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Molecular mechanisms underlying prostaglandin E_2-exacerbated inflammation and immune diseases

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Abstract

Prostaglandins (PGs) are the major lipid mediators in animals and which are biosynthesized from arachidonic acid by the cyclooxygenases (COX-1 or COX-2) as the rate-limiting enzymes. Prostaglandin E_2 (PGE_2), which is the most abundantly detected PG in various tissues, exerts versatile physiological and pathological actions via four receptor subtypes (EP1–4). Non-steroidal anti-inflammatory drugs, such as aspirin and indomethacin, exert potent anti-inflammatory actions by the inhibition of COX activity and the resulting suppression of PG production. Therefore, PGE_2 has been shown to exacerbate several inflammatory responses and immune diseases. Recently, studies using mice deficient in each PG receptor subtype have clarified the detailed mechanisms underlying PGE_2-associated inflammation and autoimmune diseases involving each EP receptor. Here, we review the recent advances in our understanding of the roles of PGE_2 receptors in the progression of acute and chronic inflammation and autoimmune diseases. PGE_2 induces acute inflammation through mast cell activation via the EP3 receptor. PGE_2 also induces chronic inflammation and various autoimmune diseases through T helper 1 (T_h1)-cell differentiation, T_h17-cell proliferation and IL-22 production from T_h22 cells via the EP2 and EP4 receptors. The possibility of EP receptor-targeted drug development for the treatment of immune diseases is also discussed.

Keywords: IL-22, mast cells, T_h1 cells, T_h17 cells, T_h22 cells

Introduction

Prostaglandin E_2 (PGE_2) is a major physiologically active lipid, which is biosynthesized from arachidonic acid (AA) by the cyclooxygenases (COX-1 and COX-2) and PGE synthases; AA is released from membrane phospholipids by phospholipase A_2 (PLA_2), and thus PGE_2 is derived from membrane phospholipids (Fig. 1) (1–4). PGE_2, which is the most abundant PG detected in various tissues, exerts versatile physiological and pathological actions via four subtypes of PGE receptors, termed E-type prostanoid receptor 1–4 (EP1–4), expressed in the cell surface membrane. Each EP subtype is coupled to a distinct signal transduction pathway: EP1 induces intracellular Ca^{2+} mobilization via the Gq protein, EP2 and EP4 increase cyclic adenosine monophosphate (cAMP) production via Gs and EP3 inhibits adenylyl cyclase (thus decreasing cAMP) via Gi and elicits Ca^{2+} mobilization and phosphoinositide 3-kinase (PI3K) activation in some cell types (Fig. 1) (1–6). Moreover, EP2 and EP4 have been shown to activate PI3K through the β-arrestin pathway (7–9).

PGE_2 produced in the cells is released into the extracellular space, and rapidly converted to 15-keto PGE_2, the inactive metabolite of PGE_2 by 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in the intercellular space or blood (10). Therefore, PGE_2 does not readily circulate throughout the body, and its actions are limited to cells in the vicinity of the PGE_2-producing cells. Non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin and indomethacin, exert potent anti-inflammatory actions by the inhibition of COX activity, and thereby the suppression of PG production (Fig. 1) (11–15).

In the late 1990s, mice deficient in each of the EP receptors were generated, and selective agonists and antagonists for each EP receptor were developed (1, 5). These mice and reagents have been powerful tools that enabled us to uncover the molecular mechanisms underlying the physiological and

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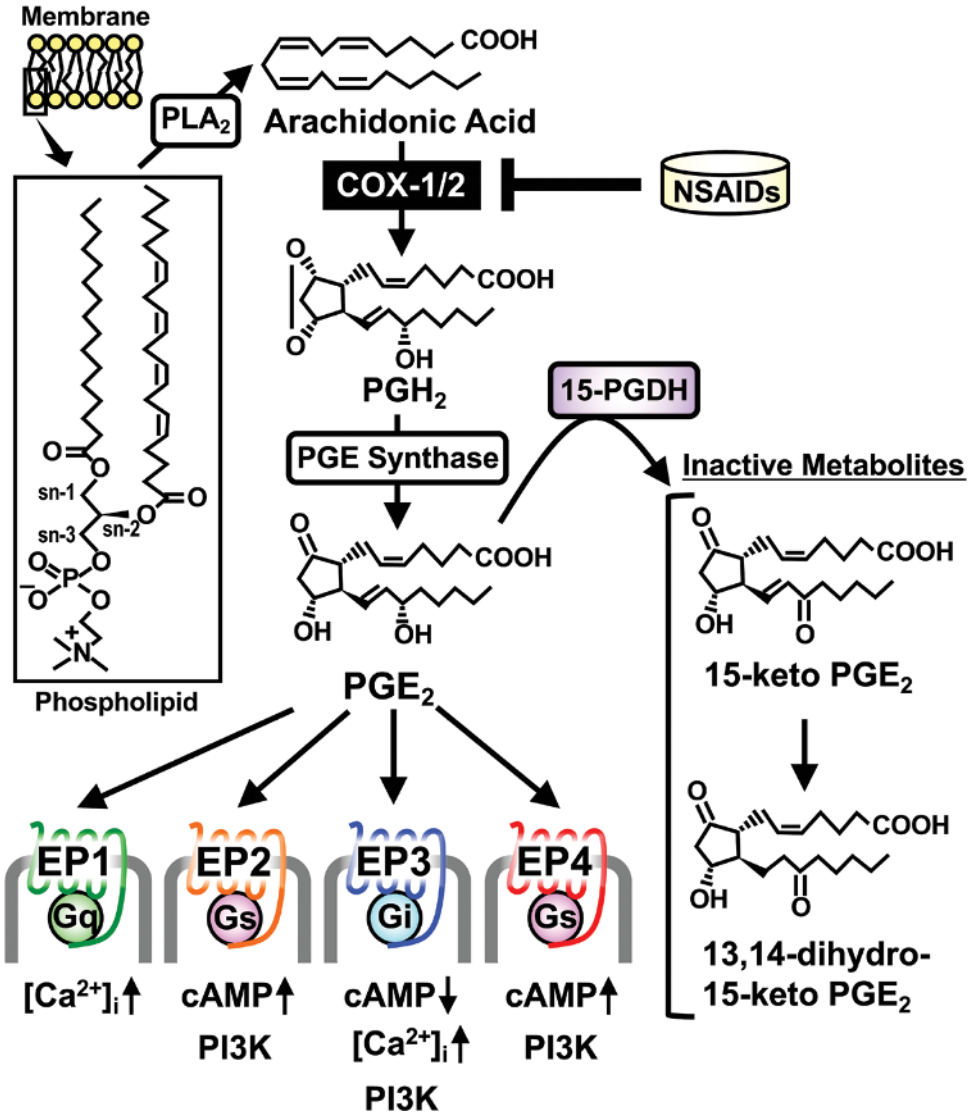

