Current Perspective

Prostaglandin E₂, an Immunoactivator

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Abstract. Diseases caused by immune inflammation, such as rheumatoid arthritis, multiple sclerosis, and Crohn’s disease, are intractable diseases to which novel therapeutics are highly demanded. Prostaglandin (PG) E₂ is the most ubiquitously produced PG with various actions. PGE₂ has been traditionally regarded as an immunosuppressant based on its inhibition of T cell activation in vitro. However, in vivo relevance of the immunosuppressant action of PGE₂ has remained obscure. Recently, several groups including ourselves have made unexpected findings that PGE₂ facilitates expansion of the Th17 subset of T helper cells of both human and mouse through elevation of cAMP via PGE receptors EP2 and EP4. We have further found that PGE₂ can induce and not suppress Th1 differentiation under certain conditions, again, through EP2 and EP4. Given the putative roles of these Th subsets in immune diseases such as the above, these findings suggest that, on the contrary to the traditional view, PGE₂ functions as a mediator of immune inflammation. Consistently, administration of an EP4 antagonist could suppress disease progression and development of antigen-specific Th17 cells in mice subjected to experimental allergic encephalomyelitis and contact hypersensitivity. In this perspective, we review these findings and discuss the prospect of EP4 antagonists as immunomodulatory drugs.

Keywords: prostaglandin (PG) E₂, PGE receptor subtype, helper T cell subset, dendritic cell, immune inflammation

Helper T cell (Th) subsets and immune diseases

Immune diseases are caused by derangement of the immune system. Normally, when foreign antigens such as various pathogens invade into a host, they are taken up by dendritic cells (DCs). DCs digest these antigens, migrate to regional lymph nodes, and present processed antigens to naïve T cells as a MHC-peptide complex. There are two types of naïve T cells, CD4⁺ T cells (helper T cells, Th) and CD8⁺ T cells (cytotoxic T cells, Tc). Dependent on types of cytokines to which they are exposed upon antigen presentation, CD4⁺ T cells differentiate into three distinct subsets of effector T cells, termed Th1, Th2, and Th17 (1). Th1, Th2, and Th17 cells are characterized by the cytokines they produce, interferon (IFN)-γ, interleukin (IL)-4, and IL-17, respectively; and they use these cytokines and facilitate pathogen elimination in a way specific to each subset (Fig. 1). IFN-γ from Th1 cells induces activation of macrophages and promotes elimination of pathogens. IL-4 from Th2 cells instructs B cells to produce IgG1 and IgE type of antibodies that bind to specific pathogens for elimination. IL-17 from Th17 cells promotes tissue inflammation to eliminate certain types of extracellular pathogens (2, 3). Thus, these Th subsets primarily serve for host defense. However, when activation of these Th cells is excessive or aberrantly diverted to self-antigens, they attack host tissues and induce various forms of immune diseases (1) (Fig. 2). The close link between Th2 cells and atopy such as atopic dermatitis and bronchial asthma is well recognized, and involvement of Th17 cells or both Th1 and Th17 cells in immune diseases, such as rheumatoid arthritis, multiple sclerosis, and Crohn’s disease, is indicated by experiments in animal models of these diseases, such as experimental autoimmune encephalomyelitis, collagen-induced arthritis, and experimental colitis, as well as accumulation of these Th cells and expression of their signature cytokines in the lesion of the respective
diseases (1 – 3). It is therefore believed that suppression of excessive generation of these Th subsets might be a means to treat various immune diseases. Currently, a major focus in this area is elucidation of regulatory mechanisms responsible for differentiation and expansion of Th17 cells, because this Th subset is a new-comer, yet is involved in many immune diseases.

