The Role of Prostaglandin D_2 as an Inflammatory Mediator Following Spinal Cord Injury

Adriana Redensek

Center for Research in Neuroscience

Department of Neurology and Neurosurgery

McGill University

Montreal, Canada

June 2009

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements of the degree of Master of Science

Dedicated with love to Stephanie Aleong

Table of Contents

Abstract

Spinal cord injury affects thousands of people every year, however, treatment options are currently limited and none are universally accepted. Inflammation has been shown to play a critical role in propagating secondary damage which exacerbates the initial trauma, therefore modulating the inflammatory response after injury has therapeutic potential. As prostaglandins are powerful inflammatory mediators in the periphery, I have examined the role of prostaglandin D_2 (PGD_2) after spinal cord injury. My thesis provides evidence that PGD_2 produced from hematopoietic prostaglandin D synthase plays a detrimental role in locomotor recovery. Blocking HPGDS or its receptor DP1, leads to reduced secondary damage, accompanied by changes in the immune response as well as improved locomotor recovery. My thesis provides new data about the inflammatory response that follows spinal cord injury as well as providing insight into possible therapeutic treatments for the future.

Résumé

Les lésions médullaires touchent des milliers de personnes chaque année. Cependant, il n'existe pas encore de consensus sur le traitement à adopter et les stratégies thérapeutiques offertes restent limitées. Il est à présent clairement établi que la réaction inflammatoire qui succède au traumatisme initial joue un rôle critique dans le développement de lésions secondaires. C'est pourquoi la modulation de la réponse inflammatoire possède un potentiel thérapeutique non négligeable. Etant donné que les prostaglandines sont de puissants médiateurs de l'inflammation dans le système périphérique, j'ai étudié le rôle de la prostaglandine D_2 (PGD_2) dans un modèle expérimental de lésion médullaire. Ma thèse démontre que PGD_2, produite par l'hématopoïétique prostaglandine D synthase joue un rôle déterminant dans la récupération fonctionnelle. Bloquer HPGDS ou son récepteur DP1 entraîne une réduction des lésions secondaires, un changement de la réponse immunitaire ainsi qu'une amélioration de la récupération fonctionnelle. Ma thèse fournit de nouvelles données sur la réponse inflammatoire consécutive aux lésions de la moelle épinière, elle ouvre également une fenêtre sur de potentielles stratégies thérapeutiques.

Acknowledgements

I would like to express my gratitude to everyone who has helped me during my Master's work. My supervisor, Dr. Sam David, I thank him immensely for giving me the opportunity to work on a great project and in a wonderful lab. I appreciate the advice and suggestions he had for me and this project.

My committee members, Dr. Giamal Luheshi and Dr. Adriana Di Polo were extremely helpful and encouraging, I am grateful I had such a wonderful committee.

I would like to extend my gratitude to Dr. Stefanie Bennet, Dr. Leigh-Ann Swayne and Ashleigh McClean from the University of Ottawa for their help with Figure 5. Dr. Leigh-Ann Swayne graciously helped me by extracting lipids from my samples using their modified bligh-dyer extraction technique. I thank Dr. Stefanie Bennet and Ashleigh McClean for analyzing the levels of prostaglandin D_2 in my spinal cord injury samples.

I thank the entire David lab, on top of being excellent scientists, I enjoyed seeing them every day. I am grateful to Delphine Bouhy for translating my abstract into French. I thank our technicians, Nia Tsatsa, Hiba Kazak, and Margaret Attiwell. I especially thank Nia and Khizr Rathore for their assistance in editing this thesis and Margaret for helping me with the figures. I thank Khizr Rathore and Dr. Ruben Lopez-Vales for assisting me in the BMS analysis. I appreciate everything Dr. Ruben Lopez-Vales has done for me. I thank him from the bottom of my heart; I could not have asked for a better mentor.

I must thank my loving family, my parents, who always thought I was smarter than I really am and my brothers, for being my brothers.

Finally, I thank Khizr Rathore, for being by my side through the rough times and for always believing in me. I could not have done this without him.

Abbreviations

AA	arachidonic acid
BBB	blood brain barrier
BDNF	brain derived neurotrophic factor
CNS	central nervous system
COX	cyclooxygenase
DHA	docosahexaenoic acid
EAE	experimental autoimmune encephalomyelitis
GFAP	glial fibrillary acidic protein
HPGDS	hematopoietic prostaglandin D synthase
IFN	interferon
IL	interleukin
iNOS	inducible nitric oxide synthase
L-PGDS	lipocalin-type prostaglandin D synthase
LFB	Luxol Fast Blue
MCP-1	monocyte chemotactic protein-1
MIP-1	macrophage inflammatory protein-1
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NGF	nerve growth factor
NSAIDs	non-steroidal anti-inflammatory drugs
PG	prostaglandin
PLA_2	phospholipase A_2
PND	post natal day
PPAR	peroxisome proliferators-activated receptor

RANTES	regulated on activation, normal T expressed and secreted (CCL5)
SCI	spinal cord injury
TLR	toll like receptor
TNF	tumor necrosis factor

Introduction

In Canada alone, approximately 1,100 people suffer spinal cord injury (SCI) accidents every year. Currently, there are approximately 41,000 Canadians who are living with SCI. At present, there are no treatments for complete recovery or cure. There are several reasons for the lack of recovery after SCI, which include failure of long distance axon regeneration, a depletion of the neuronal pool, demyelination and gliosis. In addition to the initial trauma that causes direct damage to axons, neuronal cell bodies and myelin, detrimental changes continue to occur in the days and weeks following injury and are referred to as 'secondary damage'. There is substantial evidence that inflammation following SCI contributes importantly to this secondary damage after SCI.

1.1 Inflammation and Secondary Damage

Several factors may contribute to the secondary damage that follows the initial trauma to the spinal cord. Vascular disruption and abnormalities occur after injury, glutamate mediated excitotoxicity, ischemia-reperfusion, ionic imbalance, free radicals and reactive oxygen species are some of the factors which contribute directly to secondary damage (Dumont et al., 2001; Kwon et al., 2004). Many of these factors, including excess glutamate, ionic imbalance and free radicals are produced and propagated by the inflammatory response and increasing evidence in the literature suggests that targeting the inflammatory response decreases the extent of secondary damage.

Endogenous Central Nervous System (CNS) Response

The inflammatory response after SCI is initiated by endogenous cells within the spinal cord, mainly astrocytes and microglia. The initial trauma of the injury triggers both an

astrocytic and microglial response commonly referred to as 'gliosis'. Astrocytes can be overlooked in a CNS inflammatory response as they are not considered to be an immune cell. Astrocytic contribution to the inflammatory response is not well defined or completely understood after spinal cord injury (Silver and Miller, 2004), however, several studies have demonstrated astrocytic contribution to the propagation of inflammation and secondary damage. Activated astrocytes after injury increase their production of intermediate filaments such as glial fibrillary acidic protein (GFAP), undergo hypertrophy and begin to migrate towards the lesion site (Silver and Miller, 2004). As the astrocytes migrate towards the lesioned area they release a variety of molecules, which contribute to an inhibitory environment towards axon growth around the lesion core. Reactive astrocytes produce proteoglycans as well as semaphorins and ephrins, which have been shown to inhibit axon regeneration. Digestion of some of the proteoglycans, such as chondroitin sulfate proteoglycan after SCI has been shown to reduce secondary damage, promote axon regeneration and improve functional recovery (Davies et al., 1999; Bradbury et al., 2002; Caggiano et al., 2005; Houle et al., 2006; Garcia-Alias et al., 2008). Reactive astrocytes are also key sources of cytokines and chemokines, such as IL-1β and TNF-α, (Fontana et al., 1982; Bandtlow et al., 1990; Klusman and Schwab, 1997; Kwon et al., 2004). IL-1 can help initiate the inflammatory response in various cell types, including astrocytes. In conjunction with TNF-α, IL-1β can also stimulate the production of certain chemokines, such as RANTES in microglia and astrocytes (Allan and Rothwell, 2001), thereby attracting further immune cell infiltration and exacerbating the inflammatory response. Chemokines such as MCP-1 and MIP-1α also work synergistically to increase the expression of various other cytokines and chemokines.

Recombinant MCP-1 when administered into the spinal cord with MIP-1α causes the induction of IL-1β and RANTES, as well as inducing the production of more MCP-1 and MIP-1α (Perrin et al., 2005). Increased expression of these chemokines in the spinal cord causes further immune cells such as macrophages to invade the spinal cord (Perrin et al., 2005) and cause further secondary damage. At high doses, IL-1 and TNFα can also act synergistically and are directly toxic to both neurons and glia and can cause demyelination and axon damage (Allan and Rothwell, 2001). While strong evidence supports the key role of astrocytes in the inflammatory response and secondary damage, astrogliosis has also been shown to be necessary for wound healing. This was demonstrated by the conditional ablation of reactive astrocytes following spinal cord stab or crush injuries in mice expressing a GFAP-herpes simplex virus-thymidine kinase transgene that were given ganciclovir following injury (Faulkner et al., 2004). The ablation of reactive astrocytes in these mice resulted in increased influx of inflammatory cells into the parenchyma of the injured spinal cord, increased secondary damage, prolonged permeability of the blood brain barrier (BBB), an increase in lesion volume, demyelination, and lack of motor recovery. These results confirm the need to further elucidate the various roles astrocytes have after spinal cord injury, as well as the need to modulate the contribution of astrocytes to the inflammatory response as opposed to complete abolition of the astrocytic response to injury.

Microglia are activated concomitantly with astrocytes after the initial trauma to the spinal cord. Microglia are key players in the inflammatory response following SCI, however, once they are fully reactive, they become morphologically indistinguishable from infiltrating peripheral macrophages. Before peripheral macrophages begin to enter the spinal cord by day 3,(Kigerl et al., 2006) the inflammatory response has already been

set in motion by both microglia and astrocytes. IL-1β as well as TNF-α have been shown to be produced as early as 5 minutes after injury and are both produced by microglia (Klusman and Schwab, 1997; Yang et al., 2004; Pineau and Lacroix, 2007). Within an hour after injury, microglia have also upregulated the chemokines MIP-1α and MIP-1β (Bartholdi and Schwab, 1997). Along with the cytokines they release, these chemokines attract peripheral immune cells to enter the spinal cord and propagate the inflammatory response. Hematogenous macrophages migrate into and fill the lesion core by 14 days after injury and the distinction between microglia and macrophages can no longer be made.

Infiltrating Peripheral Immune Cells

Macrophages enter the spinal cord by 3 days after injury; however, polymorphonuclear cells, mainly neutrophils infiltrate the spinal cord as early as 6 hours after injury (Stirling et al., 2009). These neutrophils release inflammatory mediators such as IL-6, TNF-α, and IL-1β, as well as producing oxidative bursts (Bao et al., 2009) and nitrosyl radicals (Saville et al., 2004), all of which contribute to tissue damage. The number of neutrophils peak around 12 hours and are reduced by 24 hours but persist for several days after injury (Stirling et al., 2009). Experiments in which neutrophils were either depleted prior to SCI by the administration of nitrogen mustard (Taoka et al., 1997), or the influx of these cells into the injured cord blocked using a monoclonal antibody against the endothelial-leukocyte adhesion molecule P-selectin (Taoka et al., 1997), which was administered after spinal cord compression injury resulted in improved hind limb motor function as well as a decrease in myeloperoxidase activity. More recent attempts to modulate this

early inflammatory response by blocking neutrophil entry into the spinal cord with an antibody against CD11d, a subunit of the Cd11d/CD18 integrin, which is needed for the extravasation of neutrophils and other immune cells through the BBB also reduced secondary damage, and improved motor recovery (Gris et al., 2004). The integrin subunit, which was blocked, is also present on macrophages as well as neutrophils and the improvement seen could be attributed to a depletion of both cell types (Mabon et al., 2000).

Macrophages that enter the cord after SCI also appear to be important contributors of secondary damage; they release cytokines and chemokines, which prolong the inflammatory response. They also contribute to secondary damage with the release of nitric oxide and superoxide free radicals (Satake et al., 2000). Proteases and glutamate (Yawata et al., 2008) are also released by macrophages/microglia and cause neurotoxicity. Depletion of hematogenous macrophages in rats with SCI leads to increased tissue sparing, reduced myelin loss and improved hind limb locomotor function (Popovich et al., 1999). In these experiments, macrophages were depleted by intravenous injection of chlordronate, a toxin, which is rapidly taken up by circulating macrophages, thereby killing them. There are, however, some studies showing that transplantation of *in vitro* activated macrophages into the injured CNS has beneficial effects (Bandtlow et al., 1990; Rapalino et al., 1998; Bomstein et al., 2003; Schwartz and Yoles, 2006). These differences are likely to be due to the type of macrophage activation, as there is evidence that after phagocytosing myelin debris macrophages acquire an anti-inflammatory phenotype (Boven et al., 2006). Nevertheless, based on the literature the general consensus is that the early macrophage response in the spinal cord after injury is detrimental. Several studies have also indicated the presence of T-cells within the injured

mouse spinal cord, beginning by day 14 (Sroga et al., 2003) and peaking at much later time points (42 days). Our lab has shown with the use of nude mice, which lack a thymus and therefore cannot generate mature T lymphocytes, that T cells do not play a significant role in affecting functional recovery after SCI. These experiments demonstrated that nude mice after SCI show no difference in locomotor recovery as compared to wildtype mice (Ghasemlou N, unpublished data). Blocking IP-10, a chemokine involved in T cell recruitment, with an intraperiotoneal injection of a blocking antibody 1 day prior to spinal cord hemisection and every other day for a total of 5 injections resulted in a decrease in T cells seen in the spinal cord as well as a decrease in the number of macrophages, an increase in angiogenesis, a reduced number of apoptotic cells and an improvement in locomotor recovery. IP-10 is produced early after SCI (Lee et al., 2000) and can act on various cell types besides T-cells, including monocytes and endothelial cells (Luster et al., 1995). Early on after SCI, IP-10 is expressed by astrocytes (Brambilla et al., 2005). These data suggest a complex role for IP-10 after SCI other than only T-cell recruitment.

1.2 Strategies to Modulate Inflammation

As mentioned above, modulating the inflammatory response leads to a reduction of secondary damage accompanied by an improvement in locomotor function. These studies confirm the necessity for modulation of the inflammatory response as opposed to complete abrogation of it. Several techniques can be employed to accomplish this; such as targeting immune cells themselves, either by preventing their trafficking to the injury site or by depletion of particular cell types, or by targeting inflammatory mediators which are produced by both hematogenous as well as endogenous cells. Several broad-spectrum anti-inflammatory agents have also been tested. Dexamethasone as well as

glucocorticoids were first used clinically to treat SCI (Tator, 1972). Methylprednisolone sodium succinate is currently the broad-spectrum anti-inflammatory drug given to most SCI patients. The beneficial effects of methylprednisolone are not universally accepted, however, and administration of the anti-inflammatory agent is not the standard treatment in all Centers (Baptiste and Fehlings, 2006). Methylprednisolone, along with other glucocorticoids was initially tested in animal models of SCI in the 1960's (Ducker and Hamit, 1969; Richardson and Nakamaura, 1971; Tator, 1972). Immediate or delayed administration of methylprednisolone after injury resulted in improved recovery and reduced pathological changes. However the effectiveness was not mirrored in humans, and while benefits of methylprednisolone treatment can be seen (Sipski and Pearse, 2006; Tator, 2006) other treatments, such as the antibody against CD11d have been shown to be more effective in rats (Weaver et al., 2005). Therefore, a need for a more effective strategy for recovery remains. Another anti-inflammatory agent which is currently in a limited clinical trial is minocycline (Baptiste and Fehlings, 2006). Minocycline, a derivative of tetracycline, has anti-inflammatory properties such as the inhibition of pro-inflammatory mediators released by activated microglia. Minocycline has also been shown to inhibit neurotoxicity as well as oxidative stress (Baptiste and Fehlings, 2006). Several animal models of SCI have demonstrated the effectiveness of minocycline as a neuroprotective agent (Wells et al., 2003; Stirling et al., 2004). Administration of minocycline after injury reduced microglial activation, decreased the number of apoptotic oligodendrocytes and reduced the lesion size (Stirling et al., 2004). Several other anti-inflammatory agents administered after SCI have been studied, which include the administration of estrogen (Sribnick et al., 2003; Sribnick et al., 2005), as well as chemokine antagonists (Eng and Lee, 2003) following injury, all of which have exhibited

promising results. While broad-spectrum anti-inflammatory agents dampen the immune response and can result in improved functional recovery, evidence in the literature suggests that a more appropriate solution would be to target and modulate specific aspects of the immune response. One such molecular target, NFκB, was inhibited in astrocytes by creating transgenic mice which over express a dominant negative form of IκBα (an endogenous inhibitor of NFκB), driven by the GFAP promoter (Brambilla et al., 2005). NFκB is activated in inflammatory conditions by cytokines such as TNF-α and promotes the expression of several inflammatory mediators, including cytokines, chemokines and COX-2. Inhibition of NFκB in only astrocytes resulted in a significant improvement in functional recovery, a reduction in lesion volume, an increase in white matter preservation, decrease in proteoglycan expression and changes in inflammatory cytokine expression after spinal cord contusion injury.