Fig. 1. Biosynthesis and inactivation of PGE_2 and EP receptor-elicited signal transduction pathways. PGE_2 is synthesized by COX-1 or COX-2 and PGE synthase. Arachidonic acid, a COX substrate, is released by PLA_2 from membrane phospholipids. PGE_2 exerts versatile actions via four receptor subtypes (EP1–4). NSAIDs exert anti-inflammatory actions by the inhibition of COX activity, and thereby the suppression of PGE_2 biosynthesis. PGE_2 released outside of the cell is rapidly converted into an inactive metabolite by 15-PGDH in the extracellular space.

pathological actions of PGE_2. In this review, we summarize the recent advances in PGE_2-EP receptor-mediated acute and chronic inflammation, as well as autoimmune diseases.

Dual pathways of PGE_2-EP signaling in acute inflammation

Acute inflammation is characterized by four major symptoms, namely, *rubor* (red flare), *calor* (heat), *tumor* (swelling) and *dolor* (pain), and is caused by tissue damage or the invasion of pathogens. The red flare and heat reactions are triggered by an increase in blood flow caused by vasodilatation, and the swelling reaction is induced by increased vascular permeability and the recruitment of leukocytes (16). PGE_2 is abundantly produced in the region of inflammation. Furthermore, NSAIDs block these major inflammatory symptoms. These results suggest that PGE_2 is involved in the induction of acute inflammation (14, 15).

In a previous report, the EP subtypes involved in PGE_2-mediated acute inflammatory responses were identified using an ultraviolet B (UVB)-irradiated skin inflammation mouse model (17). UVB irradiation to the mouse ear induced the recruitment of leukocytes, and PGE_2 was abundantly produced in the irradiated ear tissue, leading to edema. The UVB-induced recruitment of leukocytes and edema were partially suppressed in EP2-deficient mice and by treatment with an EP4 antagonist. Interestingly, treatment with an EP4 antagonist in EP2-deficient mice further suppressed UVB-induced edema formation. EP2 and EP4 have been shown to be expressed in vascular smooth muscle cells (18, 19), and their activation promotes vasodilation via cAMP signaling. It is thus likely that PGE_2 induces red flare, heat and swelling

reactions by relaxing vascular smooth muscle via the EP2/EP4 receptors, resulting in an increase in blood flow (Fig. 2; Table 1) (14, 15, 17).

Recently, Morimoto *et al.* further uncovered the mechanisms underlying PGE_2-induced acute inflammatory responses using an AA-induced dermatitis model (20). In this model, PGE_2 was abundantly synthesized within ear tissue after topical AA application (21). They monitored AA-induced inflammatory responses in mice deficient in each EP receptor and found that only EP3-deficient mice demonstrated a significantly reduction of inflammatory responses, such as vascular hyperpermeability and the recruitment of neutrophils. Conversely, treatment of mice with PGE_2 and an EP3-selective agonist induced ear swelling. A previous report showed that AA-induced inflammation was abolished in COX-1-deficient mice (22). These results suggest that COX-1-derived PGE_2 exacerbates acute inflammation via EP3 in an AA-induced dermatitis model.

Interestingly, PGE_2-induced vascular hyperpermeability was canceled by treatment of mice with a histamine H1 receptor antagonist, as well as in mast cell-depleted mice, indicating that mast cells are the key player for this inflammatory response. Moreover, reconstitution with wild-type (WT) bone marrow-derived mast cells (BMMCs) in the ears of mast cell-depleted mice recovered the PGE_2 response, but reconstitution with EP3-deficient BMMCs did not, indicating that the EP3 receptor in mast cells is crucial for this event.

