Prostaglandin E_2-induced inflammation: relevance of prostaglandin E receptors

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Abstract

Prostaglandin E_2 (PGE_2) is one of the most typical lipid mediators produced from arachidonic acid (AA) by cyclooxygenase (COX) as the rate-limiting enzyme, and acts on four kinds of receptor subtypes (EP1-EP4) to elicit its diverse actions including pyrexia, pain sensation, and inflammation. Recently, the molecular mechanisms underlying the PGE_2 actions mediated by each EP subtype have been elucidated by studies using mice deficient in each EP subtype as well as several compounds highly selective to each EP subtype, and their findings now enable us to discuss how PGE_2 initiates and exacerbates inflammation at the molecular level. Here, we review the recent advances in PGE_2 receptor research by focusing on the activation of mast cells via the EP3 receptor and the control of helper T cells via the EP2/4 receptor, which are the molecular mechanisms involved in PGE_2-induced inflammation that had been unknown for many years. We also discuss the roles of PGE_2 in acute inflammation and inflammatory disorders, and the usefulness of anti-inflammatory therapies that target EP receptors.

Keywords:

Prostanoid; Mast cell; Irritant contact dermatitis; Helper T cell; Multiple sclerosis

1. Introduction

Aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the activity of COX by binding to its active site [1, 2], and thereby inhibit the biosynthesis of prostanoids, resulting in antipyretic, analgesic, and anti-inflammatory effects. Since exogenously added PGE_2 elicits actions such as pyrexia, pain sensation, and inflammation, it was thought that the action of NSAIDs is mainly based on the inhibition of PGE_2 production. Recently, studies on mice deficient in each EP subtype as well as EP-specific agonists/antagonists have revealed the physiological functions of PGE_2 via each EP receptor [3, 4]. In this review, we summarize the molecular basis of prostanoid receptors and the recent advances in PGE_2 receptor research, by focusing on the molecular mechanism of PGE_2-induced inflammation, and discuss the pathophysiological roles of PGE_2-EP receptors as well as their usefulness as target proteins for drug design.

2. Molecular basis of prostanoid actions

2-1. Biosynthesis and structure of prostanoids

Prostanoids are a group of eicosanoids consisting of four kinds of prostaglandins (PGs) and thromboxanes (TXs): PGE_2, PGD_2, PGF_2, PGI_2, and TXA_2. Prostanoids are produced by the sequential actions of COX and the respective synthases from AA, which is released by phospholipase A_2 (PLA_2) from membrane phospholipids [1, 2, 5]. The COX protein contains two active sites: a cyclooxygenase site, where AA is converted into

hydroperoxy endoperoxide PGG_2, and a peroxidase site, responsible for the reduction of PGG_2 to PGH_2 (Fig. 1A) [1, 2]. To date, two COX isozymes are known: COX-1 and COX-2. PGs are molecules with a basic structure of prostanoic acid, which consists of a cyclopentane ring and two carbon chains, and are classified from A to J, according to the structure of their cyclopentane ring. On the other hand, the basic structure of TXs is thrombanoic acid, which contains two oxygens in a ring structure (Fig. 1B). Therefore, TXs should be strictly distinguished from PGs. However "PGs" in a broad sense refers to the products of COX that includes TXs, and in many cases the term is used as a synonym for prostanoids.

2-2. Prostanoid receptors

Receptors mediating the action of prostanoids were characterized first by pharmacological analysis, which indicated the presence of multiple receptors for PGE_2 and one receptor each for PGD_2, $PGF_{2\alpha}$, PGI_2, and TXA_2 [6, 7]. Coleman *et al*. proposed the presence of receptors specific for TX, PGI, PGE, PGF, and PGD, and named them the TP (type T Prostanoid receptor), IP, EP, FP, and DP receptors, respectively. They further classified the EP receptor into three subtypes, EP1, EP2, and EP3, all of which respond to the naturally-occurring agonist, PGE_2, but differ in their actions and in their responses to various analogues. They later reported a fourth subtype, the EP4 receptor, which, like the EP2 receptor, is positively coupled to adenylate cyclase, but differs in response to certain ligands. Molecular identification of these receptors was achieved by their cDNA cloning, which revealed that the prostanoid receptors are G-protein-coupled receptors

(GPCRs) and that there is indeed a family of eight GPCRs that correspond to the pharmacologically defined receptors (Fig. 2A). In 2001, Hirai *et al*. revealed the presence of a second receptor for PGD_2, which was originally called CRTH2 (Chemoattractant receptor-homologous molecule expressed on T helper type 2 cells) [8], and is currently known as DP2 [9]. Among the prostanoids, PGE_2 is most widely found in animal species, and exhibits the most versatile actions. Since each EP subtype has distinct signal transduction properties, PGE_2 is able to exert diverse actions; EP1 is coupled to intracellular Ca^{2+} mobilization via G_q, EP2 and EP4 are coupled to stimulation of adenylyl cyclase via G_s, and EP3 is mainly coupled to inhibition of adenylyl cyclase via G_i, respectively. EP2 and EP4 receptors also elicit the activation of phosphoinositide 3-kinase (PI3K) via the β-arrestin pathway [10, –12].

2-3. Molecular evolution of prostanoid receptors

The prostanoid receptors form clusters not according to their ligand type but according to the type of signal transduction pathway they are coupled to (Fig. 2B). Their phylogenetic tree also illustrates that the phylogenetic distance of EP2 from its ancestor is shorter than that of the two other G_s-coupled prostanoid receptors IP or DP1, and the distance of EP1 is shortest among the three G_q-coupled-prostanoid receptors EP1, FP, and TP (Fig. 2B). The evolutionary position of PGE receptors in the tree suggests that the cyclooxygenase pathway initially evolved as a system composed of PGE_2 and its receptor, and both diversification of the ligand and duplication of the receptor gene lead to the evolution of the diverse physiological functions [3]. Intriguingly, the evolutionary

position of the DP2 receptor in the tree suggests that this receptor evolved from chemoattractant receptors such as the leukotriene B_4 receptors BLT1 and BLT2 [13].

