J Comp Neurol 462:223–240.

Tanaka K, Hirai H, Takano S, Nakamura M, Nagata K. 2004. Effects of prostaglandin D2 on helper T cell functions. Biochem Biophys Res Commun 316:1009–1014.

Taniguchi H, Mohri I, Okabe-Arahori H, Aritake K, Wada K, Kanekiyo T, Narumiya S, Nakayama M, Ozono K, Urade Y, et al. 2007. Prostaglandin D2 protects neonatal mouse brain from hypoxic ischemic injury. J Neurosci 27:4303–4312.

Ujihara M, Horiguchi Y, Ikai K, Urade Y. 1988. Characterization and distribution of prostaglandin D synthetase in rat skin. J Invest Dermatol 90:448–451.

Urade Y, Fujimoto N, Hayaishi O. 1985a. Purification and characterization of rat brain prostaglandin D synthetase. J Biol Chem 260:12410–12415.

Urade Y, Kaneko T, Fujimoto N, Watanabe Y, Mizuno N, Hayaishi O. 1985b. Purification, characterization, and immunohistochemistry of rat brain prostaglandin D synthetase. Adv Prostaglandin Thromboxane Leukot Res 15:549–551.

Urade Y, Kitahama K, Ohishi H, Kaneko T, Mizuno N, Hayaishi O. 1993. Dominant expression of mRNA for prostaglandin D synthase in leptomeninges, choroid plexus, and oligodendrocytes of the adult rat brain. Proc Natl Acad Sci USA 90:9070–9074.

Urade Y, Ujihara M, Horiguchi Y, Igarashi M, Nagata A, Ikai K, Hayaishi O. 1990. Mast cells contain spleen-type prostaglandin D synthetase. J Biol Chem 265:371–375.

Urade Y, Ujihara M, Horiguchi Y, Ikai K, Hayaishi O. 1989. The major source of endogenous prostaglandin D2 production is likely antigen-presenting cells. Localization of glutathione-requiring prostaglandin D synthetase in histiocytes, dendritic, and Kupffer cells in various rat tissues. J Immunol 143:2982–2989.

West AK, Hidalgo J, Eddins D, Levin ED, Aschner M. 2008. Metallothionein in the central nervous system: Roles in protection, regeneration and cognition. Neurotoxicology 29:489–503.

Yang L, Blumbergs PC, Jones NR, Manavis J, Sarvestani GT, Ghabriel MN. 2004. Early expression and cellular localization of proinflammatory cytokines interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha in human traumatic spinal cord injury. Spine 29:966–971.