Moreover, in the *in vitro* BMMC culture system, PGE_2-EP3 signaling induced antigen-independent degranulation and interleukin-6 (IL-6) production, and both of these events were completely blocked by the depletion of extracellular Ca^{2+}, indicating that PGE_2-EP3 signaling induces mast cell activation via Ca^{2+} entry from the extracellular space. Pharmacological experiments elucidated that PGE_2-EP3 signaling-induced Ca^{2+} influx in mast cells is mediated by Gi, phospholipase C (PLC), and the stromal interaction molecule (STIM)-Orai channel pathway. Mast cell degranulation is mainly divided into two processes: translocation of granules into the plasma membrane and membrane fusion. The former event is dependent on PI3K signaling, and the latter event is dependent on intracellular Ca^{2+} levels (23–25). PGE_2-EP3 signaling also activated PI3K signaling, and inhibition of PI3K significantly repressed PGE_2-induced degranulation and IL-6 production in mast cells. These results suggest that PGE_2-EP3 signaling induces mast cell degranulation and IL-6 release by Gi-dependent Ca^{2+} mobilization and PI3K activation (Fig. 2; Table 1). Histamine released by mast cells promotes vascular permeability via H1 receptor signaling (16). On the other

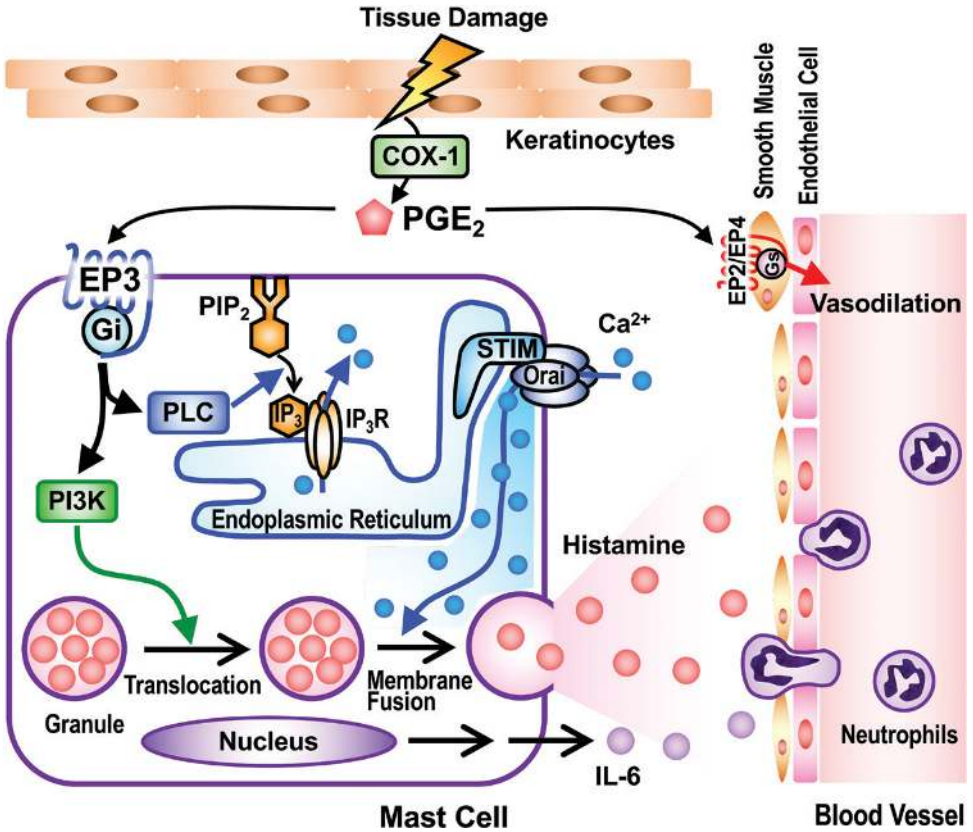

Fig. 2. Dual pathways of PGE_2-EP signaling-induced acute inflammation. Tissue damage induces COX-1-mediated PGE_2 production in the skin. PGE_2 induces vasodilatation and increases local blood flow via the EP2/EP4 receptors, leading to red flare and heat. PGE_2 also activates mast cells via the EP3 receptor. PGE_2-EP3 signaling induces Gi-dependent PLC activation, and then facilitates intracellular Ca^{2+} mobilization via STIM-Orai channels. The EP3 pathway also induces PI3K activation in a Gi-dependent manner. These two signaling pathways cooperatively induce histamine release (degranulation) and IL-6 release. Histamine stimulates vascular permeability, and IL-6 promotes the recruitment of neutrophils. IP_3, inositol 1,4,5-trisphosphate; IP_3R, IP_3 receptor; PIP_2, phosphatidylinositol 4,5-bisphosphate.

Table 1. Summary of EP receptors involved in acute and chronic inflammation

Disease and associated process	Involved or associated receptor	Reference
In vitro model		
Mast-cell degranulation, IL-6 production	EP3	(20)
T_h1-cell differentiation from naive T cells	EP2, EP4	(34, 35)
IL-23-induced T_h17-cell expansion	EP2, EP4	(34, 41)
IL-23 production from dendritic cells	EP4[a]	(34, 45)
IL-22 production from T_h22 cells	EP2, EP4	(65)
In vivo disease mouse model		
UVB-irradiated skin inflammation	EP2, EP4	(17)
AA-induced dermatitis	EP3 (mast cell[b])	(20)
DNFB-induced CHS, EAE	EP4[a]	(34)
DNFB-induced CHS, adoptive transfer colitis	EP4[a] (T cell)	(35)
IL-23-induced psoriatic skin inflammation	EP2, EP4 (T cell)	(41)
Oxazolone-induced allergic skin inflammation	EP4[c] (T cell)	(65)
Human disease		
Psoriasis	EP4[d]	(41)
AD	EP4[e]	(65)

[a]Little or no involvement of EP2.
[b]Evaluation of PGE_2-induced edema and hyperpermeability.
[c]Involvement of EP2 not evaluated.
[d]Association of EP2 gene expression not described.
[e]EP2 gene expression showed negative association with disease severity.

hand, IL-6 promotes the production of IL-8, which is a neutrophil-attracting chemokine (26).