3. Mast cell activation by PGE_2-EP3 signaling

3-1. Mechanism of acute inflammation

Four cardinal features, namely *rubor* (red flare), *calor* (heat), *tumor* (swelling), and *dolor* (pain) characterize acute inflammation. The flare and heat reactions are caused by an increase in local blood flow as a result of vasodilatation, and the swelling is elicited by an increase in vascular permeability and resultant leukocyte recruitment. These processes are triggered by tissue injury and invasion of exogenous materials and organisms [14]. Such inflammatory insults are primarily detected by Toll-like receptors (TLRs) on immune cells followed by activation of the local cytokine network such as TNF-α and IL-1β. Since these cytokines affect vascular permeability and leukocyte recruitment, such a TLR-cytokine axis in innate immunity is one factor that governs the inflammation process [15, 16]. On the other hand, using various experimental models of acute inflammation, chemical mediators such as bradykinin, histamine, thrombin, and growth factors have been found and characterized [14]. Aspirin-like drugs have been used as the first choice of drugs for acute inflammation because of their high potency to suppress the above inflammatory symptoms [17]. Since these drugs exert their actions by inhibiting COXs and thereby inhibiting the biosynthesis of PGs, endogenously synthesized PGs are believed to be involved in inflammation reactions [14, 18]. Indeed,

PGs have been shown to elicit vasodilatation and an increase in local blood flow, leading to red flare and local heat. It is believed that vascular permeability factors such as histamine and bradykinin are thereafter released into the inflammation site, leading to edema formation [14, 19]. However, the link between the initial vasodilatation and the subsequent permeability change remained unclear (Fig. 3).

3.2. Role of PGE_2-EP3 signaling in AA-induced and PGE_2-induced hyperpermeability

AA-induced inflammation is a model of antigen-independent irritant contact dermatitis in mice, and is induced by the application of AA dissolved in solvent to the skin. This model sequentially elicits the three major symptoms of inflammation (i.e., edema, increased vascular permeability, and cellular infiltration). Application of AA has been shown to induce the production of a broad range of eicosanoids, such as PGI_2, PGE_2, and leukotrienes [20]. In this model, PGE_2 is the most abundantly produced AA metabolite, and inflammation including PGE_2 production is abolished in COX-1-knock-out mice, suggesting that PGE_2 produced by COX-1 contributes to the pathogenesis of this inflammation model [21]. Recently, Morimoto and colleagues [22] applied this model to mice deficient in each of the four kinds of EP subtypes, and identified the EP3 receptor to be involved in AA-induced inflammation. The inflammation response was monitored by the amount of dye leaking into the ear (as an index of vascular hyperpermeability), ear thickness (as an index of edema formation), and the level of myeloperoxidase activity (as an index of neutrophil infiltration). Only EP3-deficient mice showed significantly

attenuated responses, suggesting that the PGE_2-EP3 receptor signal mainly contributes to this inflammation model. In addition, application of PGE_2 to ear tissue also increased vascular permeability, and its effect was abolished in EP3-deficient mice. Furthermore, only an EP3-specific agonist among the EP-selective agonists induced vascular permeability with a dose-response similar to PGE_2. Intriguingly, the PGE_2-induced vascular permeability was suppressed by histamine H_1 antagonist treatment as well as histidine decarboxylase deficiency, suggesting that histamine mediates PGE_2-induced hyperpermeability.

3.3. PGE_2-induced vascular hyperpermeability is mediated by EP3 receptors on mast cells

Mast cells (MCs) are immune cells widely distributed in various peripheral tissues including skin, and are activated in an antigen-dependent manner [23, 24]. Once activated by antigen-induced cross-linking of IgE receptors, MCs release bioactive substances in their granules such as histamine and proteases. MC-derived histamine has been shown to increase vascular permeability and elicit edema formation. Although PGE_2 has been shown to positively or negatively regulate the degranulation of MCs elicited by antigen-IgE stimulation [25, 26], there had been no studies exploring the direct effect of PGE_2 on MCs. Recently, Morimoto and colleagues [22] examined this point, and found that PGE_2-induced vascular permeability is completely abolished in mast cell-deficient mice, and the response is rescued upon reconstitution with wild-type MCs but not with EP3-

deficient MCs. PGE_2 directly elicited histamine release in mouse peritoneal MCs derived from wild-type mice, but not from EP3-deficient mice. These results indicate that PGE_2-induced vascular permeability is mediated by PGE_2-EP3 signaling on MCs. In addition, they showed that PGE_2 directly triggers degranulation and IL-6 release in mouse bone-marrow-derived MCs (BMMCs), and then investigated the mechanism underlying the PGE_2-induced MC activation using the BMMCs. Activation of MCs by PGE_2 was dependent on the EP3-G_i protein, and was mediated by both the continuous influx of extracellular Ca^{2+} [27] and the activation of PI3K-Akt pathways [28, 29] (Fig. 4). These results revealed that PGE_2-induced acute inflammation is mediated, at least in part, by EP3 on MCs. Moreover, a recent study using human cord mast cells reported that PGE_2 triggers degranulation in a G_i-dependent manner [30], suggesting that PGE_2-EP3 signaling may act as a secretagogue of human mast cells. Indeed, PGE_2 was revealed to mediate the acute inflammatory response to topical 5-aminolaevulinic acid photodynamic therapy in human skin [31]. Thus, there is a possibility that activation of MCs via PGE_2-EP3 signaling may be triggered by topical 5-aminolaevulinic acid photodynamic therapy and contact with chemical irritants, as well as infection with pathogens, and it can be considered as an exacerbating factor of skin disorders also in humans. Indeed, since it is reported that MCs play a major role in the onset of irritant contact dermatitis [32], the inhibition of EP3 receptor signaling is expected to be a strategy effective for the prevention and treatment of such skin disorders.

4. Helper T cell immune regulation by PGE_2-EP2/EP4 receptors

Helper T cells are classified into three distinct subsets of effector T cells, termed Th1, Th2, and Th17 [33]. Th1, Th2, and Th17 cells are characterized by the cytokines they produce, which are interferon-γ (IFN-γ), interleukin (IL)-4, and IL-17, respectively. Among them, Th1 and Th17 cells play major roles in inflammatory disorders and autoimmune diseases, and, in fact, they were shown to mediate tissue damage and inflammation in animal models of various immune diseases such as multiple sclerosis, rheumatoid arthritis, and contact dermatitis [34–39]. One main strategy of drug development for these inflammatory immune diseases is therefore to manipulate the function of these Th subsets.

