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Pharmacology and signaling of prostaglandin receptors: Multiple roles in inflammation and immune modulation

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Abstract

Prostaglandins are lipid-derived autacoids that modulate many physiological systems including the CNS, cardiovascular, gastrointestinal, genitourinary, endocrine, respiratory, and immune systems. In addition, prostaglandins have been implicated in a broad array of diseases including cancer, inflammation, cardiovascular disease, and hypertension. Prostaglandins exert their effects by activating rhodopsin-like seven transmembrane spanning G protein-coupled receptors (GPCRs). The prostanoid receptor subfamily is comprised of eight members (DP, EP1–4, FP, IP, and TP), and recently, a ninth prostaglandin receptor was identified—the chemoattractant receptor homologous molecule expressed on Th2 cells (CRTH2). The precise roles prostaglandin receptors play in physiologic and pathologic settings are determined by multiple factors including cellular context, receptor expression profile, ligand affinity, and differential coupling to signal transduction pathways. This complexity is highlighted by the diverse and often opposing effects of prostaglandins within the immune system. In certain settings, prostaglandins function as pro-inflammatory mediators, but in others, they appear to have anti-inflammatory properties. In this review, we will discuss the pharmacology and signaling of the nine known prostaglandin GPCRs and highlight the specific roles that these receptors play in inflammation and immune modulation.
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Keywords: Eicosanoid; G-protein-coupled receptor; Immune modulation; Inflammation; Pharmacology; Prostaglandin; Signal transduction

Abbreviations: cAMP, cyclic AMP; COX, cyclooxygenase; CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 cells; DAG, diacylglycerol; DP, D prostanoid receptor; EGFR, epidermal growth factor receptor; EP, E prostanoid receptor; ERK, extracellular signal-related kinase; FP, F prostanoid receptor; GPCR, G protein-coupled receptor; GRK, G-protein receptor kinase; GSK-3, glycogen synthase kinase-3; HEK293, human embryonic kidney 293; IP, prostacyclin receptor; IP_3, inositol 1,4,5-trisphosphate; LPS, lipopolysaccharide; MAP kinase, mitogen-activated protein kinase; NSAID, nonsteroidal anti-inflammatory drug; OVA, ovalbumin; PG, prostaglandin; PI 3-kinase, phospatidylinositol 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; PPARγ, peroxisome proliferator-activated receptor γ; TP, thromboxane receptor.

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1. Introduction

Prostaglandins (PGs) are lipid-derived autacoids generated by sequential metabolism of arachidonic acid by the cyclooxygenase (COX) and prostaglandin synthase enzymes (Fig. 1). Arachidonic acid is a 20-carbon unsaturated fatty acid normally esterified to the sn-2 position of membrane glycerophospholipids. Upon release from the membrane by phospholipase A_2, arachidonic acid undergoes oxidation by cyclooxygenase (prostaglandin endoperoxide H synthase; PGHS) to PGG_2 followed by reduction to the unstable endoperoxide PGH_2 (for a review, see Smith et al., 2000). PGH_2 serves as a substrate for the prostaglandin synthase enzymes, which are responsible for the production of the five principal bioactive prostaglandins generated in vivo, PGE_2, $PGF_{2\alpha}$, PGD_2, PGI_2 (prostacyclin), and TXA_2 (thromboxane). The prostaglandins produced by a given cell largely depends on the expression profile of the individual prostaglandin synthase enzymes. Prostaglandins are ubiquitously produced and act locally in an autacrine or juxtacrine manner to elicit a diverse set of pharmacological effects modulating many physiological systems including the CNS, cardiovascular, gastrointestinal, genitourinary, endocrine, respiratory, and immune systems. In addition, prostaglandin synthesis has been implicated in a broad array of diseases including cancer, inflammation, cardiovascular disease, and hypertension. The physiological importance of prostaglandins is highlighted by the use of the cyclooxygenase-inhibiting nonsteroidal anti-inflammatory drugs (NSAIDs) in the clinical treatment of various disorders.