The above information hence suggests that PGE_2 exacerbates acute inflammation via two different mechanisms. One is that PGE_2 relaxes vascular smooth muscle, resulting in vasodilatation via EP2 and EP4 signaling in smooth muscle cells, and consequently promotes red flare and heat reactions. The other is that PGE_2 induces vascular hyperpermeability and the recruitment of neutrophils via the EP3 receptor in mast cells, resulting in swelling (Fig. 2; Table 1) (17, 20).

PGE_2-EP2/EP4 signaling in T_h1 differentiation *in vitro*

Naive $CD4^+$ T cells are activated by antigen-induced T-cell receptor (TCR) signaling and co-stimulatory signaling via cluster of differentiation (CD)28, and then differentiate into the effector cells, such as T helper 1 (T_h1), T_h2 and T_h17 cells according to their local environment, resulting in diverse immunological responses. T_h1-cell differentiation is induced by IL-12 and interferon-γ (IFN-γ), and T_h2-cell differentiation is induced by IL-4. T_h17-cell differentiation is induced by IL-6 and transforming growth factor-β (TGF-β), and T_h17-cell expansion is stimulated by IL-23 (27–29). PGE_2 was previously reported to inhibit TCR signaling via the cAMP-protein kinase A (PKA) pathway, and to repress T_h1-cell differentiation (30–32). As Gs-deficient T cells were reported to be unable to differentiate into T_h1 cells, the significance of cAMP signaling in T_h1-cell differentiation has been controversial (33).

Yao *et al.* analyzed whether PGE_2 promotes T_h1-, T_h2- or T_h17-cell differentiation from naive $CD4^+$ T cells under priming conditions for each effector T cell (34). As a result, PGE_2 was found to strongly accelerate T_h1-cell differentiation only under conditions of both TCR and CD28 stimulation, mimicking pathogen infection, but not TCR stimulation alone. Moreover, EP2 and EP4 agonists also promoted T_h1-cell differentiation, whereas EP1 and EP3 agonists did not. Further experiments showed that although cAMP-PKA signaling inhibits TCR signaling when only TCR is activated, additional activation of CD28 cancels the inhibition of TCR signaling by PKA via PI3K activation. It is likely that PGE_2-EP2/EP4 signaling activates PI3K coordinately with CD28 signaling, leading to the up-regulation of master regulators of T_h1-cell differentiation, such as IL-2 (*Il2*), IL-2 receptor α-chain (*Il2ra*), IFN-γ (*Ifng*) and T-box transcription factor expressed in T cells (T-bet, *Tbx21*) (Fig. 3; Table 1) (35).

PGE_2 enhanced the expression of the IL-12 receptor β2 chain (*Il12rb2*) in TCR-activated naive T cells, and the effect of PGE_2 was repressed by cotreatment with a PKA inhibitor, but not a PI3K inhibitor. Moreover, PKA activator-induced gene expression of *Il12rb2* and the IFN-γ receptor α-chain (*Ifngr1*) was robustly suppressed by knockdown of the transcription factor cAMP responsive element-binding protein (CREB). PKA has been shown to promote the nuclear localization of CREB by its phosphorylation (36). It is also known that CREB-dependent transcriptional activation is promoted by a family of cAMP-regulated transcriptional coactivators (CRTCs) (37). The nuclear translocation of CRTCs is negatively regulated by its phosphorylation by salt-inducible kinase (SIK) (38, 39), and PKA is also known to suppress the kinase activity of SIK2 by its phosphorylation (40). Thus, PKA indirectly activates the nuclear translocation of CRTCs through SIK2 phosphorylation. Indeed, a cAMP analogue was shown to promote the nuclear translocation of CRTC2, which is a member of the CRTCs, in WT but not SIK2-deficient T cells. Furthermore, the expression of *Il12rb2* and *Ifngr1* induced by a cAMP analogue was suppressed by the knockdown of CRTC2. Thus, PGE_2-EP2/EP4 signaling facilitates T_h1-cell differentiation via CREB-mediated and CRTC-mediated amplification of IL-12 signaling, as well as IFN-γ signaling by cAMP and PI3K pathways (Fig. 3; Table 1) (35, 36).

PGE_2-EP2/EP4 signaling in T_h17-cell expansion *in vitro*

Yao *et al.* also investigated the roles of PGE_2 in the function of T_h17. Although PGE_2 did not promote T_h17-cell differentiation

from naive T cells in any TCR-stimulating conditions, PGE_2 stimulated T_h17-cell expansion in the presence of IL-23 (34). Moreover, an EP2 agonist, an EP4 agonist, a cAMP analogue and a PKA activator also mimic the effects of PGE_2, suggesting that PGE_2-EP2/EP4 signaling facilitates IL-23-induced T_h17-cell expansion via the cAMP-PKA pathway.