4.1. Action of PGE_2 on Th1 differentiation

T cell activation is primarily induced by stimulation of the T cell receptor (TCR) with the respective antigen, and one of the mechanisms downstream of TCR stimulation is activation of the Src-family kinase Lck. It was shown that cAMP in T cells activates protein kinase A (PKA), which subsequently phosphorylates and activates C-terminal Src kinase, which in turn phosphorylates the C-terminal tyrosine of Lck and inactivates it [40]. Thus, cAMP-producing signals such as PGE_2 were long believed to suppress TCR signaling and Th1 differentiation [41-46]. Then, is cAMP signaling unnecessary for Th1 differentiation? Intriguingly, it was reported that $G_{\alpha s}$-deficient T cells, which fail to produce cAMP, show impaired differentiation into Th1 cells, and that the addition of a

cAMP analogue restores Th1 differentiation in these cells [47]. These results suggest that cAMP signaling is a prerequisite for Th1 differentiation. The differentiation of naïve T cells into Th1 cells is driven by two critical cytokines, IL-12 and IFN-γ [48]. Yao *et al.* recently found that PGE_2 promotes the expression of the receptors for IL-12 and IFN-γ in a cAMP-dependent manner [49]. They also showed that a cAMP analogue activates PKA, which in turn directly phosphorylates the transcription factor cAMP responsive element-binding protein (CREB) and induces the dephosphorylation and nuclear translocation of cAMP-regulated transcriptional coactivator (CRTC) by inhibiting salt-inducible kinase 2 (SIK2). Activated CREB and CRTC2 together promote gene transcription of the IL-12 receptor β2 chain and IFN-γ receptor α chain. Thus, PGE_2-induced facilitation of Th1 differentiation is mediated by increased signaling of both IL-12 and IFN-γ via EP2/EP4 receptors.

Then, how does PGE_2 signaling selectively promote gene expression of cytokine receptors without PKA-mediated Lck inactivation? Intriguingly, Yao *et al.* found that PGE_2-EP2/EP4 signaling promotes Th1 differentiation only when the cells are subjected to simultaneous TCR and CD28 stimulation, but not upon TCR stimulation only [50]. They further demonstrated that cAMP-mediated inhibition of the TCR-induced expression of CD25, production of IL-2 and IFN-γ, Th1 differentiation, and cell proliferation could all be rescued by strengthening CD28 costimulation in a PI3K-dependent manner [49]. Based on these results, they proposed a mechanism underlying the PGE_2-induced promotion of Th1 differentiation, namely that PGE_2-EP2/EP4 signaling simultaneously activates PI3K and cAMP generation, and with the additional

stimulation of PI3K by CD28, cancels the cAMP-mediated inactivation of TCR signaling. Indeed, it was suggested that PKA interferes with Lck activation, and this action can be antagonized by PI3K activation following CD28 costimulation [40, 51] (Fig. 5).

It is currently unknown whether PGE_2 directly regulates the Th2 subset. In the allergic asthma model, EP2 deficiency results in the augmentation of both IL-13 production from lymph organ cells and airway inflammation, and thus PGE_2 appears to suppress allergic sensitization and lung inflammation through EP2 receptors on T cells [52]. However, such results may be due to the loss of PGE_2-driven Th1 responses.

4.2. Roles of PGE_2 in Th17 cell expansion

Then, what are the roles of PGE_2 on Th17 cell function? IL-6 and transforming growth factor-β (TGF-β) are key determinants of Th17 differentiation. Th17 cells are then expanded by IL-23 that stabilizes their fate. Yao *et al.* [50] showed that dendritic cells (DCs) produce PGE_2 which acts on their own EP4 receptors to promote IL-23 production, and that the actions of PGE_2 are mediated by exchange proteins activated by the cAMP (Epac) pathway. Furthermore, they showed that PGE_2 acting on EP2/EP4 of Th17 cells amplifies IL-23-mediated Th17 cell expansion. On the other hand, PGE_2 potently suppressed Th17 differentiation from naïve T cells by IL-6 and TGF-β, which was consistent with the report by Chen *et al.* [53]. Thus, PGE_2 appears to act on both DCs and Th17 cells, and facilitates Th17 cell expansion cooperatively.

The above studies have thus revealed novel actions of PGE_2-EP2/EP4 signaling on

the 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 the study on mouse cells [50], several groups [54, 55] have used human peripheral blood mononuclear cells and reported the actions of PGE_2 on human Th17 differentiation. Boniface *et al.* [54] reported that PGE_2 in combination with IL-1β and IL-23 promotes the production of IL-17 from differentiating Th17 cells by up-regulating IL-1β receptor and IL-23 receptor expression through the EP2/EP4-cAMP pathway. Chizzolini *et al.* [55] reported that PGE_2 synergizes with IL-23 and increases the number of Th17 cells from human memory T cells but not from naïve T cells, consistent with the report by Yao *et al.* [50] that PGE_2 cannot enhance Th17 differentiation but facilitates the action of IL-23 on Th17 expression.

4.3. Role of PGE_2-EP4 signaling in vivo in immune inflammation

Although an *in vivo* study showed that PGE_2 elicits Th1 differentiation and facilitates Th17 cell expansion, how does PGE_2 act on disease states? Two models of immune inflammation, 2,4-dinitro-1-fluorobenzene (DNFB)-induced contact hypersensitivity (CHS) [56] and experimental allergic encephalomyelitis (EAE) [57], are widely used as models of CHS and multiple sclerosis (MS), respectively. Th1 cells and Th17 cells are considered to be essential to the pathogenesis of both models [58–63]. Treatment with an EP4 antagonist suppressed disease severity and decreased the accumulation of antigen-specific Th1 and Th17 cells in regional lymph nodes in both models [50]. Only EP4-

deficient mice among the mice deficient in each of the four EPs showed significantly attenuated disease progression in the EAE model [64]. These findings indicate that the PGE_2-EP4 signaling indeed positively regulates the differentiation and expansion of Th1 and Th17 subsets, respectively and determines the extent of immune inflammation. In particular, T-cell specific deletion of the EP4 gene was also found to attenuate disease progression in the CHS model [49]. Moreover, in a colitis model that is induced by the transfer of naïve T cells into mice deficient in recombination-activation gene 2, transfer of $EP4^{+/-}$ or $EP4^{-/-}$ T cells showed weaker colonic inflammation and lower amounts of IFN-γ and IL-2 in the mesenteric lymph nodes than the transfer of T cells from wild-type littermate controls [49]. On the other hand, there was no difference in colitis development between groups that received wild-type or $EP2^{-/-}$ T cells [49]. In addition, deficiency of EP2 alone did not significantly affect disease progression in the CHS model [50]. Therefore, PGE_2 appears to facilitate the function of helper T cells mainly via EP4 *in vivo*, and plays a role in the pathogenesis of various types of chronic inflammation and autoimmune diseases (Fig. 6). These findings indicate that an EP4 antagonist should be a good therapeutic drug target for intractable immune diseases.

