

# Potential Roles of Prostaglandin E<sub>2</sub> and Interleukin-1 β in Experimental Autoimmune Encephalomyelitis

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## Abstract

Multiple sclerosis (MS) is a progressive disease that is characterized by multifocal inflammation and demyelination in a central nervous system. Experimental autoimmune encephalomyelitis (EAE) is an animal model of MS that shows ascending flaccid paralysis with inflammation of spinal cord. We focus on the potential roles of inducible prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and interleukin-1β (IL-1β) in EAE after myelin oligodendrocyte glycoprotein 35-55 peptide immunization in this review. PGE<sub>2</sub> synthesized by cyclooxygenase (COX)-2 and microsomal prostaglandin E synthase-1 (mPGES-1) in vascular endothelial cells (VECs) or macrophages/microglia aggravates inflammation, demyelination and paralysis and facilitates the activation and differentiation of CD4<sup>+</sup> T cells into interleukin-17 (IL-17)-producing helper T cells to promote neuronal dysfunction and blood-spinal cord barrier disruption in the EAE model. PGE<sub>2</sub> also causes vascularity and increases IL-1β production in VECs and CD4<sup>+</sup> T cells, and IL-1β plays a crucial role in facilitating EAE progression and stimulates the synthesis of COX-2 and mPGES-1 to produce PGE<sub>2</sub>. Thus, the local PGE<sub>2</sub>-IL-1β-PGE<sub>2</sub> signalling pathway facilitates IL-17 production in inflammatory lesions in the spinal cord of EAE animals. This pathway represents a possible mechanism by which PGE<sub>2</sub> participates in EAE pathology. Taken together, this evidence highlights the intercellular PGE<sub>2</sub> signalling pathway in the spinal cord as a therapeutic target for ameliorating MS severity after disease onset.

**Keywords:** Prostaglandin E<sub>2</sub>; Interleukin-1β; Microsomal prostaglandin synthetase-1; Multiple sclerosis; Experimental autoimmune encephalomyelitis; CD4-positive (CD4<sup>+</sup>) T cells; Vascular endothelial cells

**Abbreviations:** MS: Multiple Sclerosis; PGE<sub>2</sub>: Prostaglandin E<sub>2</sub>; COX: Cyclooxygenase; mPGES-1: Microsomal Prostaglandin E Synthetase-1; mac/mic: Macrophages/microglia; VECs: Vascular Endothelial Cells; EAE: Experimental Autoimmune Encephalomyelitis; MOG35-55: Myelin Oligodendrocyte Glycoprotein35-55 Peptide; IL-1β: Interleukin-1β; IL-1RN: Interleukin-1 Receptor Antagonist; IL-1R1: Interleukin-1 Receptor 1; mPGES-2: Microsomal Prostaglandin E Synthetase-2; cPGES: Cytosolic PGE<sub>2</sub> Synthase; EP: E-prostanoid; mPGES-1<sup>-/-</sup>: Microsomal Prostaglandin Synthetase-1-deficient; Wt: Wild Type; IL-17: Interleukin-17; BBB: Blood-Brain Barrier; CD4<sup>+</sup> T Cell: CD4-positive T cell; Th17: Interleukin-17-producing Helper T cell; Th1: Type 1 helper T cell; IFN-γ: Interferon-γ; VEGF: Vascular Endothelial Growth Factor; IL-17R: Receptor for IL-17; ASC: Apoptosis-associated Speck-like Protein Containing a Caspase Recruitment Domain.

## Introduction

Multiple sclerosis (MS) is a progressive disease showing multifocal inflammation and demyelination. Inducible prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) is an inflammatory mediator synthesized by cyclooxygenase (COX)-2 and microsomal prostaglandin E synthase-1 (mPGES-1, also known as PTGES). COX-2 is increased in macrophages/microglia (mac/mic) in the brains of MS patients and in vascular endothelial cells (VECs) in animals with experimental autoimmune encephalomyelitis (EAE), an animal model of MS [1,2]. In addition, mPGES-1 expression is induced in brain VECs in fever and neuronal injury after kainic acid injection [3,4]. Rodents with EAE induced by the myelin oligodendrocyte glycoprotein 35-55 peptide (MOG35-55) exhibit typical perivascular infiltration of mononuclear cells and inflammatory foci in the spinal cord and brain. MOG35-55-induced EAE also results in infiltration of the cerebral meninges at the third and lateral ventricles, as well as severe parenchymal infiltration in the spinal cord [5,6]. The symptomatic course of EAE involves progressive flaccid paralysis with inflammation which targets the spinal cord but accordingly, we mainly focus on spinal inflammation in EAE and its association with PGE<sub>2</sub> in this review [7,8].

PGE<sub>2</sub> is increased after EAE induction in spinal cord and treatment with selective COX-2 inhibitors prevent the development of EAE-associated paralysis. Similarly, the induction of mPGES-1 expression in infiltrating macrophages stimulates the clinical EAE progression in mice. Moreover, mPGES-1 expression is also induced in VECs located around inflammatory foci and accelerates inflammation, demyelination, and paralysis in EAE models. Thus, PGE<sub>2</sub> produced by mPGES-1 promotes disease progression in the spinal cord of animals with EAE [9-15].