Phospholipases are regulators of an inflammatory pathway which is initiated in various inflammatory disorders, including experimental autoimmune encephalomyelitis (EAE), Alzheimer's disease and CNS ischemia (Kalyvas and David, 2004; Farooqui et al., 2006). Phospholipase A2 (PLA_2) enzymes hydrolyze membrane phospholipids to produce free fatty acids such as arachidonic acid (AA) and lysophospholipids. AA mediates a variety of inflammatory responses via a variety of downstream products. In addition, lysophosphatidylcholine produced by the action of PLA_2 on membrane phosphatidylcholine is a potent demyelinating agent (Ousman and David, 2000, 2001). The secreted form of PLA_2 ($sPLA_2$) has been shown to enhance inflammation in a rat model of SCI (Liu et al., 2006). Our laboratory has also generated unpublished data that $sPLA_2$ contributes significantly to the inflammatory response after SCI in mice.

While the cleavage of membrane phospholipids into AA is a controlled and regulated response after SCI, membrane phospholipids also undergo lipid peroxidation, which can also greatly contribute to secondary damage. Lipids can undergo peroxidation due to oxidative stress and membrane phospholipids which contain polyunsaturated fatty acids, such as arachidonic acid, are extremely susceptible to peroxidation (Catala, 2009). Free radicals, lipid hydroperoxides, and reactive aldehyde derivatives, which are produced from peroxidation, can cause further damage. As oxidative stress can cause lipid peroxidation and further exacerbate secondary damage, several attempts have been made to minimize this damage by reducing oxidative stress after SCI. PLA_2 enzymes hydrolyze membrane phospholipids to give rise to AA and lysophospholipids, and a variety of end products can result from several independent pathways as depicted in the phospholipase schematic (Figure 1). Some of these end products, such as resolvins and protectins, which arise from the metabolization of docosahexaenoic acid (DHA), as well as lipoxins, which are converted from AA by 5-lipoxygenase, are endogenous anti-inflammatory mediators and are suggested to play key roles in the resolution of inflammation (Serhan, 2008). The vast majority of the AA metabolites, such as prostanoids, leukotrienes and thromboxanes are typically considered pro-inflammatory mediators. AA is converted to prostaglandin H_2 (PGH_2) by cyclooxygenases 1 and 2 (COX-1/COX-2), the latter being activated by a variety of inflammatory stimuli including cytokines and NFκB. The inhibition of COX-2 in mild forms of SCI models results in improved functional recovery, reduced lesion size and an increase in viable tissue (Faden et al., 1988; Resnick et al., 1998; Hains et al., 2001; O'Banion et al., 2002; Lopez-Vales et al., 2006).

Cyclooxygenase 1/2 (COX-1/COX-2) give rise to PGH_2, a precursor for the 4 different prostaglandins, PGD_2, PGE_2, PGI_2, PGF_2, and thromboxane A2. COX-1 is

constitutively expressed (DeWitt, 1991), while COX-2 is inducible and often implicated in inflammatory conditions. These prostaglandins and thromboxanes have multiple effects that may include detrimental and beneficial effects in inflammation. 15-deoxy-delta(12,14)-prostaglandin J_2 (15d-PGJ_2), a metabolite of PGD_2, has been implicated in the resolution of inflammation (Lawrence et al., 2002) and has been shown to be beneficial in both EAE (Diab et al., 2002) and after SCI (Kerr et al., 2008). As COX enzymes give rise to both pro and anti inflammatory mediators, maximal therapeutic benefits would arise from targeting only the pro-inflammatory mediators that COX produces. Prostaglandins are powerful inflammatory mediators in the periphery; however, their role in inflammatory conditions in the CNS is not as well defined. The role of these prostaglandins needs to be evaluated to know which of these should be modulated after SCI. There are, however, no studies published in the literature on the expression and role of prostaglandins after SCI. PGD_2, which was first defined as an inflammatory mediator due to its presence in inflamed tissue exudates, (Anhut et al., 1979) has not been extensively studied or defined in CNS inflammation nor SCI.

1.3 Prostaglandins

Prostaglandins are potent inflammatory mediators, however, they also have key roles in physiological functions, such as smooth muscle contraction, tissue remodeling, and maintenance of body temperature homeostasis (Brock and Peters-Golden, 2007); their synthesis therefore is tightly regulated via several mechanisms. Prostaglandins work at nanomolar concentrations and tend to act in close proximity to their origin of synthesis usually in a paracrine or autocrine fashion (Herlong and Scott, 2006). As potent inflammatory mediators, both in the periphery and the CNS, non-steroidal anti-

inflammatory drugs (NSAIDs) have been generated which block prostaglandin synthesis by inhibiting both forms of COX. SCI studies in animal models have shown some positive results with the administration of NSAIDs such as Indomethacin and Ibuprofen (Schwab et al., 2004; Pantovic et al., 2005) after spinal cord injury. Daily injection of indomethacin in rabbits after spinal cord injury resulted in significant improvement in their motor activity (Pantovic et al., 2005). Indomethacin and Ibuprofen have been shown to inhibit RhoA and promote axon regeneration (Schwab et al., 2004; Fu et al., 2007; Wang et al., 2009) and, subcutaneous Ibuprofen administration after spinal cord contusion in rats resulted in improved locomotor recovery (Wang et al., 2009). NSAIDs are of course in use clinically for a wide variety of inflammatory conditions such as arthritis. However chronic use of NSAIDs is also accompanied by deleterious side effects such as gastrointestinal bleeding (Hata and Breyer, 2004). Initially, prostaglandins were accepted as solely pro-inflammatory, however, recent work suggests that certain prostaglandins may also act as anti-inflammatory mediators. Prostaglandin E_2, which is often associated with the onset of inflammation in the periphery (Schuligoi et al., 2005), has been shown to be both protective and detrimental in the context of cerebral ischemia (McCullough et al., 2004; Ahmad et al., 2008) depending on which receptor PGE_2 signals through, EP2 or EP1 respectively. Recent work in our lab has also demonstrated that modifying the expression of certain upstream PLA_2s can induce PGE_2 synthase and EP1 receptor expression after SCI and result in improved functional recovery and reduced secondary injury (Lopez-Vales, unpublished data). However, blocking the PGE_2 receptor signaling alone without inhibiting the upstream PLA_2 has no effect on functional recovery. This work reveals underlying complexity of prostaglandin regulation and confirms the need to understand their roles after SCI and the appropriate modulations to their expression for

therapeutic benefits. Information on PGD_2 however in the CNS is limited, especially in the context of inflammation. PGD_2 mediates several biological functions (Kostenis and Ulven, 2006). In the CNS, PGD_2 has been linked to sleep induction, nociception and the function of nerve cells (Breyer et al., 2001; Hata and Breyer, 2004).

Prostaglandin D_2 Synthases

As mentioned above, the synthesis of prostanoids is tightly regulated. Mechanisms of regulation include tissue distribution and cell specific localization of selective prostaglandin synthases. Two specific prostaglandin D_2 synthases exist, which differ in their tissue specificity. One, 'hematopoietic prostaglandin D synthase' (HPGDS) is expressed mainly in immune cells, while the other 'lipocalin-type prostaglandin D synthase' (L-PGDS) is constitutively expressed mainly in the CNS by oligodendrocytes and meningeal cells. These structurally distinct synthases are a prime example of 'functional convergence' as they have evolved separately to serve the same function with similar efficiency and turnover. HPGDS is largely responsible for the production of PGD_2 in inflammatory conditions, due to its localization in immune and inflammatory cells such as macrophages, mast cells, and Th2 cells. Within the CNS however, HPGDS is not normally expressed in adult mice, however, HPGDS expression has been found in microglia in post natal mice (Mohri et al., 2003). HPGDS is strongly expressed in microglia at post natal day (PND) 1 and 5, however, by PND 30 the immunoreactivity to an HPGDS antibody is markedly reduced and by PND 40 HPGDS expression in the brain can no longer be seen. HPGDS expression is seen in activated microglia surrounding senile plaques in Alzheimer's brains in humans and in an Alzheimer's mouse model

(Mohri et al., 2007). Thus there is potential for HPGDS expression to be upregulated in microglia in the CNS.

Adult healthy mice synthesize PGD_2 in the CNS from L-PGDS. L-PGDS is expressed in oligodendrocytes as well as meningeal cells. Homeostatic levels of PGD_2 in the CNS are vital for the regulation of the sleep-wake cycle (Herlong and Scott, 2006) as well as tactile pain sensitivity (Eguchi et al., 1999). In addition to converting PGH_2 to PGD_2, L-PGDS is also referred to as 'beta trace' as it is secreted into the cerebrospinal fluid where it acts as a lipophilic ligand carrier (Kanaoka and Urade, 2003). While L-PGDS has been shown to play a role in some CNS disorders, such as lysosomal storage disorders (Mohri et al., 2006a), in the context of inflammation in the CNS, PGD_2 is produced mainly via HPGDS.

Prostaglandin D_2 Receptors

PGD_2 binds to two distinct high affinity receptors: Prostaglandin D Receptor (DP1) and 'chemoattractant receptor homologous molecule expressed on Th2 cells' (CRTH2 or DP2). Both seven transmembrane receptors are G-protein coupled; DP1 is Gαs/adenyl cyclase coupled while DP2 is coupled to a Gαi subunit. DP1 activation has been shown to activate adenyl cyclase and increase intracellular cAMP levels as well as PKA activity; however, the DP1 downstream signaling pathway has yet to be extensively characterized. DP2 activation can lead to a decrease in intracellular cAMP levels as well as a concomitant calcium mobilization. In immune cells this intracellular response is often associated with effector cell activation (Kostenis and Ulven, 2006). DP2 was originally discovered as aTh2-specific T cell surface receptor, but has since also been found to be present on eosinophils, basophils, monocytes, mast cells and dendritic cells, and is largely

responsible for the chemotactic effects of PGD_2. This chemotaxis along with immune cell activation thus led to DP2 being initially categorized as pro-inflammatory. As new evidence emerged, the classic categorization of DP1 and DP2 has evolved into a more complex picture where neither receptor is either solely a pro or anti inflammatory mediator. When both receptors are present, the binding of PGD_2 to each receptor tends to produce antagonistic effects within the same cell. Th2 cells express both DP1 and DP2 and stimulation with PGD_2 results in the production of IL-2, IL-4, IL-5 and IL-13 (Tanaka et al., 2004), however, this effect is only mediated via DP2. Stimulating Th2 cells with a DP1 agonist causes attenuation of cytokine production such as IFN-γ and IL-2 (Tanaka et al., 2004). Another layer of complexity of prostaglandin signaling is that receptor stimulation may result in varying responses depending on the cell type as well as the tissue. Generally, DP2 mediated signals predominate over DP1 signaling when both receptors are expressed within the same cell (Gervais et al., 2001; Monneret et al., 2001; Gallant et al., 2005), this, however could be a result of higher expression levels of DP2 (Kostenis and Ulven, 2006).

PGD_2 as an Inflammatory Mediator in the CNS

PGD_2 signaling within the CNS has not been as extensively studied as compared to the periphery. PGD_2 plays a role in homeostatic functions like the sleep-wake cycle, however, information on PGD_2 as an inflammatory mediator within the CNS is limited. In a mouse model (*twitcher*) of human globoid cell leukodystrophy (Krabbe's disease) PGD_2 has been implicated to play an important role as a pro-inflammatory mediator(Mohri et al., 2006b). In *twitcher* mice, spontaneous oligodendrocyte apoptosis occurs by PND 30 and initiates a robust inflammatory response in the CNS. The CNS of these mice exhibit

gliosis, as well as increased cytokine production, and cannot survive past PND 44. Inhibition of HPGDS in these animals, both by gene deletion as well as administration of a selective inhibitor to HPGDS, reduces severity of the symptoms and prolongs lifespan. The *twitcher* mice crossed with the HPGDS knockout mice showed attenuated activation of microglia and astrocytes, as well as reduced demyelination, along with a reduction in the number of apoptotic oligodendrocytes. This overall beneficial effect of a lack of HPGDS in the *twitcher* mice was attributed to DP1 signaling as *twitcher*/$DP^{-/-}$ had similar results as the *twitcher*/$HPGDS^{-/-}$. This data would suggest a pro-inflammatory role for PGD_2/DP1 signaling. Inflammation also has a key role in the pathology of Alzheimer's disease. Increased expression of HPGDS and DP1 were found in brains of patients who had suffered from Alzheimer's disease (Mohri et al., 2007). Both HPGDS and DP1 were found in microglia and astrocytes and were principally located around small sized plaques, indicating a possible role for PGD_2/DP1 signaling in contributing to the neuroinflammation of developing plaques.

PGD_2 has also been implicated in mediating neuronal damage by microglia (Bate et al., 2006). Microglial cells that are added to a culture of primary cortical neurons that have been incubated with $A\beta_{1\text{-}42}$ become activated and neurotoxic. Application of PGD_2 alone to neuronal cultures also causes microglial activation, although neuronal survival was not assessed. Neuronal cell death however, can be prevented by treating the neurons with a DP1 antagonist. While these studies suggest that PGD_2 via DP1 can promote inflammation and is potentially neurotoxic, it has also been shown that PGD_2 via DP1 mediates neuronal protection against glutamate toxicity (Liang et al., 2005). Cultured hippocampal slices and neurons, when given glutamate and PGD_2 or a DP1 agonist have a higher survival rate. PGD_2 has also been shown to induce nerve growth factor (NGF)

and brain derived neurotrophic factor (BDNF) secretion by astrocytes *in vitro* (Toyomoto et al., 2004), implicating an indirect role for PGD_2 in neuroprotection. However, how PGD_2 is neuroprotective or mediates neuronal damage under different conditions *in vivo* has yet to be elucidated. *In vivo* studies of PGD_2 have indicated that PGD_2/DP1 signaling plays a beneficial role after ischemia (Saleem et al., 2007; Taniguchi et al., 2007). Hypoxic ischemia in neonatal mice lacking HPGDS and L-PGDS (double knockouts) showed markedly larger infarct size. $DP1^{-/-}$ mice had a larger infarct size as well, however, $DP2^{-/-}$ showed a trend toward a smaller infarct size, indicating DP1 signaling is beneficial after ischemia. Although the double synthase knockout had the largest infarct size, measuring the PGD_2 levels in the single synthase knockouts after ischemia showed that the majority of PGD_2 produced after ischemia was generated by HPGDS, implicating the HPGDS-PGD_2-DP1 signaling pathway as mediating ischemic protection. These experiments in the CNS indicate a complex role for PGD_2. The effects of PGD_2 in an inflammatory response appear to be context related, as HPGDS-PGD_2-DP1 signaling has been shown to be detrimental in an inflammatory condition (*twitcher* mice) as well as being protective (neonatal hypoxic ischemia). Further complications of understanding PGD_2 actions arise from actions of its downstream metabolites, which are capable of eliciting pro or anti-inflammatory responses on their own. Other work done in our laboratory has shown that the downstream metabolite 15d-PGJ2, has a protective effect after SCI. However, whether its protective effects are a result of binding to DP2 is unknown. It is therefore of particular therapeutic interest to assess the potential role of PGD_2 produced by HPGDS in inflammation induced secondary damage that is triggered after spinal cord contusion injury.

Hypothesis

The hypothesis to be tested is that PGD_2 produced from HPGDS is a pro-inflammatory mediator that contributes to secondary damage and locomotor deficits after spinal cord injury.