5. Concluding remarks

As mentioned above, PGE_2 was found to be involved in acute inflammation as well as inflammatory immune diseases via different mechanisms including distinct receptors and molecules; PGE_2 elicits vascular permeability and edema formation via EP3 on MCs,

and facilitates Th1 differentiation and Th17 expansion via EP4 on T cells and DCs. The latter is particularly important as it also shows that PGE_2 is deeply involved in the immune response itself by skillfully potentiating cytokine signaling via gene regulation, in contrast to the traditional view that PGE_2 regulates biological functions by eliciting acute inflammation, as represented by smooth muscle contraction. It is expected that in the future, the meaning of EP3 and EP4 signaling in various skin disorders, chronic inflammation, and inflammatory diseases will be revealed, and that their validity as a prevention or therapeutic targets will be evaluated.

6. Acknowledgments

We thank Professors Kazuhisa Nakayama, Atsushi Ichikawa, and Shuh Narumiya for precious advice. We also thank Toshiko Sugimoto and Yue Kanagawa for their technical support. We are grateful to Dr. H. Akiko Popiel for careful reading of the manuscript.

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Figure legends

Fig. 1. Cyclooxygenase pathway and prostanoids. A, Arachidonic acid, which is released by phospholipase A_2 (PLA_2) from membrane phospholipids, is converted to PGG_2 and then to PGH_2 by cyclooxygenase (COX), and then each prostanoid is produced by the action of their specific synthases. B, Chemical structure of prostanoic acid and thrombanoic acid.

Fig. 2. Prostanoid receptors and their molecular (evolutionary) phylogenetic tree. A, Prostanoids exert various actions by acting on each specific receptor, which is coupled to a specific G protein. PGE_2 acts on four kinds of receptor subtypes (EP1-EP4), each of which has distinct signal transduction properties, and exerts diverse physiological functions. PGD_2 also acts on two different receptors, DP1 and DP2. B, Phylogenetic tree of the human prostanoid DP1, EP1, EP2, EP3, EP4, FP, IP, and TP receptors, together with the human DP2 receptor and leukotriene B_4 receptors BLT1 and BLT2. Each branch length indicates the phylogenetic distance. The eight prostanoid receptors form clusters not according to their ligand but according to the signal transduction pathway that they are coupled to. The DP2 receptor belongs to the chemoattractant receptor family that includes BLT1 and BLT2, rather than the prostanoid receptor family.

Fig. 3. Process of acute inflammation, and the roles of PGE_2 considered traditionally. Acute inflammation involves processes such as (i) hyperpermeability, (ii) plasma leakage,

and (iii) neutrophil infiltration, and is induced by the activation of TLR on immune cells upon stimuli such as tissue injury or infection of an exogenous organism, and subsequent triggering of the cytokine network. PGE_2 was thought to potentiate inflammation through eliciting vasodilatation and an increase in local blood flow, leading to red flare and local heat. PGE_2 was thought to potentiate inflammation through such actions. However, it remained unknown whether PGE_2 is directly involved in vascular permeability.

Fig. 4. A role of PGE_2-EP3 signaling in acute inflammation. When AA is released by the activation of PLA_2 induced by contact stimuli with irritants or stimulation with pathogen-associated molecular patterns (PAMPs), PGE_2 is produced by COX-1 that is expressed on skin keratinocytes. PGE_2 acts on the EP3 receptor of nearby MCs, and elicits (i) accumulation of PIP_3 and (ii) continuous Ca^{2+} increase in a G_i-dependent manner. The former process is mediated by PI3K, and the latter is mediated by activation of the Stim1-Orai channel induced by depletion of Ca^{2+} in the endoplasmic reticulum via PLC-activation. PGE_2-EP3 finally induces degranulation and IL-6 production. Histamine released by degranulation is thought to potentiate vascular permeability, and IL-6 promotes the migration of neutrophils, leading to inflammatory swelling.

Fig. 5. Facilitation of Th1-cell differentiation by PGE_2-EP2/EP4 signaling. In naïve T cells, PGE_2-EP2/EP4 signaling activates both the PKA and PI3K pathways. PKA phosphorylates CREB and dephosphorylates CRTC2 through phosphorylation of SIK2. Activated CREB and CRTC2 translocate to the nucleus and induce the expression of

Il12rb2, *Ifngr1*, and possibly *Il2rb*. While PKA inhibits T-cell activation by suppressing TCR signalling, coactivation of PI3K by EP2/EP4 and the coreceptor CD28 cancels this inhibition. PGE_2-EP2/EP4 signaling facilitates Th1 differentiation through the amplification of cytokine signaling via the cooperative action of PKA and PI3K pathways.

Fig. 6. Regulation of helper T cells by PGE_2. PGE_2 is thought to contribute to the pathogenesis of contact dermatitis/ulcerative colitis and multiple sclerosis/rheumatoid arthritis, by inducing Th1 differentiation and Th17 expansion, respectively, by the following steps: (i) Th1 differentiation via EP4, (ii) Th17 expansion via EP2/EP4, and (iii) IL-23 production from activated dendritic cells via EP4 (resulting in acceleration of Th17 expansion).