Interleukin-1β (IL-1β) is also an important inflammatory and pathological mediator of EAE mice. The administration of a recombinant interleukin-1 receptor antagonist (IL-1RN) delays disease onset and decreases EAE severity [16]. Additionally, the expression of a defective interleukin-1 receptor 1 (IL-1R1) gene in mice show complete resistance to EAE [17]. IL-1β is generally known to stimulate PGE<sub>2</sub> production; whereas, evidence showing that PGE<sub>2</sub> stimulates IL-1β production is unavailable. However, we recently find that IL-1β is a mediator or component of the mechanism by which PGE<sub>2</sub> promotes the progression of EAE [18].

In this review article, we summarize the roles of PGE<sub>2</sub> and IL-1β in EAE and their relations with inflammatory molecules related to spinal cord inflammation. Furthermore, we discuss intercellular interactions among VECs, CD4<sup>+</sup> T cells, and monocytes mediated by PGE<sub>2</sub> and autocrine IL-1β signaling in EAE mice.

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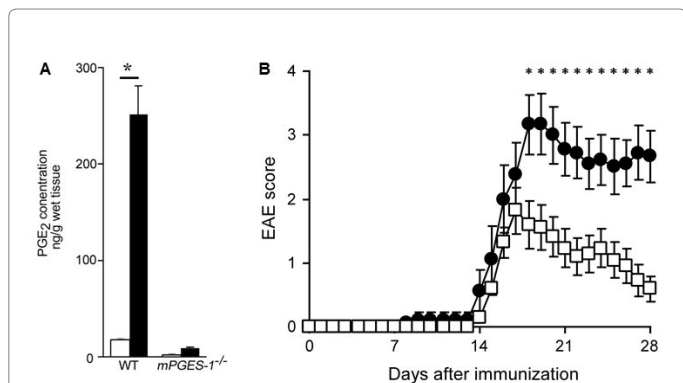
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## PGE<sub>2</sub> Induction in EAE Models

Patients with definite MS have higher baseline PGE expression in leukocyte cultures than healthy control subjects and PGE<sub>2</sub> expression is similarly elevated in peripheral blood monocytes from chronic MS patients [19-20]. Based on these reports, peripheral PGE<sub>2</sub> expression might be related to MS progression. PGE<sub>2</sub> is synthesized from arachidonic acid by COX and PGES, and COX exists as constitutively active (COX-1) and inducible (COX-2) isoforms. MS patients show COX-2 induction in chronic, active lesions in the brain [1]. COX-2 expression also appears in mac/mic in the spinal cord 16 days after EAE induction [21]. In EAE models, COX-2 is localized to ECs in the spinal cord, particularly 14-25 days after immunization [2]. Thus, COX-2 in central nervous system (CNS) is a crucial mediator of MS and EAE pathology. PGE<sub>2</sub> concentration is elevated in the spinal cord after EAE induction (Figure 1A) [12].

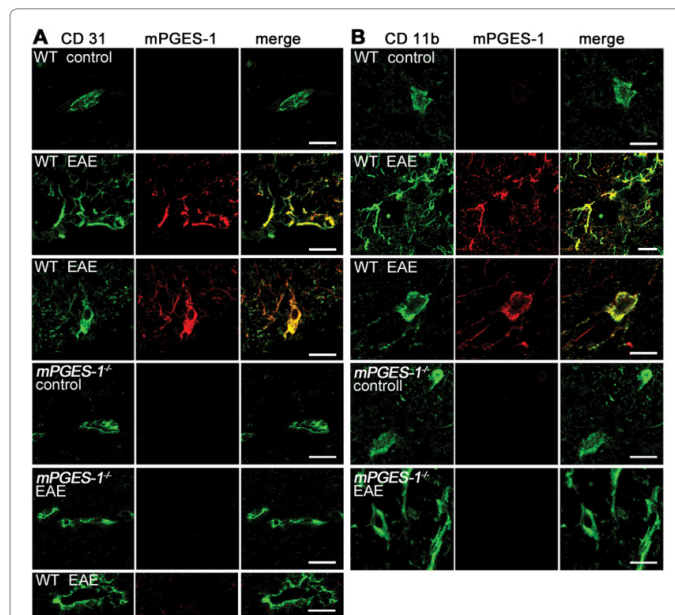
Three isoforms of PGES have been identified: mPGES-1, microsomal prostaglandin E synthase-2 (mPGES-2) and cytosolic PGE<sub>2</sub> synthase (cPGES). Notably, mPGES-2 and cPGES are constitutively appeared, while mPGES-1 expression is induced in a manner functionally coupled to COX-2 in macrophages or osteoblasts following pro-inflammatory stimulation [22-25]. In the brain, mPGES-1 expression is induced by COX-2 in VECs during fever, kainic acid-induced neuronal injury, and cerebral ischemia [3,4,26]. In EAE models, mPGES-1 is expressed in ECs macrophages and mac/mic (Figure 2) but not in CD4<sup>+</sup> T cells in the spinal cord [11,12,18]. COX-2 expression is also induced in ECs, but conflicting information about the induction of COX-2 expression in mac/mic has been reported [2,21]. Therefore, VECs in CNS are undoubtedly important sources of PGE<sub>2</sub> synthesized by COX-2 and mPGES-1 in response to pro-inflammatory changes in the spinal cord of EAE animals.