This hypothesis will be tested in a model of spinal cord contusion injury in adult mice.

Objectives

1. To characterize the expression of PGD_2 synthases in the adult mouse spinal cord after contusion injury.
2. To assess the role of HPGDS in mediating inflammation, secondary damage and locomotor loss after spinal cord contusion injury in adult mice.

Materials and Methods

Animals

HPGDS$^{-/-}$ and HPGDS$^{+/+}$ mice (littermates) on a C57BL/6 background were obtained from Yoshihiro Urade (Osaka Bioscience Institute, Japan) and were generated by the Osaka Bioscience Institute and Japan Tobacco Inc (Japan) (Mohri et al., 2006b). C57BL/6 mice (Charles River Canada) showed no differences in functional recovery compared to HPGDS$^{+/+}$ littermate mice after spinal cord contusion injury and therefore were used whenever HPGDS$^{+/+}$ littermates were unavailable. DP1$^{-/-}$ mice on a C57BL/6 background were obtained from Shuh Narumiya (Kyoto University) (Matsuoka et al., 2000). All animal procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the McGill University Animal Care Committee.

Spinal cord contusion injury

Female adult mice ranging from 18-22g were anesthetized with a cocktail of 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 with an impact force of either 50 ± 3 kdynes or 60 ± 3 kdynes; and displacement of the spinal cord tissue at the time of impact was 400-500µm or 500-600µm for the two impact forces, respectively. The 50kdyne force was used for only the RT-PCR, Western blotting, and immunofluorescence experiments. Locomotor analysis was performed using the

Basso Mouse Scale (BMS) (Basso et al., 2006) which is a 9-point scale that was designed for evaluating locomotor control after contusion injuries in mice. Individual mice were rated by two observers that had been trained at the Basso laboratory in Ohio University. The BMS scoring as well as the subsequent histological analysis were performed blind.

Reverse transcriptase polymerase chain reaction (RT-PCR) and Semi-quantitative PCR

Spinal cord contusion injuries were made in adult C57BL/6 mice. A 4 mm section 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). Spinal cord sections were homogenized in QIAzol reagent (Qiagen) and total RNA extracted using the RNeasy Lipid Mini Tissue Kit (Qiagen). RNA concentrations were determined by spectophotometry and 1μg of RNA was converted to cDNA using the Omniscript RT Kit (Qiagen) according to the manufacturer's protocol. Semi-quantitative PCR was performed using HSTaq Master Mix (Qiagen). Primers and conditions for HPGDS, L-PGDS, DP1 and DP2 were the same as that used in a previous publication (Mohri et al., 2006b). 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 (QRT-PCR)

Spinal cord contusion injuries were made on adult HPGDS$^{-/-}$ and C57BL/6 mice. Spinal cord sections were collected as stated for RT-PCR 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.

0.5µg 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) while following the protocols of the manufacturer. 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'- TGTGTGCCTTAGGAGTGGGAAACT-3';

Mac-2 reverse, 5'- AGAACACTTGCCTAGCAGTCACGA-3';

TNF-α forward, 5' – AGACCCTCACACTCAGATCATCTTC-3';

TNF-α reverse, 5'– CCTCCACTTGGTGGTTTGCT-3';

IL-1β forward, 5'- GCTTCAGGCAGGCAGTATCACT-3';

IL-1β reverse, 5'- CACGGGAAAGACACAGGTAGCT-3';

GAPDH forward, 5'- TCAACAGCAACTCCCACTCTTCCA-3';

GAPDH reverse, 5'- ACCCTGTTGCTGTAGCCGTATTCA-3'.

Annealing temperature was 60°C for all primer sets. Each time point was compared to naïve uninjured controls and normalized to glyceraldehydes 3-phosphate dehydrogenase (GAPDH).

Western blotting

Contusion injuries were made on adult C57BL/6 mice and a 5mm section of spinal cord centered on the lesion collected on 1, 3, 7, 14 21 and 28 days after injury (n=3 for each time point). Protein was extracted by homogenizing in 500ul of RIPA buffer (1% NP-40,

1% SDS, 0.15M NaCl, 0.01M sodium phosphates buffer, 0.02M EDTA) and centrifuged at 14,000 RPM for 12 minutes at 4°C. Protein samples (25µg) were separated by 4-12% Novex-Bis Tris (Invitrogen) gel electrophoresis and transferred to a polyvinylidene fluoride membrane (Millipore). Membranes were blocked for 1 hour at room temperature with 5% nonfat powdered milk in PBS Tween-20 (0.05%) and incubated overnight at 4°C with the following antibodies: rabbit anti-HPGDS (1:500, Cayman Chemical), rabbit anti L-PGDS (1:1000; Cayman Chemical), rabbit anti DP1 (3.5µg/ml; Cayman Chemical), rabbit anti DP2 (0.5µg/ml; Cayman Chemical). The membranes were washed and incubated with horseradish peroxidase conjugated secondary antibodies (1:300,000; Jackson ImmunoResearch) and detected with Western Lightning Chemiluminescence Reagent Plus (Perkin-Elmer). Membranes were subsequently re-probed with rabbit anti-β-actin (1:600; Sigma-Aldrich) to ensure equal loading volumes. Densitometric analysis was carried out on scanned blots using ImageQuant 5.0 (Molecular Dynamics).

Immunofluorescence, immunohistochemical and histological analyses

Mice were sacrificed with an overdose of ketamine/xylazine/acepromazine cocktail used for contusion surgeries as described above and perfused with 0.1M phosphate buffer (PB) followed by 4% paraformaldehyde in 0.1M PB. A 1 cm length of spinal cord centered on the lesion was removed and processed for cryostat sectioning (12µm). Tissue sections were blocked with 1% bovine serum albumin (BSA) and 0.1% Triton-x-100 in PBS and subsequently washed and incubated overnight at 4°C with the following primary antibodies: rabbit anti HPGDS (1:500; Cayman Chemical), rabbit anti L-PGDS (1:2000; Cayman Chemical), rabbit anti DP1 (1:250; Cayman Chemical), and rabbit anti DP2

(1:100; Cayman Chemical). Primary antibodies used for cell localization for double immunofluorescence were as follows: rat anti-GFAP (1:400; Zymed; for astrocytes), rat anti-CD11b (1:200; Serotec, for macrophage/microglia), mouse anti-APC (1:50; Calbiochem, for oligodendrocytes), mouse anti-NeuN (1:50; Chemicon, for neurons), and rabbit anti 5-HT (1:5000; Sigma Aldrich, for serotonergic innervation). Tissue sections were subsequently washed and incubated for 1 hour at room temperature with the following secondary antibodies: Alexa Fluor 488 goat anti rabbit IgG (1:600; Invitrogen) and either Alexa Fluor 594 donkey anti rat (1:600; Invitrogen) or rhodamine conjugated goat anti mouse IgG (1:500; Jackson ImmunoResearch). Specificity of the HPGDS and DP1 antibodies were verified by repeating all stains on $HPGDS^{-/-}$ and $DP1^{-/-}$ mice respectively after contusion injury. Myelin was visualized by staining with Luxol Fast Blue (LFB; Fisher) overnight at 37°C followed by dehydration through ascending alcohols and Hemo-De (Thermo Fisher Scientific). Motor neurons were stained with cresyl violet (Sigma-Aldrich) for 10 minutes at room temperature followed by dehydration through ascending alcohols and Hemo-De (Thermo Fisher Scientific).

Quantification of histological results

All images were captured with a QImaging Retiga 1300C camera and viewed using a Zeiss Axioskop2 Plus microscope. Histological analyses of LFB and 5-HT was performed using the BioQuant Image Analysis System (BioQuant) using the threshold function of the BioQuant Nova Prime tissue analysis software.

Cytokine protein expression

Contusion injuries were done in adult HPGDS$^{-/-}$ and wildtype mice (n=4) and a 4mm length of spinal cord centered on the lesion collected at 12 hours 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/volume. The protein levels of 20 different cytokines (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, KC, MCP-1, MIG, MIP-1α, TNF-α, VEGF) were then analyzed using the BioSource Mouse Cytokine 20-Plex Multiplex Bead Immunoassay (Invitrogen) on a Luminex-100LS system (Luminex Corp.) as per manufacturers' protocol. Results were analyzed using Beadview multiplex data analysis software (Upstate).

PGD_2 enzyme immunoassay

Contusion injuries were done in HPGDS$^{-/-}$ and wildtype mice (n=4) and 4mm 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 PGD_2 levels analyzed using a Prostaglandin D_2 Enzyme Immunoassay Kit (Cayman Chemical) as per manufacturer's protocol.

Results

PGD_2 synthase and receptor expression in the normal uninjured spinal cord

Expression and localization of PGD_2 synthases and receptors were examined in naïve uninjured adult female C57BL/6 mice. Cross sections of the thoracic spinal cord were immunostained for HPGDS, L-PGDS, DP1 and DP2. HPGDS expression was undetectable before injury; however L-PGDS was moderately expressed in oligodendrocytes (Figure 2A) as previously reported. Both DP1 and DP2 were expressed at low levels in astrocytes (Figure 2B, D). DP1 was also found to be expressed in neurons of the dorsal horn (Figure 2C).

HPGDS is upregulated after SCI

Expression levels of both PGD_2 synthases, HPGDS and L-PGDS, were analyzed at several time points after SCI at both the mRNA and protein level. L-PGDS remained relatively stable after contusion injury (Figure 3A, C). The slight decrease in levels early after injury could likely be a result of oligodendrocytic death, which is known to occur after SCI (Crowe et al., 1997; Emery et al., 1998). However, HPGDS expression at the mRNA and protein level increased slowly after injury beginning on day 3. Expression levels peaked at 7-14 days after injury and remained high for at least 28 days. mRNA expression levels were found to be significant on days 7 and 14, however the protein samples used for the Western blots had a large amount of variability and no significant differences were found, although a similar trend was seen. This up regulation could be the result of active transcriptional increase, or as a result of the influx of macrophages, whose time course mirrors that of the increase seen in HPGDS. Double immunofluorescence

labeling shows that HPGDS is found within macrophages (Figure 4A) as early as 3 days after injury and as late as 14 days after injury within the lesion core (Figure 4B).

PGD_2 levels after SCI

To assess the changes in expression of PGD_2 after SCI and in particular to assess the contribution of HPGDS to the increases in PGD_2 levels after SCI, the level of PGD_2 in spinal cord tissue was quantified at days 1, 5, and 14 after injury in $HPGDS^{-/-}$ and wildtype mice. Differences in the PGD_2 levels in wildtype and $HPGDS^{-/-}$ will therefore indicate how much prostaglandin is produced after injury from HPGDS and L-PGDS. No significant changes were found early after injury, although PGD_2 levels were slightly higher at 1 and 5 days. At 14 days after contusion injury, PGD_2 levels in wildtype mice rose significantly to triple the naïve level (Figure 5). Interestingly, this increase in PGD_2 was completely abrogated in the $HPGDS^{-/-}$ mice. The increase in PGD_2 levels after SCI can therefore be attributed solely to production via HPGDS.

PGD_2 receptor expression after SCI

The mRNA and Western blotting protein levels of DP1 and DP2 did not change significantly following contusion injury (Figure 6A, C). Slight increases in DP1 were seen on days 3 and 7; however, they were not significant.

Double immunofluorescence labeling at 14 days post SCI showed DP1 was expressed markedly in astrocytes surrounding the lesion area (Figure 7A) as well as in reactive astrocytes in the white matter (Figure 7B). DP1 continued to be expressed in neurons in the dorsal horn similar to that seen in uninjured cord (Figure 7F).A marked increase in DP2 expression was also found at 14 days post SCI mainly in astrocytes

(Figure 8A, B) and some neurons in the ventral gray matter (Figure 8C). DP2 expression in astrocytes was localized to reactive astrocytes surrounding the lesion core (Fig 8A). Although quantification of Western blots for DP1 and DP2 did not show a change in receptor levels after injury, immunofluorescence labeling showed a marked increase in expression levels of both receptors as compared to expression in the uninjured spinal cord. Expression was principally located around the lesion area but gradually diminished farther away (Figure 8A). As samples taken for Western blotting include regions rostral and caudal to the lesion epicenter, this pattern of expression could explain why the increase was not detected on the Western blots.

Induction of PGD_2 via HPGDS after SCI is detrimental

The experiments described above indicate that the induction of PGD_2 in the spinal cord after contusion injury was mainly attributable to HPGDS, which is expressed in macrophages occupying the lesion core. On the other hand, the DP1 and DP2 receptors were highly expressed in the reactive astrocytes surrounding the macrophages in the lesion epicenter. This pattern of expression would be consistent with the notion that PGD_2 acts in close proximity to its origin of synthesis. To determine what role PGD_2 has after SCI, contusion injuries were done in $HPGDS^{+/+}$ and $HPGDS^{-/-}$ mice and their locomotor recovery assessed at several time points using the BMS analysis (Figure 9A). Significant improvement of locomotor control was observed in $HPGDS^{-/-}$ starting from 5 days after injury and continuing until day 28. $HPGDS^{-/-}$ mice which were shown to have reduced levels of PGD_2 as compared to wildtype mice, reached an average BMS score of 4.5, which indicates stepping on both hind limbs. $HPGDS^{+/+}$ mice in contrast, reached an average score of 3, which indicates the mice can only place their hind limb paws in the

correct placement, but may or may not be able to weight bare. This significant improvement seen in the HPGDS$^{-/-}$ indicates a detrimental role for PGD_2 after SCI. These results were confirmed with the daily administration of a selective inhibitor for HPGDS, HQL-79 after SCI (Figure 9B). Wildtype mice that were given daily subcutaneous injections of HQL-79 showed a significant improvement in locomotor recovery as judged by the BMS analysis as compared to the vehicle treated animals Similar to the HPGDS$^{-/-}$ mice, wildtype mice given HQL-79 reached an average score of 5 by 28 days after injury, which indicates frequent stepping on both hind limbs. Wildtype mice given vehicle performed similar to HPGDS$^{+/+}$ mice, being 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.

Secondary tissue damage after SCI is reduced in HPGDS$^{-/-}$ mice

Since HPGDS$^{-/-}$ mice showed improved locomotor recovery, we next examined whether the lack of HPGDS also has an impact on secondary tissue damage. For this, the extent of myelin loss after SCI was examined by staining cross sections of the spinal cord with Luxol Fast Blue. HPGDS$^{-/-}$ mice showed significantly greater sparing of myelin as compared to wildtype mice (Figure 10A, B). Less myelin was lost at the epicenter and at distances rostral and caudal. Neuronal cell death also occurs rostral and caudal to the epicenter as a result of secondary damage. We therefore assessed the number of neurons in the ventral gray matter of the spinal cord at varying distances from the epicentre. HPGDS$^{-/-}$ mice had significantly more neurons caudal to the epicentre as compared to HPGDS$^{+/+}$ mice (Figure 10C, D). We also assessed the serotonergic innervation in the ventral gray matter 1000μm caudal to the epicenter at 28 days after SCI. Serotonergic

axons descend from the raphe nuclei in the brainstem and innervate the ventral horn and are required for locomotor control. HPGDS$^{-/-}$ had a significantly higher amount of 5-HT innervation as compared to the injured wildtype controls (Figure 10E, F).

PGD_2 generated via HPGDS mediates Inflammation after SCI

As PGD_2 is a potent inflammatory mediator in the periphery, we therefore examined the expression levels of several inflammatory cytokines. Cytokines levels in the spinal cord are increased early after injury with the majority of cytokines peaking at 12 hours after SCI. Cytokine levels were analyzed in wildtype and HPGDS$^{-/-}$ using a Cytokine 20-Plex Multiplex Bead Immunoassay. At this time in both wildtype and HPGDS$^{-/-}$ mice, a marked increase in IL-5, IL-1α, KC, MCP-1, and MIP-1α was seen compared to naïve basal levels (Figure 11A,B). Interestingly, HPGDS$^{-/-}$ showed a significant decrease in IP-10 levels and a significant increase in IL-6 as compared to wildtype mice. Unexpectedly, neither TNF-α nor IL-1β levels were detected in either genotype after injury which potentially was due to a technical problem as both these cytokines have been reported to be expressed at this time point after SCI (Pineau and Lacroix, 2007). Therefore, QPCR analysis of the mRNA levels of IL-1β, TNF-α and TGF-β1 were also analyzed at 24 hours and 14 days after injury. 14 days after injury was chosen as a second time point as not all cytokines peak at 12 hours and several inflammatory cytokines have also been reported to peak at 2 weeks after injury (Pineau and Lacroix, 2007). Although HPGDS$^{-/-}$ showed a trend for less TNF-α and IL-1β at later time points after injury (Figure 12A,B), these differences were not significant. A significant induction was seen in TGF-β1 mRNA at 14 days after injury in the HPGDS$^{-/-}$ mice (Figure 12C). As both cytokines and chemokines can influence the activation and infiltration of peripheral immune cells, the level of Mac-

2, a galectin expressed by activated macrophages and microglia (Liu et al., 1995) was also analyzed at the mRNA level by QPCR at 24 hours and 14 days after injury and was found to be significantly decreased in $HPGDS^{-/-}$ by 14 days after spinal cord injury (Figure 12D). Collectively these data suggest that PGD_2 produced via HPGDS after SCI may mediate inflammatory responses in the spinal cord.

