Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Prostaglandins and Inflammation

Emanuela Ricciotti and Garret A. FitzGerald

Arterioscler Thromb Vasc Biol. 2011;31:986-1000
doi: 10.1161/ATVBAHA.110.207449
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636

ATVB in Focus
Inflammation

Series Editor: Alain Tedgui

Prostaglandins and Inflammation

Emanuela Ricciotti, Garret A. FitzGerald

Abstract—Prostaglandins are lipid autacoids derived from arachidonic acid. They both sustain homeostatic functions and mediate pathogenic mechanisms, including the inflammatory response. They are generated from arachidonate by the action of cyclooxygenase isoenzymes, and their biosynthesis is blocked by nonsteroidal antiinflammatory drugs, including those selective for inhibition of cyclooxygenase-2. Despite the clinical efficacy of nonsteroidal antiinflammatory drugs, prostaglandins may function in both the promotion and resolution of inflammation. This review summarizes insights into the mechanisms of prostaglandin generation and the roles of individual mediators and their receptors in modulating the inflammatory response. Prostaglandin biology has potential clinical relevance for atherosclerosis, the response to vascular injury and aortic aneurysm. **(*Arterioscler Thromb Vasc Biol*. 2011;31:986-1000.)**

Key Words: eicosanoids ■ prostacyclin ■ prostaglandins ■ thromboxanes

Inflammation is the immune system's response to infection and injury and has been implicated in the pathogeneses of arthritis, cancer, and stroke, as well as in neurodegenerative and cardiovascular disease. Inflammation is an intrinsically beneficial event that leads to removal of offending factors and restoration of tissue structure and physiological function. The acute phase of inflammation is characterized by the rapid influx of blood granulocytes, typically neutrophils, followed swiftly by monocytes that mature into inflammatory macrophages that subsequently proliferate and thereby affect the functions of resident tissue macrophages. This process causes the cardinal signs of acute inflammation: rubor (redness), calor (heat), tumor (swelling), and dolor (pain). Once the initiating noxious stimulus is removed via phagocytosis, the inflammatory reaction can decrease and resolve. During the resolution of inflammation, granulocytes are eliminated, and macrophages and lymphocytes return to normal preinflammatory numbers and phenotypes. The usual outcome of the acute inflammatory program is successful resolution and repair of tissue damage, rather than persistence and dysfunction of the inflammatory response, which can lead to scarring and loss of organ function. It may be anticipated, therefore, that failure of acute inflammation to resolve may predispose to autoimmunity, chronic dysplastic inflammation, and excessive tissue damage.[1]

Prostaglandins (PGs) play a key role in the generation of the inflammatory response. Their biosynthesis is significantly increased in inflamed tissue, and they contribute to the development of the cardinal signs of acute inflammation. Although the proinflammatory properties of individual PGs during the acute inflammatory response are well established, their role in the resolution of inflammation is more controversial.

In this review, we discuss the biosynthesis of and response to PGs and the pharmacology of their blockade in orchestrating the inflammatory response, with particular regard to cardiovascular disease.

Biosynthesis of PGs

PGs and thromboxane A_2 (TXA_2), collectively termed prostanoids, are formed when arachidonic acid (AA), a 20-carbon unsaturated fatty acid, is released from the plasma membrane by phospholipases and metabolized by the sequential actions of PGG/H synthase or by cyclooxygenase (COX) and their respective synthases.

There are 4 principal bioactive PGs generated in vivo: prostaglandin E_2 (PGE_2), prostacyclin (PGI_2), prostaglandin D_2 (PGD_2), and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$).

They are ubiquitously produced—usually each cell type generates 1 or 2 dominant products—and act as autocrine and paracrine lipid mediators to maintain local homeostasis in the body. During an inflammatory response, both the level and the profile of PG production change dramatically. PG production is generally very low in uninflamed tissues but increases immediately in acute inflammation before the recruitment of leukocytes and the infiltration of immune cells.

PG production (Figure 1) depends on the activity of PGG/H synthases, colloquially known as COXs , bifunctional enzymes that contain both COX and peroxidase activity and that exist as distinct isoforms referred to as COX-1 and COX-2.[2]

COX-1, expressed constitutively in most cells, is the dominant source of prostanoids that subserve housekeeping

Received on: December 4, 2010; final version accepted on: December 31, 2010.
From the Institute for Translational Medicine and Therapeutics, University of Pennsylvania, Philadelphia, PA.
Correspondence to Garret A. FitzGerald, 153 Johnson Pavilion, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104. E-mail garret@upenn.edu

Arterioscler Thromb Vasc Biol **is available at http://atvb.ahajournals.org** **DOI: 10.1161/ATVBAHA.110.207449**

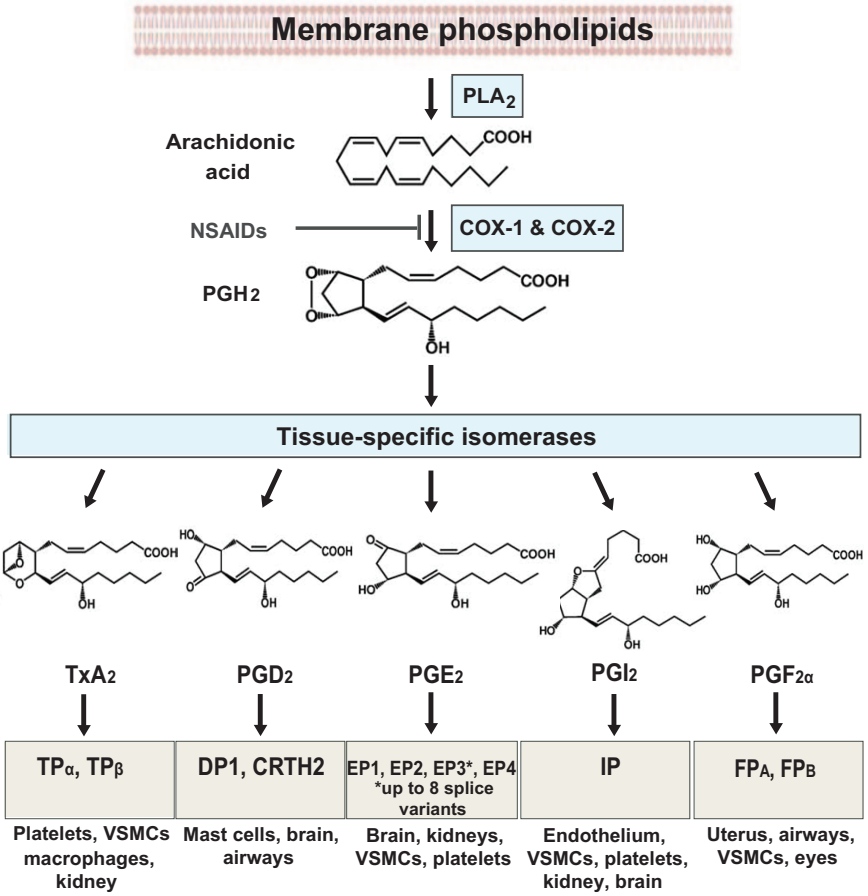

Figure 1. Biosynthetic pathway of prostanoids.

functions, such as gastric epithelial cytoprotection and homeostasis.[3] COX-2, induced by inflammatory stimuli, hormones, and growth factors, is the more important source of prostanoid formation in inflammation and in proliferative diseases, such as cancer.[3] However, both enzymes contribute to the generation of autoregulatory and homeostatic prostanoids, and both can contribute to prostanoid release during inflammation.

PGH_2 is produced by both COX isoforms, and it is the common substrate for a series of specific isomerase and synthase enzymes that produce PGE_2, PGI_2, PGD_2, $PGF_{2\alpha}$, and TXA_2. COX-1 couples preferentially, but not exclusively, with thromboxane synthase, PGF synthase, and the cytosol (c) PGE synthase (PGES) isozymes.[4] COX-2 prefers prostaglandin I synthase (PGIS) and the microsomal (m) PGES isozymes, both of which are often coinduced along with COX-2 by cytokines and tumor promoters.[4]

The profile of prostanoid production is determined by the differential expression of these enzymes within cells present at sites of inflammation. For example, mast cells predominantly generate PGD_2, whereas macrophages produce PGE_2 and TXA_2.[5] In addition, alterations in the profile of prostanoid synthesis can occur on cellular activation. Although resting macrophages produce TXA_2 in excess of PGE_2, this ratio changes to favor PGE_2 production after bacterial lipopolysaccharide (LPS) activation.[5]

PG Receptors

PGs exert their effects by activating rhodopsin-like 7-transmembrane-spanning G protein-coupled receptors (Table). The prostanoid receptor subfamily is composed of 8 members: E prostanoid receptor (EP) 1, EP2, EP3, and EP4 subtypes of the PGE receptor; PGD receptor (DP1); PGF receptor (FP); PGI receptor (IP); and thromboxane receptor (TP).[6] Two additional isoforms of the human TP (TP_α, TP_β) and FP (FP_A, FP_B) and 8 EP3 variants are generated through alternative splicing, which differ only in their C-terminal tails.[7] In addition, there is another G protein-coupled receptor termed chemoattractant receptor-homologous molecule ex-

Table. Signal Transduction of Prostanoid Receptors

Class	Subtype	G-Protein Coupled	Second Messenger
PGE_2	EP1	G_q	↑ IP_3
	EP2	G_s	↑ cAMP
	EP3	G_i, G_{12}, G_{Rho}	↓ cAMP, ↑ Ca^{2+}
	EP4	G_s	↑ cAMP
PGD_2	DP	G_s	↑ cAMP
	CRTH2	G_i	↓ cAMP, ↑ Ca^{2+}
$PGF_{2\alpha}$	FP_A, FP_B	G_q, G_{Rho}	↑ IP_3
PGI_2	IP-IP	G_s, G_q, G_i	↑ ↓ cAMP, ↑ IP_3
	IP-TP_α	G_s	↑ cAMP
TxA_2	TP_α,TP_β	G_q, G_s (α), G_i (β), G_h, $G_{12/13}$	↑ IP_3, ↑ ↓ cAMP, ↑ Ca^{2+}

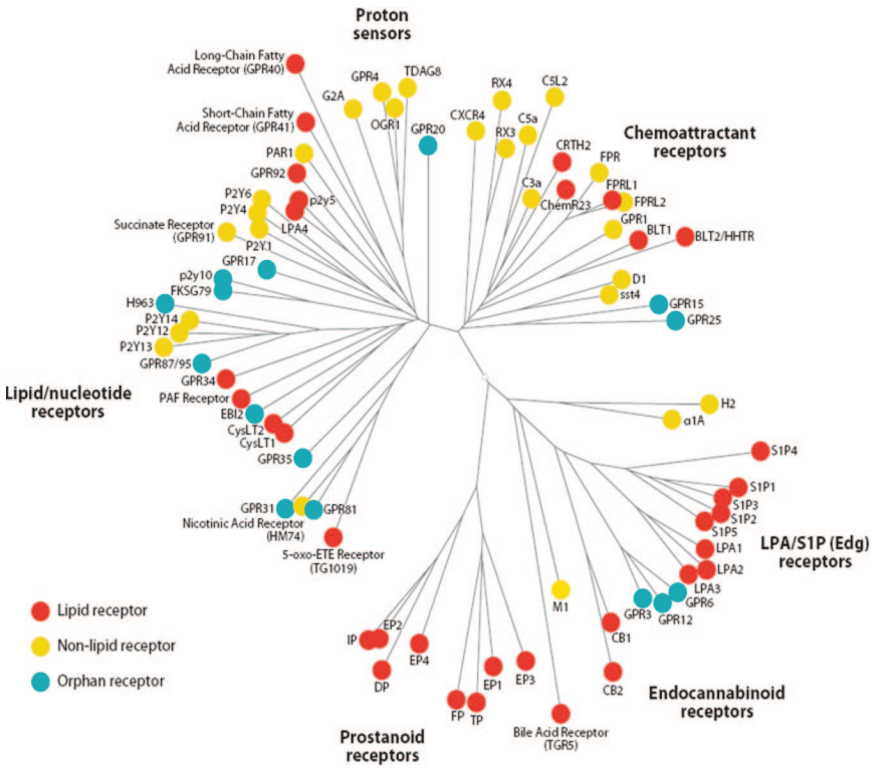

Figure 2. Phylogenetic tree of lipid G protein–coupled receptors. Figure modified with permission from Shimizu.[181]

pressed on T helper 2 cells (CRTH2 or DP2) that responds to PGD_2 but belongs to the family of chemokine receptors.[8] CRTH2 is a member of the N-formyl-methionyl-leucyl-phenylalanine chemoattractant receptor superfamily (Figure 2).