Fig. 1

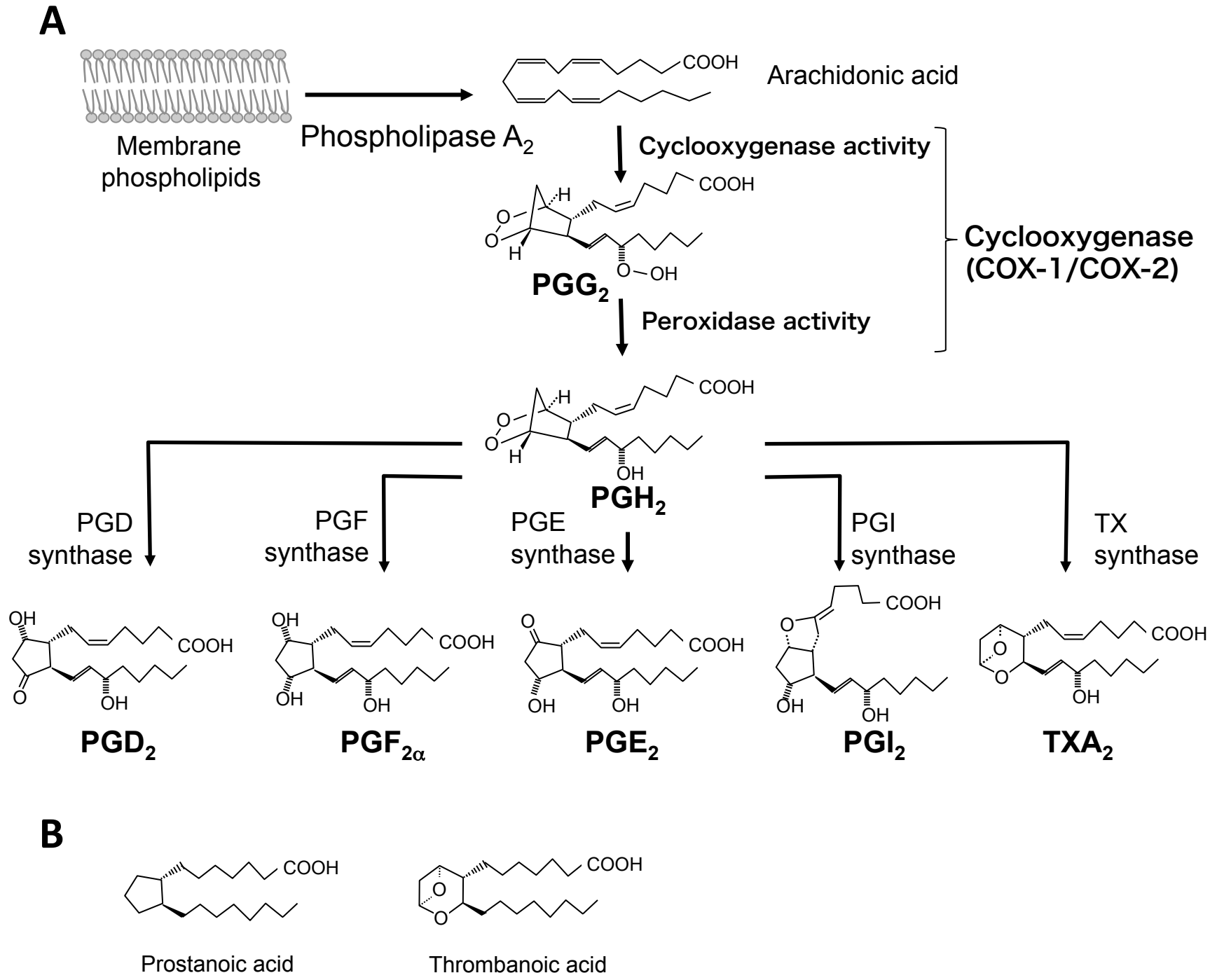
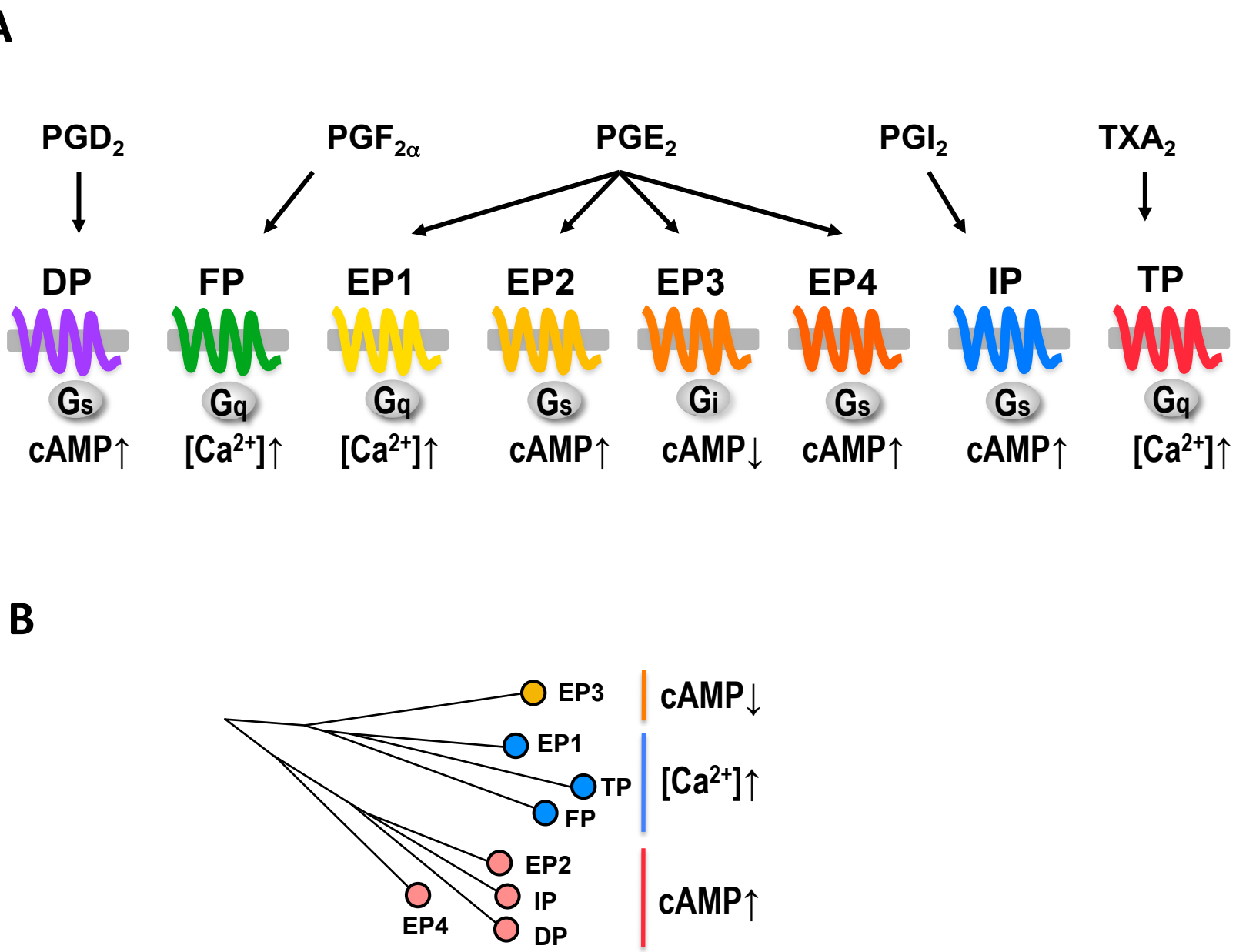