PGD2 mediated effects after SCI are via the DP1 receptor

PGD_2 can bind and induce signaling via two distinct receptors, DP1 and DP2. To assess which receptor signaling pathway is responsible for the detrimental effects mediated by the increased PGD_2 produced after SCI, we assessed locomotor recovery after SCI in $DP1^{-/-}$ and wildtype mice. Interestingly, $DP1^{-/-}$ mice showed a pattern of locomotor recovery as assessed by the BMS analysis similar to that seen in both (i) $HPGDS^{-/-}$ mice, and (ii) wildtype mice treated with HQL-79, the HPGDS inhibitor . A significant improvement in locomotor recovery was seen in the $DP1^{-/-}$ mice as compared to wildtype mice beginning on day 5 after SCI (Figure 13). This improvement in locomotor recovery suggests strongly that the detrimental effects of PGD_2 after SCI are likely to be mediated via DP1 receptor signaling.

Discussion

There is considerable evidence in the literature that inflammation contributes to secondary damage after SCI (Tator, 1972; Tator and Fehlings, 1991; Dumont et al., 2001; Kwon et al., 2004; Baptiste and Fehlings, 2006). A number of factors have been shown to be implicated in triggering the inflammatory response after SCI which contribute to secondary damage. 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. The objective of my thesis was therefore to assess the expression of prostaglandin D_2 (PGD_2), its synthases (HPGDS and L-PGDS), its receptors (DP1 and DP2) and the functional role of PGD_2 produced by HPGDS and DP1 in contributing to the secondary damage after SCI. I first examined the expression of the two synthetic enzymes that synthesize PGD_2, namely, HPGDS, which has been shown to be expressed by hematopoietic immune cells, and L-PGDS, which is expressed by oligodendrocytes and meningeal cells. I also assessed the expression of the PGD_2 receptors, DP1 and DP2. Finally, the functional role of PGD_2 and DP1 after SCI was examined using gene knockout mice and a small molecular inhibitor of HPGDS.

My studies show that HPGDS expression gradually increases in the spinal cord after injury, beginning on day 3, peaking between the first two weeks, and remains elevated for up to 28 days post SCI. In contrast, there were no significant changes in L-PGDS expression after SCI. The immunofluorescence labeling studies showed that HPGDS is expressed by macrophages that aggregate at and near the spinal cord lesion site. Oligodendrocytes also continue to express L-PGDS as they do in the uninjured cord. These results suggest HPGDS is the principal producer of PGD_2 after SCI. To confirm this, I assessed the PGD_2 levels in $HPGDS^{-/-}$ and $HPGDS^{+/+}$ mice after SCI. This analysis

suggests that the upregulation of PGD_2 observed after SCI, is produced solely from HPGDS. Since PGD_2 can both promote and resolve inflammation (Rajakariar et al., 2007) its role in modulating inflammation after SCI using $HPGDS^{-/-}$ mice was examined. Analysis of locomotor recovery after contusion injury revealed that $HPGDS^{-/-}$ mice recover markedly better than the wildtype mice. This significant increase in locomotor function was accompanied by a reduction in myelin loss, a reduction in neuronal loss, and an increase in serotonergic innervation caudal to the lesion. $HPGDS^{-/-}$ mice showed reduced IP-10 and increased IL-6 expression, as well as a reduction in the macrophage activation marker (Mac-2). The effects on locomotor recovery seen in the $HPGDS^{-/-}$ mice after SCI was further confirmed by blocking HPGDS in wildtype mice using a selective inhibitor (HQL-79). Since PGD_2 can mediate its effects by binding to either DP1 or DP2 receptors, the contribution of DP1 in SCI was assessed using the $DP1^{-/-}$ mice. Interestingly, there was a significant improvement in locomotor recovery in $DP1^{-/-}$ as compared to wildtype mice suggesting that the DP1 receptor signaling may be responsible for the PGD_2 mediated detrimental effects after spinal cord contusion injury. These results offer new insight into prostaglandins and their role after SCI as well as determining a potential therapeutic target to limit inflammation and secondary damage after injury.

PGD_2 mediates Inflammation

The reduction of secondary damage seen after SCI may have been mediated by PGD_2 effects on the inflammatory response, since a significant decrease in Mac-2, as well as a reduction in the pro-inflammatory chemokine IP-10 was found. Interestingly, IL-6 and TGF-β1 were markedly increased in $HPGDS^{-/-}$ mice after SCI.

PGD_2 has previously been shown to mediate microglial activation in the twitcher mouse mutant, which is a model of human Krabbes disease. These authors found that the double *twitcher/HPGDS*$^{-/-}$ mice had reduced microglial activation (Mohri et al., 2006b). The *twitcher/HPGDS* knockout mice also exhibited signs of reduced inflammation in the CNS as well as a reduction in iNOS expression. However in our study, mRNA analysis of iNOS expression on days 1, 3, or 14 in HPGDS$^{-/-}$ mice after SCI did not show differences from wildtype mice (data not shown). These *Twitcher/HPGDS* mice showed no differences in levels of IL-6, in contrast to the increase we have seen in HPGDS$^{-/-}$ after SCI. Although both models highlight PGD_2 as a pro-inflammatory mediator in the CNS, the mechanisms of action might be distinct in these two models. Although some studies have suggested that IL-6 may have pro-inflammatory effects after SCI (Okada et al., 2004), 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 (Cao et al., 2006; Hannila and Filbin, 2008). Increased levels of IL-6 have also been found after contusion injury in both transgenic mice lacking active NFκB (Brambilla et al., 2005) as well as wildtype mice injected with 15d-PGJ2 (Kerr et al., 2008) in which secondary damage was reduced along with an 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). The increase in IL-6 found in the HPGDS$^{-/-}$ may therefore have a positive impact on regenerating axons or in neuroprotection after injury, both of which we found in the HPGDS$^{-/-}$ mice, i.e., increased serotonergic innervation and improved survival of ventral neurons. The expression of the

chemokine IP-10 (CXCL10) was also significantly reduced in the $HPGDS^{-/-}$ after contusion injury. IP-10 is regarded as a potent recruiter of T lymphocyte, and has been shown to play a role in the recruitment of macrophages as well after SCI (Gonzalez et al., 2003; Glaser et al., 2004; Glaser et al., 2006; Gonzalez et al., 2007). These studies used hemisections as an injury model, in which the inflammatory response is not as robust as compared to contusion injury. Inhibition of IP-10 signaling not only reduced the amount of T cells and macrophages, but also had a role in promoting angiogenesis (Glaser et al., 2004), reducing apoptosis and increasing axon sprouting (Glaser et al., 2006). Whether the induction of IL-6 or reduction of IP-10 is a result of direct or indirect PGD_2 signaling is unknown. PGD_2 has been shown to attenuate cytokine production directly via DP1 (Tanaka et al., 2004) in $CD4^+$ T cells *in vitro*. PGD_2 or a DP1 agonist attenuated production of IFN-γ and reduced production of IL-2. PGD_2 was also shown to stimulate IL-2, IL-4, IL-5 and IL-13 in Th2 cells via DP2 (Tanaka et al., 2004). There is evidence thus that PGD_2 can directly influence the production of several cytokines, however most of the literature has examined this effect in T cells and *in vitro*. Its effects on spinal cord tissue has not been previously examined. There are no previous studies showing PGD_2 can directly influence IL-6 or IP-10, either *in vitro* or *in vivo*. PGD_2 may directly alter the expression of only one of these inflammatory mediators which in turn results in the modification of the other. We have found PGD_2 receptors only on astrocytes and neurons after injury and thus the effect must be mediated via one or both of these cell types. Astrocytes have been shown to produce IL-6 *in vitro* and the IL-6 produced by astrocytes was found to protect neurons against oxidative stress (Fujishita et al., 2009).

Prostaglandin Balance

PGD_2 is a product of a tightly regulated and complex pathway which begins with the hydrolysis of membrane phospholipids by PLA_2. Arachidonic acid which is produced is then converted into PGG_2 and PGH_2 via the action of the COX enzymes. Determination of which prostaglandin is produced from PGH_2 depends on a variety of factors. Certain cell types only express one type of prostaglandin synthase and therefore are not capable of producing several different prostaglandins. One type of regulation is by altering the expression of only one prostaglandin synthase in order to bias the pathway toward production of that prostaglandin. Microglia/macrophages produce both PGD_2 as well as PGE_2. Besides altering the expression level of each synthase, the intracellular milieu can also affect the activity level of each synthase. PGE_2 synthase operates at optimal efficiency with reduced glutathione levels (Brock and Peters-Golden, 2007), while PGD_2 synthase requires high levels of glutathione (Herlong and Scott, 2006). Different inflammatory stimuli are able to induce the synthesis of specific prostaglandins (Stachowska et al., 2009). Several studies have shown that the stimulation of macrophages *in vitro* with inflammatory stimuli such as LPS causes the induction of a prostaglandin E synthase and the release of PGE_2 (Tajima et al., 2008). LPS mediates signals via toll like receptor 4 (TLR 4), however, after contusion injury, evidence suggests that inflammatory signals exist for both TLR2 as well as TLR4 and depending on which receptor is activated could bias microglia/macrophages to produce either PGD_2 or PGE_2. The reduction in inflammation and secondary damage that we have found after inhibiting HPGDS after contusion injury is likely caused by attenuation of PGD_2-DP1 signaling. It is also possible, however, that the beneficial recovery that we see may be a result of increased PGE_2 signaling. As a result of inhibiting HPGDS activity, there could be an abundance of PGH_2, the precursor for all terminal eicosanoids. As

microglia/macrophages also possess the ability to produce and release PGE_2, it is possible that there is an increase in PGE_2 signaling when we inhibit HPGDS. The changes in inflammation could therefore be attributed to an increase in PGE_2 levels and signaling as well as the reduction of PGD_2-DP1 signaling. This however, has yet to be shown.

Differences in locomotor recovery were seen as early as 5 days after injury with the $HPGDS^{-/-}$ mice, and changes in cytokine and chemokine levels between genotypes were found as early as 12 hours after injury, however, no significant differences in PGD_2 levels between $HPGDS^{+/+}$ and $HPGDS^{-/-}$ were seen until 14 days after injury, although there is a trend towards an increase at 5 days in the wildtype mice. PGD_2 is rapidly metabolized and can therefore be difficult to measure accurately *ex vivo*. Several assays rely on converting PGD_2 in the sample to a stable compound in order for precise measurements. However, this type of assay is not suitable for tissue, and therefore it is possible that small but functionally important changes in PGD_2 levels in the lesion epicenter may have been missed.

HPGDS-PGD_2-DP1 signaling is detrimental after SCI

PGD_2-DP1 signaling has been shown to be protective in neonatal mice after hypoxic ischemia (Taniguchi et al., 2007), while we have found that PGD_2-DP1 signaling is detrimental after SCI. Although both models involve an inflammatory response, the protective effect after hypoxic ischemia was attributed to DP1 signaling in endothelial cells rather then a change in the inflammatory response, which is poor in the neonate. We did not observe any DP1 staining in cells which resembled endothelial cells based on nuclear morphology and DAPI staining. Most of the DP1 reactive cells we observed around the injury co-localized with GFAP; thus the majority of $DP1^{+}$ cells around the

lesion epicenter seen after SCI are astrocytes. Although both DP1 and DP2 are highly expressed in the same cells after injury, our locomotor analysis indicate that PGD_2-DP1 pathway predominated over that of DP2. An overall reduction in PGD_2 resulting in improved locomotor function was mirrored in the $DP1^{-/-}$ mice. However from this data we can not conclude what role, if any, DP2 plays after SCI. Reducing the levels of PGD_2 by inhibiting HPGDS would result in an amelioration of both DP1 and DP2 signaling.$DP1^{-/-}$ mice showed a similar recovery to $HPGDS^{-/-}$ mice and thus we can conclude that DP1 signaling is detrimental. However, it is also possible that in the absence of DP1, heightened DP2 signaling may occur. In the periphery, it has been shown that DP1 and DP2 within the same cell can produce antagonistic effects; however, it is common for one receptor to dominate signaling over the other. It is therefore possible that agonizing DP2 after SCI may produce beneficial effects, and that modulation of both receptors would therefore be a valid potential therapeutic approach. Several DP2 agonists are available for mice, however many of them also possess the ability to bind and signal through several other receptors, such as a thromboxane receptor and PPARγ. In addition, these agonists have only been used *in vitro* or administered in the periphery and therefore it is unclear whether they would be effective *in vivo*. I attempted to administer two DP2 agonists to wildtype and $HPGDS^{-/-}$ and $DP1^{-/-}$ mice with inconclusive results (data not shown). It would be of interest to determine whether DP2 has a role after SCI and whether it is beneficial to modulate its signaling after SCI but this would likely require development of new agonists and more in depth studies.

Conclusion

The data presented here show a pivotal role for prostaglandin D_2 and the DP1 receptor in promoting the inflammatory response after spinal cord contusion injury. Future work needs to focus on delineating the downstream mechanism of DP1 signaling in the injured spinal cord, as well as other beneficial mechanisms which might result from blocking HPGDS, such as improved axonal regeneration and reduced oxidative damage. The inhibition of PGD_2 via blocking HPGDS or DP1 show a marked improvement in both locomotor function and reduction of secondary damage, and thus provides two possible targets for therapeutic intervention for the treatment of spinal cord injury.

References

Ahmad AS, Yun YT, Ahmad M, Maruyama T, Dore S (2008) Selective blockade of PGE2 EP1 receptor protects brain against experimental ischemia and excitotoxicity, and hippocampal slice cultures against oxygen-glucose deprivation. Neurotox Res 14:343-351.

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

Anhut H, Brune K, Frolich JC, Peskar BA (1979) Prostaglandin D2 is the prevailing prostaglandin in the acute inflammatory exudate of urate arthritis in the chicken. Br J Pharmacol 65:357-359.

Bandtlow CE, Meyer M, Lindholm D, Spranger M, Heumann R, Thoenen H (1990) Regional and cellular codistribution of interleukin 1 beta and nerve growth factor mRNA in the adult rat brain: possible relationship to the regulation of nerve growth factor synthesis. J Cell Biol 111:1701-1711.

Bao F, Bailey CS, Gurr KR, Bailey SI, Rosas-Arellano MP, Dekaban GA, Weaver LC (2009) Increased oxidative activity in human blood neutrophils and monocytes after spinal cord injury. Exp Neurol 215:308-316.

Baptiste DC, Fehlings MG (2006) Pharmacological approaches to repair the injured spinal cord. J Neurotrauma 23:318-334.

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-beta or prions which activates microglial cells. Neuropharmacology 50:229-237.

Bomstein Y, Marder JB, Vitner K, Smirnov I, Lisaey G, Butovsky O, Fulga V, Yoles E (2003) Features of skin-coincubated macrophages that promote recovery from spinal cord injury. J Neuroimmunol 142:10-16.

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.

Boven LA, Van Meurs M, Van Zwam M, Wierenga-Wolf A, Hintzen RQ, Boot RG, Aerts JM, Amor S, Nieuwenhuis EE, Laman JD (2006) Myelin-laden macrophages are anti-inflammatory, consistent with foam cells in multiple sclerosis. Brain 129:517-526.

Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW, McMahon SB (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416:636-640.

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

Breyer RM, Bagdassarian CK, Myers SA, Breyer MD (2001) Prostanoid receptors: subtypes and signaling. Annu Rev Pharmacol Toxicol 41:661-690.