Prostanoid receptors couple to a range of intracellular signaling pathways that mediate the effects of receptor activation on cell function. EP2, EP4, IP, and DP1 receptors activate adenylyl cyclase via G_s, increasing intracellular cAMP. EP1 and FP activate phosphatidylinositol metabolism via G_q, leading to the formation of inositol trisphosphate with mobilization of intracellular free calcium. In addition to signaling through G_q, the FP receptor couples to the small G-protein Rho via a G_q-independent mechanism.[9] TP couples mainly to 2 types of G-proteins, the G_q (G_q, G_{11}, G_{15}, G_{16}) and the G_{13} (G_{12}, G_{13}) families, resulting in the activation of phospholipase C and guanine nucleotide exchange factor of the small G protein Rho, respectively. In addition, TP can also be coupled via G_h to phospholipase C, as well as via G_i and G_s to adenylate cyclase. Both TP isoforms are coupled to phospholipase C activation, but TPα stimulates adenylyl cyclase, whereas TPβ inhibits it. EP3 isoforms can couple via G_i or G_{12} to elevation of intracellular Ca^{2+}, inhibition of cAMP generation, and activation of the small G protein Rho.[10] The DP2/CRTH2 couples to a G_i-type G protein to inhibit cAMP synthesis and elevate intracellular Ca^{2+}. However, the effects of prostanoids on these G protein–coupled signaling pathways may change as a function of ligand concentration or structure.[6]

Some but not all prostanoid receptors exhibit the capacity to dimerize, which may alter ligand affinity or preference for downstream signaling pathways. Thus, although DP1/DP2 dimers appear not to form, under similar conditions, IP and TP receptors can associate to form homo- and heterodimers. TPα-TPβ heterodimers enhance the response to activation by free radical–catalyzed isoprostanes.[11] Furthermore, dimerization of IP and TPα enables cAMP formation through TP receptor activation, a cellular outcome typically observed with IP activity.[12] Moreover, EP1 receptor activation has been shown to modulate β2-adrenoreceptor function in bronchial airways via formation of a heterodimeric complex.[13]

COXs and Inflammation

The 2 COX isoforms, COX-1 and COX-2, are targets of nonsteroidal antiinflammatory drugs (NSAIDs). These drugs are competitive active site inhibitors of both COXs. Although both COXs exist as homodimers, only 1 partner is used at a time for substrate binding.[14] COX-1/COX-2 heterodimers may also exist, but their role in biology remains to be established.[15] NSAIDs bind to and inactivate the COX site at only 1 of the monomers of the COX dimer, and this is sufficient to shut down prostanoid formation.[14] The other monomer appears to play an allosteric function. The peroxidase capacity of both proteins is unaltered by NSAIDs.

The clinical efficacy of structurally distinct NSAIDs, all of which share this capacity for prostanoid inhibition, points to the importance of these mediators in the promotion of pain, fever, and inflammation.[16] The dramatic increase of COX-2 expression on provocation of inflammatory cells, its expression in inflamed tissues, and the assumption that inhibition of COX-1-derived prostanoids in platelets and gastric epithelium explain NSAID-evoked gastrointestinal adverse effects and provide a rationale for development of NSAIDs designed to be selective for inhibition of COX-2 for treating arthritis and other chronic inflammatory diseases.[17]

Although COX-2 appears to be the dominant source of PG formation in inflammation, there is some suggestion that both isoforms of the human enzyme may contribute to the acute inflammatory response. COX-1 is constitutively expressed in resident inflammatory cells, and there is evidence for induction of COX-1 during LPS-mediated inflammatory response and cellular differentiation.[18] Both COX isoforms are coexpressed in circulating inflammatory cells ex vivo and in both inflamed rheumatoid arthritis (RA) synovium and atherosclerotic plaques obtained from patients.[19,20] Human data are compatible with COX-1-derived products driving the initial phase of an acute inflammation, with COX-2 upregulation occurring within several hours.[4] However, controlled clinical trials to test the comparative efficacy of NSAIDs that inhibited both COXs versus COX-2 alone were never performed at scale. Such trials were designed to seek divergence in the incidence of gastrointestinal adverse effects rather than to assess comparative clinical efficacy.

Studies in both COX-1- and COX-2-knockout (KO) mice reveal impaired inflammatory responses, although the impacts of gene deletion diverge in time course and intensity.

Mice deficient in COX-1 but not COX-2 exhibit a reduction in AA-induced ear edema, although AA induces an equivalent inflammatory response in wild-type (WT) and COX-2-deficient mice.[21–23] By contrast, the level of edema induced by the tumor promoter tetradecanoyl phorbol acetate was not significantly different among WT, COX-1-deficient, and COX-2-deficient mice.[21–23] The ear inflammation studies indicate that COX-1, as well as COX-2, may contribute to inflammatory responses, and the isoform responsible for the inflammation may depend on the type of inflammatory stimulus or the relative levels of each isoform in the target tissue.

Similarly, arthritis models exhibit a significant reliance on either the COX-1 or COX-2 isoforms for the development of clinical synovitis. This varies, depending on the experimental model used. Thus, in the K/BxN serum–transfer model of arthritis, COX-1-derived PGs, in particular PGI_2, make a striking contribution to the initiation and perpetuation of arthritis.[24] In a collagen-induced arthritis model, COX-2 deletion considerably suppresses synovial inflammation and joint destruction, whereas arthritis in COX-1-deficient mice is indistinguishable from that of controls.[25,26]

COX-2 deletion also suppresses acute inflammation in the air pouch model. Here, the COX-2 inhibitor NS-398, administered 6 hours after carrageenan treatment, reduced PG production in WT mice to levels comparable to those seen in COX-2-KO mice and was also effective during the early stages of inflammation.[27] Compared with WT mice, the deficiency of COX-2 reduces the level of PGE_2 production by approximately 75%, whereas the deficiency of COX-1 reduces the PGE_2 level by 25% during this early stage. By day 7 following carrageenan treatment, higher numbers of inflammatory cells were present in the pouch fluid of COX-2-KO mice, and little resolution of inflammation was apparent compared with WT or COX-1-KO mice.[27] These findings indicate that both COX isoforms contribute to PG production during inflammation and also that COX-2-derived PGs appear to be important in both the acute inflammatory process and in the resolution phase. A contribution of COX-2 to both phases of inflammation was also reported in other models. Gilroy et al reported that COX-2 expression and PGE_2 levels increased transiently early in the course of carrageenan-induced pleurisy in rats.[28] Later in the response, COX-2 was induced again to even greater levels and generated antiinflammatory PGs, such as PGD_2 and 15-deoxy-Δ^{12-14}-PGJ_2 (15d-PGJ_2), but only low levels of the proinflammatory PGE_2. Further support for an antiinflammatory role of COX-2 in this model was the finding that late administration of the COX-2-selective inhibitor NS-398 exacerbates the inflammatory response. Furthermore, Wallace et al observed that in the paw carrageenan model, the resultant inflammation resolves within 7 days in WT mice but is unaltered over this period in COX-2-deficient mice.[29] An antiinflammatory role of COX-2-derived prostanoids has also been reported in models of inflammatory colitis and allergic airway disease.[30,31] Thus, COX-2 appears to have a dual role in the inflammatory process, initially contributing to the onset of inflammation and later helping to resolve the process. Although COX-2 does play a role in supporting resolution of this process in some models of inflammation, it is unclear which products of the enzyme might contribute in which settings to this effect. This is exemplified by the case of 15d-PGJ_2. Long touted as an endogenous ligand to peroxisome proliferator–activated receptor-γ (PPARγ), it remains to be established that endogenous concentration sufficient to subserve this function are formed in any model of resolving inflammation. Erroneously elevated levels have been reported using a variety of immunoassays, but the concentrations of bound and free compound documented to be formed by quantitative mass spectrometry fall far short of the EC_{50} for PPARγ activation.[32] It is one thing to show that putative proresolution products can be formed in vitro and that the synthetic compounds do exert proresolution actions when administered in vivo and another to document that the concentrations formed in vivo in the setting of inflammation are sufficient and necessary to mediate resolution.

In the case of atherosclerosis, deletion or inhibition of COX-2 has been shown variously to retard, accelerate, or leave unaltered atherogenesis in mouse models.[5] This may reflect an impact postnatally of disruption of the many roles of the enzyme in development in COX-2 KOs, differences in timing of interventions with COX-2 inhibitors, or a failure in most cases to define biochemically the selectivity for inhibition of COX-2 of the drug regimen deployed. In contrast, COX-1 deletion markedly attenuated lesion development in the apolipoprotein E–KO mouse, as does inhibition of COX-1 and COX-2 together in low-density lipoprotein receptor–KO model.[33,34] Thus, products of COX-1, such as TXA_2, promote atherogenesis, whereas there is more ambiguity around the role of COX-2. Nevertheless, deletion of the IP, the receptor for the major COX-2 product, PGI_2, fosters the initiation and early development of atherosclerosis in hyperlipidemic mice.[35]

PGE_2 and Inflammation

PGE_2 is one of the most abundant PGs produced in the body, is most widely characterized in animal species, and exhibits

versatile biological activities. Under physiological conditions, PGE_2 is an important mediator of many biological functions, such as regulation of immune responses, blood pressure, gastrointestinal integrity, and fertility. Dysregulated PGE_2 synthesis or degradation has been associated with a wide range of pathological conditions.[36] In inflammation, PGE_2 is of particular interest because it is involved in all processes leading to the classic signs of inflammation: redness, swelling, and pain.[37] Redness and edema result from increased blood flow into the inflamed tissue through PGE_2-mediated augmentation of arterial dilatation and increased microvascular permeability.[37] Pain results from the action of PGE_2 on peripheral sensory neurons and on central sites within the spinal cord and the brain.[37]

PGE_2 is synthesized from PGH_2 by cPGES or mPGES-1 and mPGES-2.[38] cPGES is constitutively and abundantly expressed in the cytosol of various tissues and cells and it requires glutathione as a cofactor.[39] The role of cPGES and even its ability to form PGE_2 is controversial. cPGES seems capable of converting COX-1-derived but not COX-2-derived PGH_2 to PGE_2 in cells, particularly during the immediate PGE_2-biosynthetic response elicited by Ca^{2+} evoked stimuli. Localization of cPGES in the cytosol may allow coupling with proximal COX-1 in the endoplasmic reticulum in preference to distal COX-2 in the perinuclear envelope.[39] Functional coupling of cPGES with COX-1 suggests that the functions of cPGES in vivo overlap significantly, if not entirely, with COX-1. cPGES-deficient mice were developed, but they have not been particularly informative in addressing the importance of cPGES-derived PGE_2, because deletion of this enzyme results in perinatal lethality.[40]

mPGES-1 is a member of the membrane-associated proteins involved in eicosanoid and glutathione metabolism superfamily, and like cPGES, it requires glutathione as cofactor.[41] mPGES-1 is a perinuclear protein that is markedly induced by cytokines and growth factors and downregulated by antiinflammatory glucocorticoids, as in the case of COX-2.[42,43] It is functionally coupled with COX-2 in marked preference to COX-1.[44] Constitutive expression of mPGES-1 in certain tissues and cell types was also reported.

The generation of mPGES-1-deficient mice has revealed the dominant role of this enzyme in PGE_2 generation relevant to promotion of inflammation. In collagen-induced arthritis, a disease model of human RA, mPGES-1-null mice exhibited a reduced incidence and severity of disease compared with WT controls.[45] This difference was not associated with alterations in interleukin (IL)-6 production by peritoneal macrophages or significant differences in circulating IgG2a anticollagen antibodies. Likewise, in collagen antibody–induced arthritis, another model of RA that does not involve the activation of the immune system, mPGES-1-null mice had a similar incidence but a lesser severity of arthritis than WT mice, as well as a 50% reduction in paw levels of PGE_2.[46] In the same study, it was also observed that the migration of macrophages following peritoneal injection of thioglycollate was strikingly reduced in mPGES-1-null mice relative to WT mice.[46]

The formation of inflammatory granulation tissue and attendant angiogenesis in the dorsum induced by subcutaneous implantation on the paw of a cotton thread was significantly reduced in mPGES-1-KO mice as compared with WT mice.[46] In this model, mPGES-1 deficiency was also associated with reduced induction of vascular endothelial cell growth factor in the granulation tissue. These results indicate that mPGES-1-derived PGE_2, in cooperation with vascular endothelial growth factor, may play a critical role in the development of inflammatory granulation and angiogenesis, thus eventually contributing to tissue remodeling.