A
Membrane phospholipids
Phospholipase A2
Arachidonic acid
COOH
Cyclooxygenase activity
PGG2
Peroxidase activity
Cyclooxygenase (COX-1/COX-2)
PGH2
PGD synthase
PGF synthase
PGE synthase
PGI synthase
TX synthase
PGD2
PGF2α
PGE2
PGI2
TXA2
B
Prostanoic acid
Thrombanoic acid

Fig. 2


A
PGD2
PGF2α
PGE2
PGI2
TXA2
DP
FP
EP1
EP2
EP3
EP4
IP
TP
Gs
Gq
Gq
Gs
Gi
Gs
Gs
Gq
cAMP↑
[Ca2+]↑
[Ca2+]↑
cAMP↑
cAMP↓
cAMP↑
cAMP↑
[Ca2+]↑
B
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EP1
TP
FP
EP2
IP
DP
EP4
cAMP↓
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cAMP↑

Fig. 3

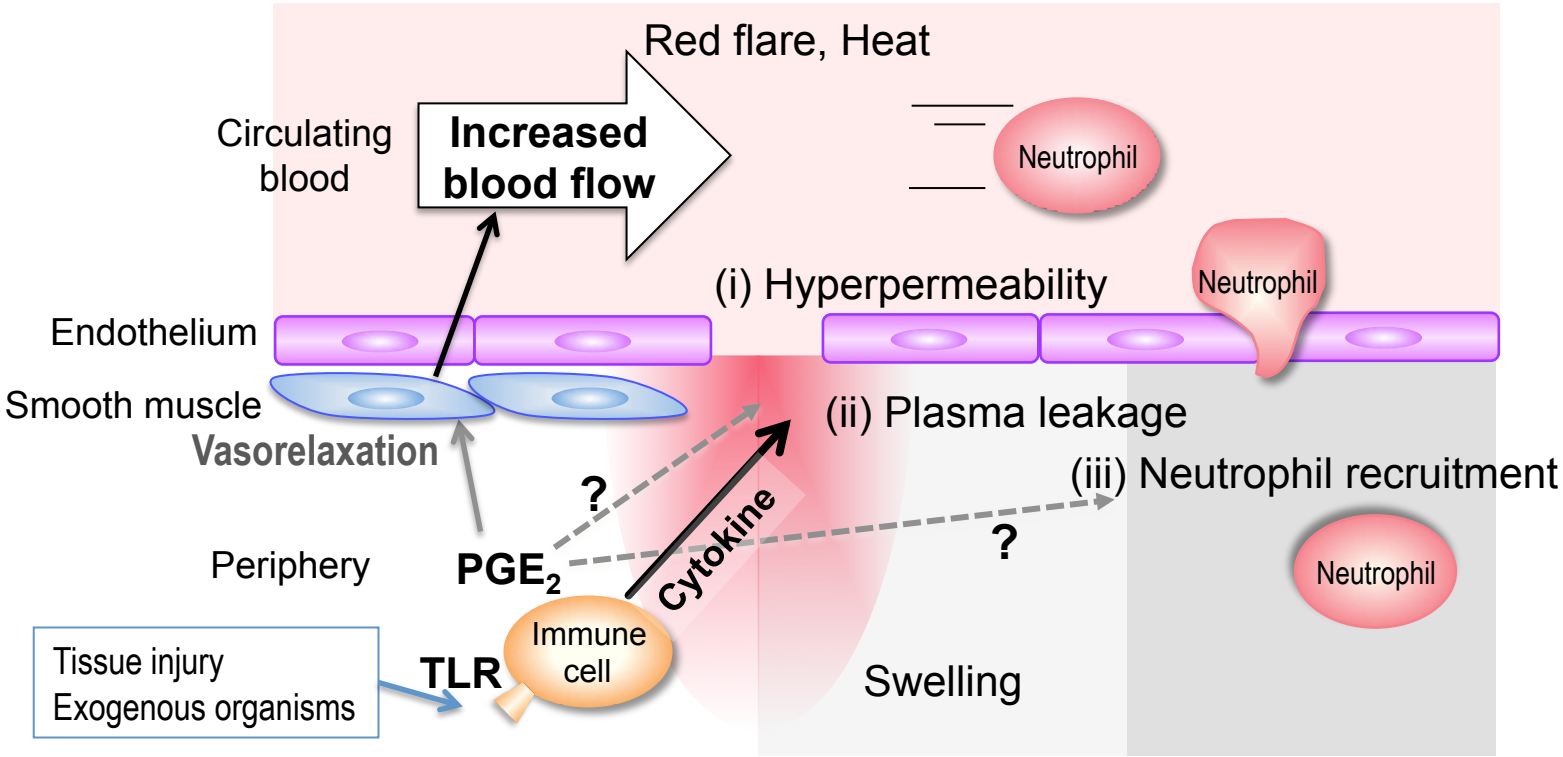

Red flare, Heat
Circulating blood
Increased blood flow
Neutrophil
(i) Hyperpermeability
Neutrophil
Endothelium
Smooth muscle
Vasorelaxation
(ii) Plasma leakage
(iii) Neutrophil recruitment
?
?
Periphery
PGE2
Cytokine
Neutrophil
Tissue injury
Exogenous organisms
TLR
Immune cell
Swelling