**Figure 1:** Spinal prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) concentration and the development of experimental autoimmune encephalomyelitis (EAE) progression after myelin oligodendrocyte glycoprotein 35-55 peptide immunization.

(A) The tissue PGE<sub>2</sub> concentration in lumbar spinal cords 19 days after EAE induction. Spinal cords from wild-type (wt) control, wt EAE, microsomal PGE synthase-1 (mPGES-1) deficient (mPGES-1<sup>-/-</sup>) control and mPGES-1<sup>-/-</sup> EAE mice are used for the analysis. The black bars and white bars represent EAE and control mice. The data reveals a significant increase in the PGE<sub>2</sub> concentration in wt EAE mice compared to the wt control, whereas there is no increase in the mPGES-1<sup>-/-</sup> EAE mice.

(B) The time course of EAE progression in wt (closed circles) and mPGES-1<sup>-/-</sup> (open squares) mice. mPGES-1<sup>-/-</sup> mice are resistant to development of EAE. A repeated measure ANOVA reveals that the mPGES-1<sup>-/-</sup> mice exhibit significantly lower EAE scores 18 days after immunization. (Figure first published in *Neurochemistry International* 62(3): 271-280, 2013, Takeuchi C et al., *Microsomal prostaglandin E synthase-1 aggravates inflammation and demyelination in a mouse model of multiple sclerosis*. Copyright © 2012 by the authors).



**Figure 2:** Induction of microsomal prostaglandin E synthase-1 (mPGES-1) in the spinal cord.

(A) Double immunostaining for CD31 (green) and mPGES-1 (red). In the wild-type (wt) experimental autoimmune encephalomyelitis (EAE) mice after myelin oligodendrocyte glycoprotein 35-55 peptide immunization, CD31-positive endothelial cells (ECs) are double stained for mPGES-1 (merge), whereas the ECs of the anterior spinal artery does not express mPGES-1 (bottom panels). The mPGES-1-deficient (mPGES-1<sup>-/-</sup>) EAE mice do not express any detectable mPGES-1 protein.

(B) Double immunostaining for CD11b (green) and mPGES-1 (red). CD11b-positive cells in wt EAE mice are double labeled with mPGES-1 (merge). Scale bar: 20  $\mu$ m in A and second top panel of B, 10  $\mu$ m in all other panels in B. (Figure first published in *Neurochemistry International* 62(3): 271-280, 2013, Takeuchi C et al., *Microsomal prostaglandin E synthase-1 aggravates inflammation and demyelination in a mouse model of multiple sclerosis*. Copyright © 2012 by the authors).

## Effects of PGE<sub>2</sub> on Neuro Inflammation and EAE Models

In the brain and spinal cord, mPGES-1 activates several neuroinflammatory processes in neurodegenerative disease states and under physiological conditions. For example, mPGES-1 exacerbates hippocampal neuron injury induced by seizure and cerebral ischemia after transient occlusion of the hemilateral middle cerebral artery [4,26-29]. Moreover, mPGES-1 expression is associated with the  $\beta$ -amyloid plaque density, microglial accumulation, and learning impairment in subjects with Alzheimer's disease [30]. The expression of mPGES-1 is also induced by lipopolysaccharide treatment and plays a crucial role in amyotrophic lateral sclerosis [3,31-34]. Finally, mPGES-1 stimulates macrophage activation in the spinal cord and is related with anoxia and the maintenance of wakefulness. According to these reports, PGE<sub>2</sub> synthesized by mPGES-1 is a pathological mediator of and therapeutic target for neurodegenerative diseases and CNS injury [35-37].

Several studies have investigated the effects of PGE<sub>2</sub> inhibition on EAE. COX-2 inhibitors limit the extent of EAE severity and T cell responses [13-15]. The administration of COX-2 inhibitors starting on the day of immunization are more effective at reducing EAE severity than administration starting 8 days or 14 days after immunization. Four subtypes of PGE<sub>2</sub> receptors have been identified (E-prostanoid (EP) receptors EP1-4), and EP4-deficient mice show a significant attenuation of EAE symptoms. Moreover, the treatment of EP2-deficient mice with an EP4 antagonist from days 3 to 7 after

immunization completely blocks the onset of EAE [13,15,38]. In addition, mPGES-1-deficient (mPGES-1<sup>-/-</sup>) mice show a delayed, brief period of symptomatic disease followed by recovery rather than progression, and exhibit the impairment for 26 days or 28 days after immunization (Figure 1B). Compared to wild-type (wt) mice, mPGES-1<sup>-/-</sup> mice exhibit a delayed EAE onset [11,12,18]. Moreover, mPGES-1 aggravates the inflammation and demyelination associated with EAE (Table 1 and Figure 3). Thus, PGE<sub>2</sub> synthesized by COX-2 and mPGES-1 plays important roles in early disease onset and later during disease maintenance. Because PGE<sub>2</sub> is a generalized production, the source of PGE<sub>2</sub> is not limited in spinal cord and brain in acute inflammation of EAE.