**Prostaglandin (PG) E₂, an immunosuppressant: a traditional view**

PG-E₂ is the most ubiquitously produced PG under both physiological and pathophysiological conditions (4, 5) and is long known as an immunosuppressant. This is partly because it inhibits production of inflammatory cytokines such as IL-1β and TNFα by macrophages and also because of its well-documented inhibitory action on Th1 differentiation (6). It was already known in the 1980’s that PGE₂ is produced by APCs, inhibits production of IL-2 and IFN-γ, and suppresses proliferation of murine as well as human T cells in vitro (7, 8). Betz and Fox (9) then examined the effect of PGE₂ on cytokine production from Th1, Th2, and Th0 clones and found that PGE₂ inhibited production of IL-2 and IFN-γ, two Th1 cytokines, while it spared production of the Th2 cytokines, IL-4 and IL-5. This differential action of PGE₂ on Th1 and Th2 cells has been confirmed by many studies (10, 11). Because the best known action of PGE₂ at that time was elevation of intracellular cAMP, and cAMP exerted similar Th1-selective suppression (12, 13), most, if not all, studies assigned PGE₂ as a modulator of T cells raising the intracellular cAMP level. Among the four subtypes of PGE receptor, EP2 and EP4 are coupled to a rise in cAMP (14). Nataraj et al. (15) used T cells obtained from mice deficient in each EP subtype individually and found that immunosuppressive action of PGE₂ was significantly attenuated in T cells obtained either from EP2⁻/⁻ or EP4⁻/⁻ mice, suggesting that both EP2 and EP4 mediate suppression of PGE₂ on T cells. Thus there is ample literature suggesting the Th1-suppressive action of PGE₂. Curiously, however, these T cell suppressive effects of PGE₂ have been shown mostly by in vitro studies and are rarely seen in vivo, leaving the action of PGE₂ in vivo in the immune system enigmatic.

**PGE₂ action in Th1 differentiation revisited**

As mentioned above, it has been known that cAMP signaling induces suppression of T cell proliferation (16). However, the mechanism of this action remained obscure. T cell activation is primarily induced by stimulation of T cell receptor (TCR) with the respective antigen, and one of the mechanisms downstream of TCR stimulation is activation of a Src-family kinase, Lck. It was shown recently that cAMP in T cells activates PKA, which then phosphorylates and activates C-terminal Src kinase (CSK) that phosphorylates the C-terminal tyrosine of Lck and inactivates it. cAMP and TCR thus compete with each other in Lck activation (17). These results have not only identified the suppression mechanism of T cell proliferation by cAMP, but also indicate a possibility that strong TCR stimulation may overcome the suppressive action of cAMP (17). Indeed, experimentally, increased TCR stimulation with higher amount of anti-CD3 and anti-CD28 antibodies could overcome the cAMP-mediated suppression of T cells (18). We exploited these findings and re-examined the effects of PGE₂ on differentiation of mouse naïve T cells to the Th₁ subset under strengthened TCR stimulation, and we found that under these conditions, PGE₂ facilitated IL-12-subset strengthened TCR stimulation, and we found that under these conditions, PGE₂ facilitated Th1 differentiation significantly at nM concentrations without affecting T cell proliferation (19). Furthermore, we found that this PGE₂ effect was mimicked by EP2 and an EP4 agonists, and the action of these agonists was not seen in T cells from mice lacking the respective receptor. Interestingly, the inhibitor study suggested that this effect of PGE₂ via EP2 and EP4 depended on the PI3K pathway and not the cAMP pathway. Our results were thus opposite to the previous findings and suggest a possibility that PGE₂ can function as an immunoactivator to facilitate Th1 differentiation via EP2 and EP4 under some conditions (19).