Brock TG, Peters-Golden M (2007) Activation and regulation of cellular eicosanoid biosynthesis. ScientificWorldJournal 7:1273-1284.

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.

Caggiano AO, Zimber MP, Ganguly A, Blight AR, Gruskin EA (2005) Chondroitinase ABCI improves locomotion and bladder function following contusion injury of the rat spinal cord. J Neurotrauma 22:226-239.

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.

Catala A (2009) Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions. Chem Phys Lipids 157:1-11.

Crowe MJ, Bresnahan JC, Shuman SL, Masters JN, Beattie MS (1997) Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. Nat Med 3:73-76.

Davies SJ, Goucher DR, Doller C, Silver J (1999) Robust regeneration of adult sensory axons in degenerating white matter of the adult rat spinal cord. J Neurosci 19:5810-5822.

DeWitt DL (1991) Prostaglandin endoperoxide synthase: regulation of enzyme expression. Biochim Biophys Acta 1083:121-134.
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.
Ducker TB, Hamit HF (1969) Experimental treatments of acute spinal cord injury. J Neurosurg 30:693-697.
Dumont RJ, Okonkwo DO, Verma S, Hurlbert RJ, Boulos PT, Ellegala DB, Dumont AS (2001) Acute spinal cord injury, part I: pathophysiologic mechanisms. Clin Neuropharmacol 24:254-264.
Eguchi N, Minami T, Shirafuji N, Kanaoka Y, Tanaka T, Nagata A, Yoshida N, Urade Y, Ito S, Hayaishi O (1999) Lack of tactile pain (allodynia) in lipocalin-type prostaglandin D synthase-deficient mice. Proc Natl Acad Sci U S A 96:726-730.
Emery E, Aldana P, Bunge MB, Puckett W, Srinivasan A, Keane RW, Bethea J, Levi AD (1998) Apoptosis after traumatic human spinal cord injury. J Neurosurg 89:911-920.
Eng LF, Lee YL (2003) Response of chemokine antagonists to inflammation in injured spinal cord. Neurochem Res 28:95-100.
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.
Farooqui AA, Ong WY, Horrocks LA (2006) Inhibitors of brain phospholipase A2 activity: their neuropharmacological effects and therapeutic importance for the treatment of neurologic disorders. Pharmacol Rev 58:591-620.
Faulkner JR, Herrmann JE, Woo MJ, Tansey KE, Doan NB, Sofroniew MV (2004) Reactive astrocytes protect tissue and preserve function after spinal cord injury. J Neurosci 24:2143-2155.
Fontana A, Kristensen F, Dubs R, Gemsa D, Weber E (1982) Production of prostaglandin E and an interleukin-1 like factor by cultured astrocytes and C6 glioma cells. J Immunol 129:2413-2419.
Fu Q, Hue J, Li S (2007) Nonsteroidal anti-inflammatory drugs promote axon regeneration via RhoA inhibition. J Neurosci 27:4154-4164.
Fujishita K, Ozawa T, Shibata K, Tanabe S, Sato Y, Hisamoto M, Okuda T, Koizumi S (2009) Grape Seed Extract Acting on Astrocytes Reveals Neuronal Protection Against Oxidative Stress via Interleukin-6-mediated Mechanisms. Cell Mol Neurobiol.
Gallant MA, Samadfam R, Hackett JA, Antoniou J, Parent JL, de Brum-Fernandes AJ (2005) Production of prostaglandin D(2) by human osteoblasts and modulation of osteoprotegerin, RANKL, and cellular migration by DP and CRTH2 receptors. J Bone Miner Res 20:672-681.
Garcia-Alias G, Lin R, Akrimi SF, Story D, Bradbury EJ, Fawcett JW (2008) Therapeutic time window for the application of chondroitinase ABC after spinal cord injury. Exp Neurol 210:331-338.
Gervais FG, Cruz RP, Chateauneuf A, Gale S, Sawyer N, Nantel F, Metters KM, O'Neill G P (2001) Selective modulation of chemokinesis, degranulation, and apoptosis in

eosinophils through the PGD2 receptors CRTH2 and DP. J Allergy Clin Immunol 108:982-988.
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, 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.
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.
Gonzalez R, Glaser J, Liu MT, Lane TE, Keirstead HS (2003) Reducing inflammation decreases secondary degeneration and functional deficit after spinal cord injury. Exp Neurol 184:456-463.
Gonzalez R, Hickey MJ, Espinosa JM, Nistor G, Lane TE, Keirstead HS (2007) Therapeutic neutralization of CXCL10 decreases secondary degeneration and functional deficit after spinal cord injury in mice. Regen Med 2:771-783.
Gris D, Marsh DR, Oatway MA, Chen Y, Hamilton EF, Dekaban GA, Weaver LC (2004) Transient blockade of the CD11d/CD18 integrin reduces secondary damage after spinal cord injury, improving sensory, autonomic, and motor function. J Neurosci 24:4043-4051.
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.
Hata AN, Breyer RM (2004) Pharmacology and signaling of prostaglandin receptors: multiple roles in inflammation and immune modulation. Pharmacol Ther 103:147-166.
Herlong JL, Scott TR (2006) Positioning prostanoids of the D and J series in the immunopathogenic scheme. Immunol Lett 102:121-131.
Houle JD, Tom VJ, Mayes D, Wagoner G, Phillips N, Silver J (2006) Combining an autologous peripheral nervous system "bridge" and matrix modification by chondroitinase allows robust, functional regeneration beyond a hemisection lesion of the adult rat spinal cord. J Neurosci 26:7405-7415.
Kalyvas A, David S (2004) Cytosolic phospholipase A2 plays a key role in the pathogenesis of multiple sclerosis-like disease. Neuron 41:323-335.
Kanaoka Y, Urade Y (2003) Hematopoietic prostaglandin D synthase. Prostaglandins Leukot Essent Fatty Acids 69:163-167.
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.
Kigerl KA, McGaughy VM, Popovich PG (2006) Comparative analysis of lesion development and intraspinal inflammation in four strains of mice following spinal contusion injury. J Comp Neurol 494:578-594.
Klusman I, Schwab ME (1997) Effects of pro-inflammatory cytokines in experimental spinal cord injury. Brain Res 762:173-184.

Kostenis E, Ulven T (2006) Emerging roles of DP and 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.
Lawrence T, Willoughby DA, Gilroy DW (2002) Anti-inflammatory lipid mediators and insights into the resolution of inflammation. Nat Rev Immunol 2:787-795.
Lee YL, Shih K, Bao P, Ghirnikar RS, Eng LF (2000) Cytokine chemokine expression in contused rat spinal cord. Neurochem Int 36:417-425.
Liang X, Wu L, Hand T, Andreasson K (2005) Prostaglandin D2 mediates neuronal protection via the DP1 receptor. J Neurochem 92:477-486.
Liu FT, Hsu DK, Zuberi RI, Kuwabara I, Chi EY, Henderson WR, Jr. (1995) Expression and function of galectin-3, a beta-galactoside-binding lectin, in human monocytes and macrophages. Am J Pathol 147:1016-1028.
Liu NK, Zhang YP, Titsworth WL, Jiang X, Han S, Lu PH, Shields CB, Xu XM (2006) A novel role of phospholipase A2 in mediating spinal cord secondary injury. Ann Neurol 59:606-619.
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.
Luster AD, Greenberg SM, Leder P (1995) The IP-10 chemokine binds to a specific cell surface heparan sulfate site shared with platelet factor 4 and inhibits endothelial cell proliferation. J Exp Med 182:219-231.
Mabon PJ, Weaver LC, Dekaban GA (2000) Inhibition of monocyte/macrophage migration to a spinal cord injury site by an antibody to the integrin alphaD: a potential new anti-inflammatory treatment. Exp Neurol 166:52-64.
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.
McCullough L, Wu L, Haughey N, Liang X, Hand T, Wang Q, Breyer RM, Andreasson K (2004) Neuroprotective function of the PGE2 EP2 receptor in cerebral ischemia. J Neurosci 24:257-268.
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, Taniike M, Okazaki I, Kagitani-Shimono K, Aritake K, Kanekiyo T, Yagi T, Takikita S, Kim HS, Urade Y, Suzuki K (2006a) Lipocalin-type prostaglandin D synthase is up-regulated in oligodendrocytes in lysosomal storage diseases and binds gangliosides. J Neurochem 97:641-651.
Mohri I, Kadoyama K, Kanekiyo T, Sato Y, Kagitani-Shimono K, Saito Y, Suzuki K, Kudo T, Takeda M, Urade Y, Murayama S, Taniike 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

(2006b) Prostaglandin D2-mediated microglia/astrocyte interaction enhances astrogliosis and demyelination in twitcher. J Neurosci 26:4383-4393.
Monneret G, Gravel S, Diamond M, Rokach J, Powell WS (2001) Prostaglandin D2 is a potent chemoattractant for human eosinophils that acts via a novel DP receptor. Blood 98:1942-1948.
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.
Pantovic R, Draganic P, Erakovic V, Blagovic B, Milin C, Simonic A (2005) Effect of indomethacin on motor activity and spinal cord free fatty acid content after experimental spinal cord injury in rabbits. Spinal Cord 43:519-526.
Perrin FE, Lacroix S, Aviles-Trigueros M, David S (2005) Involvement of monocyte chemoattractant protein-1, macrophage inflammatory protein-1alpha and interleukin-1beta in Wallerian degeneration. Brain 128:854-866.
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.
Popovich PG, Guan Z, Wei P, Huitinga I, van Rooijen N, Stokes BT (1999) Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. Exp Neurol 158:351-365.
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-deoxyDelta12 14 PGJ2. Proc Natl Acad Sci U S A 104:20979-20984.
Rapalino O, Lazarov-Spiegler O, Agranov E, Velan GJ, Yoles E, Fraidakis M, Solomon A, Gepstein R, Katz A, Belkin M, Hadani M, Schwartz M (1998) Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. Nat Med 4:814-821.
Resnick DK, Graham SH, Dixon CE, Marion DW (1998) Role of cyclooxygenase 2 in acute spinal cord injury. J Neurotrauma 15:1005-1013.
Richardson HD, Nakamaura S (1971) An electron microscopic study of spinal cord edema and the effect of treatment with steroids, mannitol, and hypothermia. Proc Veterans Adm Spinal Cord Inj Conf 18:10-16.
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.
Satake K, Matsuyama Y, Kamiya M, Kawakami H, Iwata H, Adachi K, Kiuchi K (2000) Nitric oxide via macrophage iNOS induces apoptosis following traumatic spinal cord injury. Brain Res Mol Brain Res 85:114-122.
Saville LR, Pospisil CH, Mawhinney LA, Bao F, Simedrea FC, Peters AA, O'Connell PJ, Weaver LC, Dekaban GA (2004) A monoclonal antibody to CD11d reduces the

inflammatory infiltrate into the injured spinal cord: a potential neuroprotective treatment. J Neuroimmunol 156:42-57.
Schuligoi R, Grill M, Heinemann A, Peskar BA, Amann R (2005) Sequential induction of prostaglandin E and D synthases in inflammation. Biochem Biophys Res Commun 335:684-689.
Schwab JM, Conrad S, Elbert T, Trautmann K, Meyermann R, Schluesener HJ (2004) Lesional RhoA+ cell numbers are suppressed by anti-inflammatory, cyclooxygenase-inhibiting treatment following subacute spinal cord injury. Glia 47:377-386.
Schwartz M, Yoles E (2006) Immune-based therapy for spinal cord repair: autologous macrophages and beyond. J Neurotrauma 23:360-370.
Serhan CN (2008) Controlling the resolution of acute inflammation: a new genus of dual anti-inflammatory and proresolving mediators. J Periodontol 79:1520-1526.
Silver J, Miller JH (2004) Regeneration beyond the glial scar. Nat Rev Neurosci 5:146-156.
Sipski ML, Pearse DD (2006) Methylprednisolone and other confounders to spinal cord injury clinical trials. Nat Clin Pract Neurol 2:402-403.
Sribnick EA, Wingrave JM, Matzelle DD, Ray SK, Banik NL (2003) Estrogen as a neuroprotective agent in the treatment of spinal cord injury. Ann N Y Acad Sci 993:125-133; discussion 159-160.
Sribnick EA, Wingrave JM, Matzelle DD, Wilford GG, Ray SK, Banik NL (2005) Estrogen attenuated markers of inflammation and decreased lesion volume in acute spinal cord injury in rats. J Neurosci Res 82:283-293.
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.
Stachowska E, Dolegowska B, Dziedziejko V, Rybicka M, Kaczmarczyk M, Bober J, Rac M, Machalinski B, Chlubek D (2009) Prostaglandin E2 (PGE2) and thromboxane A2 (TXA2) synthesis is regulated by conjugated linoleic acids (CLA) in human macrophages. J Physiol Pharmacol 60:77-85.
Stirling DP, Liu S, Kubes P, Yong VW (2009) Depletion of Ly6G/Gr-1 leukocytes after spinal cord injury in mice alters wound healing and worsens neurological outcome. J Neurosci 29:753-764.
Stirling DP, Khodarahmi K, Liu J, McPhail LT, McBride CB, Steeves JD, Ramer MS, Tetzlaff W (2004) Minocycline treatment reduces delayed oligodendrocyte death, attenuates axonal dieback, and improves functional outcome after spinal cord injury. J Neurosci 24:2182-2190.
Tajima T, Murata T, Aritake K, Urade Y, Hirai H, Nakamura M, Ozaki H, Hori M (2008) Lipopolysaccharide induces macrophage migration via prostaglandin D(2) and prostaglandin E(2). J Pharmacol Exp Ther 326:493-501.
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, Taniike M (2007) Prostaglandin D2 protects neonatal mouse brain from hypoxic ischemic injury. J Neurosci 27:4303-4312.

Taoka Y, Okajima K, Uchiba M, Murakami K, Kushimoto S, Johno M, Naruo M, Okabe H, Takatsuki K (1997) Role of neutrophils in spinal cord injury in the rat. Neuroscience 79:1177-1182.

Tator CH (1972) Acute spinal cord injury: a review of recent studies of treatment and pathophysiology. Can Med Assoc J 107:143-145 passim.

Tator CH (2006) Review of treatment trials in human spinal cord injury: issues, difficulties, and recommendations. Neurosurgery 59:957-982; discussion 982-957.

Tator CH, Fehlings MG (1991) Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg 75:15-26.

Toyomoto M, Ohta M, Okumura K, Yano H, Matsumoto K, Inoue S, Hayashi K, Ikeda K (2004) Prostaglandins are powerful inducers of NGF and BDNF production in mouse astrocyte cultures. FEBS Lett 562:211-215.

Wang X, Budel S, Baughman K, Gould G, Song KH, Strittmatter SM (2009) Ibuprofen Enhances Recovery from Spinal Cord Injury by Limiting Tissue Loss and Stimulating Axonal Growth. J Neurotrauma.

Weaver LC, Gris D, Saville LR, Oatway MA, Chen Y, Marsh DR, Hamilton EF, Dekaban GA (2005) Methylprednisolone causes minimal improvement after spinal cord injury in rats, contrasting with benefits of an anti-integrin treatment. J Neurotrauma 22:1375-1387.

Wells JE, Hurlbert RJ, Fehlings MG, Yong VW (2003) Neuroprotection by minocycline facilitates significant recovery from spinal cord injury in mice. Brain 126:1628-1637.

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.

Yawata I, Takeuchi H, Doi Y, Liang J, Mizuno T, Suzumura A (2008) Macrophage-induced neurotoxicity is mediated by glutamate and attenuated by glutaminase inhibitors and gap junction inhibitors. Life Sci 82:1111-1116.