Recently, Lee *et al.* investigated the mechanism therein (41). They showed that PGE_2-induced T_h17-cell expansion is mediated by the up-regulation of CREB-dependent IL-23 receptor (*Il23r*). Furthermore, IL-23 enhanced the expression of COX-2 (*Ptgs2*), as well as PGE_2 production. IL-23-induced up-regulation of *Il23r* was suppressed by cotreatment with indomethacin, suggesting that IL-23 triggers endogenous PGE_2 signaling. Several genomic studies showed that Janus kinase 2 and signal transducer and activator of transcription 3 (STAT3) are involved in IL-23 signaling (42–44). Indeed, IL-23 promoted the phosphorylation of STAT3, and IL-23-induced up-regulation of *Il23r* was canceled by cotreatment with a STAT3 inhibitor. Interestingly, IL-23-induced phosphorylation of STAT3 was inhibited by cotreatment with a PKA inhibitor. These results suggest that PGE_2 secreted by IL-23-stimulated T_h17 cells synergistically promotes *Il23r* expression via CREB and STAT3 pathways activated by PKA, resulting in the amplification of IL-23 signaling (Fig. 4; Table 1) (34, 41).

Dendritic cells have been shown to produce IL-23 upon their activation. Interestingly, PGE_2 was also shown to induce IL-23 production from dendritic cells (34, 45). CD40 induced the production of IL-23 from dendritic cells, and this effect was up-regulated by cotreatment with PGE_2, an EP4 agonist and a cAMP analogue but not an EP2 agonist. Although EP2 (*Ptger2*) and EP4 (*Ptger4*) mRNAs were detected at similar levels in dendritic cells, an EP2 agonist hardly increased intracellular cAMP levels, whereas an EP4 agonist did so considerably. Therefore, the EP2 receptor is unlikely to be functionally coupled to cAMP production in dendritic cells, although the mechanism remains unclear. Moreover, IL-23 production from CD40-activated dendritic cells was prominently blocked by cotreatment with indomethacin or an EP4 antagonist. These results suggest that CD40 signaling induces endogenous PGE_2 production, and secreted PGE_2 promotes IL-23 production in dendritic cells via the EP4-cAMP pathway. Thus, these results suggest that PGE_2-EP4 signaling promotes T_h17-cell expansion not only by inducing IL-23 production from dendritic cells, but also by up-regulating *Il23r* expression in T_h17 cells (Fig. 4; Table 1) (34, 45).

PGE_2-EP2/EP4 signaling in T_h1-/T_h17-associated autoimmune diseases

Among the three effector T_h cells, both T_h1 and T_h17 cells mediate inflammation and autoimmune diseases (46–50). Thus, the next question was whether PGE_2 drives T_h1-cell differentiation and T_h17-cell expansion *in vivo* as well as *in vitro*, and moreover, whether such functions of PGE_2 would contribute to the progression of these diseases.

Yao *et al.* aimed to answer these questions using the following two autoimmune disease models, in which both T_h1 and T_h17 cells are deeply associated with the observed pathogenesis: a dinitrofluorobenzene- (DNFB-) induced contact hypersensitivity (CHS) model and an experimental autoimmune encephalomyelitis (EAE) model that is induced by a peptide fragment of the myelin oligodendrocyte glycoprotein and is a model of multiple sclerosis (34, 51, 52). In both models, an EP4 antagonist greatly attenuated disease severity and repressed the production of IFN-γ and IL-17 in regional lymph nodes in a dose-dependent manner. On

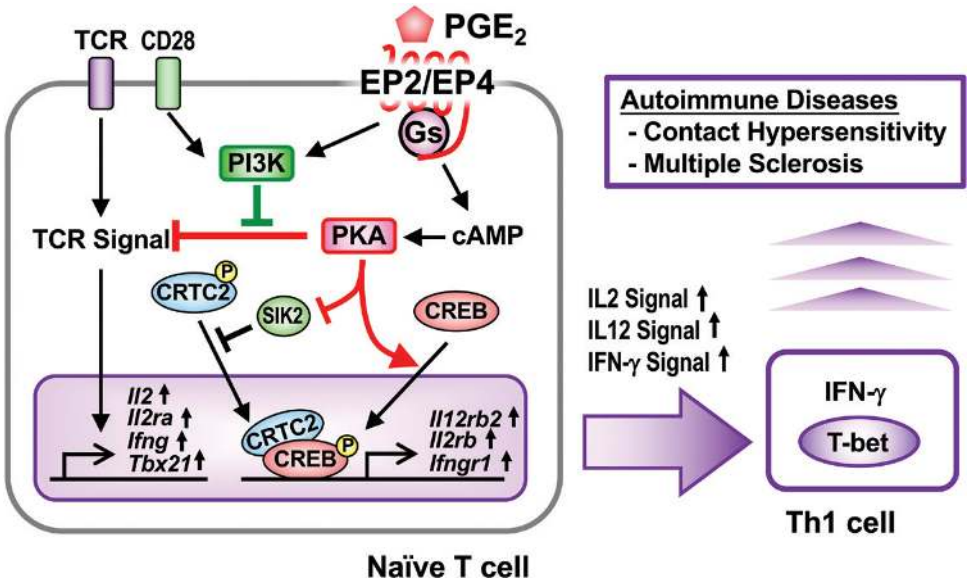

Fig. 3. PGE_2-EP2/EP4 signaling-induced T_h1 differentiation. PGE_2-EP2/EP4 signaling accelerates T_h1 differentiation under both TCR-stimulated and CD28-stimulated conditions. PKA signaling on its own inhibits TCR signaling, but simultaneous PI3K activation by CD28 and EP2/EP4 cancels the inhibitory effects of PKA on TCR signaling, leading to increased gene expression of *Il2*, *Il2ra*, *Ifng* and *Tbx21*. PGE_2-EP2/EP4 signaling promotes the nuclear localization of CREB and CRTC2 by the inhibition of SIK2-derived phosphorylation of CRTC2 via the cAMP-PKA pathway. CREB and CRTC2 cooperatively stimulate the gene expression of *Il12rb2* and *Ifngr1*. PGE_2-induced T_h1 differentiation is involved in the progression of autoimmune diseases, such as CHS and multiple sclerosis.