Together, these findings illustrate that deletion or inhibition of mPGES-1 markedly reduces inflammatory response, in several mouse models. The proinflammatory effect of mPGES-1-derived PGE_2 was also observed in atherosclerosis. Deletion of mPGES-1 retarded atherogenesis in fat-fed hyperlipidemic mice, in both sexes.[47] Interestingly, in addition to the expected depression of PGE_2 production, deletion of mPGES-1 permits rediversion of the PGH_2 substrate to other PG synthases, with, for example, augmented formation of PGI_2 and PGD_2.[47] This complicates the selection of mPGES-1 as a drug target. Thus, elevated PGI_2 may contribute to the more benign cardiovascular profile of mPGES-1 deletion compared with COX-2 deletion or inhibition: less predisposition to hypertension and thrombogenesis. However, this same effect may attenuate relief of pain, in the case of augmented PGI_2, or may mediate adverse effects on bronchial tone, allergic inflammatory disease, or the sleep-wakefulness cycle, in case of elevated PGD_2. This may account for the less impressive effectiveness of disrupting mPGES-1 versus COX-2 in some models of pain.[48] Recently, Brenneis et al have provided evidence that mPGES-1 derived PGE_2 may contribute both to promotion and resolution of neuroinflammation in mice.[49] Finally, the major substrate products of rediversion will be influenced by the dominant cell type in a particular setting. For example, augmented PGI_2 may contribute to the restraint of atherogenesis in hyperlipidemic mice consequent to mPGES-1 deletion.[47] However, it remains to be seen whether mPGES-1 inhibition in the setting of established atherosclerosis causes regression or possibly accelerates further progression of disease because of endoperoxide rediversion to TXA_2 in macrophage-rich plaques.

mPGES-2 is synthesized as a Golgi membrane–associated protein, and the proteolytic removal of the N-terminal hydrophobic domain leads to the formation of a mature cytosolic enzyme. This enzyme is constitutively expressed in various cells and tissues and is functionally coupled with both COX-1 and COX-2.[37] mPGES-2-deficient mice showed no specific phenotype and no alteration in PGE_2 levels in several tissues or in LPS-stimulated macrophages.[50] Studies with PGES-null mice have revealed that cross-regulation between the different PGES isoforms may function, on occasion, as a compensatory mechanism. For example, mPGES-1-null mice exhibit a delayed increase in urinary PGE_2 excretion in response to acute water loading, coincident with enhanced renal medullary expression of cPGES but not of mPGES-2.[51] Similar evidence suggestive of cross-regulation was observed with the COXs. Thus, deletion of COX-2 in macrophages is associated with upregulated expression of COX-2 in vascular smooth muscle cells (VSMCs) in atherosclerotic plaque.[52]

After PGE_2 is formed, it is actively transported through the membrane by the ATP-dependent multidrug resistance

protein-4 or diffuses across the plasma membrane to act at or near its site of secretion.[53]

PGE_2 then acts locally through binding of 1 or more of its 4 cognate receptors (Table), termed EP1–EP4.[45] Among the 4 EPs, EP3 and EP4 receptors are the most widely distributed, with their mRNAs being expressed in almost all mouse tissues, and have the highest affinity for binding PGE_2. In contrast, the distribution of EP1 mRNA is restricted to several organs, such as the kidney, lung, and stomach, and EP2 is the least abundant of the EP receptors. Both EP1 and EP2 bind PGE_2 with lower affinity.[54] Each EP subtype shows a distinct cellular localization within tissues.[54]

One of the lessons learned from the KO mouse studies is that PGE_2 can exert both proinflammatory and antiinflammatory responses, and these actions are often produced through regulation of receptor gene expression in relevant tissues. For example, hyperalgesia, a classic sign of inflammation, is mediated mainly by PGE_2 through EP1 receptor signaling that acts on peripheral sensory neurons at the site of inflammation, as well as on central neuronal sites.[55] Other studies have also implicated the EP3 receptor in the inflammatory pain response mediated by low doses of PGE_2.[56]

EP2 and EP4 redundantly mediate development of paw swelling associated with collagen-induced arthritis.[57] Likewise, studies of carrageenan-induced paw edema and carrageenan-induced pleurisy both revealed participation of EP2 and EP3 in inflammatory exudation.[58] The EP4 receptor also appears to play a proinflammatory role in the pathogenesis of RA. PGE_2 produced by rheumatoid synovium has been implicated in IL-6 production and joint destruction.[59,60] Mice deficient in the EP4 but not in the EP1, EP2, or EP3 receptors exhibit an attenuated response in the collagen antibody–induced arthritis model, with significantly lower levels of the inflammatory cytokines IL-6 and IL-1 and a dramatic reduction in the clinical signs of disease.[61] Antiinflammatory actions of PGs are seen typically in allergic or immune inflammation and are usually balanced by proinflammatory actions of other PGs. Such contrasting biology is evident between the PGI_2-IP and TXA_2-TP pathways in cardiovascular disease and between the PGD_2-DP and the PGE_2-EP3 pathways in elicitation of allergic asthma.[62,63]

PGE_2, binding to different EP receptors, can regulate the function of many cell types including macrophages, dendritic cells (DCs) and T and B lymphocytes, leading to both pro- and antiinflammatory effects. As proinflammatory mediator, PGE_2 contributes to the regulation of the cytokine expression profile of DCs and has been reported to bias T cell differentiation toward a T helper (Th) 1 or Th2 response.[35] A recent study showed that PGE_2-EP4 signaling in DCs and T cells facilitates Th1 and IL-23-dependent Th17 differentiation.[64] In addition, PGE_2 is fundamental to induction of a migratory DC phenotype permitting their homing to draining lymph nodes.[65,66] Simultaneously, PGE_2 stimulation early during maturation induces the expression of costimulatory molecules of the tumor necrosis factor superfamily on DCs resulting in an enhanced T-cell activation.[67] In contrast, PGE_2 has also been demonstrated to suppress Th1 differentiation, B-cell functions, and allergic reactions.[68] Moreover, PGE_2 can exert antiinflammatory actions on innate immune cells, such as neutrophils, monocytes, and natural killer cells.[68]

PGE_2 can thus modulate various steps of inflammation in a context-dependent manner and coordinate the whole process in both proinflammatory and antiinflammatory directions.

This dual role of PGE_2 and its receptors in modulating the inflammatory response has been observed in several disorders. In atherosclerosis, EP4 deficiency promotes macrophage apoptosis and suppresses early atherosclerosis in low-density lipoprotein receptor$^{-/-}$ mice chimeric for EP4$^{-/-}$ in hematopoietic cells after 8 weeks on a Western diet.[69] EP2 deficiency in hematopoietic cells revealed a trend for similar, but modest, effects on atherosclerosis.[69] In the same study macrophage EP4 appeared to play a proinflammatory role in the early stages of atherosclerosis by regulating production of inflammatory cytokines ,such as IL-1β, IL-6, and monocyte chemotactic protein-1.[69] In contrast, EP4 deletion in bone marrow-derived cells enhanced local inflammation (increased expression of chemotactic proteins, including monocyte chemotactic protein-1 and IL-10, and increased inflammatory cells, such as macrophages and T cells) and altered lesion composition (increased smooth muscle cells within plaque) but did not alter plaque size or morphology in established atherosclerosis (after 10 weeks of high-fat diet).[70]

PGE_2 also plays contrasting roles during neuroinflammation. LPS-induced PGE_2 synthesis causes deleterious effects in neurons resulting in lesions or enhanced pain transmission.[71–73]

However, PGE_2 also has antiinflammatory properties. It mediates bradykinin-induced neuroprotection and blocks LPS and ATP-induced cytokine synthesis in cultured microglia and in neuron-glia cocultures.[74,75] The antiinflammatory and neuroprotective effects of PGE_2 are mediated via microglial EP2 and EP4 receptors. Recently, it has been reported that PGE_2 limits cytokine and PG synthesis mainly through EP2 activation in a model of LPS-induced neuroinflammation and that mPGES-1 is a critical enzyme in this negative feedback regulation.[49]

PGI_2 and Inflammation

PGI_2 is one of the most important prostanoids that regulates cardiovascular homeostasis. Vascular cells, including endothelial cells, VSMCs, and endothelial progenitor cells, are the major source of PGI_2.[76]

PGI_2 is generated by the sequential action of COX and PGIS, a member of the cytochrome P450 superfamily that specifically converts PGH_2 to PGI_2. PGIS colocalizes with COX in the endoplasmic reticulum, plasma membrane, and nuclear membrane.[77] PGIS is constitutively expressed in endothelial cells, where it couples with COX-1,[78] although COX-2-dependent PGI_2 production by endothelial cells has been reported to be modulated in vitro by thrombin, shear stress, oxidized low-density lipoprotein, hypoxia, and inflammatory cytokines, and it is synchronized by upregulation of COX-2.[79,80] In vivo studies in mice and humans showed that COX-2 was the dominant source of PGI_2.[81]

Once generated, PGI_2 is released to act on neighboring VSMCs, as well as circulating platelets. Indeed, PGI_2 exerts

its effects locally, is not stored, and is rapidly converted by nonenzymatic processes to an inactive hydrolysis product, 6-keto-$PGF_{1\alpha}$.[82]

PGI_2 is a potent vasodilator and an inhibitor of platelet aggregation, leukocyte adhesion, and VSMC proliferation.[75] PGI_2 is also antimitogenic and inhibits DNA synthesis in VSMC.[83]

These actions of PGI_2 are mediated through specific IP receptors (Table). This receptor is expressed in kidney, liver, lung, platelets, heart, and aorta.[84] There is inconclusive evidence that some effects of PGI_2 on the vasculature might be mediated by the PPARδ pathway, in addition to the classical IP-cAMP signaling pathway.[85] PGI_2 can indeed activate PPARδ; however, just as with 15d-PGD_2 and PPARγ, it is unclear that it represents a biological target at concentrations of the ligand attained in vivo.[86]

Although IP-deficient mice mature normally without experiencing spontaneous thrombosis, both the response to thrombogenic stimuli and VSMC proliferation in response to vascular injury are enhanced compared with control littermates.[87] Mice lacking IP are also sensitive to dietary salt induced hypertension[88] and exhibit accelerated atherogenesis with enhanced platelet activation and increased adhesion of leukocytes on the vessel walls in both the low-density lipoprotein receptor and apolipoprotein E KO models.[35,89]

In addition to its cardiovascular effects, PGI_2 is an important mediator of the edema and pain that accompany acute inflammation. PGI_2 is rapidly produced following tissue injury or inflammation and it is present at high concentrations in inflammatory milieus.[90] PGI_2 is the most abundant prostanoid in synovial fluid in human arthritic knee joints, as well as in peritoneal cavity fluid from mice injected with irritants.[91,92] In IP receptor–deficient mice, potentiation of bradykinin-induced microvascular permeability by PGI_2 is abolished; in addition, these mice have substantially reduced carrageenan-induced paw edema.[93] The level of paw edema observed in IP-deficient mice was equivalent to that of indomethacin-treated controls, and indomethacin treatment of IP-deficient animals did not induce a further decrease in swelling, indicating that PGI_2-IP receptor signaling is the major prostanoid pathway that mediates the acute inflammatory response in this model. It has also been suggested that bradykinin induces PGI_2 formation leading to enhancement of microvascular permeability and edema.[94] The IP receptor has been shown to mediate nociceptive pain during acute inflammation. IP receptor mRNA is present in dorsal root ganglion neurons including those that express substance P, a marker for nociceptive sensory neurons.[95] IP receptor-deficient mice have an attenuated writhing response following intraperitoneal injection of either acetic acid or PGI_2, indicating that the IP receptor plays a role in mediating peripheral nociceptive sensitization to inflammatory stimuli.[93] The IP receptor is also expressed in the spinal cord and has been implicated in spinal pain transmission in response to peripheral inflammation.[96] IP antagonists were shown to reduce pain responses in several models, including acetic acid–induced abdominal constriction, mechanical hyperalgesia produced by carrageenan, and pain associated with models of osteoarthritis and inflammatory arthritis.[97,98] In contrast to the proinflammatory effects of IP receptor activation in nonallergic acute inflammation, some studies have suggested that IP receptor signaling suppresses Th2-mediated allergic inflammatory responses.[99] IP receptor mRNA is upregulated in CD4+ Th2 cells, and inhibition of PGI_2 formation by the COX-2 inhibitor NS-398 during antigen-induced airway inflammation results in greater lung Th2-mediated lung inflammation.[99] PGI_2 has been suggested to exert this effect in part by enhancing Th2 cell production of the antiinflammatory cytokine IL-10. This immunosuppressive role for the IP receptor in Th2-mediated inflammation is supported by the observation that in ovalbumin (OVA)-induced asthma, IP deletion results in increased antigen-induced leukocyte accumulation in bronchoalveolar lavage fluid and peribronchiolar and perivascular inflammatory infiltration.[100] Thus, PGI_2 may shift the balance within the immune system away from a Th2 dominant response and inhibit allergic inflammation.

PGD_2 and Inflammation

PGD_2 is a major eicosanoid that is synthesized in both the central nervous system and peripheral tissues and appears to function in both an inflammatory and homeostatic capacity.[101] In the brain, PGD_2 is involved in the regulation of sleep and other central nervous system activities, including pain perception.[102,103] In peripheral tissues, PGD_2 is produced mainly by mast cells but also by other leukocytes, such as DCs and Th_2 cells.[104–106] Two genetically distinct PGD_2-synthesizing enzymes have been identified, including hematopoietic- and lipocalin-type PGD synthases (H-PGDS and L-PGDS, respectively). H-PGDS is generally localized to the cytosol of immune and inflammatory cells, whereas L-PGDS is more restrained to tissue-based expression.[107]

PGD_2 can be further metabolized to $PGF_{2\alpha}$, 9α,11β-PGF_2 (the stereoisomer of $PGF_{2\alpha}$) and the J series of cyclopentanone PGs, including PGJ_2, Δ^{12}-PGJ_2, and 15d-PGJ_2.[108] Synthesis of J series PGs involves PGD_2 undergoing an initial dehydration reaction to produce PGJ_2 and 15d-PGJ_2, after which PGJ_2 is converted to 15d-PGJ_2 and Δ^{12}-PGJ_2 via albumin-dependent and albumin-independent reactions, respectively.[109]

PGD_2 activity is principally mediated through DP or DP1 and CRTH2 or DP2, as described previously (Table). Also, 15d-PGJ_2 binds with low affinity the nuclear PPARγ.[110]

PGD_2 has long been associated with inflammatory and atopic conditions, although it might exert an array of immunologically relevant antiinflammatory functions as well.