However, researchers have not clearly determined the source of PGE<sub>2</sub>: VECs in CNS and mac/mic in the spinal cord or peripheral blood monocytes. Interestingly, based on the findings from bone marrow transplantation models, which enable EP expression or PGE<sub>2</sub> synthesis to be blocked in peripheral immune cells, EP4 or COX-2 deletion in bone marrow-derived cells causes a significant delay in the EAE onset, but the animals ultimately experience serious paralysis, similar to the controls. Moreover, the deletion reduces the number of T cells and levels of IL-6 and interleukin-17 (IL-17) in the blood [39]. Therefore, the peripheral PGE<sub>2</sub>/EP4 pathway is very important during EAE onset but does not control the severity of EAE. Researchers have hypothesized

that inducible PGE<sub>2</sub> synthesized by COX-2 and mPGES-1 in VECs in CNS and mac/mic facilitates pathological changes in the spinal cord of EAE models that lead to symptom manifestation.

### Effects of IL-1 $\beta$ on EAE and IL-1 $\beta$ Production

In a clinical study, MS patients presented significantly higher levels of IL-1 $\beta$  in the serum and cerebrospinal fluid than healthy control subjects [40]. IL-1 $\beta$  has consistently been shown to play an important role in EAE. The administration of recombinant IL-1RN to EAE animals delays the onset of the disease and decreases its severity. Moreover, a mutation in the IL-1R1 gene in mice show complete resistance to EAE [16,17]. IL-1 $\beta$  also regulates blood-brain barrier (BBB) permeability through endothelial IL-1R1 and induces BBB disruption. IL-1 $\beta$  also exacerbates neuronal inflammation and is showed at high levels in infiltrating macrophages [41-48]. The expression of the IL-1 $\beta$  mRNA is induced in peritoneal leukocytes right after the initial induction of EAE [49]. In addition, macrophages have a minor contribution to the total IL-1 $\beta$  production during EAE pathogenesis and CD4<sup>+</sup> T cells are thought to be a major source of IL-1 $\beta$  in EAE pathogenesis [44,48-50]. IL-1 $\beta$  levels are increased in activated CD4<sup>+</sup> T cells in inflammatory lesion in the spinal cord of EAE animals, and the increased IL-1 $\beta$  levels are mediated by mPGES-1 (Figure 4). Moreover, IL-1R1 expression in CD4<sup>+</sup> T cells in EAE models is also controlled by mPGES-1. IL-1R1 is expressed on IL-17-producing helper T cells (Th17), but not type 1 helper T cells (Th1), in EAE mice and early Th17 differentiation is regulated by IL-1 $\beta$  signaling through IL-1R1 [18,50-52].

IL-1 $\beta$  also stimulates IL-1R1 expressed on ECs and induces the production of IL-6 and chemokine ligand 2 in inflammatory lesions in EAE models whereas, no studies have evaluated the IL-1 $\beta$  production in ECs. Recently, we investigated whether IL-1 $\beta$  expression was increased in ECs by PGE<sub>2</sub> synthesized from mPGES-1. When we compared IL-1 $\beta$  levels in ECs from wt and mPGES-1<sup>-/-</sup> mice, IL-1 $\beta$  levels were significantly elevated in wt EAE mice than in mPGES-1<sup>-/-</sup> EAE mice. Therefore, IL-1 $\beta$  also plays an important role in ECs, and IL-1 $\beta$  expression is regulated by PGE<sub>2</sub> during EAE [44,53].

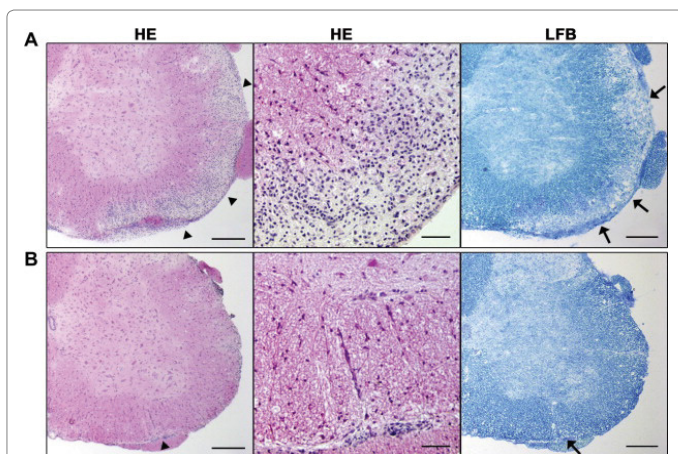
### Relationship between IL-1 $\beta$ and COX-2/mPGES-1