**PGE₂ action on Th17 cell expansion revealed**

The Th17 subset is currently thought to be a principal Th subset involved in immune inflammation (2, 3). Th17 cells are differentiated from naïve T cells by IL-6 and TGF-β and become stabilized and expand in the presence of IL-23 (3). IL-6 and TGF-β are provided, for example, by DCs that have recognized apoptotic cells in tissue injury, and IL-23 is also provided by DCs after certain activation. We examined effects of PGE₂ on mouse Th17 development in vitro (19) and first found that PGE₂ potently suppresses Th17 differentiation from naïve T cells by IL-6 and TGF-β, which was consistent with the report by Chen et al. (20). We then examined PGE₂ effects on Th17 expansion and found that PGE₂ facilitated Th17 expansion in the presence of IL-23. This effect of PGE₂ was again mimicked by EP2 and EP4 agonists. However, contrary to its effect on Th1 differentiation, this PGE₂ effect is elicited through the cAMP pathway and not through the PI3K pathway. We further examined the effect of PGE₂ on IL-23 production by DCs and found that treatment with indomethacin almost completely suppressed IL-23 production by DCs activated with anti-CD40
Regulation of Immune System by PGE$_2$

Fig. 1. Roles of Th cells in normal immune response. Differentiation of naïve T cells into each Th subset is regulated by specific cytokines in association with antigen stimulation. IL-12, IL-4, or IL-6 and transforming growth factor (TGF)-β are key determinants for differentiation into Th1, Th2, and Th17 cells, respectively. Th17 cells are then stabilized and expand in the presence of IL-23. Th1 cells produce IFN-γ, which activate macrophages and promotes elimination of pathogens. Th17 cells also promote elimination of certain extracellular pathogens by inducing the expression of inflammatory cytokines and chemokines, resulting in recruitment of inflammatory cells such as neutrophils. On the other hand, Th2 cells produce IL-4, and activate B cells to facilitate production of antibodies.

Fig. 2. Roles of Th cells and their differentiation in the disease condition. In diseases such as autoimmune disease or allergy, Th cells are excessively or abnormally activated and/or react with self antigens, resulting in tissue inflammation and destruction.

Fig. 3. Regulation of Th1 differentiation and Th17 expansion by PGE$_2$. PGE$_2$ acts on DCs and T cells to mobilize distinct intracellular signaling pathways for Th1 differentiation and Th17 expansion. PGE$_2$ is produced by DCs and regulates IL-23 production from activated DCs through the EP4-cAMP-Epac pathway. IL-23 then acts on Th17 cells to stabilize and expand these cells. PGE$_2$ promotes the IL-23–induced Th17 cell expansion through the EP2/EP4–cAMP–PKA pathway. PGE$_2$, on the other hand, also uses EP2 and EP4 to promote IL-12–driven Th1 cell differentiation from naïve T cells using PI3K signals.
antibody, which was restored by the addition of PGE₂ or an EP4 agonist, whereas an EP2 agonist showed variable effects. In addition, an EP4-specific antagonist, ONO-AE3-208, also completely suppressed IL-23 production. Consistent with our findings, Ganea and collaborators (21, 22) found that exogenously added PGE₂ enhanced lipopolysaccharide-induced IL-23 production by bone marrow-derived DCs and that DCs differentiated with GM-CSF in the presence of exogenously added PGE₂ acquired a Th17-inducing phenotype, producing higher amounts of IL-1β, IL-6, TNF-α, IL-10, and IL-23.

Thus, PGE₂ can facilitate both Th1 differentiation and Th17 expansion of mouse T cells in vitro through the same receptors EP2 and EP4 (Fig. 3).

**PGE₂ in human Th17 differentiation and expansion**

The above studies have thus revealed novel actions of PGE₂-EP2/EP4 signaling on expansion of mouse Th17 cells, which are exerted both on primed T cells and activated DCs. The next question is whether the same mechanism operates in human cells. Indeed, concomitant with our report on mouse cells, several groups have used human peripheral blood mononuclear cells and reported the actions of PGE₂ on human Th17 differentiation. Boniface et al. (23) reported that PGE₂ in the combination with IL-1β and IL-23 promote production of IL-17 from differentiating Th17 cells by up-regulating the IL-1βR and IL-23R expression through the EP2/EP4-cAMP pathway. In addition, they also found that the combination of PGE₂ with IL-1β and IL-23 induces CCR6 expression, a chemokine receptor preferentially expressed on Th17 cells. Chizzolini et al. (24) reported that PGE₂ synergizes with IL-23 and increases the number of Th17 cells from human CD4⁺CD45RO⁺ (memory) T cells but not from CD4⁺CD45RO⁻ (naïve) T cells, consistent with our finding in mouse T cells that PGE₂ cannot enhance Th17 differentiation but facilitates the action of IL-23 on Th17 expansion. Again, IL-1β is required for this PGE₂ action. They also observed that PGE₂ itself facilitates the CCR6 expression on CD4⁺ T cells. Napolitani et al. (25) used human peripheral memory T cells and obtained results similar to Boniface et al. and Chizzolini et al.