PGD_2 is the predominant prostanoid produced by activated mast cells, which initiate IgE-mediated type I acute allergic responses.[104,111] It is well established that the presence of an allergen triggers the production of PGD_2 in sensitized individuals. In asthmatics, PGD_2, which can be detected in the bronchoalveolar lavage fluid within minutes, reaches biologically active levels at least 150-fold higher than preallergen levels.[112] PGD_2 is produced also by other immune cells, such as antigen-presenting DCs and Th_2 cells, suggesting a modulatory role for PGD_2 in the development of antigen-specific immune system responses.[104,105] PGD_2 challenge elicits several hallmarks of allergic asthma, such as bronchoconstriction and airway eosinophil infiltration,[113,114] and mice that over-

express L-PGDS have elevated PGD_2 levels and an increased allergic response in the OVA-induced model of airway hyperreactivity.[115]

The proinflammatory effects of PGD_2 appear to be mediated by both DP1 and DP2/CRTH2 receptors. Because both receptors bind PGD_2 with similar high affinity, PGD_2 produced by activated mast cells or T cells would be capable of activating multiple signaling pathways leading to different effects, depending on whether the DP1 or DP2/CRTH2 receptors or both are locally expressed.

The DP1 receptor is expressed on bronchial epithelium and has been proposed to mediate production of chemokines and cytokines that recruit inflammatory lymphocytes and eosinophils, leading to airway inflammation and hyperreactivity seen in asthma.[116] Mice deficient in DP1 exhibit reduced airway hypersensitivity and Th2-mediated lung inflammation in the OVA-induced asthma model, suggesting that the DP1 plays a key role in mediating the effects of PGD_2 released by mast cells during an asthmatic response.[62] Furthermore, PGD_2 may inhibit eosinophil apoptosis via the DP1 receptor.[117]

DP1 antagonists exert antiinflammatory properties in several experimental models, including inhibition of antigen-induced conjunctival microvascular permeability in guinea pigs and OVA-induced airway hyperreactivity in mice.[118,119]

DP2/CRTH2 receptors contribute largely to pathogenic responses by mediating inflammatory cell trafficking and by modulating effector functions. PGD_2 released from mast cells may mediate recruitment of Th2 lymphocytes and eosinophils directly via the DP2/CRTH2 receptor. In humans, the DP2/CRTH2 receptor is expressed on Th2 lymphocytes, eosinophils, and basophils,[8,120,121] and an increase in DP2/CRTH2$^+$ T cells has been positively associated with certain forms of atopic dermatitis.[122] The DP2/CRTH2 receptor has been demonstrated to mediate PGD_2-stimulated chemotaxis of these cells in vitro and leukocyte mobilization in vivo.[123]

In contrast to the proinflammatory role of PGD_2 in allergic inflammation, PGD_2 may act to inhibit inflammation in other contexts. The DP1 receptor is expressed on DCs that play a key role in initiating an adaptive immune response to foreign antigens. PGD_2 activation of the DP1 receptor inhibits DC migration from lung to lymph nodes following OVA challenge, leading to reduced proliferation and cytokine production by antigen specific T cells.[124] DP1 activation also reduces eosinophilia in allergic inflammation in mice and mediates inhibition of antigen-presenting Langerhans cell function by PGD_2.[125,126] As mentioned, PGD_2 and its degradation product 15d-PGJ_2 have been suggested as the COX-2 products involved in the resolution of inflammation.[28,127] Administration of a COX-2 inhibitor during the resolution phase exacerbated inflammation in a carrageenan-induced pleurisy model [28]. In a zymosan-induced peritonitis model, deletion of H-PGDS induced a more aggressive inflammatory response and compromised resolution, findings that were moderated by addition of a DP1 agonist or 15d-PGJ_2.[123] Although these data appear to implicate PGD_2 and 15d-PGJ_2 in resolution, there is a large disparity between the nanomolar to picomolar amounts of 15d-PGJ_2 detected by physicochemical methodology in in vivo settings and the amount needed to have a biological effect in vitro on PPARγ or nuclear factor-κB (micromolar amounts).[32,128,129] This discrepancy is supported by recent data reported in zymosan-induced peritonitis, where we observed evoked biosynthesis of PGD_2 only during the proinflammatory phase and not during resolution. Consistent with this observation, DP2/CRTH2 deletion reduced the severity of acute inflammation, but neither DP1 or DP2/CRTH2 deletion affected resolution. Although 15d-PGJ_2 is readily detected when synthetic PGD_2 is infused into rodents,[130] endogenous biosynthesis of 15d-PGJ_2 was not detected during promotion or resolution of inflammation (J. Mehta et al, unpublished data, 2010).

PGD_2 may play a role in the evolution of atherosclerosis. In the context of inflamed intima, PGD_2 in part derives from H-PGDS-producing inflammatory cells that are chemotactically compelled to permeate the vasculature.[131,132] Additionally, L-PGDS expression is induced by laminar sheer stress in vascular endothelial cells and is actively expressed in synthetic smooth muscle cells of atherosclerotic intima and coronary plaques of arteries with severe stenosis.[133–135] PGD_2 has been shown to inhibit expression of proinflammatory genes, such as inducible nitric oxide synthase and plasminogen activator inhibitor.[136,137] Indeed, L-PGDS deficiency accelerates atherogenesis.[138]

In summary, studies with COX-2 inhibitors suggest that products of this enzyme may play a role in resolution in several models of inflammation. However, the identity of such products, whether formed directly by COX-2 or because of substrate diversion consequent to COX-2 inhibition, remains, in many cases, to be established.

$PGF_{2\alpha}$ and Inflammation

$PGF_{2\alpha}$ is synthesized from PGH_2 via PGF synthase, and it acts via the FP, which couples with G_q protein to elevate the intracellular free calcium concentration (Table). Two differentially spliced variants of the sheep FP receptor ortholog have been reported: FP_A and FP_B, which differ from each other in the length of their C-terminal tails.[139] The FP receptor is the least selective of the prostanoid receptors in binding the principal endogenous PGs; both PGD_2 and PGE_2 ligate the FP with EC_{50} values in the nanomolar range.[140] PGF ring compounds can be formed as minor products from other PGs. For example, enzymatic reduction of 9-keto group of PGE compounds by 9-ketoreductases results in either 9a-hydroxyl, yielding PGF_α compounds, or more rarely a 9b-hydroxyl, yielding PGF_β compounds.[141] PGF ring metabolites may also be formed from PGD ring compounds by 11-keto reductases.[142]

15-Keto-dihydro-$PGF_{2\alpha}$, a major stable metabolite of $PGF_{2\alpha}$ that reflects in vivo $PGF_{2\alpha}$ biosynthesis, is found in larger quantities first in the peripheral plasma and later on in the urine both in basal physiological conditions and in certain physiological and pathophysiological situations, such as acute and chronic inflammation.[143]

$PGF_{2\alpha}$, derived mainly from COX-1 in the female reproductive system, plays an important role in ovulation, luteolysis, contraction of uterine smooth muscle, and initiation of parturition.[144,145] Recent studies have shown that $PGF_{2\alpha}$ also plays a significant role in renal function,[146] contraction of

arteries,[147] myocardial dysfunction,[148,149] brain injury,[150] and pain.[151] Analogs of $PGF_{2\alpha}$ have previously been developed for estrus synchronization and abortion in domestic animals[152,153] and to influence human reproductive function.[154] FP agonists are being widely used worldwide to reduce intraocular pressure in the treatment of glaucoma.[155]

Administration of $PGF_{2\alpha}$ leads to acute inflammation, and NSAIDs inhibit $PGF_{2\alpha}$ biosynthesis both in vitro and in vivo.[144] In models of acute inflammation, evoked biosynthesis of $PGF_{2\alpha}$ may coincide with free radical catalyzed generation of F ring isoprostanes, indices of lipid peroxidation.[156,157] The tachycardia induced in WT mice by injection of LPS is greatly attenuated in FP-deficient or TP-deficient mice and is completely absent in mice lacking both of these receptors.[148] A recent study reported that deletion of FP selectively attenuates pulmonary fibrosis without a change in alveolar inflammation after microbial invasion.[158]

Elevated biosynthesis of $PGF_{2\alpha}$ has been reported in patients experiencing RA, psoriatic arthritis, reactive arthritis, and osteoarthritis.[159]

Cardiovascular risk factors, such as diabetes, obesity, smoking, and thickening of intima-media ratio in the carotid artery, have been variably associated with elevations in $PGF_{2\alpha}$ metabolites, together with IL-6 and acute phase proteins in body fluids.[160,161] Deletion of the FP reduces blood pressure and retards the attendant atherogenesis in hyperlipidemic mice despite the absence of detectable FP in large blood vessels and their atherosclerotic plaques.[162] $PGF_{2\alpha}$ is the most abundant prostanoid formed by human umbilical cord endothelial cells in response to laminar shear stress that upregulates expression of COX-2.[163]

The emerging role of $PGF_{2\alpha}$ in acute and chronic inflammation opens opportunities for the design of new antiinflammatory drugs.

Thromboxane and Inflammation

TXA_2, an unstable AA metabolite with a half-life of about 30 seconds, is synthesized from PGH_2 via thromboxane synthase, and it is nonenzymatically degraded into biologically inactive TXB_2. TXA_2 is predominantly derived from platelet COX-1, but it can also be produced by other cell types, including macrophage COX-2.[164,165]

TXA_2 activity is principally mediated through the TP, which couples with G_q, $G_{12/13}$, and multiple small G proteins, which in turn regulate several effectors, including phospholipase C, small G protein Rho, and adenylyl cyclase (Table).[166] TPα and TPβ, 2 spliced isoforms of TP in humans, communicate with different G proteins and undergo heterodimerization, resulting in changes in intracellular traffic and receptor protein conformations. Only the TPα protein is expressed in mice.

TP activation mediates several physiological and pathophysiological responses, including platelet adhesion and aggregation, smooth muscle contraction and proliferation, and activation of endothelial inflammatory responses.[167] TP function is regulated by several factors, such as oligomerization, desensitization and internalization, glycosylation, and crosstalk with receptor tyrosine kinases.[167]

Although TXA_2 is the preferential physiological ligand of the TP receptor, PGH_2, in particular, can also activate this receptor.[168] In addition, isoprostanes (nonenzymatic free radical-catalyzed peroxidative products of polyunsaturated fatty acids) and hydroxyeicosatetraenoic acids (generated by lipoxygenases and cytochrome P450 monooxygenases or formed by nonenzymatic lipid peroxidation) are also potent agonists at TP receptors.[169,170] Epoxyeicosatrienoic acid (cytochrome P450 metabolites of AA) dihydro derivatives, in contrast, are selective endogenous antagonists of TP.[171] However, whether PGH_2, isoprostanes, or hydroxyeicosatetraenoic acids significantly contribute to the responses attributed to TP activation in vivo is still to be investigated. For example, TP activation by isoprostanes may play an important role in clinical settings of oxidative stress, such as during reperfusion after organ transplant.

TP-deficient mice are normotensive but have blunted vascular responses to TP agonists and show a tendency to bleeding.[172]

The deletion of TP decreases vascular proliferation and platelet activation in response to vascular injury, delays atherogenesis, and prevents angiotensin II– and L-NAME-induced hypertension and the associated cardiac hypertrophy.[87,89,173,174]

In septic shock models, TP deletion or TP antagonism protected against various LPS-induced responses, such as the increase in inducible nitric oxide synthase expression, acute renal failure, and inflammatory tachycardia,[175,176] suggesting a potential role of TXA_2 as proinflammatory mediator.

The phenotype of thromboxane synthase–deficient mice is much less pronounced, perhaps because TXA_2 is only 1 of the endogenous ligands of TP and more likely because the deletion of this enzyme may redirect the PGH_2 toward other countervailing synthases.[177]

Prostanoids in Translation

This review has described a stunning complexity of evidence about the role of prostanoids in inflammation. Different products have conflicting effects on both the promotion and resolution of inflammation. The same product formed by different enzymes—COX-1 or COX-2—may either promote or resolve inflammation. Products of the same enzyme may promote or resolve inflammation in different models. Different cell types that predominate at varying stages of disease evolution generate prostanoids that have contrasting effects on inflammation. Individual prostanoids overlap considerably in their biological effects with other mediators.

These observations prompt several questions.

First, given this complex array of biological effects mediated by prostanoids, how does their general inhibition, high up in the cascade, result in drugs that are reasonably well tolerated and reasonably effective? Aspirin and the myriad NSAIDs, including acetaminophen and those developed to inhibit selectively COX-2, are among the most commonly consumed drugs on the planet. Hard evidence with which to address this question is in short supply, but let us speculate. Aspirin at doses less than 100 mg per day or greater than 1 g per day have equivalent effects on platelet COX-1-derived TXA_2. They both suppress it completely. However, increas-

ing daily doses of aspirin increasingly inhibit PGI_2 coincidentally with this effect. We do not have direct randomized comparisons across doses, but indirect comparisons suggest that the cardioprotective efficacy of aspirin may be progressively attenuated as the dose is increased. Similarly, locally formed, COX-1-derived PGE_2 and PGI_2 are protective of gastroduodenal epithelial integrity, and platelet COX-1-derived TXA_2 contributes to hemostatic competence. Disruption of these pathways by NSAIDs that inhibit COX-1 is thought to account for NSAID induced ulcers. However, NSAIDs selective for inhibition of COX-2 only halve the comparative incidence of serious gastrointestinal events. This may in part reflect their impact on gastroduodenal epithelial COX-2-dependent prostanoids that accelerate ulcer healing. In these examples, inhibitors high up in the pathway confer benefit, but it is a net benefit, and of course the margin of that benefit may vary substantially between individuals. We only poorly understand interindividual differences in antiinflammatory efficacy among the reversibly acting NSAIDs.