COX-2 expression is facilitated by IL-1 $\beta$ . IL-1 $\beta$  induces COX-2 expression in synovial fibroblasts and an intraperitoneal IL-1 $\beta$  injection induces the COX-2 mRNA expression in VECs in the brain [54-55]. The main protein that upregulates the COX-2 expression in the spinal cord is IL-1 $\beta$ , which promotes PGE<sub>2</sub> production and contributes to pain hypersensitivity. IL-1 $\beta$  also plays a role on mediating the activity of nuclear factor kappa B and the COX-2 transcription in cells of the BBB in response to inflammation. COX-2 inhibitors prevent IL-1 $\beta$ -induced increases in PGE<sub>2</sub> production in the brain. It suggests that IL-1 $\beta$  controls PGE<sub>2</sub> production by modulating the synthesis of COX-2. Moreover, crosstalk between microvascular ECs and tumor cells increases COX-2 and mPGES-1, which are strongly inhibited by an IL-1R antagonist [54-59]. Thus, IL-1 $\beta$  plays an important role in the induction of COX-2 and mPGES-1 to produce the pathophysiological protein PGE<sub>2</sub>. In contrast, IL-1 $\beta$  levels in CD4<sup>+</sup> T cells were elevated by PGE<sub>2</sub> derived from mPGES-1 in EAE spinal cords (Figure 4) [18]. Moreover, IL-1 $\beta$  was also increased in ECs present in inflammatory lesions in EAE models therefore, IL-1 $\beta$  expression in CD4<sup>+</sup> T cells and ECs is stimulated by PGE<sub>2</sub> in the spinal cord of EAE animals [53].

	WT EAE (n=7)	mPGES-1 <sup>-/-</sup> EAE (n=7)	p
Inflammatory ratio*	5.33 ± 4.86	0.24 ± 0.12	<0.01
Demyelinating score	2.0 ± 0.81	0.1 ± 0.48	<0.01

\* Area of the inflammatory lesion / spinal cord area (%)

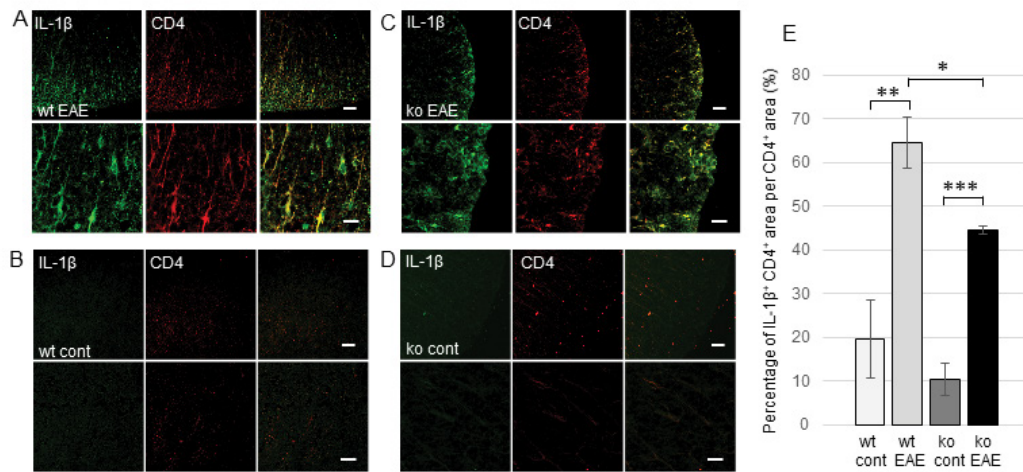
**Table 1:** The pathological analysis during the acute phase of EAE (19 days after immunization).



**Figure 3:** Histopathology of a spinal cord of experimental autoimmune encephalomyelitis mice during the acute phase of the disease (19 days after immunization).

(A) Sections of lumbar spinal cord from wild type (wt) mice. Inflammation is shown with H-E staining (left and middle panels), and demyelination is shown with LFB staining (right panel). Note the massive infiltration of mononuclear cells into the parenchyma (left panel, arrowhead) and the diffuse demyelination (right panel, arrow) in wt mice.

(B) Sections of lumbar spinal cord from microsomal PGE<sub>2</sub> synthase -1 (mPGES) - deficient (mPGES-1<sup>-/-</sup>) EAE mice. mPGES-1<sup>-/-</sup> mice have less severe infiltration, which is mainly localized to the intravascular or leptomeningeal space (left and middle panels, arrowhead), with little demyelination (right panel, arrow). Scale bar: 200  $\mu$ m in the left and right panels of parts A and B and 50  $\mu$ m in the middle panels of parts A and B. (Figure first published in *Neurochemistry International* 62(3): 271-280, 2013, Takeuchi C et al., Microsomal prostaglandin E synthase-1 aggravates inflammation and demyelination in a mouse model of multiple sclerosis. Copyright © 2012 by the authors).