Prostaglandins are generally considered to be potent pro-inflammatory mediators, as indicated by the term *non-steroidal anti-inflammatory drugs*, used to describe phar-

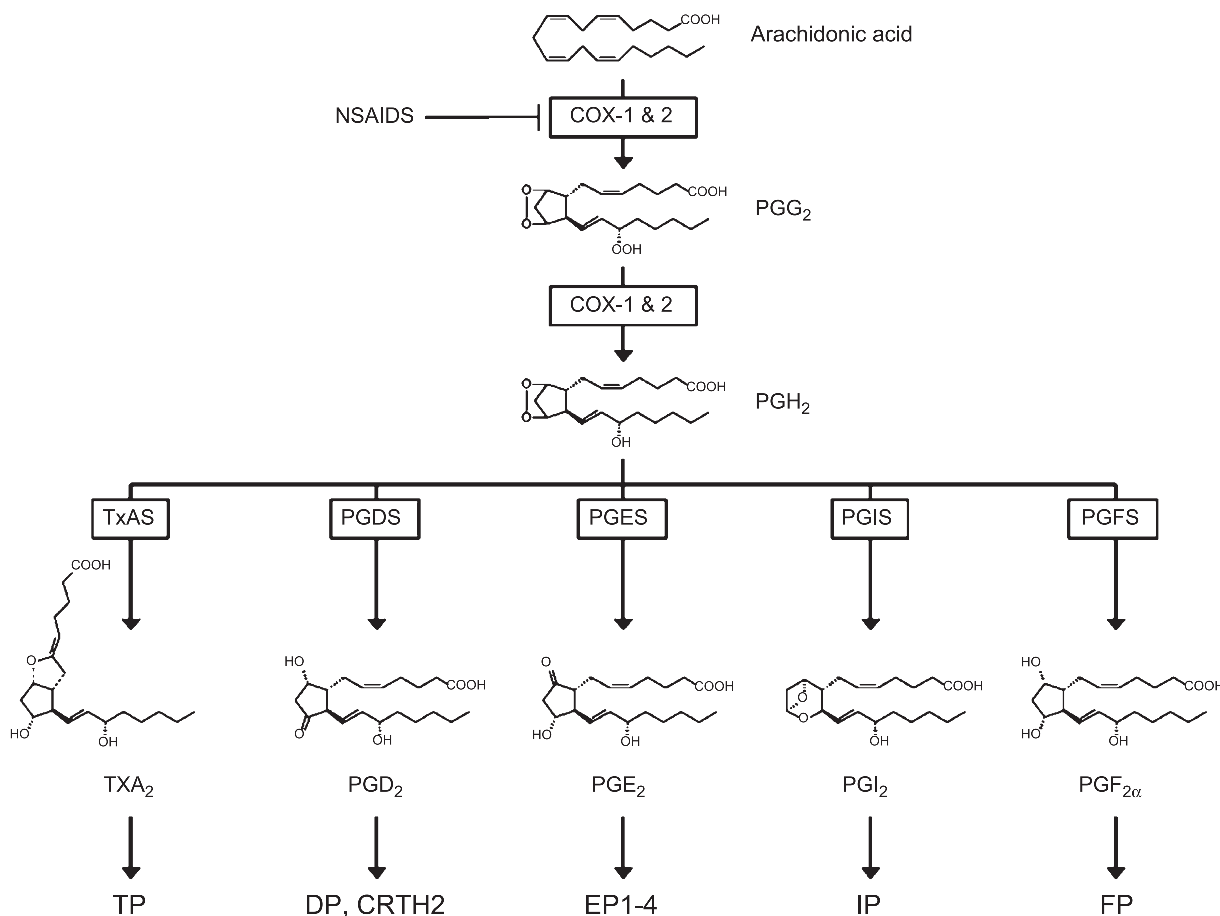

Fig. 1. Biosynthesis of prostaglandins. Arachidonic acid is metabolized by cyclooxygenase-1 or -2 to the unstable endoperoxide PGH_2, the common precursor for the five principal prostaglandins. Thromboxane A_2, PGD_2, PGE_2, PGI_2, and $PGF_{2\alpha}$ are generated by individual prostaglandin synthase enzymes (TxAS, PGDS, PGES, PGIS, and PGFS) and elicit their biological effects by activating cell surface G protein-coupled receptors.