Prostanoids tend to be relatively weak agonists in systems where their blockade has resulted in clinical efficacy. For example, TXA_2 is a relatively weak platelet agonist compared with thrombin, and there is a considerable amount of redundancy in the system.

Why does blockade of just 1 of the many pathways of platelet activation result in an effect so great that its impact can be detected by as crude and instrument as a randomized clinical trial? Similarly, why does blockade of sulfidopeptide leukotrienes alone among many bronchoconstrictors result in clinical efficacy in asthma, or why do other mediators, such as NO, not substitute for the cardioprotective effects of PGI_2, suppressed by NSAIDs selective for inhibition of COX-2? Perhaps drug efficacy is realized because eicosanoids often tend to function as amplifying signals for other, more potent agonists. Activate platelets with thrombin, ADP, or collagen and release of TXA_2 amplifies and sustains the aggregation response. Perhaps this is why aspirin is so effective in the secondary prevention of myocardial infarction or stroke. As for why other mediators do not step in to substitute for suppressed prostanoids, this may speak to their singular importance in circumstances of phenotypic perturbations, as discussed next.

Given the myriad biological effects of these compounds, how are drugs that shut down their synthesis even tolerated? NSAIDs may indeed result in life-threatening gastrointestinal or cardiovascular adverse events, but only in a small minority (perhaps 1% to 2%) of patients exposed. This may reflect the fact that prostanoid formation is a homeostatic response system. Under physiological conditions, trivial amounts of these compounds are formed, and their biological importance is, in many cases, marginal. However, when a system is stressed, they may become pivotal. Examples include their essential role in the maintenance of renal blood flow under renoprival conditions, their antihypertensive effects in patients infused with vasopressors, or their antithrombotic effects in patients at increased risk of thrombogenesis. For example, deletion of the IP does not result in spontaneous thrombosis but rather accentuates the response to thrombogeneic stimuli. Similarly, although preexisting cardiovascular disease was often used to dilute the legal liability of the sponsor in cases where patients experienced myocardial infarctions while taking coxibs, the relative risk of myocardial infarctions on celecoxib relates to the underlying burden of cardiovascular disease, an expected consequence of suppression of COX-2 derived PGI_2.[164]

Given the contrasting effects of prostanoids, should we move down the pathway to get a more targeted and safer response? Presently, we have almost no data that address this question. In the case of downstream PG synthase versus COX-2 inhibition, the experience with mPGES-1 deletion highlights the complexity of the comparison. Here, substrate rediversion to PGIS may attenuate the cardiovascular risk of COX-2 inhibition but dilute analgesic efficacy. Another example is the comparison between low-dose aspirin for cardioprotection and TP antagonism. The latter strategy avoids PGI_2 suppression, but this is very modest, on average $\approx$15%, with low-dose aspirin. One would need an enormous clinical trial to detect that theoretical benefit. Alternatively, the antagonist, unlike aspirin, might block TP activation by unconventional ligands, such as isoprostanes and hydroxyeicosatetraenoic acids. However, although these compounds can activate the TP, their relevance in vivo, even in settings of tissue reperfusion where they are formed in excess, remains speculative. One might of course model this comparison and use COX-1 knockdown mice, which mimic the asymmetrical impact on platelet COX-1 of low-dose aspirin, to address the question. Finally, as the overlap in the biological consequences of activating several prostanoid receptors—the IP, EP2, EP4, and DP1 group or the FP, TP, and EP1 group, for example—hint at the possibility that efficacy might be diluted as one moves downstream to target just 1 prostanoid in the pathway. Our poor understanding of such potential functional redundancy at the receptor level, never mind insight into the implications of receptor dimerization, leave these as open questions for drug development.

Surely, this complexity suggests that experiments in animals are going to be of virtually no value when it comes to predicting drug effects in vivo. Certainly the limitations of standard experimental paradigms apply in this pathway, as in others. We study mouse models of atherosclerosis that fail to undergo spontaneous plaque fissure and thrombotic occlusion of vital arteries to reach conclusions about prevention or provocation of myocardial infarction. We extrapolate from drug action on murine tolerance of a hotplate to patients undergoing molar extraction in an effort to divine therapies for elderly women with osteoarthritis of the knees. These and many such examples inspire caution in the field of translational therapeutics. However, consistency of evidence from different model systems across multiple species can, especially when integrated with independent lines of evidence, predict with some confidence the outcome in randomized clinical trials of drug action in this pathway. Such was the case in the prediction and mechanistic elucidation of the cardiovascular hazard from NSAIDs.[178]

Finally, is it likely that this pathway will yield more therapeutic opportunities? Besides drugs already approved for various indications (like aspirin and the myriad NSAIDs; analogues of PGE_2, PGF_{2a}, and PGI_2; TP and leukotriene

antagonists) currently inhibitors of mPGES-1, 5–lipoxygenase and its activating factor (five lipoxygenase activating protein) and antagonists of DP1, DP2, and EP4 are all undergoing clinical evaluation. Many other targets in the eicosanoid pathway are undergoing preclinical evaluation, just as others, such as the multiple secretory phospholipases and the soluble epoxide hydrolase, emerge. This coincides with new information about old targets.

Why do we need to inhibit both COXs, not just COX-1, to see gastrointestinal injury in model systems?[29] Why is a form of aspirin confined to platelet inhibition in the presystemic circulation associated with a reduction in the incidence of colon cancer?[179,180] Much remains to be discovered about the biology of the eicosanoids, and from this is likely to come new therapeutic opportunity.

Conclusion

Prostanoids can promote or restrain acute inflammation. Products of COX-2 in particular may also contribute to resolution of inflammation in certain settings. Presently, we have little information on which products of COX-2 might subserve this role or indeed whether the dominant factors reflect rediversion of the AA substrate to other metabolic pathways consequent to deletion or inhibition of COX-2. As with cyclopentanone prostanoids, many arachidonate derivatives, including transcellular products, when synthesized and administered as exogenous compounds, can promote resolution in models of inflammation. However, rigorous physicochemical evidence for the formation of the endogenous species in relevant quantities to subserve this role in vivo is limited. Elucidation of whether and how prostanoids might restrain inflammation and how substrate modification, such as with fish oils, might exploit this understanding is currently a focus of much research from which novel therapeutic strategies are likely to emerge.

Sources of Funding

The work that formed the basis for opinions expressed in this review was supported by National Institutes of Health Grants HL062250, HL083799, and HL054500 (to G.A.F). Dr FitzGerald is the McNeil Professor of Translational Medicine and Therapeutics.

Disclosures

Dr FitzGerald has consulted in the past year for Astra Zeneca, Daiichi Sankyo, Logical Therapeutics, Lilly, and Nicox on NSAIDs and related compounds. The other authors report no conflicts.

References

1. Nathan C. Points of control in inflammation. *Nature*. 2002;420:846–885.
2. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. *Annu Rev Biochem*. 2000;69:145–182.
3. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. *FASEB J*. 1998;12:1063–1073.
4. Smyth EM, Grosser T, Wang M, Yu Y, FitzGerald GA. Prostanoids in health and disease. *J Lipid Res*. 2009;50:423–428.
5. Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest*. 2001;108:15–23.
6. Narumiya S, FitzGerald GA. Genetic and pharmacological analysis of prostanoid receptor function. *J Clin Invest*. 2001;108:25–30.
7. Breyer RM, Bagdassarian CK, Myers SA, Breyer MD. Prostanoid receptors: subtypes and signaling. *Annu Rev Pharmacol Toxicol*. 2001;41:661–690.
8. Hirai H, Tanaka K, Yoshie O, Ogawa K, Kenmotsu K, Takamori Y, Ichimasa M, Sugamura K, Nakamura M, Takano S, Nagata K. Prostaglandin D2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J Exp Med*. 2001;193:255–261.
9. Pierce KL, Fujino H, Srinivasan D, Regan JW. Activation of FP prostanoid receptor isoforms leads to Rho-mediated changes in cell morphology and in the cell cytoskeleton. *J Biol Chem*. 1999;274:35944–35949.
10. Hatae N, Sugimoto Y, Ichikawa A. Prostaglandin receptors: advances in the study of EP3 receptor signaling. *J Biochem*. 2002;131:781–784.
11. Wilson SJ, McGinley K, Huang AJ, Smyth EM. Heterodimerization of the α and β isoforms of the human thromboxane receptor enhances isoprostane signaling. *Biochem Biophys Res Commun*. 2007;352:397–403.
12. Wilson SJ, Roche AM, Kostetskaia E, Smyth EM. Dimerization of the human receptors for prostacyclin and thromboxane facilitates thromboxane receptor-mediated cAMP generation. *J Biol Chem*. 2004;279:53036–53047.
13. McGraw DW, Mihlbachler KA, Schwarb MR, Rahman FF, Small KM, Almoosa KF, Liggett SB. Airway smooth muscle prostaglandin-EP1 receptors directly modulate β2-adrenergic receptors within a unique heterodimeric complex. *J Clin Invest*. 2006;116:1400–1409.
14. Yuan C, Sidhu RS, Kuklev DV, Kado Y, Wada M, Song I, Smith WL. Cyclooxygenase allosterism, fatty acid-mediated cross-talk between monomers of cyclooxygenase homodimers. *J Biol Chem*. 2009;284:10046–10055.
15. Yu Y, Fan J, Chen XS, Wang D, Klein-Szanto AJ, Campbell RL, FitzGerald GA, Funk CD. Genetic model of selective COX2 inhibition reveals novel heterodimer signaling. *Nat Med*. 2006;12:699–704.
16. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat New Biol*. 1971;231:232–235.
17. Mardini IA, FitzGerald GA. Selective inhibitors of cyclooxygenase-2: a growing class of anti-inflammatory drugs. *Mol Interv*. 2001;1:30–38.
18. McAdam BF, Mardini IA, Habib A, Burke A, Lawson JA, Kapoor S, FitzGerald GA. Effect of regulated expression of human cyclooxygenase isoforms on eicosanoid and isoeicosanoid production in inflammation. *J Clin Invest*. 2000;105:1473–1482.
19. Crofford LJ, Wilder RL, Ristimaki AP, Sano H, Remmers EF, Epps HR, Hla T. Cyclooxygenase-1 and -2 expression in rheumatoid synovial tissues: effects of interleukin-1β, phorbol ester, and corticosteroids. *J Clin Invest*. 1994;93:1095–1101.
20. Schonbeck U, Sukhova GK, Graber P, Coulter S, Libby P. Augmented expression of cyclooxygenase-2 in human atherosclerotic lesions. *Am J Pathol*. 1999;155:1281–1291.
21. Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD, Kim HS, Smithies O. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell*. 1995;83:483–492.
22. Morham SG, Langenbach R, Loftin CD, Tiano HF, Vouloumanos N, Jennette JC, Mahler JF, Kluckman KD, Ledford A, Lee CA, Smithies O. Prostaglandin synthase-2 gene disruption causes severe renal pathology in the mouse. *Cell*. 1995;83:473–482.
23. Dinchuk JE, Car BD, Focht RJ, Johnston JJ, Jaffee BD, Covington MB, Contel NR, Eng VM, Collins RJ, Czerniak PM, Gorry SA, Trzaskos JM. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature*. 1995;378:406–409.
24. Chen M, Boilard E, Nigrovic PA, Clark P, Xu D, Fitzgerald GA, Audoly LP, Lee DM. Predominance of cyclooxygenase 1 over cyclooxygenase 2 in the generation of proinflammatory prostaglandins in autoantibody-driven K/BxN serum-transfer arthritis. *Arthritis Rheum*. 2008;58:1354–1365.
25. Myers LK, Kang AH, Postlethwaite AE, Rosloniec EF, Morham SG, Shlopov BV, Goorha S, Ballou LR. The genetic ablation of cyclooxygenase 2 prevents the development of autoimmune arthritis. *Arthritis Rheum*. 2000;43:2687–2693.
26. Ochi T, Ohkubo Y, Mutoh S. Role of cyclooxygenase-2, but not cyclooxygenase-1, on type II collagen-induced arthritis in DBA/1J mice. *Biochem Pharmacol*. 2003;66:1055–1060.