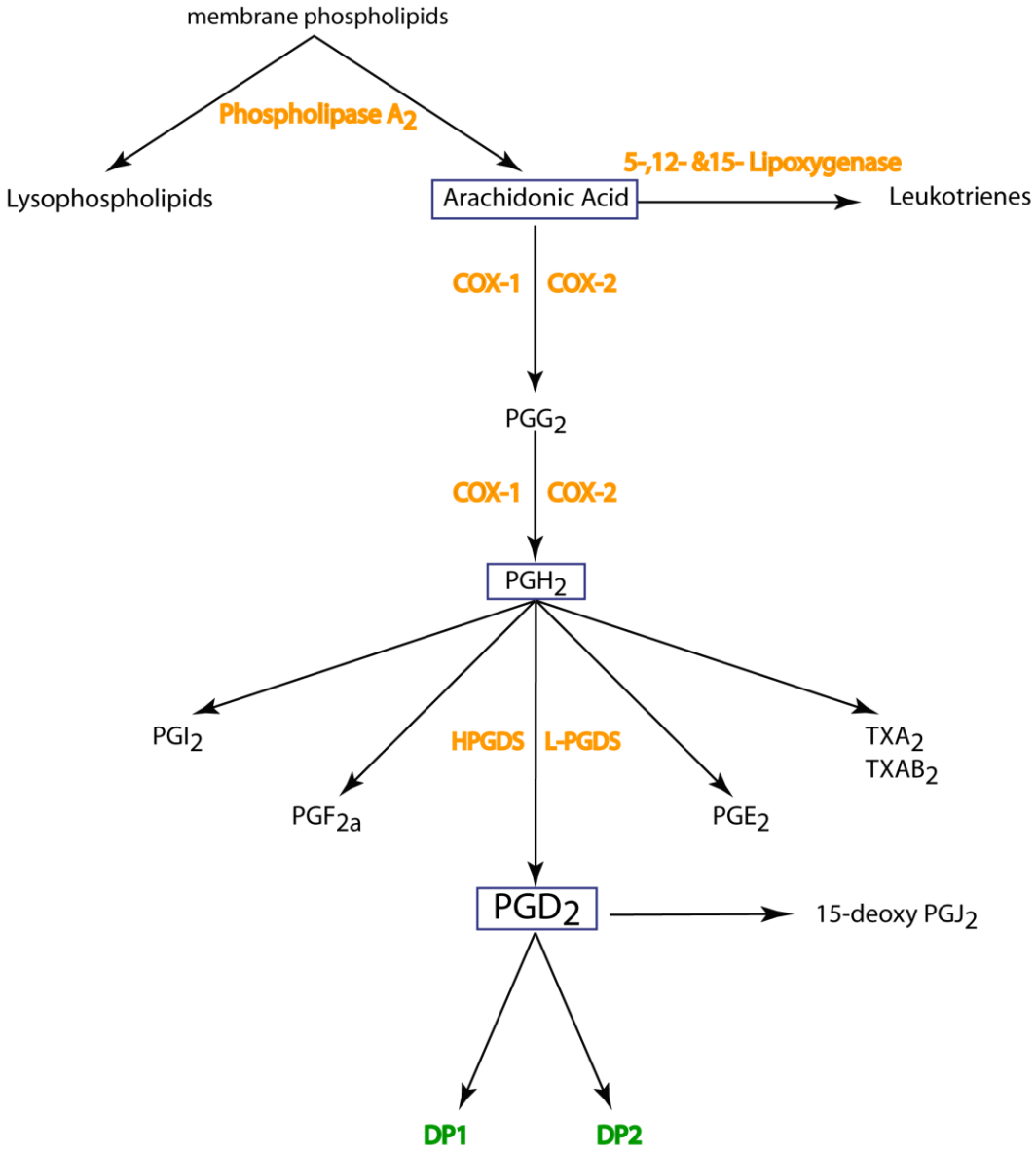

Figure 1

Schematic of Phospholipase A_2 pathway

Membrane phospholipids are hydrolyzed by phospholipase A_2 to give rise to lysophosholipids and free fatty acids such as arachidonic acid. Arachidonic acid can be converted to leukotrienes by lipoxygenases or converted to a prostanoid precursor in a two-step conversion by either COX-1 or COX-2. Prostaglandin H_2 (PGH_2) is the precursor for prostaglandin I_2 (PGI_2), prostaglandin F_{2a} (PGF_{2a}), prostaglandin D_2 (PGD_2), prostaglandin E_2 (PGE_2), and thromboxanes (TXA_2, TXB_2). Specific synthases convert PGH_2 into each specific prostaglandin. PGH_2 is converted to PGD2 by two specific synthases, hematopoietic prostaglandin D synthase (HPGDS) or lipocalin-type prostaglandin D synthase (L-PGDS). PGD_2 can signal via two distinct G-protein coupled receptors, DP1 and DP2. PGD_2 may also be further metabolized into 15-deoxy Prostaglandin J_2.

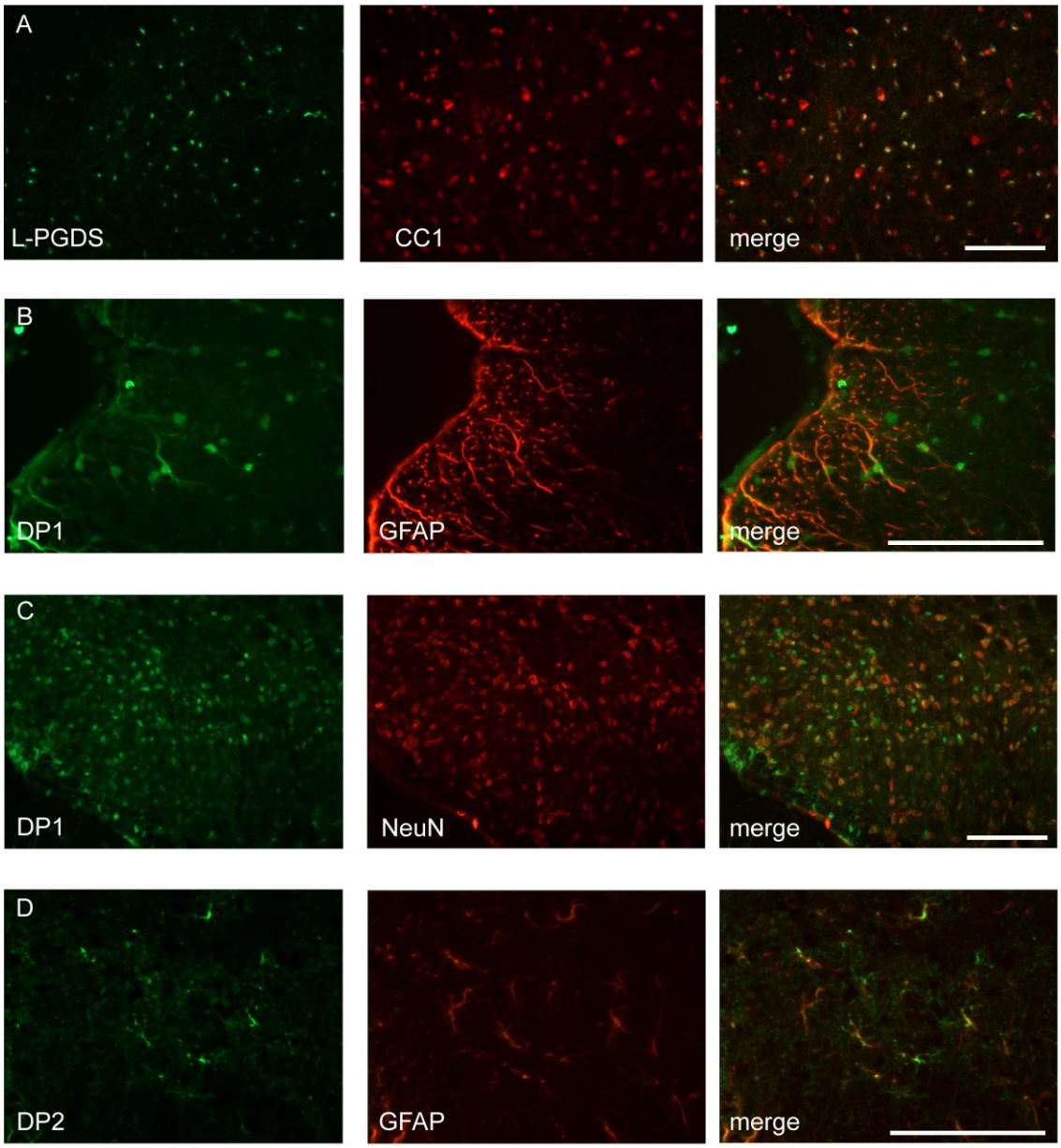

Figure 2

Expression of L-PGDS and DP1 and DP2 receptors in uninjured adult mouse spinal cord. Double immunofluorescence labeling of cross sections of adult mouse thoracic spinal cord. (**A**) Note that L-PGDS is relatively abundant in $CC1^+$ oligodendrocytes. (**B**) DP1 receptor is expressed in some $GFAP^+$ astrocytes, and in neurons identified by their $NeuN^+$ labeling (**C**) located in the dorsal horn. (**D**) DP2 is expressed at low levels in some astrocytes. HPGDS expression is not detected in the uninjured mouse spinal cord. Scale bars, 100µm.

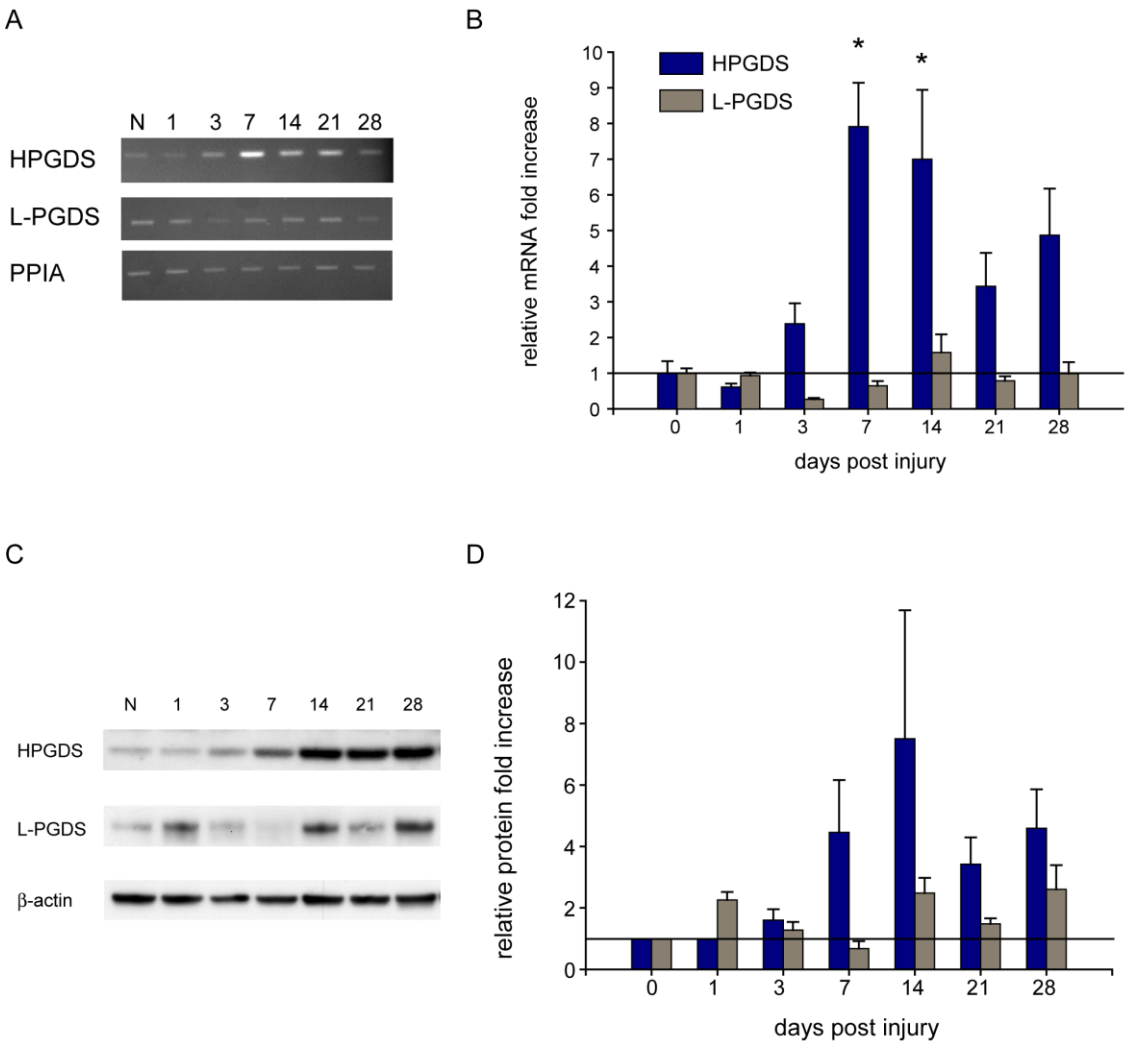

Figure 3

HPGDS expression is increased after spinal cord contusion injury. The 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 at both the mRNA and protein levels. (**A**) Representative agarose gel of RT-PCR products. (**B**) Quantification of relative mRNA fold increases over 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. (**C**) Representative Western blot of total protein extracted from contused spinal cord at several time points after injury and stained for HPGDS and L-PGDS. β-actin was used as a loading control. (**D**) Quantification of Western blots show that HPGDS begins to rise at 7 days after injury and peaks at 14 days and appears almost 4-fold higher at day 28. The blots of the L-PGDS were more variable between experiments but the overall levels remained relatively stable. n=3 for all analyses. Values are represented as mean ± SEM; * $p<0.05$.

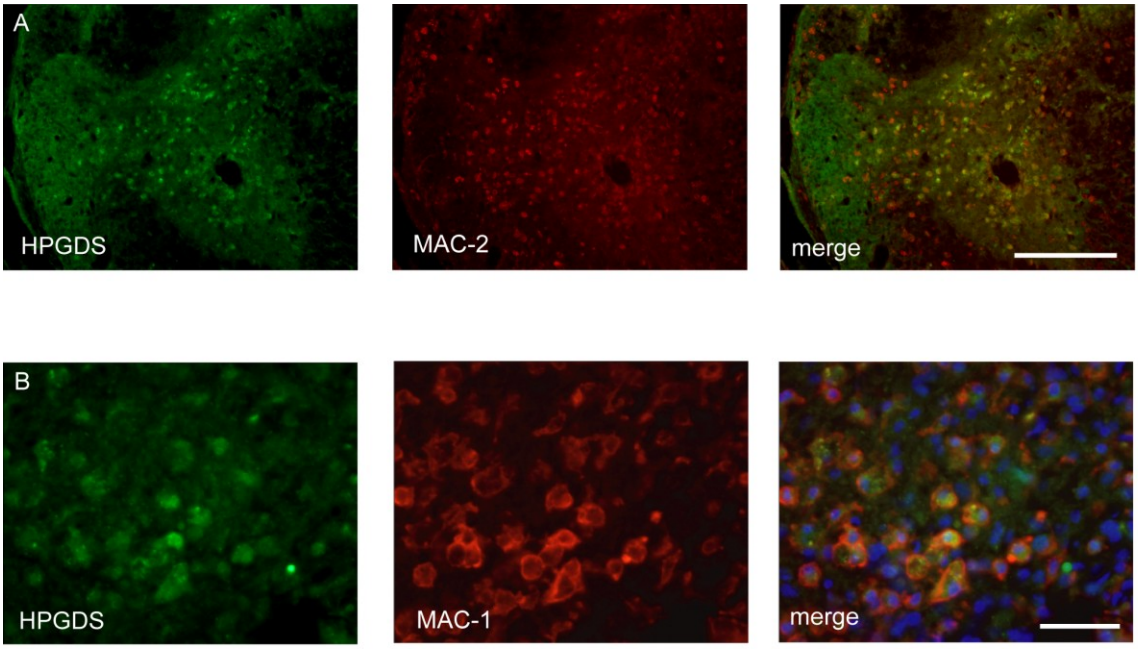

Figure 4

HPGDS expression is increased after injury. (**A**) Double immunofluorescence labeling showed that HPGDS is localized to activated Mac-2^{+} macrophages/microglia as early as 3 days after injury. (**B**) Mac-1^{+} macrophages/microglia that express HPGDS are also found within the lesion core 7 days after injury. Scale bars: (**A**), 250µm; (**B**) 50µm.