the other hand, EP2-deficient mice did not demonstrate any changes in the onset and progression of both models. These results suggest that PGE_2 enhances T_h1-cell differentiation and T_h17-cell expansion via the EP4 receptor in *in vivo* models, leading to the exacerbation of T_h1-/T_h17-associated autoimmune diseases. In contrast, endogenous PGE_2-EP2 signaling does not appear to contribute to the pathogenesis of EAE and CHS models, although the mechanism is unclear (Table 1) (34).

Yao *et al.* also analyzed the significance of PGE_2-EP4 signaling in T cells for autoimmune diseases using T-cell-specific EP4-deficient mice (35, 53, 54). In the CHS model, EP4 depletion in T cells reduced the population of T_h1 cells in lymph nodes as well as ear swelling. Moreover, the role of PGE_2-EP4 signaling in T cells was confirmed by an adoptive transfer colitis experiment, which creates a model of inflammatory bowel disease, particularly Crohn's disease (55, 56). Transfer of naive $CD4^+$ T cells isolated from WT mice into T-/B-cell-deficient mice (recombination-activating gene 2-deficient mice) induced severe inflammatory colitis, whereas the transfer of naive $CD4^+$ T cells isolated from $EP4^{+/-}$ mice demonstrated milder body weight loss and weaker colonic inflammation than those of WT T cells. In contrast, $EP2^{-/-}$ T cells did not show the attenuation of colitis development observed in WT T cells. These results indicate that the exacerbating actions of PGE_2 on T-cell-mediated autoimmune diseases is mediated by the EP4 receptor, but not by the EP2 receptor, as shown using CHS and inflammatory colitis models (Table 1) (35).

Recently, Lee *et al.* analyzed the contribution of PGE_2-EP2/EP4 signaling in IL-23-induced skin inflammation, which is a model of T_h17-regulated psoriasis (41). IL-23 administration into the skin up-regulated the expression of PGE_2 biosynthesis-associated enzymes, including COX-2 (*Ptgs2*), microsomal prostaglandin E synthase-1 (mPGES-1, *Ptges1*) and mPGES-2 (*Ptges2*). IL-23-induced ear swelling was reduced by 50% in EP2-deficient mice or WT mice treated with an EP4 antagonist. Furthermore, when EP2-deficient mice were treated with an EP4 antagonist, IL-23-induced skin inflammation was completely blocked. They also showed that this skin inflammation was attenuated in T-cell-specific EP2- or EP4-deficient mice, and was also completely inhibited in both EP2-deleted and EP4-deleted conditions. These results suggest that PGE_2-EP2/EP4 signaling in T cells cooperatively exacerbates the pathogenesis of T_h17-associated skin inflammation (Table 1) (41).