27. Langenbach R, Loftin C, Lee C, Tiano H. Cyclooxygenase knockout mice-Models for elucidating isoform-specific functions. *Biochem Pharmacol*. 1999;58:1237–1246.
28. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat Med.* 1999;5:698–701.
29. Wallace JL, McKnight W, Reuter BK, Vergnolle N. NSAID-induced gastric damage in rats: requirement for inhibition of both cyclooxygenase 1 and 2. *Gastroenterology*. 2000;119:706–714.
30. Morteau O, Morham SG, Sellon R, Dieleman LA, Langenbach R, Smithies O, Sartor RB. Impaired mucosal defense to acute colonic injury in mice lacking cyclooxygenase-1 or cyclooxygenase-2. *J Clin Invest*. 2000;105:469–478.
31. Gavett SH, Madison SL, Chulada PC, Scarborough PE, Qu W, Boyle JE, Tiano HF, Lee CA, Langenbach R, Roggli VL, Zeldin DC. Allergic lung responses are increased in prostaglandin H synthase-deficient mice. *J Clin Invest*. 1999;104:721–732.
32. Bell-Parikh LC, Ide T, Lawson JA, McNamara P, Reilly M, FitzGerald GA. Biosynthesis of 15-deoxy-δ12,14-PGJ2 and the ligation of PPARγ. *J Clin Invest*. 2003;112:945–955.
33. McClelland S, Gawaz M, Kennerknecht E, Konrad CS, Sauer S, Schuerzinger K, Massberg S, Fitzgerald DJ, Belton O. Contribution of cyclooxygenase-1 to thromboxane formation, platelet-vessel wall interactions and atherosclerosis in the ApoE null mouse. *Atherosclerosis*. 2009;202:84–91.
34. Pratico D, Tillmann C, Zhang ZB, Li H, FitzGerald GA, Acceleration of atherogenesis by COX-1-dependent prostanoid formation in low density lipoprotein receptor knockout mice. *Proc Natl Acad Sci U S A*. 2001;98:3358–3363.
35. Egan KM, Lawson JA, Fries S, Koller B, Rader DJ, Smyth EM, FitzGerald GA. COX-2-derived prostacyclin confers atheroprotection on female mice. *Science*. 2004;306:1954–1957.
36. Legler DF, Bruckne M, Uetz-von Allmen E, Krause P. Prostaglandin E_2 at new glance: novel insights in functional diversity offer therapeutic chances. *Int J Biochem Cell Biol*. 2010;42:198–201.
37. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science*. 2001;294:1871–1875.
38. Samuelsson B, Morgenstern R, Jakobsson PJ. Membrane prostaglandin E synthase-1: a novel therapeutic target. *Pharmacol Rev*. 2007;59:207–224.
39. Tanioka T, Nakatani Y, Semmyo N, Murakami M, Kudo I, Molecular identification of cytosolic prostaglandin E2 synthase that is functionally coupled with cyclooxygenase-1 in immediate prostaglandin E2 biosynthesis. *J Biol Chem*. 2000;275:32775–32782.
40. Nakatani Y, Hokonohara Y, Kakuta S, Sudo K, Iwakura Y, Kudo I. Knockout mice lacking cPGES/p23, a constitutively expressed PGE2 synthetic enzyme, are perinatally lethal. *Biochem Biophys Res Commun*. 2007;362:387–392.
41. Jakobsson PJ, Thorén S, Morgenstern S, Samuelsson B. Identification of human prostaglandin E synthase: a microsomal, glutathione-dependent, inducible enzyme, constituting a potential novel drug target. *Proc Natl Acad Sci U S A*. 1999;96:7220–7225.
42. Mancini JA, Blood K, Guay J, Gordon R, Claveau D, Chan CC, Riendeau D. Cloning, expression, and up-regulation of inducible rat prostaglandin E synthase during lipopolysaccharide-induced pyresis and adjuvant-induced arthritis. *J Biol Chem*. 2001;276:4469–4475.
43. Thorén S, Jakobsson PJ. Coordinate up- and down-regulation of glutathione dependent prostaglandin E synthase and cyclooxygenase-2 in A549 cells: inhibition by NS-398 and leukotriene C4. *Eur J Biochem*. 2000;267:6428–6434.
44. Murakami M, Naraba H, Tanioka T, Semmyo N, Nakatani Y, Kojima F, Ikeda T, Fueki M, Ueno A, Oh S, Kudo I. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. *J Biol Chem*. 2000;275:32783–32792.
45. Trebino CE, Stock JL, C.P. Gibbons CP, Naiman BM, Wachtmann TS, Umland JP, Pandher K, Lapointe JM, Saha S, Roach ML, Carter D, Thomas NA, Durtschi BA, McNeish JD, Hambor JE, Jakobsson PJ, Carty TJ, Perez JR, Audoly LP. Impaired inflammatory and pain responses in mice lacking an inducible prostaglandin E synthase. *Proc Natl Acad Sci U S A*. 2003;100:9044–9049.
46. Kamei D, Yamakawa K, Takegoshi Y, Mikami-Nakanishi M, Nakatani Y, Oh-Ishi S, Yasui H, Azuma Y, Hirasawa N, Ohuchi K, Kawaguchi H, Ishikawa Y, Ishii T, Uematsu S, Akira S, Murakami M, Kudo I. Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. *J Biol Chem*. 2004;279:33684–33695.
47. Wang M, Zukas AM, Hui Y, Ricciotti E, Pure E, FitzGerald GA. Deletion of microsomal prostaglandin E synthase-1 augments prostacyclin and retards atherogenesis. *Proc Natl Acad Sci U S A*. 2006;103:14507–14512.
48. Brenneis C, Coste O, Schmidt R, Angioni C, Popp L, Nusing RM, Becker W, Scholich K, Geisslinger G. Consequences of altered eicosanoid patterns for nociceptive processing in mPGES-1-deficient mice. *J Cell Mol Med*. 2008;12:639–648.
49. Brenneis C, Coste O, Altenrath K, Angioni C, Schmidt H, Schuh CD, Zhang DD, Henke M, Weigert A, Brüne B, Rubin B, Nusing R, Scholich K, Geisslinger G. Anti-inflammatory role of microsomal prostaglandin E synthase-1 in a model of neuroinflammation. *J Biol Chem.* 2011;286:2331–2342.
50. Jania LA, Chandrasekharan S, Backlund MG, Foley NA, Snouwaert J, Wang IM, Clark P, Audoly LP, Koller BH. Microsomal prostaglandin E synthase-2 is not essential for in vivo prostaglandin E2 biosynthesis. *Prostaglandins Other Lipid Mediat*. 2009;88:73–81.
51. Soodvilai S, Jia Z, Wang MH, Dang Z, Yang T. mPGES-1 deletion impairs diuretic response to acute water loading. *Am J Physiol*. 2009;296:1129–1135.
52. Hui Y, Ricciotti E, Crichton I, Yu Z, Wang D, Stubbe J, Wang M, Puré E, FitzGerald GA. Targeted deletions of cyclooxygenase-2 and atherogenesis in mice. *Circulation*. 2010;121:2654–2660.
53. Park JY, Pillinger MH, Abramson SB. Prostaglandin E2 synthesis and secretion: the role of PGE_2 synthases. *Clin Immunol*. 2006;119:229–240.
54. Sugimoto Y, Narumiya S. Prostaglandin E receptors. *J Biol Chem*. 2007;282:11613–11617.
55. Moriyama T, Higashi T, Togashi K, Iida T, Segi E, Sugimoto Y, Tominaga T, Narumiya S, Tominaga M. Sensitization of TRPV1 by EP1 and IP reveals peripheral nociceptive mechanism of prostaglandins. *Mol Pain*. 2005;1:3.
56. Minami T, Nakano H, Kobayashi T, Sugimoto Y, Ushikubi F, Ichikawa A, Narumiya S, Ito S. Characterization of EP receptor subtypes responsible for prostaglandin E2-induced pain responses by use of EP1 and EP3 receptor knockout mice. *Br J Pharmacol*. 2001;133:438–444.
57. Honda T, Segi-Nishida E, Miyachi Y, Narumiya S. Prostacyclin-IP signaling and prostaglandin E2-EP2/EP4 signaling both mediate joint inflammation in mouse collagen-induced arthritis. *J Exp Med*. 2006;203:325–335.
58. Yuhki K, Ueno A, Naraba H, Kojima F, Ushikubi F, Narumiya S, Oh-ishi S. Prostaglandin receptors EP2, EP3, and IP mediate exudate formation in carrageenin-induced mouse pleurisy. *J Pharmacol Exp Ther*. 2004;311:1218–1224.
59. Portanova JP, Zhang Y, Anderson GD, Hauser SD, Masferrer JL, Seibert K, Gregory SA, Isakson PC. Selective neutralization of prostaglandin E2 blocks inflammation, hyperalgesia, and interleukin 6 production in vivo. *J Exp Med*. 1996;184:883–891.
60. Dayer JM, Krane SM, Russell RG, Robinson DR. Production of collagenase and prostaglandins by isolated adherent rheumatoid synovial cells. *Proc Natl Acad Sci U S A*. 1976;73:945–949.
61. McCoy JM, Wicks JR, Audoly LP. The role of prostaglandin E2 receptors in the pathogenesis of rheumatoid arthritis. *J Clin Invest*. 2002;110:651–658.
62. Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kabashima K, Sugimoto Y, Kobayashi T, Ushikubi F, Aze Y, Eguchi N, Urade Y, Yoshida N, Kimura K, Mizoguchi A, Honda Y, Nagai H, Narumiya S. Prostaglandin D2 as a mediator of allergic asthma. *Science*. 2000;287:2013–2017.
63. Kunikata T, Yamane H, Segi E, Matsuoka T, Sugimoto Y, Tanaka S, Tanaka H, Nagai H, Ichikawa A, Narumiya S. Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. *Nat Immunol*. 2005;6:524–531.
64. Yao C, Sakata D, Esaki Y, Li Y, Matsuoka T, Kuroiwa K, Sugimoto Y, Narumiya S. Prostaglandin E(2)-EP4 signaling promotes immune inflammation through T(H)1 cell differentiation and T(H)17 cell expansion. *Nat Med*. 2009;15:633–640.
65. Kabashima K, Sakata D, Nagamachi M, Miyachi Y, Inaba K, Narumiya S. Prostaglandin E2-EP4 signaling initiates skin immune responses by promoting migration and maturation of Langerhans cells. *Nat Med*. 2003;9:744–749.