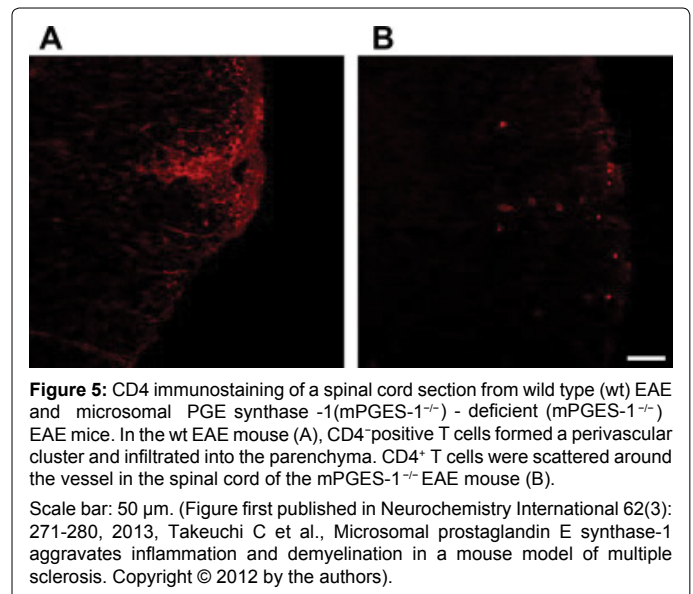
**Figure 4:** Interleukin-1 $\beta$  (IL-1 $\beta$ ) expression in CD4-positive (CD4<sup>+</sup>) T cells in the spinal cords of experimental autoimmune encephalomyelitis (EAE) mice after myelin oligodendrocyte glycoprotein 35–55 peptide immunization and control mice. IL-1 $\beta$  (green) and CD4 (red) in the inflammatory region of the spinal cords of wild-type (wt) EAE (A), wt control (B), ko EAE (C), and ko control (D) mice. Percentage of the IL-1 $\beta$ <sup>+</sup> CD4<sup>+</sup> area in the CD4<sup>+</sup> area of half spinal cords (E). Scale bar (50  $\mu$ m) for upper panels and (20  $\mu$ m) for lower panels of (A–D). wt, wild-type; ko, microsomal PGE synthase-1 (mPGES-1)-deficient (mPGES-1<sup>-/-</sup>); cont, naive mice; EAE, EAE mice. \*  $p < 0.05$ , \*\*  $p < 0.005$ , \*\*\*  $p < 0.0005$ . (Figure first published in Int J Mol Sci 18(12), 2017, Takemiya T et al., Microsomal Prostaglandin E Synthase-1 Facilitates an Intercellular Interaction between CD4<sup>+</sup> T Cells through IL-1 $\beta$  Autocrine Function in Experimental Autoimmune Encephalomyelitis. Copyright © 2017 by the authors).

### T cell Activation by PGE<sub>2</sub> and IL-1 $\beta$ in EAE Models

PGE<sub>2</sub> also regulates T cell activation and differentiation, depending on the cellular environment. PGE<sub>2</sub> enhances the Th17 responses of CD4<sup>+</sup> T cells via EP4 and EP2 promotes Th17 differentiation and function through EP4 and EP2 and works with IL-23 and IL-1 $\beta$  to enhance IL-17A expression through EP4 [60-63].

In wt EAE mice, CD4<sup>+</sup> T cells form perivascular clusters and infiltrate the spinal cord parenchyma, whereas they are scattered around vessels in the spinal cord of mPGES-1<sup>-/-</sup> EAE mice and no T cells are detected in the spinal cords of both control mice (Figure 5). Researchers have postulated that CD4<sup>+</sup> T cells infiltrate the spinal cord of animals with EAE through the disrupted blood-spinal cord barrier, and the infiltration and/or the activation of CD4<sup>+</sup> T cells is regulated by PGE<sub>2</sub>. Because CD4<sup>+</sup> T cells almost completely colocalize with IL-1 $\beta$  in wt EAE mice, whereas IL-1 $\beta$  is expressed at very low levels in the spinal cord of mPGES-1<sup>-/-</sup> EAE mice, this pathway may represent the mechanism by which PGE<sub>2</sub> directly activates CD4<sup>+</sup> T cells to increase IL-1 $\beta$  production in EAE models [12,18] (Figure 4).