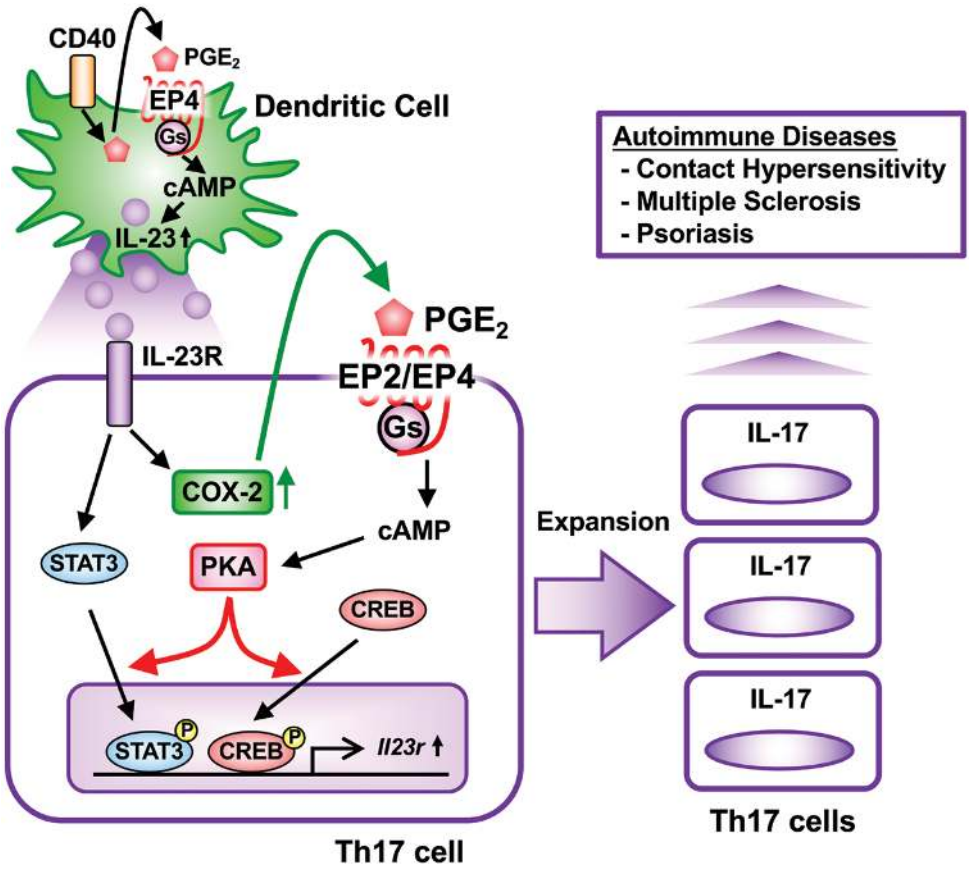

Fig. 4. PGE_2-EP2/EP4 signaling-induced T_h17 expansion. CD40 signaling induces endogenous PGE_2 production in dendritic cells, and PGE_2 promotes IL-23 production from dendritic cells via the EP4-cAMP pathway in an autocrine manner. IL-23, secreted from dendritic cells, promotes COX-2-derived PGE_2 production in T_h17 cells via IL-23R signaling. PGE_2-EP2/EP4 signaling facilitates the nuclear localization of CREB and IL-23-activated STAT3 via the cAMP-PKA pathway. CREB and STAT3 cooperatively induce the gene expression of *Il23r*. PGE_2-induced T_h17 expansion is involved in the pathology of autoimmune diseases, such as CHS, multiple sclerosis and psoriasis. IL-23R, IL-23 receptor.

PGE_2-EP2/EP4 signaling in IL-22 production from T_h22 cells

Atopic dermatitis (AD) and allergic contact dermatitis (ACD) are both inflammatory skin diseases with chronic eczema, and are histologically characterized by epidermal hyperplasia and the infiltration of immune cells, such as T cells, dendritic cells and eosinophils (57–59). Recent studies have shown that IL-22, a cytokine that induces epidermal hyperplasia and inhibits keratinocyte maturation, is deeply involved in the initiation and progression of AD and ACD (60, 61). Indeed, serum IL-22 levels are increased in patients with AD and ACD compared with those in healthy subjects (62, 63). Importantly, a clinical trial for a neutralizing anti-IL-22 antibody is being performed at present. T_h17 cells are known to produce IL-22, but the IL-22-producing $CD4^+$ effector T cell subsets have been identified as T_h22 cells. To date, T_h22 cells as well as T_h17 cells are considered to play crucial roles in the pathogenesis of AD and ACD (63).

PGE_2 has been shown to promote IL-22 production from type 3 innate lymphoid cells, but it was unknown whether PGE_2 affects T_h22 function (64). Robb *et al.* found that PGE_2 promotes IL-22 production in $CD4^+$ T cells cultured in T_h22-priming conditions (65). This effect of PGE_2 was mimicked by both an EP2 agonist and an EP4 agonist, and conversely, PGE_2-induced IL-22 production was suppressed by cotreatment with an EP2 antagonist and an EP4 antagonist. Although IL-23 on its own failed to augment *Il22* expression, simultaneous activation of the PKA pathway enhanced *Il22* transcription. Furthermore, PGE_2 accelerated the expression of the aryl hydrocarbon receptor (AHR, *Ahr*), an important transcription factor for IL-22 gene expression, and cAMP signaling-induced IL-22 induction was completely blocked by cotreatment with an AHR inhibitor (66). Therefore, these results suggest that the PGE_2-EP2/EP4-PKA pathway up-regulates the expression of *Ahr*, which in turn cooperatively enhances IL-23-driven *Il22* transcription (Fig. 5; Table 1) (65).

Robb *et al.* analyzed whether PGE_2-EP4 signaling promotes IL-22 production from T cells *in vivo*, and consequently exacerbates IL-22-associated skin disease. In T-cell-specific EP4-deficient mice, IL-22-producing T cells in the lymph nodes were attenuated after DNFB sensitization. Furthermore, the significance of PGE_2-induced IL-22 production was analyzed in an oxazolone-induced allergic skin inflammation model, which mimics human ACD pathogenesis (67). Indomethacin as well as T-cell-specific EP4-deficiency markedly suppressed oxazolone-induced ear swelling and the histological features of eczema, suggesting that PGE_2-EP4 signaling exacerbates ACD development by stimulating IL-22 production from T_h22 cells (Fig. 5; Table 1) (65). On the other hand, the pathogenesis of EP2-deficient mice was not analyzed, and therefore the significance of endogenous PGE_2-EP2 signaling in ACD remains to be determined.