66. Legler DF, Krause P, Scandella E, Singer E, Groettrup M. Prostaglandin E2 is generally required for human dendritic cell migration and exerts its effect via EP2 and EP4 receptors. *J Immunol*. 2006;176:966–973.
67. Krause P, Bruckner M, Uermosi C, Singer E, Groettrup M, Legler DF. Prostaglandin E2 enhances T cell proliferation by inducing the co-stimulatory molecules OX40L, CD70 and 4–1BBL on dendritic cells. *Blood*. 2009;113:2451–2460.
68. Harris SG, Padilla J, Koumas L, Ray D, Phipps RP. Prostaglandins as modulators of immunity. *Trends Immunol*. 2002;23:144–150.
69. Babaev VR, Chew JD, Ding L, Davis S, Breyer MD, Breyer RM, Oates JA, Fazio S, Linton MF. Macrophages EP4 deficiency increases apoptosis and suppresses early atherosclerosis. *Cell Metab*. 2008;8: 492–501.
70. Tang EH, Shimizu K, Christen T, Rocha VZ, Shvartz E, Tesmenitsky Y, Sukhova G, Shi GP, Libby P. Lack of EP4 receptors on bone marrow-derived cells enhances inflammation in atherosclerotic lesions. *Cardiovasc Res*. 2011;81:234–243.
71. Lehnardt S, Massillon L, Follett P, Jensen FE, Ratan R, Rosenberg PA, Volpe JJ, Vartanian T. Activation of innate immunity in the CNS triggers neurodegeneration through a Toll-like receptor 4-dependent pathway. *Proc Natl Acad Sci U S A*. 2003;100:8514–8519.
72. Montine TJ, Milatovic D, Gupta C, Valyi-Nagy T, Morrow JD, Breyer RM. Neuronal oxidative damage from activated innate immunity is EP2 receptor-dependent. *J Neurochem*. 2002;83:463–470.
73. Reinold H, Ahmadi S, Depner UB, Layh B, Heindl C, Hamza M, Pahl A, Brune K, Narumiya S, Muller U, Zeilhofer HU. Spinal inflammatory hyperalgesia is mediated by prostaglandin E receptors of the EP2 subtype. *J Clin Invest*. 2005;115:673–679.
74. Caggiano AO, Kraig RP. Prostaglandin E2 and 4-aminopyridine prevent the lipopolysaccharide-induced outwardy rectifying potassium current and interleukin-1 β production. *J Neurochem*. 1999;72:565–575.
75. Noda M, Kariura Y, Pannasch U, Nishikawa K, Wang L, Seike T, Ifuku M, Kosai Y, Wang B, Nolte C, Aoki S, Kettenmann H, Wada K. Neuroprotective role of bradykinin because of the attenuation of pro-inflammatory cytokine release from activated microglia. *J Neurochem*. 2007;101:397–410.
76. Kawabe J, Ushikubi F, Hasebe N. Prostacyclin in vascular diseases. *Circ J*. 2010;74:836–843.
77. Smith WL, DeWitt DL, Allen ML. Bimodal distribution of the prostaglandin I2 synthase antigen in smooth muscle cells. *J Biol Chem*. 1983;258:5922–5926.
78. Liou JY, Shyue SK, Tsai MJ, Chung CL, Wu KK. Colocalization of prostacyclin synthase with prostaglandin H synthase-1 (PGHS-1) but not phorbol ester-induced PGHS-2 in cultured endothelial cells. *J Biol Chem*. 2000;275:15314–15320.
79. Miyata A, Hara S, Yokoyama C, Inoue H, Ullrich V, Tanabe T. Molecular cloning and expression of human prostacyclin synthase. *Biochem Biophys Res Commun*. 1994;200:1728–1734.
80. Caughey GE, Cleland LG, Gamble JR, James MJ. Up-regulation of endothelial cyclooxygenase-2 and prostanoid synthesis by platelets: role of thromboxane A2. *J Biol Chem*. 2001;276:37839–37845.
81. McAdam BF, Catella-Lawson F, Mardini IA, Kapoor S, Lawson JA, FitzGerald GA. Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: the human pharmacology of a selective inhibitor of COX-2. *Proc Natl Acad Sci U S A*. 1999;96:272–277.
82. Wu KK, Liou JY. Cellular and molecular biology of prostacyclin synthase. *Biochem Biophys Res Commun*. 2005;338:45–52.
83. Libby P, Warner SJ, Friedman GB. Interleukin 1: a mitogen for human vascular smooth muscle cells that induces the release of growth-inhibitory prostanoids. *J Clin Invest*. 1988;81:487–498.
84. Smyth EM, FitzGerald GA. Human prostacyclin receptor. *Vitam Horm*. 2002;65:149–165.
85. Lim H, Dey SK. A novel pathway of prostacyclin signaling-hanging out with nuclear receptors. *Endocrinology*. 2002;143:3207–3210.
86. Huang JC, Wun WS, Goldsby JS, Egan K, FitzGerald GA, Wu KK. Prostacyclin receptor signaling and early embryo development in the mouse. *Hum Reprod*. 2007;22:2851–2856.
87. Cheng Y, Austin SC, Rocca B, Koller BH, Coffman TM, Grosser T, Lawson JA, FitzGerald GA. Role of prostacyclin in the cardiovascular response to thromboxane A2. *Science*. 2002;296:539–541.
88. Francois H, Athirakul K, Howell D, Dash R, Mao L, Kim HS, Rockman HA, Fitzgerald GA, Koller BH, Coffman TM. Prostacyclin protects against elevated blood pressure and cardiac fibrosis. *Cell Metab*. 2005; 2:201–207.
89. Kobayashi T, Tahara Y, Matsumoto M, Iguchi M, Sano H, Murayama T, Arai H, Oida H, Yurugi-Kobayashi T, Yamashita JK, Katagiri H, Majima M, Yokode M, Kita T, Narumiya S. Roles of thromboxane A(2) and prostacyclin in the development of atherosclerosis in apoE-deficient mice. *J Clin Invest*. 2004;114:784–794.
90. Bombardieri, S. Bombardieri S, Cattani P, Ciabattoni G, Di Munno O, Pasero G, Patrono C, Pinca E, Pugliese F. The synovial prostaglandin system in chronic inflammatory arthritis: differential effects of steroidal and nonsteroidal anti-inflammatory drugs. *Br J Pharmacol*. 1981;73: 893–901.
91. Higgs GA, Moncada S, Salmon JA, Seager K. The source of thromboxane and prostaglandins in experimental inflammation. *Br J Pharmacol*. 1983; 79:863–868.
92. Berkenkopf JW, Weichman BM. Production of prostacyclin in mice following intraperitoneal injection of acetic acid, phenylbenzoquinone and zymosan: its role in the writhing response. *Prostaglandins*. 1988; 36:693–709.
93. Murata T, Ushikubi F, Matsuoka T, Hirata M, Yamasaki A, Sugimoto Y, Ichikawa A, Aze Y, Tanaka T, Yoshida N, Ueno A, Oh-ishi S, Narumiya S. Altered pain perception and inflammatory response in mice lacking prostacyclin receptor. *Nature*. 1997;388:678–682.
94. Ueno A, Naraba H, Ikeda Y, Ushikubi F, Murata T, Narumiya S, Oh-ishi S. Intrinsic prostacyclin contributes to exudation induced by bradykinin or carrageenin: a study on the paw edema induced in IP-receptor-deficient mice. *Life Sci*. 2000;66:155–160.
95. Oida H, Namba T, Sugimoto Y, Ushikubi F, Ohishi H, Ichikawa A, Narumiya S. In situ hybridization studies of prostacyclin receptor mRNA expression in various mouse organs. *Br J Pharmacol*. 1995;116: 2828–2837.
96. Doi Y, Minami T, Nishizawa M, Mabuchi T, Mori H, Ito S. Central nociceptive role of prostacyclin (IP) receptor induced by peripheral inflammation. *NeuroReport*. 2002;13:93–96.
97. Bley KR, Bhattacharya A, Daniels DV, Gever J, Jahangir A, O'Yang C, Smith S, Srinivasan D, Ford AP, Jett MF. RO1138452 and RO3244794: characterization of structurally distinct, potent and selective IP (prostacyclin) receptor antagonists. *Br J Pharmacol*. 2006;147:335–345.
98. Pulichino AM, Rowland S, Wu T, Clark P, Xu D, Mathieu MC, Riendeau D, Audoly LP. Prostacyclin antagonism reduces pain and inflammation in rodent models of hyperalgesia and chronic arthritis. *J Pharmacol Exp Ther*. 2006;319:1043–1050.
99. Jaffar Z, Wan KS, Roberts K. A key role for prostaglandin I2 in limiting lung mucosal Th2, but not Th1, responses to inhaled allergen. *J Immunol*. 2002;169:5997–6004.
100. Takahashi Y, Tokuoka S, Masuda T, Hirano Y, Nagao M, Tanaka H, Inagaki N, Narumiya S, Nagai H. Augmentation of allergic inflammation in prostanoid IP receptor deficient mice. *Br J Pharmacol*. 2002; 137:315–322.
101. Jowsey IR, Thomson AM, Flanagan JU, Murdock PR, Moore GB, Meyers DJ, et al. Mammalian class Sigma glutathione *S* transferases: catalytic properties and tissue-specific expression of human and rat GSH-dependent prostaglandin D2 synthases. *Biochem J*. 2001;359: 507–516.
102. Urade Y, Hayaishi O. Prostaglandin D2 and sleep regulation. *Biochim. Biophys Acta*. 1999;1436:606–615.
103. Eguchi N, Minami T, Shirafuji N, Kanaoka Y, Tanaka T, Nagata A, Yoshida N, Urade Y, Ito S, Hayaishi O. Lack of tactile pain (allodynia) in lipocalin-type prostaglandin D synthase-deficient mice. *Proc Natl Acad Sci USA*. 1999;96:726–730.
104. Lewis RA, Soter NA, Diamond PT, Austen KF, Oates JA, Roberts II LJ. Prostaglandin D2 generation after activation of rat and human mast cells with anti-IgE. *J Immunol*. 1982;129:1627–1631.
105. Urade Y, Ujihara M, Horiguchi Y, Ikai K, Hayaishi O. The major source of endogenous prostaglandin D2 production is likely antigenpresenting cells: localization of glutathione-requiring prostaglandin D synthetase in histiocytes, dendritic, and Kupffer cells in various rat tissues. *J Immunol*. 1989;143:2982–2989.
106. Tanaka K, Ogawa K, Sugamura K, Nakamura M, Takano S, Nagata K. Differential production of prostaglandin D2 by human helper T cell subsets. *J Immunol*. 2000;164:2277–2280.
107. Herlong JL, Scott TR. Positioning prostanoids of the D and J series in the immunopathogenic scheme. *Immunol Lett*. 2006;102:121–131.
108. Fitzpatrick FA, Wynalda MA. Albumin-catalyzed metabolism of prostaglandin D2. *J Biol Chem*. 1983;258:11713–11718.
109. Shibata T, Kondo M, Osawa T, Shibata N, Kobayashi M, UchidaK. 15-Deoxy-δ 12,14-prostaglandin J2: a prostaglandin D2 metabolite gen-

erated during inflammatory processes. *J Biol Chem*. 2002;277:10459–10466.
110. Forman BM, Tontonoz P, Chen J, Brun RP, Spiegelman BM, Evans RM. 15-deoxy-Δ12, 14-prostaglandin J2 is a ligand for the adipocyte determination factor PPARγ. *Cell*. 1995;83:803–812.
111. Roberts II LJ, Sweetman BJ, Lewis RA, Austen KF, Oates JA. Increased production of prostaglandin D2 in patients with systemic mastocytosis. *N Engl J Med*. 1980;303:1400–1404.
112. Murray JJ, Tonnel AB, Brash AR, Roberts LJ II, Gosset P, Workman R, Capron A, Oates JA. Release of prostaglandin D_2 into human airways during acute antigen challenge. *N Engl J Med*. 1986;315:800–804.
113. Hardy CC, Robinson C, Tattersfield AE, Holgate ST. The bronchoconstrictor effect of inhaled prostaglandin D2 in normal and asthmatic men. *N Engl J Med*. 1984;311:209–213.
114. Emery DL, Djokic TD, Graf PD, Nadel JA. Prostaglandin D2 causes accumulation of eosinophils in the lumen of the dog trachea. *J Appl Physiol*. 1989;67:959–962.
115. Fujitani Y, Kanaoka Y, Aritake K, Uodome N, Okazaki-Hatake K, Urade Y. Pronounced eosinophilic lung inflammation and Th2 cytokine release in human lipocalin-type prostaglandin D synthase transgenic mice. *J Immunol*. 2002;168:443–449.
116. Kabashima K, Narumiya S. The DP receptor, allergic inflammation and asthma. *Prostaglandins Leukot Essent Fatty Acids*. 2003;69:187–194.
117. Gervais FG, Cruz RP, Chateauneuf A, Gale S, Sawyer N, Nantel F, Metters KM, O'Neill GP. Selective modulation of chemokinesis, degranulation, and apoptosis in eosinophils through the PGD2 receptors CRTH2 and DP. *J Allergy Clin Immunol*. 2001;108:982–988.
118. Tsuri T, Honma T, Hiramatsu Y, Okada T, Hashizume H, Mitsumori S, Inagaki M, Arimura A, Yasui K, Asanuma F, Kishino J, Ohtani M. Bicyclo[2.2.1]heptane and 6,6-dimethylbicyclo[3.1.1]heptane derivatives: orally active, potent, and selective prostaglandin D2 receptor antagonists. *J Med Chem*. 1997;40:3504–3507.
119. Arimura A, Yasui K, Kishino J, Asanuma F, Hasegawa H, Kakudo S, Ohtani M, Arita H. Prevention of allergic inflammation by a novel prostaglandin receptor antagonist, S-5751. *J Pharmacol Exp Ther*. 2001;298:411–419.
120. Nagata K, Hirai H, Tanaka K, Ogawa K, Aso T, Sugamura K, Nakamura M, Takano S. CRTH2, an orphan receptor of T-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s). *FEBS Lett*. 1999;459:195–199.
121. Nagata K, Tanaka K, Ogawa K, Kemmotsu K, Imai T, Yoshie O, Abe H, Tada K, Nakamura M, Sugamura K, Takano S. Selective expression of a novel surface molecule by human Th2 cells in vivo. *J Immunol*. 1999;162:1278–1286.
122. Iwasaki M, Nagata K, Takano S, Takahashi K, Ishii N, Ikezawa Z. Association of a new-type prostaglandin D2 receptor CRTH2 with circulating T helper 2 cells in patients with atopic dermatitis. *J Invest Dermatol*. 2002;119:609–616.
123. Shichijo M, Sugimoto H, Nagao K, Inbe H, Encinas JA, Takeshita K, Bacon KB, Gantner F. Chemoattractant receptorhomologous molecule expressed on Th2 cells activation in vivo increases blood leukocyte counts and its blockade abrogates 13,14-dihydro-15-keto-prostaglandin D2-induced eosinophilia in rats. *J Pharmacol Exp Ther*. 2003;307:518–525.
124. Hammad H, de Heer HJ, Soullie T, Hoogsteden HC, Trottein F, Lambrecht BN. Prostaglandin D2 inhibits airway dendritic cell migration and function in steady state conditions by selective activation of the D prostanoid receptor 1. *J Immunol*. 2003;171:3936–3940.
125. Spik I, Brenuchon C, Angeli V, Staumont D, Fleury S, Capron M, Trottein F, Dombrowicz D. Activation of the prostaglandin D2 receptor DP2/CRTH2 increases allergic inflammation in mouse. *J Immunol*. 2005;174:3703–3708.
126. Angeli V, Faveeuw C, Roye O, Fontaine J, Teissier E, Capron A, Wolowczuk I, Capron M, Trottein F. Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. *J Exp Med*. 2001;193:1135–1147.
127. Rajakariar R, Hilliard M, Lawrence T, Trivedi S, Colville-Nash P, Bellingan G, Fitzgerald D, Yaqoob MM, Gilroy DW. Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxy-Δ12,14 PGJ2. *Proc Natl Acad Sci U S A*. 2007;104:20979–20984.
128. Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature*. 1998;391:79–82.
129. Straus DS, Pascual G, Li M, Welch JS, Ricote M, Hsiang CH, Sengchanthalangsy LL, Ghosh G, Glass CK. 15-deoxy-Δ12,14-prostaglandin J2 inhibits multiple steps in the NF-κB signaling pathway. *Proc Natl Acad Sci U S A*. 2000;97:4844-4949.
130. Song WL, Wang M, Ricciotti E, Fries S, Yu Y, Grosser T, Reilly M, Lawson JA, FitzGerald GA. Tetranor PGDM, an abundant urinary metabolite reflects biosynthesis of prostaglandin D2 in mice and humans. *J Biol Chem*. 2008;283:1179–1188.
131. Tsukada T, Rosenfeld M, Ross R, Gown AM. Immunocytochemical analysis of cellular components in lesions of atherosclerosis of the Watanabe and fat-fed rabbit using monoclonal antibodies. *Arteriosclerosis*. 1986;6:601–613.
132. Kaartinen M, Penttila A, Kovanen PT. Mast cells of two types differing in neutral protease composition in the human aortic intima: demonstration of tryptase- and tryptase/chymase-containing mast cells in normal intimas, fatty streaks, and the shoulder region of atheromas. *Arterioscler Thromb Vasc Biol*. 1994;14:966–972.
133. Taba Y, Sasaguri T, Miyagi M, Abumiya T, Miwa Y, Ikeda T, Mitsumata M. Fluid shear stress induces lipocalin-type prostaglandin D2 synthase expression in vascular endothelial cells. *Circ Res*. 2000;86:967–973.
134. Eguchi Y, Eguchi N, Oda H, Seiki K, Kijima Y, Matsu-ura Y, Urade Y, Hayaishi O. Expression of lipocalin-type prostaglandin D synthase (β-trace) in human heart and its accumulation in the coronary circulation of angina patients. *Proc Natl Acad Sci U S A*. 1997;94:14689–14694.
135. Hirawa N, Uehara Y, Yamakado M, Toya Y, Gomi T, Ikeda T, Eguchi Y, Takagi M, Oda H, Seiki K, Urade Y, Umemura S. Lipocalin-type prostaglandin dsynthase in essential hypertension. *Hypertension*. 2002;39:449–454.
136. Nagoshi H, Uehara Y, Kanai F, Maeda S, Ogura T, Goto A, Toyo-oka T, Esumi H, Shimizu T, Omata M. Prostaglandin D2 inhibits inducible nitric oxide synthase expression in rat vascular smooth muscle cells. *Circ Res*. 1998;82:204–209.
137. Negoro H, Soo Shin W, Hakamada-Taguchi R, Eguchi N, Urade Y, Goto A, Toyo-Oka T, Fujita T, Omata M, Uehara Y. Endogenous prostaglandin D2 synthesis reduces an increase in plasminogen activator inhibitor-1 following interleukin stimulation in bovine endothelial cells. *J Hypertens*. 2002;20:1347–1354.
138. Tanaka R, Miwa Y, Mou K, Tomikawa M, Eguchi N, Urade Y, Takahashi-Yanaga F, Morimoto S, Wake N, Sasaguri T. Knockout of the L-PGDS gene aggravates obesity and atherosclerosis in mice. *Biochem Biophys Res Commun*. 2009;378:851–856.
139. Pierce KL, Bailey TJ, Hoyer PB, Gil DW, Woodward DF, Regan JW. Cloning of a carboxyl-terminal isoform of the prostanoid FP receptor. *J Biol Chem*. 1997;272:883–887.
140. Abramovitz M, Adam M, Boie Y, Carriere M, Denis D, Godbout C, Lamontagne S, Rochette C, Sawyer N, Tremblay NM, Belley M, Gallant M, Dufresne C, Gareau Y, Ruel R, Juteau H, Labelle M, Ouimet N, Metters KM. The utilization of recombinant prostanoid receptors to determine the affinities and selectivities of prostaglandins and related analogs. *Biochim Biophys Acta*. 2000;1483:285–293.
141. Samuelsson B, Granstrom E, Green K, Hamberg M, Hammarstrom S.*Prostaglandins Annu Rev Biochem*. 1975;44:669–695.
142. Liston TE, Roberts LJ II. Transformation of prostaglandin D2 to 9 α,11 β-(15S)-trihydroxyprosta-(5Z,13E)-dien-1-oic acid (9 α, 11 β-prostaglandin F2): a unique biologically active prostaglandin produced enzymatically in vivo in humans. *Proc Natl Acad Sci U S A*. 1985;82:6030–6034.
143. Basu S. Novel cyclooxygenase-catalyzed bioactive prostaglandin F2α from physiology to new principles in inflammation. *Med Res Rev*. 2007;27:435–468.
144. Sugimoto Y, Yamasaki A, Segi E, Tsuboi K, Aze Y, Nishimura T, Oida H, Yoshida N, Tanaka T, Katsuyama M, Hasumoto K, Murata T, Hirata M, Ushikubi F, Negishi M, Ichikawa A, Narumiya S. Failure of parturition in mice lacking the prostaglandin F receptor. *Science*. 1997;277:681–683.
145. Saito O, Guan Y, Qi Z, Davis LS, Kömhoff M, Sugimoto Y, Narumiya S, Breyer RM, Breyer MD. Expression of the prostaglandin F receptor (FP) gene along the mouse genitourinary tract. *Am J Physiol-Renal*. 2003;284:1164–1170.
146. Breyer MD, Breyer RM. G protein-coupled prostanoid receptors and the kidney. *Annu Rev Physiol*. 2001;63:579–605.
147. Nakahata K, Kinoshita H, Tokinaga Y, Ishida Y, Kimoto Y, Dojo M, Mizumoto K, Ogawa K, Hatano Y. Vasodilation mediated by inward