**Role of PGE₂–EP4 signaling in vivo in immune inflammation**

Thus, evidence now accumulates that PGE₂ facilitates Th17 expansion, and, in some instances, Th1 differentiation. Given the involvement of these Th subsets, particularly Th17, in immune inflammation (3), this suggests that PGE₂ acting on EP2 and EP4 amplifies immune pathology. To test this hypothesis, we subjected wild-type and EP2-deficient mice to two models of immune inflammation, 2,4-dinitro-1-fluorobenzene–induced contact hypersensitivity (CHS) and experimental allergic encephalomyelitis (EAE), and assessed effects of an EP4 antagonist on disease progression and development of Th1 and Th17 subsets (19). We found that treatment with the EP4 antagonist suppressed disease severity and decreased accumulation of antigen-specific Th1 and Th17 cells in regional lymph nodes in both models. Deficiency of EP2 alone did not affect significantly the disease progression, and no augmented suppression was found with administration of the EP4 antagonist to EP2-deficient animals in the CHS model. These findings indicate that the PGE₂–EP4 signaling indeed positively regulates development of Th1 and Th17 subsets to specific antigens and determine the extent of immune inflammation. The fact that the EP2-deficiency alone was unable to lessen the disease and did not exhibit additive suppression in combination the EP4 antagonism in CHS indicates that the EP4 signaling is a dominant, if not the sole, mechanism operating in the body. Converse to the findings by us, Sheibanie et al. (26, 27) administered various PGE analogs to animals subjected to either TNBS-induced colitis or collagen-induced arthritis and found that administration of misoprostol, an EP3/EP4 agonist, aggravated the disease in both models and induced higher expressions of IL-23 and IL-17.

**Conclusion: potential of EP4 antagonist as an immunomodulatory drug**

In this perspective, we have reviewed recent progress in research on the role of PGE₂ in immune inflammation. On the contrary to the traditional belief, PGE₂ has now emerged as an immuno-activator that acts on the EP4 receptor and facilitates Th1 differentiation and Th17 expansion, two Th subsets involved in immune inflammation. Given a variety of evidence for involvement of these Th subsets in various immune diseases of humans, an EP4 antagonist can be a good therapeutic drug target for diseases such as rheumatoid arthritis, multiple sclerosis, psoriasis, and Crohn’s disease. One finding supporting this assumption is the genomic analysis identifying EP4 as a causative gene of Crohn’s disease (28). On the other hand, Kabashima et al. previously reported that the PGE₂–EP4 signaling protects mice from dextran sodium sulfate–induced mouse colitis (29), and a recent clinical trial of an EP4 agonist, ONO-4819CD, in ulcerative colitis patients showed some beneficial effects (30). We assume that the discrepancy may come from the difference in pathophysiology between ulcerative colitis and Crohn’s disease. While the two diseases are categorized
together as inflammatory bowel diseases, ulcerative colitis could be a disease of impaired barrier function of intestinal epithelium, which the PGE2–EP4 signaling improves as shown in the DSS model, while Crohn’s disease is primarily caused by a disorder of the immune system. Thus, analysis of the role of the PGE2 signaling may provide deeper insight into the pathological mechanisms underlying each disease, which should be fully taken into account in developing an EP4 antagonist as a therapeutic agent and its clinical application.

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