An analysis of Th17 and Th1 cells in culture supernatants revealed high IL-17 expression in Th17 cells and high interferon- $\gamma$  (IFN- $\gamma$ ) expression in Th1 cells in EAE models [64]. IL-17 staining is colocalized with CD4<sup>+</sup> T cells in wt EAE mice, whereas morphological changes are restricted and IL-17 staining is weak in mPGES-1<sup>-/-</sup> EAE mice. In contrast, IFN- $\gamma$  is co-expressed in few CD4<sup>+</sup> T cells in either wt or mPGES-1<sup>-/-</sup> EAE mice [18]. Thus, mPGES-1 stimulates the production of IL-17 in CD4<sup>+</sup> T cells. In contrast, IFN- $\gamma$  staining is partially colocalized with CD4<sup>+</sup> T cells in both wt and mPGES-1<sup>-/-</sup> EAE mice, suggesting that PGE<sub>2</sub> does not regulate the production of IFN- $\gamma$ , which means an activity of Th1. The EAE incidence and scores are reduced when EAE mice are mediated with anti-IL-17 antibodies prior to the observed increase in CD4<sup>+</sup> IL-17<sup>+</sup> T cells, suggesting that CD4<sup>+</sup> T cells control tissue inflammation by inducing IL-17 production [65]. Because PGE<sub>2</sub> regulates IL-17 expression in EAE models, we postulate that this mechanism is a potential explanation for the participation of PGE<sub>2</sub> in EAE pathology.



**Figure 5:** CD4 immunostaining of a spinal cord section from wild type (wt) EAE and microsomal PGE synthase -1(mPGES-1<sup>-/-</sup>) - deficient (mPGES-1<sup>-/-</sup>) EAE mice. In the wt EAE mouse (A), CD4<sup>+</sup> positive T cells formed a perivascular cluster and infiltrated into the parenchyma. CD4<sup>+</sup> T cells were scattered around the vessel in the spinal cord of the mPGES-1<sup>-/-</sup> EAE mouse (B).

Scale bar: 50  $\mu$ m. (Figure first published in Neurochemistry International 62(3): 271-280, 2013, Takeuchi C et al., Microsomal prostaglandin E synthase-1 aggravates inflammation and demyelination in a mouse model of multiple sclerosis. Copyright © 2012 by the authors).

### Vasculature Induced by PGE<sub>2</sub> and IL-1 $\beta$ in EAE Models

Inflammation induces the vasodilation of small blood vessels, subsequent more perfusion and resulting in an obvious increase in vessel density, which is known as vasculature. After EAE induction, vasculature is caused by vasodilation and angiogenesis. Angiogenesis is facilitated by vascular endothelial growth factor (VEGF), which leads to the degeneration of the vascular basement membrane and BBB breakdown [66,67]. In MS patients, BBB dysregulation and the trans-endothelial migration of activated leukocytes are the earliest signs of cerebrovascular dysfunction. VEGF is involved in EAE process during the acute phase [66,68,69]. In addition, inhibition of VEGF receptor 2 reduces clinical signs of EAE in the acute phase of the EAE progression. Furthermore, the number of blood vessels increases

during the relapse phase of EAE animals [66,69]. Thus, angiogenesis aggravates inflammation in the spinal cord of EAE animal [70,71]. Vasculature is triggered upon EAE induction, regardless of the presence of mPGES-1. In addition, the PGE<sub>2</sub> produced by mPGES-1 stimulates vasculature in the spinal cord of animal models of EAE, and aggravates inflammation, demyelination, and paralysis. Therefore, vasculature is one mechanism by which PGE<sub>2</sub> aggravates EAE. Furthermore, IL-1 $\beta$  expression is significantly increased in ECs from EAE mice, and the increase in IL-1 $\beta$  levels is mediated by mPGES-1 [12,18,53]. Furthermore, endothelial IL-1 $\beta$  stimulates COX-2 and mPGES-1 expression to produce PGE<sub>2</sub>; therefore, the IL-1 $\beta$ -PGE<sub>2</sub> autocrine signaling pathway in ECs stimulates vasculature. In contrast, the induction of angiogenesis after inflammation promotes neuronal remodeling through the production of prostaglandin I<sub>2</sub> during the EAE chronic phase [70,71].

### IL-17 and Th17 in EAE

IL-17 is a proinflammatory cytokine produced by activated memory T cells, and the IL-17s are secreted proteins of 150–180 amino acids. There are at least six members of the IL-17 family (IL-17 (IL-17A), IL-17B, IL-17C, IL-17D, IL-25 (IL-17E) and IL-17F) in mice and humans [72,73]. The receptor for IL-17 (IL-17R) is a transmembrane protein of approximately 130 kDa. The IL-17 is expressed only by T-cells, whereas its receptor is expressed in all tissues. Moreover, four receptors are identified which share partial sequence homology to IL-17R. EAE development is suppressed in IL-17 knockout mice. The severity of EAE in mice immunized by PLP is reduced when IL-17 is neutralized in vivo, demonstrating the crucial role of IL-17 in EAE induction [74-76]. In addition, T cell-intrinsic apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) is required for the effector stage of EAE, and ASC deficiency in T cells impaired Th17- but not Th1-mediated EAE. Moreover, IL-1 $\beta$  is produced by a Th17 cell-intrinsic ASC-NOD-like receptor 3-Caspase-8 inflammasome during CNS inflammation. There is an autocrine action of Th17-derived IL-1 $\beta$  through IL-1R [50]. We found that PGE<sub>2</sub> facilitates this autocrine function of IL-1 $\beta$  [18]. It is clear that IL-17 produced in Th17 cells has

an important role on EAE. In contrast, IL-17 is also produced in CD8<sup>+</sup> positive T cell,  $\gamma\delta$ T cells, neutrophil or monocyte, therefore in the future we need to investigate the role and the regulatory mechanisms of IL-17 produced by other cells [77].