rectifier K+ channels in cerebral microvessels of hypertensive and normotensive rats. *Anesth Analg*. 2006;102:571–576.
148. Takayama K, Yuhki K, Ono K, Fujino T, Hara A, Yamada T, Kuriyama S, Karibe H, Okada Y, Takahata O, Taniguchi T, Iijima T, Iwasaki H, Narumiya S, Ushikubi F. Thromboxane A2 and prostaglandin F2a mediate inflammatory tachycardia. *Nat Med*. 2005;11:562–566.
149. Jovanovic N, Pavlovic M, Mircevski V, Du Q, Jovanovic A. An unexpected negative inotropic effect of prostaglandin F2a in the rat heart. *Prostaglandins Other Lipid Mediat*. 2006;80:110–119.
150. Saleem S, Ahmad AS, Maruyama T, Narumiya S, Doré S. PGF(2α) FP receptor contributes to brain damage following transient focal brain ischemia. *Neurotox Res*. 2009;15:62–70.
151. Kunori S, Matsumura S, Mabuchi T, Tatsumi S, Sugimoto Y, Minami T, Ito S. Involvement of prostaglandin F 2 α receptor in ATP-induced mechanical allodynia. *Neuroscience*. 2009;163:362–371.
152. Seguin B. Use of prostaglandin in cows with unobserved oestrus. *Acta Vet Scand*. 1981;77:343–352.
153. Schultz RH, Copeland DD. Induction of abortion using prostaglandins. *Acta Vet Scand*. 1981;77:353–361.
154. Karim SM. Prostaglandins in fertility control. *Lancet*. 1970;2:610.
155. Alexander CL, Miller SJ, Abel SR. Prostaglandin analog treatment of glaucoma and ocular hypertension. *Ann Pharmacother*. 2002;36:504–511.
156. Basu S, Eriksson M. Oxidative injury and survival during endotoxemia. *FEBS Lett*. 1998;438:159–160.
157. Basu S. Oxidative injury induced cyclooxygenase activation in experimental hepatotoxicity. *Biochem Biophys Res Commun*. 1999;254:764–767.
158. Oga T, Matsuoka T, Yao C, Nonomura K, Kitaoka S, Sakata D, Kita Y, Tanizawa K, Taguchi Y, Chin K, Mishima M, Shimizu T, Narumiya S. Prostaglandin F(2α) receptor signaling facilitates bleomycin-induced pulmonary fibrosis independently of transforming growth factor-β. *Nat Med*. 2009;15:1426–1430.
159. Basu S, Whiteman M, Mattey DL, Halliwell B. Raised levels of F(2)-isoprostanes and prostaglandin F(2α) in different rheumatic diseases. *Ann Rheum Dis*. 2001;60:627–631.
160. Helmersson J, Larsson A, Vessby B, Basu S. Active smoking and a history of smoking are associated with enhanced prostaglandin F(2α), interleukin-6 and F2-isoprostane formation in elderly men. *Atherosclerosis*. 2005;181:201–207.
161. Helmersson J, Vessby B, Larsson A, Basu S. Association of type 2 diabetes with cyclooxygenase-mediated inflammation and oxidative stress in an elderly population. *Circulation*. 2004;109:1729–1734.
162. Yu Y, Lucitt MB, Stubbe J, Cheng Y, Friis UG, Hansen PB, Jensen BL, Smyth EM, FitzGerald GA. Prostaglandin F2α elevates blood pressure and promotes atherosclerosis. *Proc Natl Acad Sci U S A*. 2009;106:7985–7990.
163. Di Francesco L, Totani L, Dovizio M, Piccoli A, Di Francesco A, Salvatore T, Pandolfi A, Evangelista V, Dercho RA, Seta F, Patrignani P. Induction of prostacyclin by steady laminar shear stress suppresses tumor necrosis factor-α biosynthesis via heme oxygenase-1 in human endothelial cells. *Circ Res*. 2009;104:506–513.
164. Funk CD, FitzGerald GA. COX-2 inhibitors and cardiovascular risk. *J Cardiovasc Pharmacol*. 2007;50:470–479.
165. Félétou M, Verbeuren TJ, Vanhoutte PM. Endothelium-dependent contractions in SHR: a tale of prostanoid TP and IP receptors. *Br J Pharmacol*. 2009;156:563–574.
166. Félétou M, Vanhoutte PM, Verbeuren TJ. The thromboxane/endoperoxide receptor (TP): the common villain. *J Cardiovasc Pharmacol*. 2010;55:317–332.
167. Nakahata N. Thromboxane A2: physiology/pathophysiology, cellular signal transduction and pharmacology. *Pharmacol Ther*. 2008;118:18–35.
168. Gluais P, Lonchampt M, Morrow JD, Vanhoutte PM, Félétou M. Acetylcholine-induced endothelium-dependent contractions in the SHR aorta: the Janus face of prostacyclin. *Br J Pharmacol*. 2005;146:834–845.
169. Audoly LP, Rocca B, Fabre JE, Koller BH, Thomas D, Loeb AL, Coffman TM, FitzGerald GA. Cardiovascular responses to the isoprostanes iPF(2α)-III and iPE(2)-III are mediated via the thromboxane A(2) receptor in vivo. *Circulation*. 2000;101:2833–2840.
170. Montuschi P, Barnes PJ, Roberts LJ II. Isoprostanes: markers and mediators of oxidative stress. *FASEB J*. 2004;18:1791–1800.
171. Behm DJ, Ogbonna A, Wu C, Burns-Kurtis CL, Douglas SA. Epoxyeicosatrienoic acids function as selective, endogenous antagonists of native thromboxane receptors: identification of a novel mechanism of vasodilation. *J Pharmacol Exp Ther*. 2009;328:231–239.
172. Thomas DW, Mannon RB, Mannon PJ, Latour A, Oliver JA, Hoffman M, Smithies O, Koller BH, Coffman TM. Coagulation defects and altered hemodynamic responses in mice lacking receptors for thromboxane A2. *J Clin Invest*. 1998;102:1994–2001.
173. Francois H, Athirakul K, Mao L, Rockman H, Coffman TM. Role for thromboxane receptors in angiotensin-II-induced hypertension. *Hypertension*. 2004;43:364–369.
174. Francois H, Makhanova N, Ruiz P, Ellison J, Mao L, Rockman HA, Coffman TM. A role for the thromboxane receptor in L-NAME hypertension. *Am J Physiol Renal Physiol*. 2008;295:1096–1102.
175. Yamada T, Fujino T, Yuhki K, Hara A, Karibe H, Takahata O, Okada Y, Xiao CY, Takayama K, Kuriyama S, Taniguchi T, Shiokoshi T, Ohsaki Y, Kikuchi K, Narumiya S, Ushikubi F. Thromboxane A2 regulates vascular tone via its inhibitory effect on the expression of inducible nitric oxide synthase. *Circulation*. 2003;108:2381–2386.
176. Boffa JJ, Just A, Coffman TM, Arendshorst WJ. Thromboxane receptor mediates renal vasoconstriction and contributes to acute renal failure in endotoxemic mice. *J Am Soc Nephrol*. 2004;15:2358–2365.
177. Yu IS, Lin SR, Huang CC, Tseng HY, Huang PH, Shi GY, Wu HL, Tang CL, Chu PH, Wang LH, Wu KK, Lin SW. TXAS-deleted mice exhibit normal thrombopoiesis, defective hemostasis, and resistance to arachidonate induced death. *Blood*. 2004;104:135–142.
178. Grosser T, Yu Y, FitzGerald GA. Emotion recollected in tranquility: lessons from the COX-2 saga. *Ann Rev Med*. 2010;61:17–33.
179. FitzGerald GA, Lupinetti M, Charman SA, Charman WN. Presystemic acetylation of platelets by aspirin: reduction in rate of drug delivery to improve biochemical selectivity for thromboxane A2. *J Pharmacol Exp Ther*. 1991;259:1043–1049.
180. Rothwell PM, Wilson M, Elwin CE, Norrving B, Algra A, Warlow CP, Meade TW. Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet*. 2010;376:1741–1750.
181. Shimizu T. Lipid mediators in health and disease: enzymes and receptors as therapeutic targets for the regulation of immunity and inflammation. *Annu Rev Pharmacol Toxicol*. 2009;49:123–150.