macological agents that block prostaglandin biosynthesis. The cyclooxygenase enzyme exists as two major isozymes that differ in tissue distribution and regulation of expression (Dubois et al., 1998). Although exceptions exist, COX-1 is considered to be constitutively expressed in many tissues while the expression of COX-2 is inducible, particularly in response to inflammatory cytokines and stimuli such as bacterial lipopolysaccharide (LPS). Therefore, prostaglandins produced via COX-1 are usually ascribed a role in physiological homeostasis while those generated via COX-2 are responsible for the inflammatory effects. Traditional NSAIDs, popularly used for their antipyretic, analgesic, and anti-inflammatory properties, inhibit both COX-1 and -2 and are associated with deleterious side effects such as gastrointestinal bleeding due to suppression of both COX isozymes. The more recently developed COX-2-selective inhibitors retain effectiveness in reducing inflammation and pain in rheumatoid and osteoarthritis but have a lower incidence of gastrointestinal adverse events (FitzGerald & Patrono, 2001). Yet, despite the relative clinical effectiveness of COX inhibitors for the treatment of inflammation, emerging evidence now suggests a more complicated picture in which certain prostaglandins may also exert anti-inflammatory effects in some settings.

The physiological effects of prostaglandins are mediated in part by G-protein-coupled prostanoid receptors, a family of rhodopsin-like seven transmembrane spanning receptors (GPCRs). The prostanoid receptor subfamily is comprised of eight members (DP, EP1–4, FP, IP and TP), which are classified according to the prostanoid ligand that each binds with greatest affinity (for a review, see Breyer et al., 2001). Individual prostanoid receptors share ~20–30% sequence identity with each other and encode specific motifs common only to members of the subfamily. Recently, a ninth prostaglandin receptor was identified—the chemoattractant receptor homologous molecule expressed on Th2 cells (CRTH2)—which binds PGD_2 (Hirai et al., 2001). Surprisingly, the CRTH2 receptor is more closely related to chemoattractant receptors rather than the other prostanoid receptors (Fig. 2). Prostanoid receptors couple to classic heterotrimeric G protein-mediated signal transduction pathways, and the repertoire of signaling pathways that transduce prostanoid receptor activation into biological effects are complex (Table 1).

The precise roles of prostaglandin receptors in physiologic and pathologic settings are determined by an intricate set of ligand-receptor interactions that depend on multiple factors such as ligand affinity, receptor expression profile, differential coupling to signal transduction pathways, and the cellular context in which the receptor is expressed. Activation of a given prostaglandin receptor by its cognate ligand may elicit varying responses in different cell types and tissues. Moreover, as outlined below, the existence of multiple receptors coupling to different signal transduction pathways for a given prostaglandin (e.g., EP1–4 for PGE_2 and DP/CRTH2 for PGD_2) allows for potential synergism or