Fig. 4

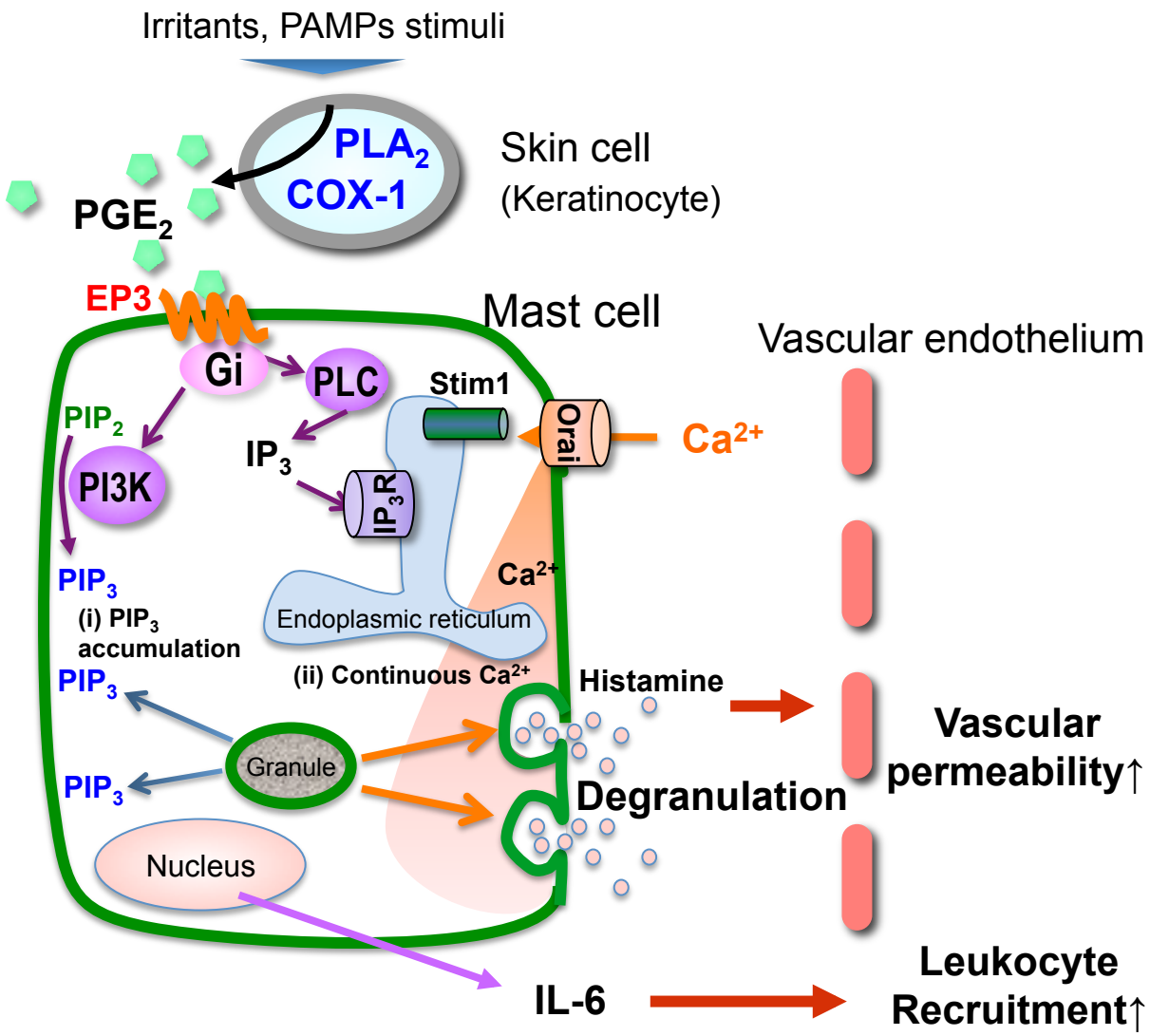

Irritants, PAMPs stimuli
PLA2
COX-1
Skin cell
(Keratinocyte)
PGE2
EP3
Mast cell
Vascular endothelium
Gi
PLC
Stim1
Orai
Ca2+
PIP2
IP3
PI3K
IP3R
PIP3
(i) PIP3 accumulation
Ca2+
Endoplasmic reticulum
(ii) Continuous Ca2+
Histamine
PIP3
Granule
PIP3
Degranulation
Vascular permeability↑
Nucleus
IL-6
Leukocyte Recruitment↑