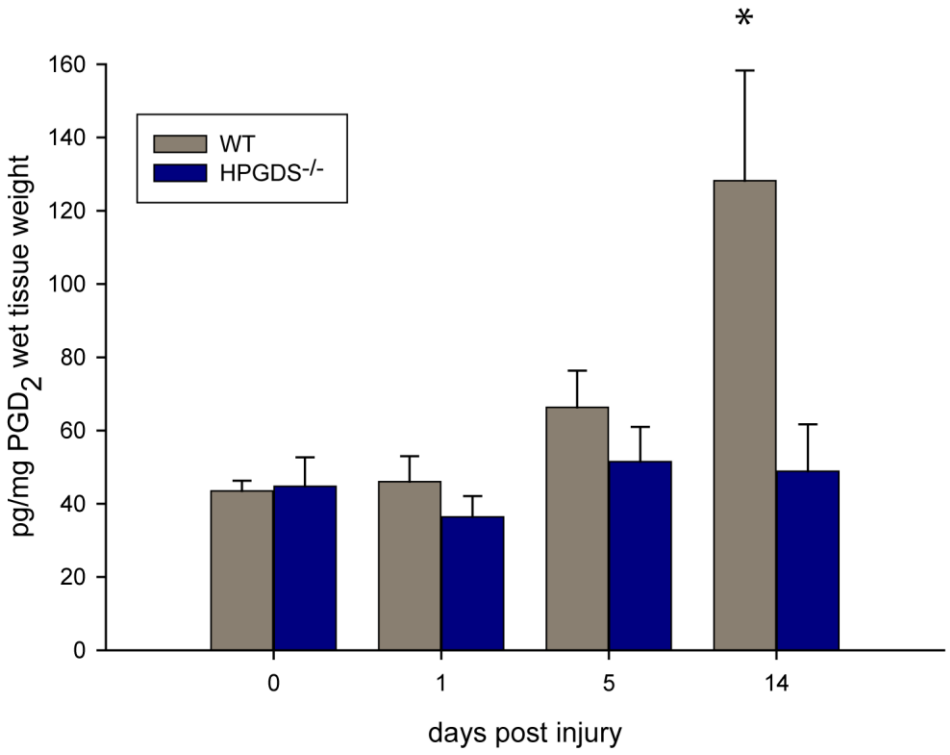

Figure 5

Increased levels of PGD_2 after contusion injury is synthesized by HPGDS. The level of PGD_2 in spinal cord tissue from uninjured and contused spinal cord, 1, 5 and 14 days after SCI from wildtype and $HPGDS^{-/-}$ mice were analyzed by ELISA. In wildtype mice, PGD_2 levels begin to rise at 5 days post-injury and are significantly greater than control levels at 14 days after injury. This increase is completely abrogated in the $HPGDS^{-/-}$ mice. n=4; values are represented as mean ± SEM; * $p < 0.05$.

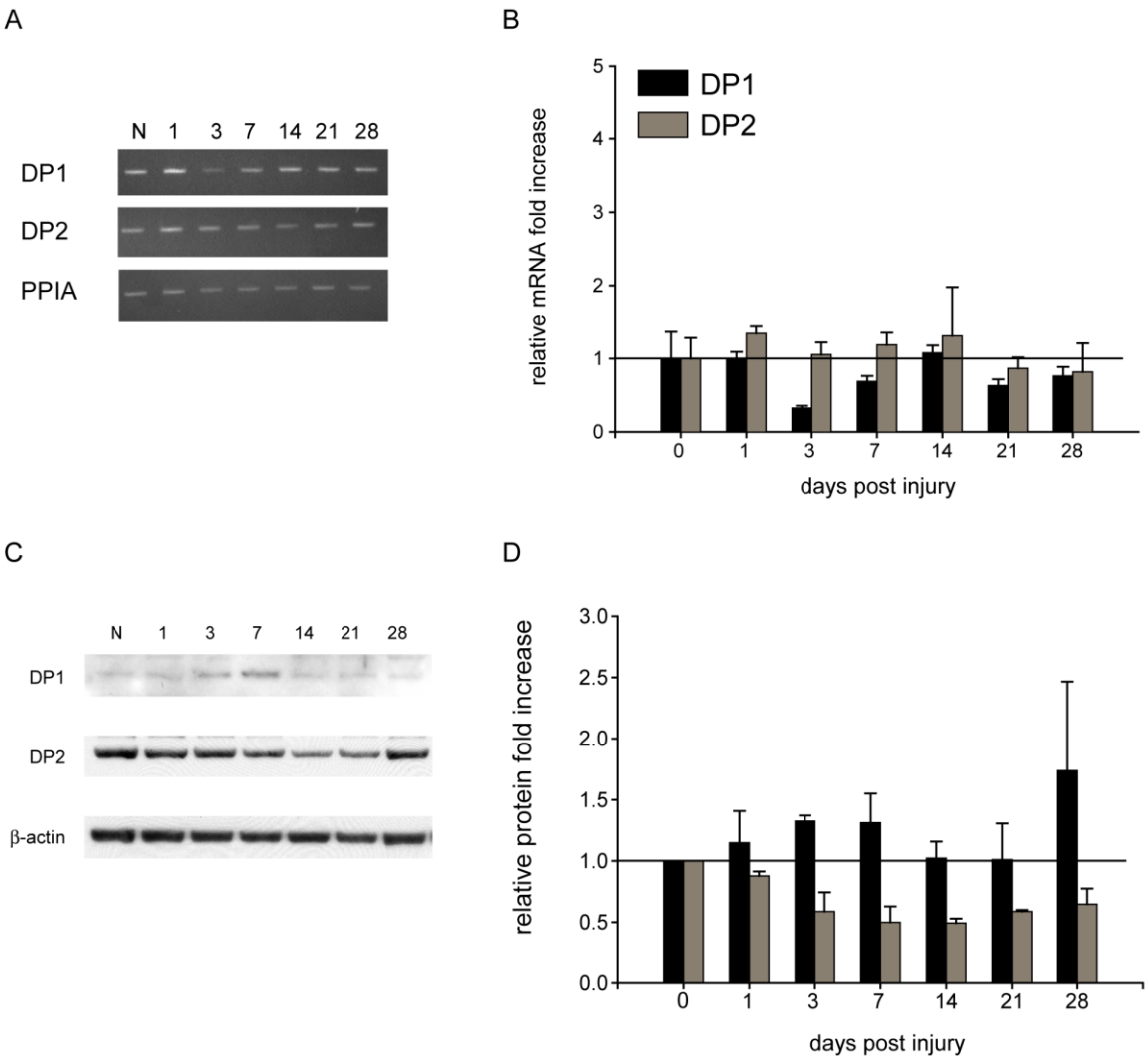

Figure 6

Expression levels of PGD_2 receptors, DP1 and DP2, after SCI. Uninjured and injured spinal cord tissue taken at several time points after contusion injury were analyzed at the mRNA and protein level for changes in expression of DP1 and DP2 receptors. (**A**) mRNA levels of both receptors remained relatively constant after SCI. (**B**) Quantification of mRNA levels. (**C**) Representative Western blots of total protein extracted from contused spinal cord at several time points after injury stained for DP1 and DP2. (**D**) Quantification of Western blots. Note that there is little change in the expression of these receptors after injury. n=3 for RT-PCR and Western blot experiments; values are represented as mean ± SEM.

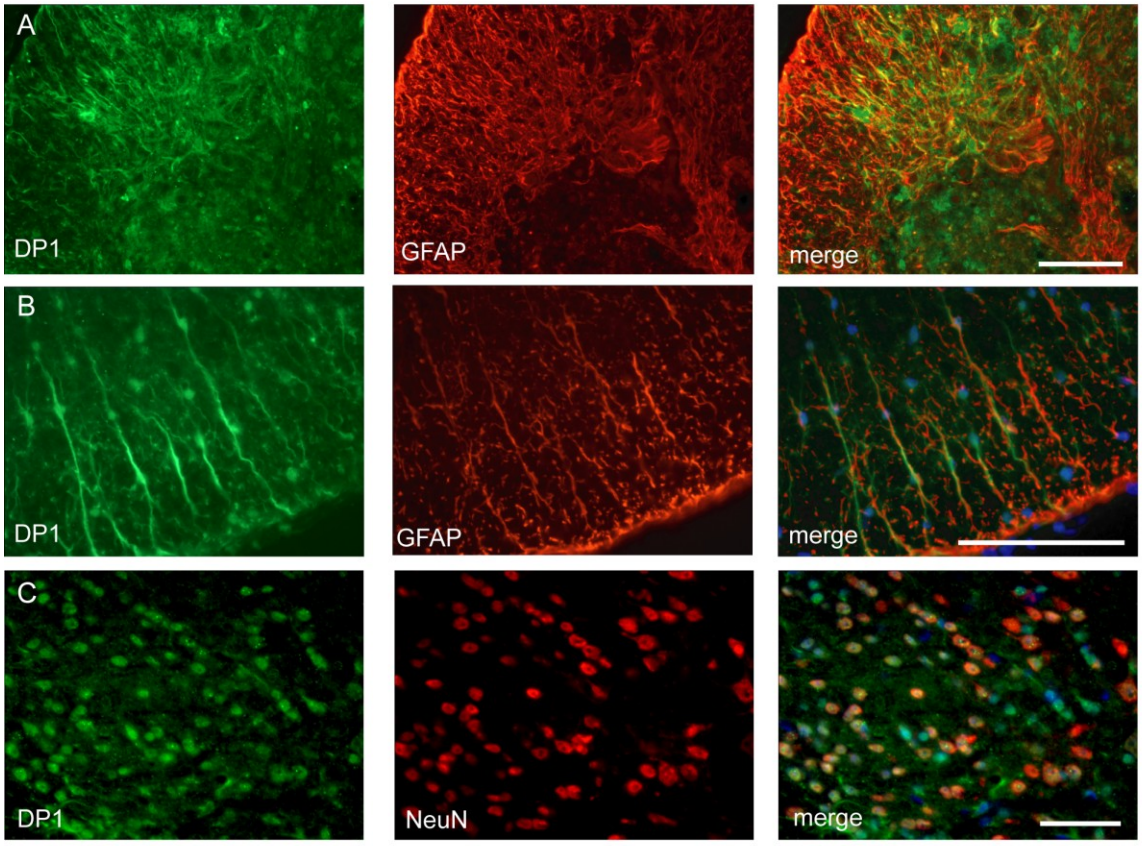

Figure 7

DP1 Expression is localized to astrocytes and some neurons after contusion injury. Double immunofluorescence labeling shows that 14 days after contusion injury DP1 expression is localized principally to GFAP$^+$ astrocytes surrounding the lesion core (**A**), and in some astrocytes in the adjacent white matter (**B**). Similar to expression seen in naïve uninjured mice, DP1 expression was also found in NeuN$^+$ neurons of the dorsal horn (**C**). Scale bars: (**A**),(**B**), 100µm; (**C**) 50µm.

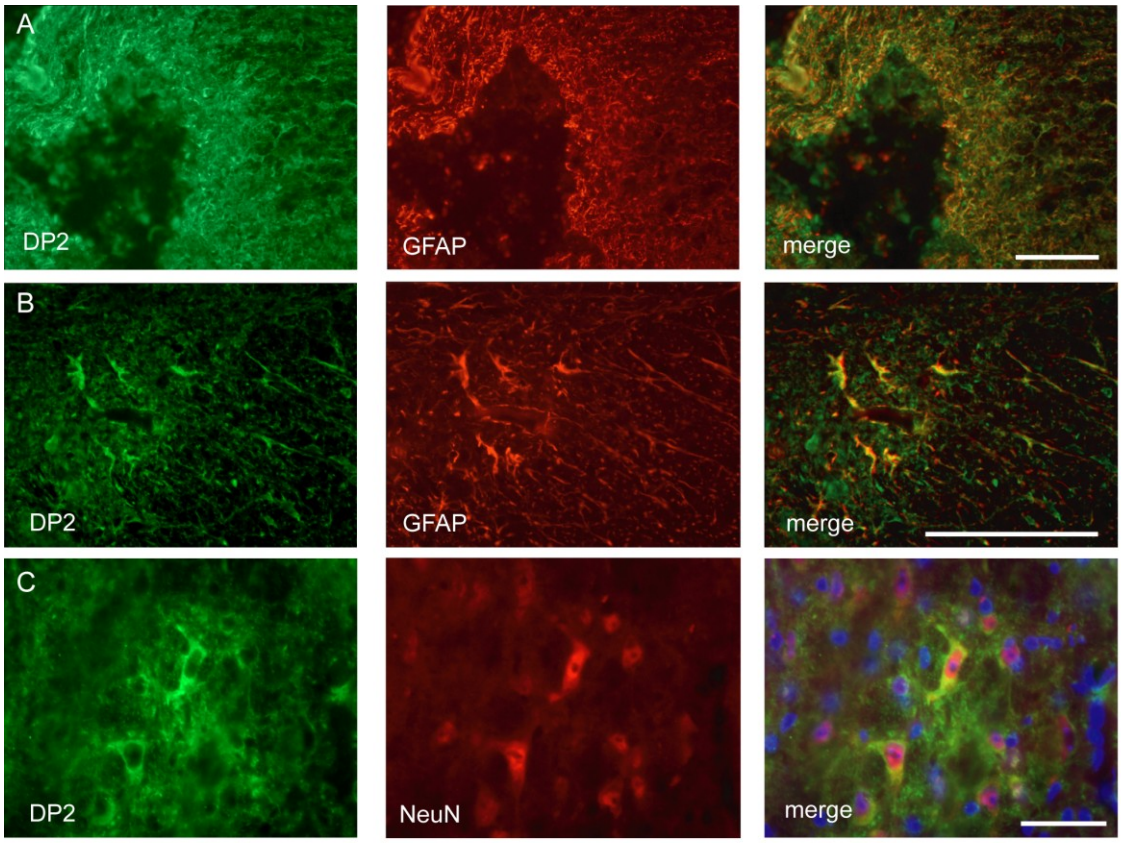

Figure 8

DP2 expression is localized to astrocytes and neurons after contusion injury. Double immunofluorescence labeling shows that 14 days after contusion injury DP2 expression is localized principally to $GFAP^{+}$ astrocytes surrounding the lesion core (**A**), and in reactive astrocytes in the gray matter (**B**). After injury, DP2 is also expressed in motor neurons of the ventral horn (**C**), identified by their NeuN labeling. Scale bars: (**A**), (**B**), 100µm; (**C**), 50µm.

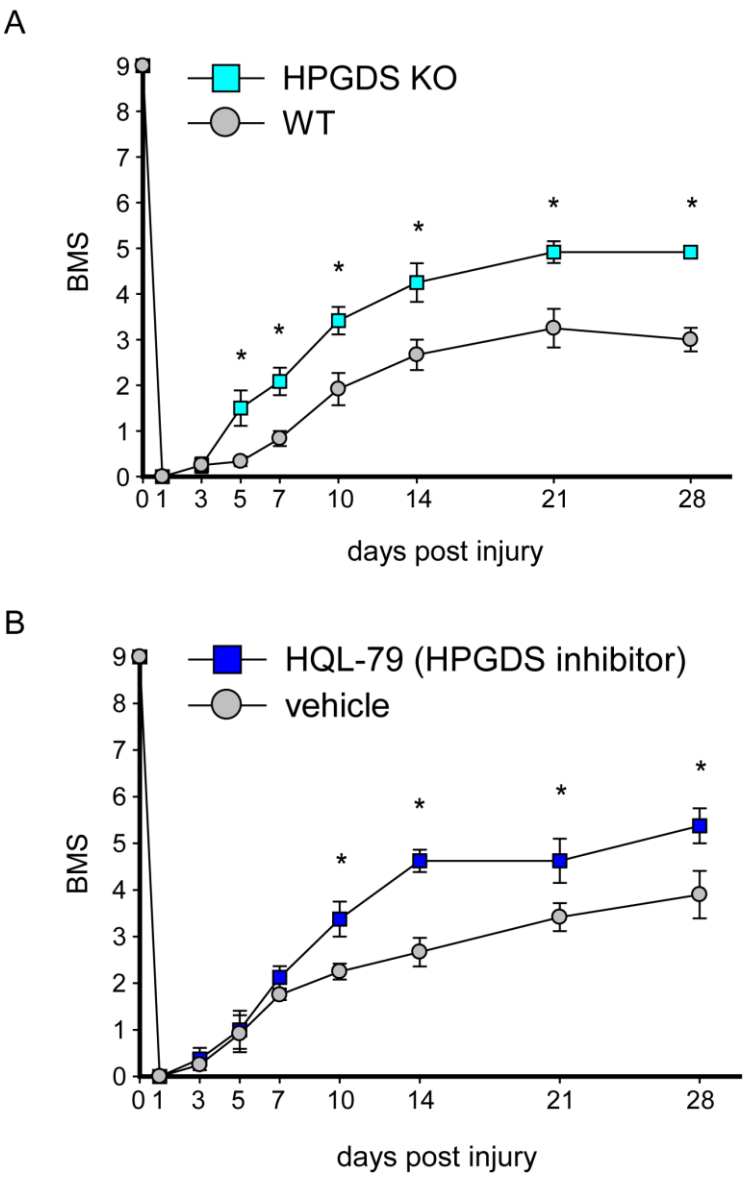

Figure 9

Lack or inhibition of HPGDS promotes locomotor recovery after SCI. (**A**) HPGDS$^{-/-}$ mice showed significant improvement in locomotor recovery as compared to wildtype controls assessed using the Basso Mouse Scale (BMS). This improvement was seen beginning on day 5 after SCI. (**B**) Wildtype C57BL/6 mice administered HQL-79 daily (50mg/kg), a HPGDS inhibitor, exhibited significant improvement in locomotor recovery as compared to vehicle-treated controls. The improvement in the BMS seen in HQL-79 administered mice was similar to that seen with HPGDS$^{-/-}$ after contusion injury. N=6 for both HPGDS$^{-/-}$ and WT; N= 4 (HQL-79); N= 5 (vehicle). Values are represented as mean ± SEM; *p<0.05.