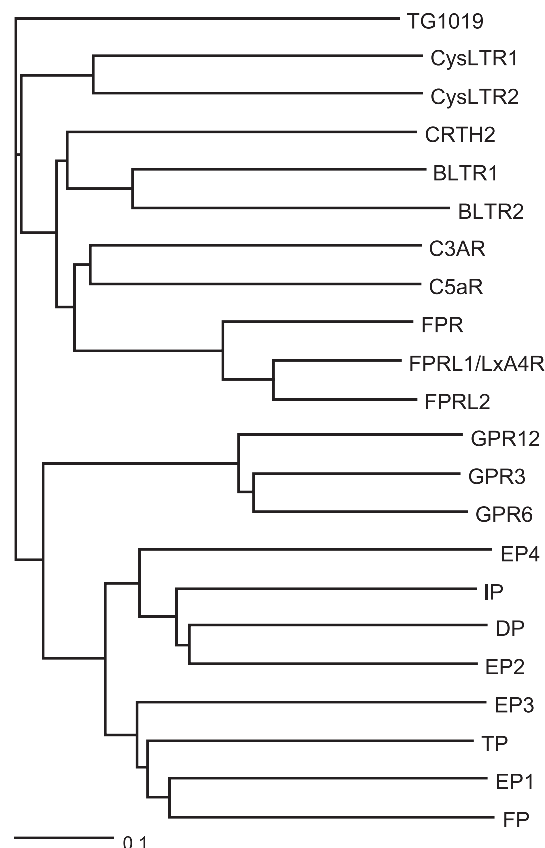

Fig. 2. Phylogenetic tree of prostanoid, selected chemoattractant, and lipid GPCRs. The DP, EP1–4, FP, IP, and TP receptors form the prostanoid subfamily of Class A (rhodopsin-like) GPCRs. The PGD_2 receptor CRTH2 is most closely related to other chemoattractant receptors. TG1019 and GPR3, GPR6 and GPR12 are novel lipid-binding GPCRs that bind 5-oxo-ETE and sphingophospholipids, respectively (for a review, see Im, 2004). The tree was generated using Clustal X based on full-length protein sequences.

antagonism between prostanoid receptor-Scale bar indicates 0.1 amino acid replacement per site mediated effects upon elaboration of a single prostanoid species. It is not surprising, given their structural similarities, that prostaglandins may activate more than one subtype of prostaglandin receptor. Pharmacologic and genetic dissection of prostaglandin receptor function has begun to reveal a complex picture in which prostaglandins serve to both promote and inhibit inflammation (Table 2). In this review, we will discuss the pharmacology and function of the nine known prostaglandin GPCRs and highlight the specific roles that these receptors play in settings of inflammation and immune system activation.

2. Thromboxane A_2 receptor (TP)

Thromboxane A_2 (TXA_2) has been most extensively characterized for its role in modulating hemodynamics and cardiovascular function. It is a potent mediator of platelet shape change and aggregation, and defective TP receptor signaling has been linked to bleeding disorders (Hirata et al.,

Table 1
Pharmacological properties of prostanoid receptors

Class	Subtype	Agonists	Antagonists	Signaling	
TXA_2	TP	U46619, I-BOP, STA_2	SQ29548, S-145, ramatroban	G_q, G_s (α), G_i (β), G_h (α), G_{12}	↑ IP_3/DAG/Ca^{2+}, ↑ cAMP, ↓ cAMP
PGD_2	DP	BW245C, L-644,698, ZK110841	BWA868C,[a] S-5751	G_s	↑ cAMP, ↑ Ca^{2+}
	CRTH2	13,15-Dihydro-15-keto-PGD_2, indomethacin, 15-*R*-methyl-PGD_2, 15d-PGJ_2	Ramatroban	G_i	↓ cAMP, ↑ Ca^{2+}, PLC, PI3K, MAPK
PGE_2	EP1	ONO-KI-004, iloprost, 17-phenyl-trinor PGE_2, sulprostone	SC51322, SC51089, ONO-8713	?	↑ Ca^{2+}
	EP2	Butaprost, 11-deoxy PGE_1, AH13205, ONO-AEI-259		G_s	↑ cAMP, EGFR transactivation, β-catenin
	EP3	Sulprostone, MB28767, misoprostol, SC46275, ONO-AE-249	ONO-AE3-240, L-826266	G_i, G_q, G_s	↓ cAMP, ↑ IP_3/DAG, ↑ cAMP
	EP4	PGE_1-OH, misoprostol, ONO-AEI-329	AH23848B ONO-AE3-208	G_s	↑ cAMP, PI3K, ERK1/2, β-catenin
PGI_2	IP	Iloprost, cicaprost, carbacyclin		G_s, G_q, G_i	↑ cAMP, ↑ IP_3/DAG, ↓ cAMP
$PGF_{2\alpha}$	FP	Fluprostenol, latanoprost		G_q	↑ IP_3/DAG, Rho, EGFR transactivation, β-catenin

[a] Partial agonist.