PGE_2-EP4 signaling in human skin diseases

PGE_2-EP4 signaling has been shown to be involved in skin diseases by stimulating T_h17-cell expansion and IL-22 production from T_h22 cells. The next question was hence whether EP4 signaling also plays a role in human diseases. Lee *et al.* analyzed public microarray data in skin biopsy specimens from psoriasis patients and healthy control subjects (41). Expression levels of the T_h17-associated genes *IL23A*, *IL23R*, *IL17A*, *IL17F* and *STAT3* were increased in psoriatic skin. Interestingly, expression levels of the PGE_2-associated genes *PTGS2* (COX-2), *PTGES1* (mPGES-1), *PTGES2* (mPGES-2) and *PTGER4* (EP4) were increased. In contrast, the expression level of *HPGD* (15-PGDH, the PG-inactivating enzyme) was decreased in psoriatic skin. In addition, expression levels of T_h17-associated genes were positively correlated with those of PGE_2-associated genes, including *PTGER4*, and were negatively correlated with that of *HPGD*. Although there was no description regarding whether *PTGER2* expression was altered, at least

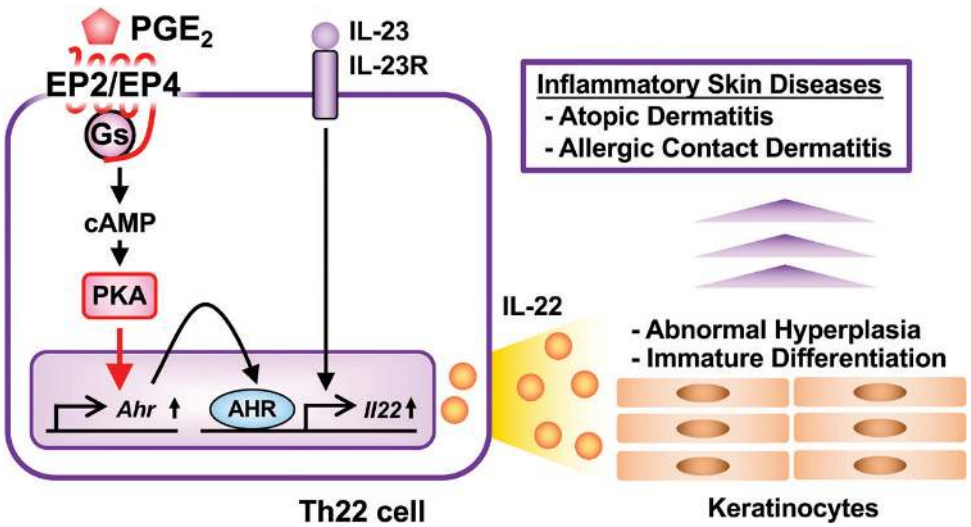

Fig. 5. PGE_2-EP2/EP4 signaling-induced IL-22 production from T_h22 cells. PGE_2-EP2/EP4 signaling promotes the gene expression of *Ahr* through the cAMP-PKA pathway. By the addition of IL-23 signaling, AHR synergistically activates *Il22* gene expression. PGE_2-induced IL-22 production from T_h22 cells is involved in the pathology of inflammatory skin diseases, such as AD and ACD.

the PGE_2-EP4 pathway appears to be up-regulated together with the T_h17-associated pathway in human psoriatic skin inflammation (Table 1) (41).

Robb *et al.* also analyzed public microarray data of skin biopsy specimens from AD patients and healthy control subjects (65). Expression levels of the T_h22-associated genes *IL22*, *AHR* and *IL23R*, as well as genes of the IL-22-induced pro-inflammatory factors *S100A7*, *S100A8* and *S100A9* were increased in AD skin. Notably, expression levels of PGE_2-associated genes, including *PTGER4* were also increased, and *HPGD* and *PTGER2* (EP2 gene) were decreased in AD skin. Furthermore, the expression level of *IL22* positively correlated with the expression levels of *PTGES1*, *PTGES2* and *PTGER4*, and negatively correlated with that of *HPGD* in AD skin (65). Robb *et al.* further analyzed whether betamethasone (a corticosteroid) may alter PGE_2-associated gene expression in human AD skin. They found that treatment of human subjects with betamethasone for 3 weeks markedly suppressed the expression levels of both IL-22-associated and PGE_2-associated genes, including *PTGER4*, compared with their expression levels before the treatment. Interestingly, UVB irradiation, an effective treatment for AD, also attenuated the expression of PGE_2-associated genes in AD skin. These results may reflect the involvement of PGE_2-EP4 signaling in IL-22 production also in human AD skin (Table 1) (65).

Conclusions

Studies using mice deficient in each of the PG receptors have enabled us to understand the detailed mechanisms of PG-regulated acute and chronic inflammation, as well as autoimmune diseases at the molecular and cellular levels. For a long time, although the role of PGE_2 in acute inflammation was understood to be vasodilation of vascular smooth muscle cells via EP2/EP4 signaling, it was subsequently demonstrated that PGE_2 induces mast cell activation via EP3 receptor signaling, and consequently enhances vascular permeability, contributing to PGE_2-induced acute inflammation (20, 68). PGE_2 promotes T_h1-cell differentiation, T_h17-cell proliferation and IL-22 production from T_h22 cells *in vitro* via EP2 and EP4 receptors, and exacerbates chronic inflammation and various autoimmune diseases, mainly via the EP4 receptor in most cases (Table 1) (34, 35, 41, 45, 65).

EP2-deficient female mice and EP4-deficient neonate mice demonstrated impaired fertilization and patent ductus arteriosus, respectively, indicating that EP2 and EP4 play crucial roles in successful fertilization and neonatal adaptation of the vascular system, respectively. However, no severe phenotypes were found in adult mice deficient for EP3 and EP4 (18, 69–73). Therefore, specific antagonists for EP3 or EP4 are expected to be safe and effective therapeutic drugs for acute and chronic inflammation, as well as for autoimmune diseases in non-pregnant adults. Just recently, Morimoto *et al.* and Toyoda *et al.* clarified the crystal structure of the human EP3 and EP4 receptors, respectively (74, 75). This information will enable us to develop more efficient and specific drugs targeting human EP3 and EP4.

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