Fig. 5

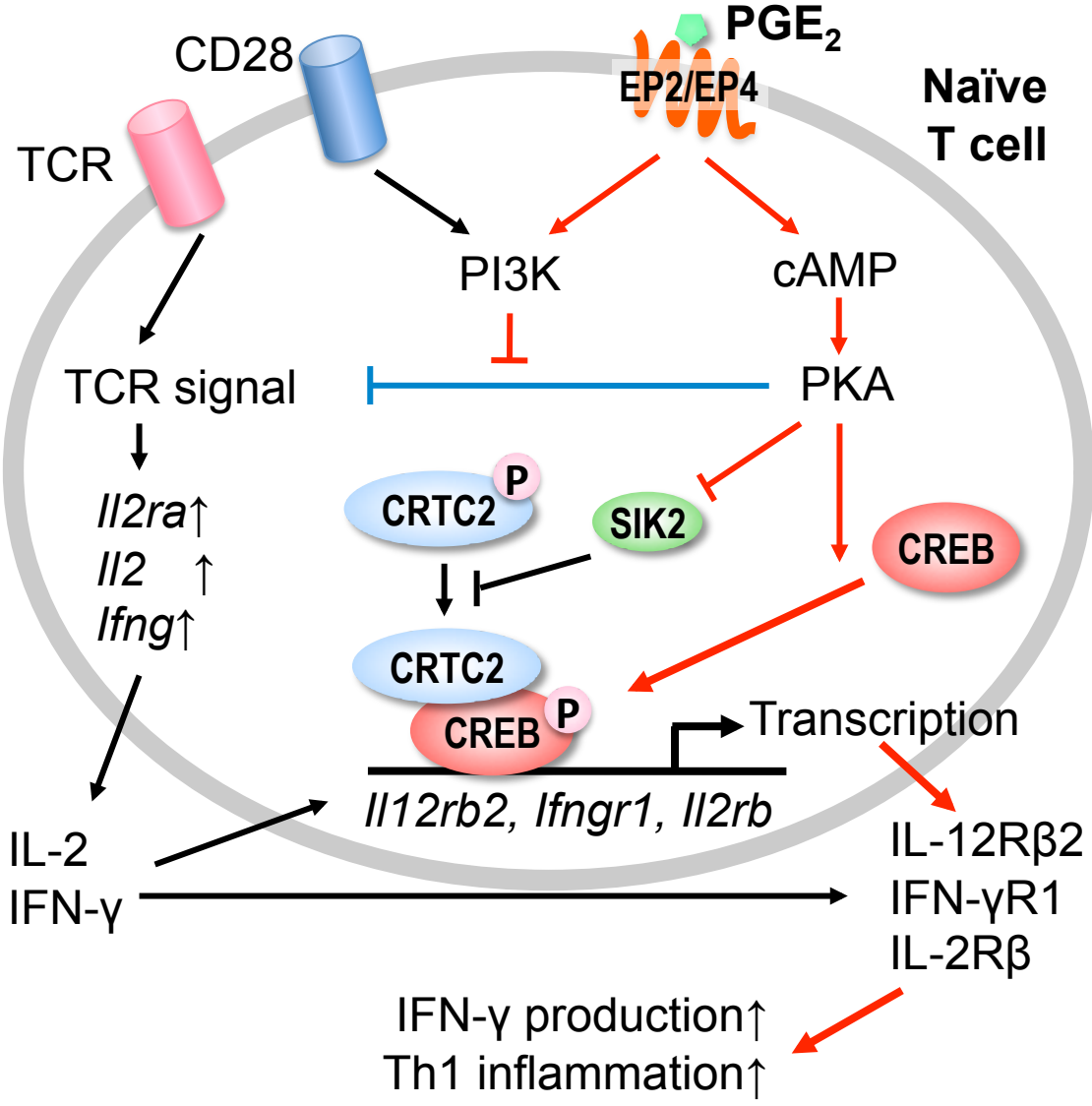
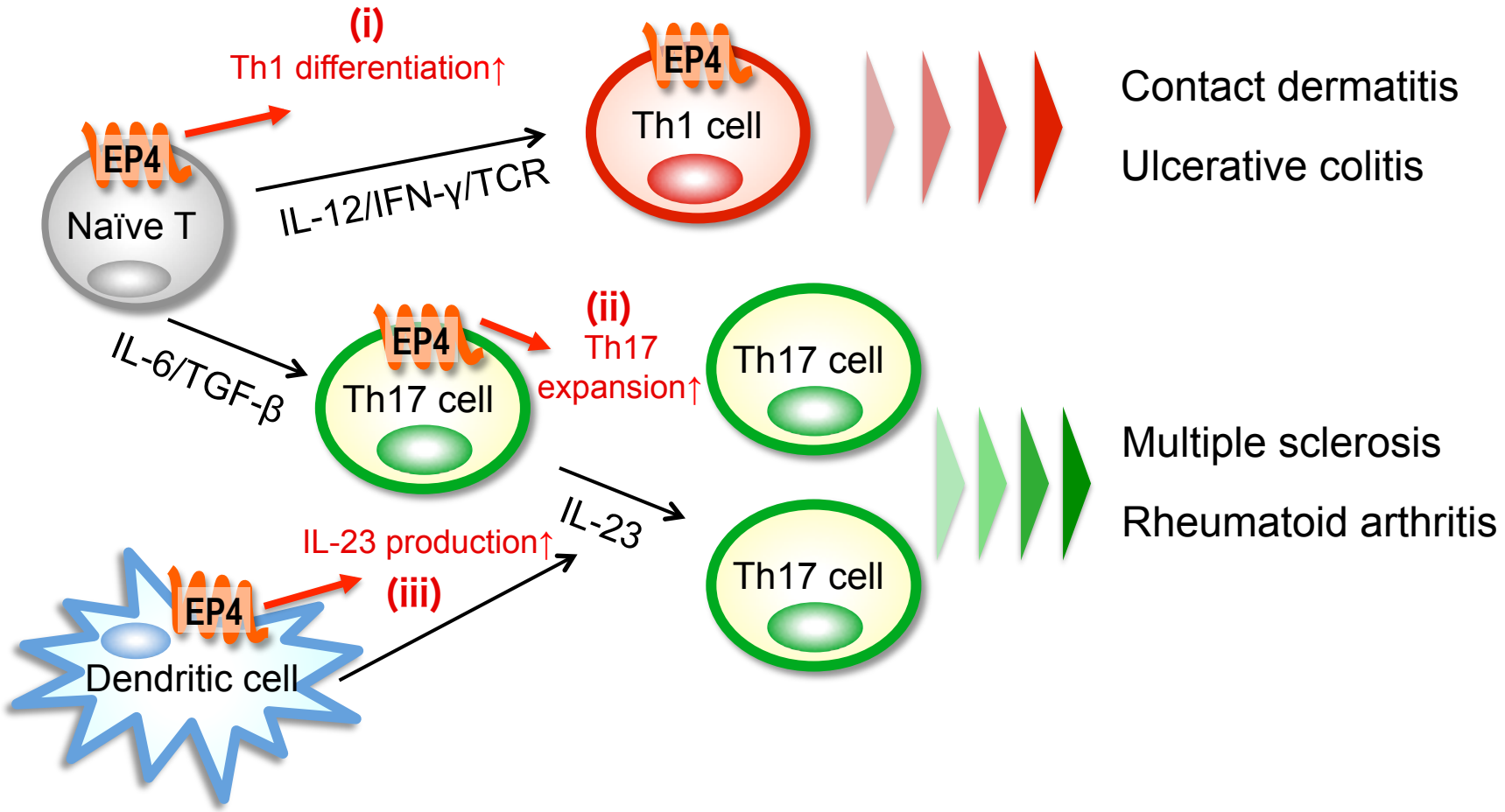

CD28
PGE2
EP2/EP4
Naïve
T cell
TCR
PI3K
cAMP
TCR signal
PKA
Il2ra↑
Il2 ↑
Ifng↑
CRTC2
P
SIK2
CREB
CRTC2
CREB
P
Transcription
Il12rb2, Ifngr1, Il2rb
IL-2
IFN-γ
IL-12Rβ2
IFN-γR1
IL-2Rβ
IFN-γ production↑
Th1 inflammation↑

Fig. 6


(i)
Th1 differentiation↑
EP4
Naïve T
IL-12/IFN-γ/TCR
EP4
Th1 cell
Contact dermatitis
Ulcerative colitis
IL-6/TGF-β
EP4
Th17 cell
(ii)
Th17
expansion↑
Th17 cell
IL-23
IL-23 production↑
(iii)
EP4
Dendritic cell
Th17 cell
Multiple sclerosis
Rheumatoid arthritis