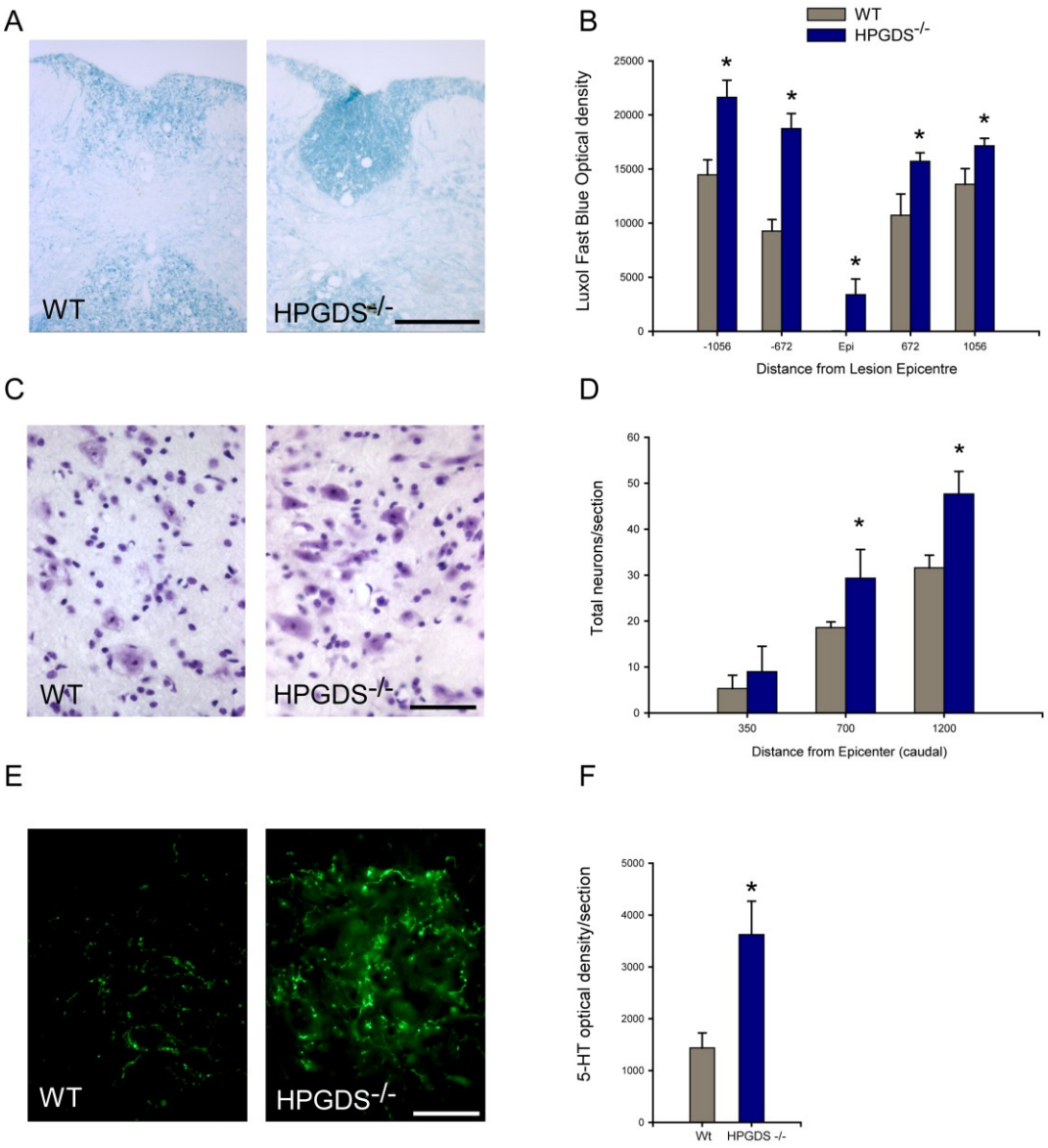

Figure 10

HPGDS$^{-/-}$ have reduced secondary damage after contusion injury as compared to WT mice. Secondary damage was assessed after contusion injury on day 28 by analyzing myelin loss, neuronal cell counts and 5-HT innervation. (**A**) Myelin loss was assessed by staining cross sections of the lesioned spinal cord of WT and HPGDS$^{-/-}$ mice with LFB. Representative micrographs were taken 672µm caudal to the epicenter. (**B**) HPGDS$^{-/-}$ showed a significant sparing of myelin in the area of the dorsal columns at several distances rostral and caudal to the epicenter of injury. (**C**) Representative micrographs of cresyl violet stained sections showing the ventral horn of the spinal cord from HPGDS$^{-/-}$ and WT mice at 1150 µm caudal to epicenter. (**D**) Quantification of cresyl violet stains showed HPGDS$^{-/-}$ mice have significantly more neurons in the ventral horn as compared to WT mice. (**E**) Representative micrographs of 5-HT staining in the ventral gray matter of WT and HPGDS$^{-/-}$ mice 1000µm caudal to injury epicenter. (**F**) HPGDS$^{-/-}$ mice showed significantly greater 5-HT innervation as compared to WT mice 28 days after contusion injury. n=3; values are represented as means ± SEM; *p<0.05. Scale bars: (**A**), 250µm; (**C**), (**E**), 50µm.

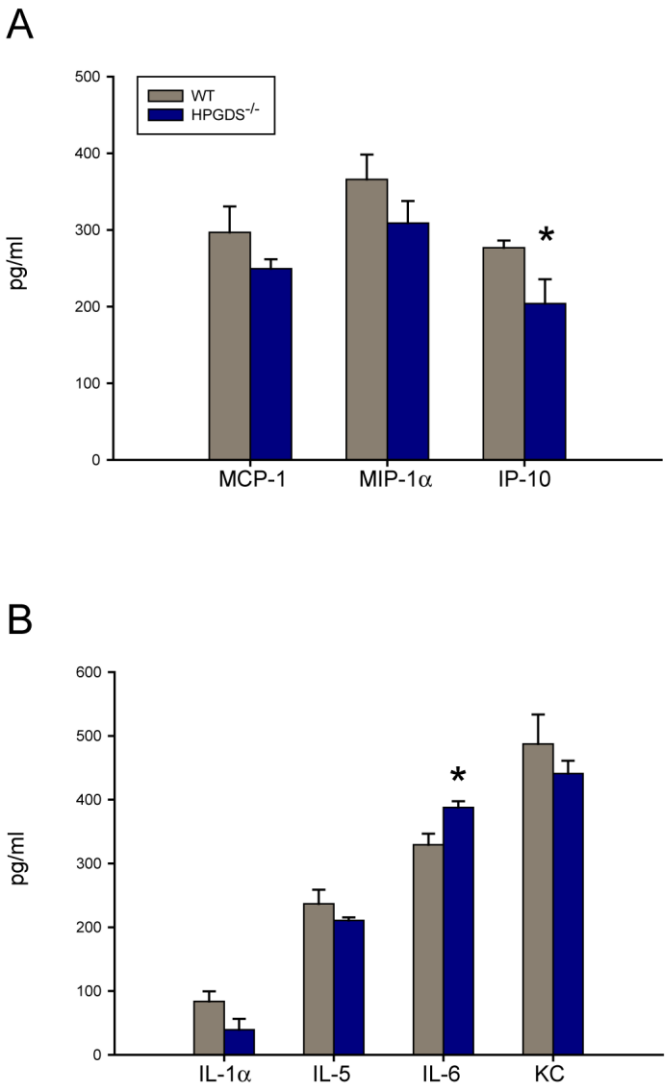

Figure 11

Changes in inflammatory mediators after contusion injury in HPGDS$^{-/-}$ and WT mice. The levels of several chemokines and cytokines were analyzed at the protein level. Twelve hours after contusion injury, reduced levels of IP-10 protein (**A**) and increased levels of IL-6 protein (**B**) were found in HPGDS$^{-/-}$ mice as compared to wildtype controls. Protein levels were assayed using the BioSource Mouse Cytokine 20-Plex Multiplex Bead Immunoassay. n=4; values are represented as means ± SEM; *p<0.05.

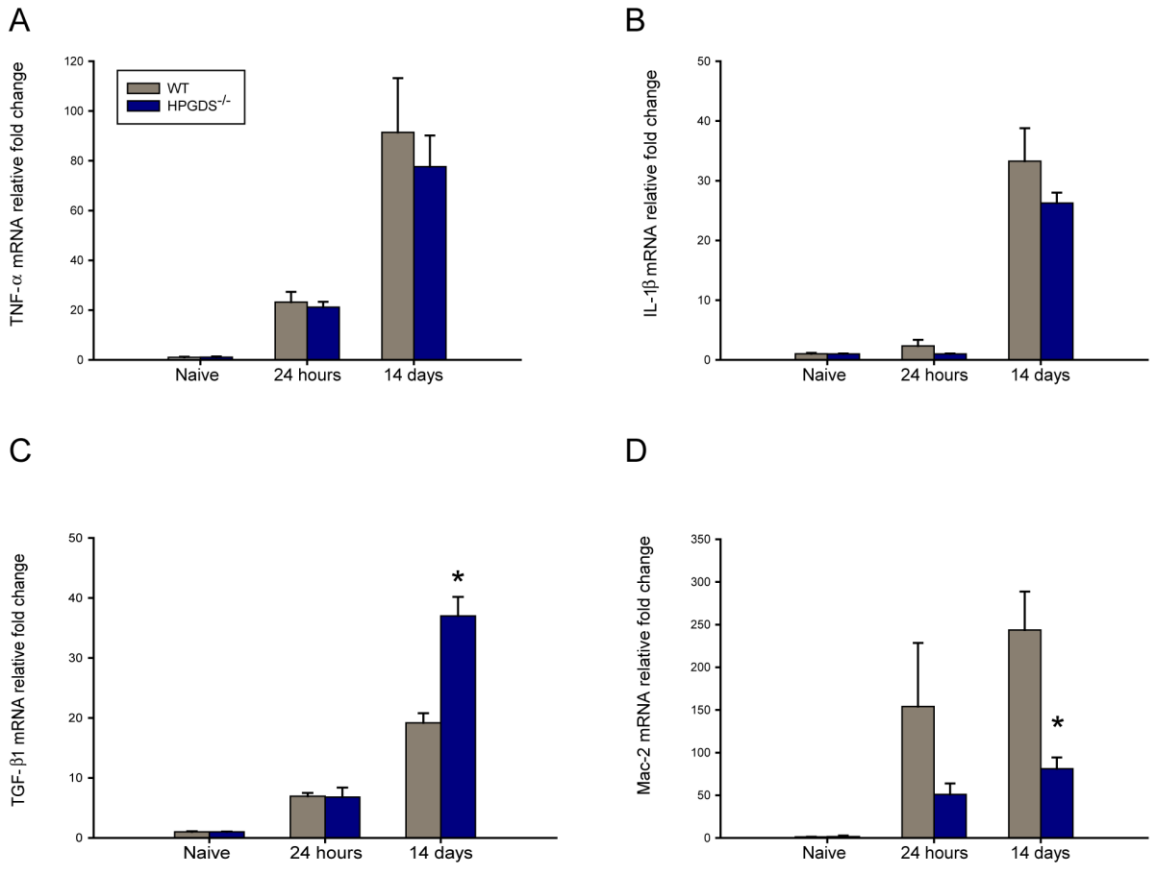

Figure 12

Changes in inflammatory mediators after contusion injury in HPGDS$^{-/-}$ and WT mice. The mRNA expression by QPCR analysis of TNF-α (**A**), IL-1β (**B**) and TGF-β1 (**C**) was examined at 24 hours and 14 days after SCI. Note that although there is a delayed increase in TNF-α (**A**), and IL-1β (**B**) in both strains of mice at 14 days, there are no differences between the two genotypes. In contrast, there was a significant increase in the expression of TGF-β1 in HPGDS$^{-/-}$ mice at 14 days post SCI (**C**). The mRNA levels of Mac-2 (a galectin expressed on activated microglia/macrophages) was significantly decreased at 14 days in HPGDS$^{-/-}$ mice as compared to WT mice, suggesting a reduction in macrophage/microglial activation. n=3; values are represented as means ± SEM; *$p<0.05$.

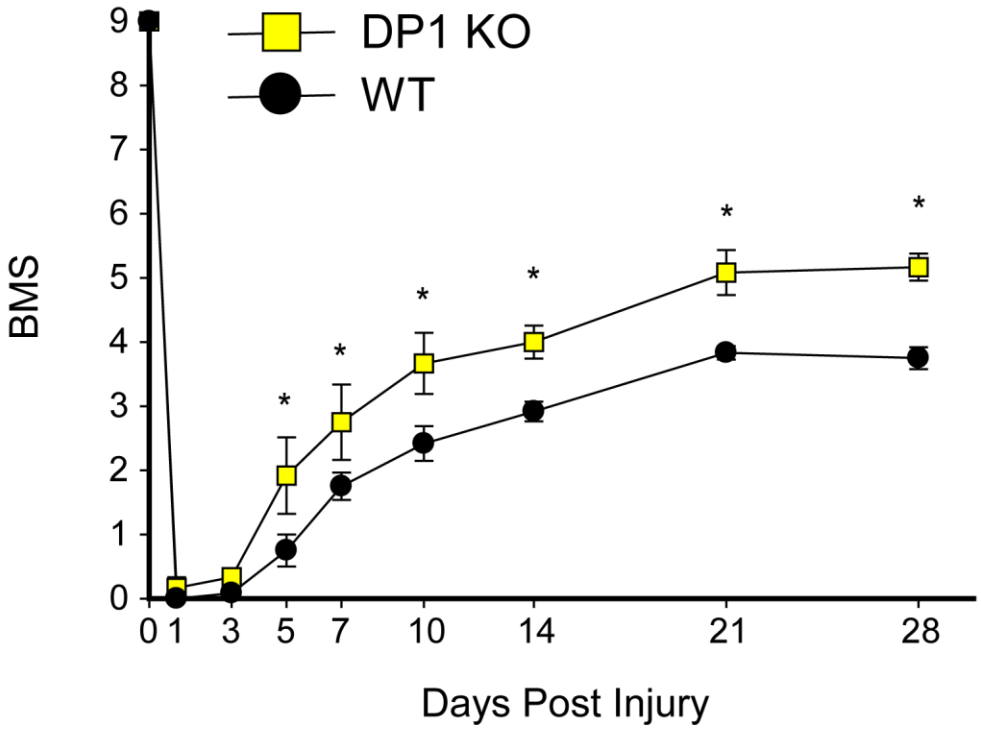

Figure 13

PGD_2 signaling via DP1 is detrimental after SCI. Locomotor recovery after SCI was assessed in $DP1^{-/-}$ mice using the BMS analysis. Note that mice lacking the DP1 receptor showed a significant improvement in locomotor recovery as compared to WT mice beginning at day 5. This result is similar to that seen with $HPGDS^{-/-}$ and HQL-79 administered to WT mice. n=6; values are represented as means ± SEM; *$p<0.05$.