Next, I discuss other cytokines concerning with EAE and Th17. IL-12p40 knockout mice show no clinical symptoms with no inflammation, whereas IL-12p35 knockout mice show more severe symptoms with severe inflammation. It suggests that p40-containing cytokine distinct from IL-12 is essential for the development of EAE. Moreover, IL-23 is a heterodimeric cytokine composed of a p19 subunit and the p40 subunit of IL-12, and IL-23p19 knockout mice are resistant to EAE. IL-23 also promotes an activation of distinct CD4 T cell producing IL-17. In addition, mice whose T cells cannot respond to signalling of TGF- $\beta$  lack Th17 cells and do not develop EAE. Local administration of antibody to block TGF- $\beta$  prevents the differentiation of Th17 cell and the onset of EAE [78-82]. On the contrary, mice whose T cells overexpress TGF- $\beta$  develop more severe EAE and the T cells produce massive amounts of IL-17. These reports suggest that TGF- $\beta$  regulates the differentiation of Th17 cell. Furthermore, IL-6 knockout mice do not develop a Th17 response. The orphan nuclear receptor ROR $\gamma$ t expression is induced by IL-6 and TGF- $\beta$  and ROR $\gamma$ t induces transcription of the genes encoding IL-17 [83-85]. Moreover, IL-21 potently induces Th17 differentiation. IL-21 knockout mice impair the generation of Th17 cells and protect against EAE. IL-9 knockout mice are resistant to the induction of EAE and exhibit fewer inflammatory infiltrates in the CNS, with lower levels of IL-17 and IFN- $\lambda$  expression. Th17 cells express IL-22, an IL-10 family member, at substantially higher amounts than Th1 or Th2 cells. Similar to IL-17, IL-22 expression is regulated by TGF- $\beta$  in the context of IL-6 and other proinflammatory cytokines [86-88]. In addition, IL-27 suppresses the development of Th17 cells mediated by IL-6 and TGF- $\beta$ , and IL-27 suppresses IL-6-mediated T cell proliferation. IFN- $\beta$  treated animals show a decrease of IL-17 expression and IFN- $\beta$  knockout mice exhibit earlier onset and more rapid progression in EAE. Furthermore, CD4 T cells in Tyk2 knockout mice reduce the IL-17A level in response to MOG35-55 [89-93]. Many cytokines including IL-17 are related

	First Author	Year	EAE mice (transgenic or general)	Antigen type PLP/ MOG/ MBP	Findings
1	Becher B	2002	IL-12p40 (-/-) mice IL-12p35 (-/-) mice	MOG 35-55	IL-12p40 (-/-) mice does not show any clinical symptoms with no inflammation, and IL-12p35 (-/-) mice shows more severe symptoms and severe inflammation.
2	Gran B	2002	IL-12p40 (-/-) mice IL-12p35 (-/-) mice	MOG 35-55	
3	Cua DJ	2003	IL-12p40 (-/-) mice IL-12p35 (-/-) mice IL-23p19 (-/-) mice	MOG 35-55	IL-23 p19 (-/-) and IL-12 p40 (-/-) mice are resistant to EAE. IL-12p35 (-/-) mice is susceptible to EAE.
4	Komiyama Y	2006	IL-17 (-/-) mice	MOG 35-55	EAE development is suppressed in IL-17 (-/-) mice with ameliorated histological changes.
5	Veldhoen M	2006	C57BL/6 mice	MOG 35-55	Mice whose T cells cannot respond to TGF- $\beta$ signaling lack Th17 cells and do not develop EAE.
6	Bettelli E	2006	2D2 x TgTGF- $\beta$ mice	MOG 35-55	Mice whose T cells overexpress TGF- $\beta$ develop more severe EAE and the T cells produce massive amounts of IL-17.
7	Nurieva R	2007	IL-21 (-/-) mice	MOG 35-55	IL-21 potently induces Th17 differentiation. IL-21 (-/-) mice impair the Th17 cells generation and protect against EAE.
8	Korn T	2007	IL-6 (-/-) mice	MOG 35-55	IL-6 (-/-) mice does not develop a Th17 response.
9	Martín-Saavedra FM	2008	SJL mice	MBP	IFN- $\beta$ treated animals show a decrease of IL-17 expression.
10	Korn T	2008	T cell-conditional gp130 (-/-) mice	MOG35-55	T cell-conditional gp130 (-/-) mice, which fail to induce IL-6, show no clinical EAE development with lower levels of IL-17.
11	Tigno-Aranjuez JT	2009	SJL/J mice	PLP 139-151	The severity of EAE in PLP in CFA-immunized mice is reduced when IL-17 is neutralized in vivo.
12	Du C	2009	C57BL/6 mice	MOG 35-55	miR-326 result in fewer Th17 cells and mild EAE, and its overexpression led to more Th17 cells and severe EAE.



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