1994b; Mitsui et al., 1997). Increased thromboxane synthesis has been linked to cardiovascular diseases including acute myocardial ischemia (Oates et al., 1988) and heart failure (Castellani et al., 1997), and inhibition of platelet thromboxane production by aspirin is widely used to reduce the risk of myocardial infarction. A role for TXA_2 in hemostasis is supported by the observation of coagulation deficiencies in mice deficient in the TP receptor (Thomas et al., 1998). In addition to its cardiovascular role, TXA_2 is a potent bronchoconstrictor and can stimulate proliferation of airway smooth muscle cells, suggesting a role in the pathogenesis of asthma (Devillier & Bessard, 1997).

The human TP receptor was the first eicosanoid receptor cloned (Hirata et al., 1991). In humans, the TP receptor exists as two alternatively spliced variants, TPα (placental/platelet) and TPβ (endothelial), that differ in the length and sequence of the carboxyl terminal tail distal to Arg328 (Raychowdhury et al., 1994, 1995). Although these TP receptor splice variants are often described according to the tissue/cell from which they were cloned, the mRNAs for both splice variants have been detected in most tissues that express the TP receptor including platelets, placenta, vascular smooth muscle, brain, small intestine, and thymus, although there is conflicting evidence for expression of both splice variants in endothelial cells (Raychowdhury et al., 1994; Hirata et al., 1996; Miggin & Kinsella, 1998).

The endogenous TP receptor ligand TXA_2 is unstable in aqueous solution, being rapidly hydrolyzed to TXB_2, and is therefore not used in receptor binding and signal transduction assays. However, a number of synthetic agonists (I-BOP, U46619) and antagonists (SQ29548, S-145) have been developed and used extensively to probe TP receptor function. The TP antagonist BAY u3405 (ramatroban) has been developed for clinical use and is currently marketed in Japan for the treatment of allergic rhinitis. Recently, ramatroban was demonstrated to also be an antagonist at the CRTH2 receptor and inhibit PGD_2-induced chemotaxis of eosinophils (discussed below; Sugimoto et al., 2003). Recently, it has been demonstrated that 8-iso-$PGF_{2\alpha}$, one of a number of prostaglandin-like compounds (isoprostanes) formed by free radical-catalyzed peroxidation of arachidonic acid, is a TP receptor agonist (Elmhurst et al., 1997; Audoly et al., 2000). These isoprostane metabolites may play an important role in activating prostaglandin receptors in settings of oxidative stress. No differences in ligand binding affinities have been observed between the TP receptor splice variants, with the exception of PBT-3, a dual TXA_2 synthesis inhibitor and TP antagonist, which possesses 6-fold greater affinity for TPα (Qiao et al., 2003).

The TP receptor was initially characterized as coupling to a G_q-type G protein (Shenker et al., 1991) leading to activation of PLC-β, IP_3/DAG generation, and mobilization of intracellular calcium. This pathway is responsible for TP-mediated platelet aggregation, and bleeding disorders caused by deficiencies in TP receptor signaling exhibit disrupted IP_3 production (Hirata et al., 1994b; Mitsui et al., 1997). Subsequently, the coupling of the TP receptor to other G proteins such as G_s, G_i, G_h, and G_{12} has been reported. Both splice variants appear to couple equally to G_q family members (G_{11} and G_{16}) as well as the G_{12} family (G_{12} and G_{13}; Offermanns et al., 1994; Becker et al., 1999; Walsh et al., 2000a). In contrast, differential G protein coupling of TPα and TPβ has been observed for G_i, G_s, and G_h. When expressed in CHO cells, TPα mediates an increase in intracellular cAMP upon I-BOP stimulation, whereas activation of TPβ results in pertussis toxin-sensitive inhibition of cAMP (Hirata et al., 1996). The high molecular weight G-protein G_h, has been demonstrated to physically interact with both splice variants, but functionally couple only to α (Vezza et al., 1999). G_h is present in TP-expressing cells such as platelets and vascular smooth muscle cells, and may be an important mediator of splice variant-specific signaling. However, the physiological role

for differences in coupling between the TP receptor splice variants remains to be determined.

In addition to variability in signaling repertoire, functional differences between the TP receptor splice variants may be achieved by differential desensitization and internalization following agonist stimulation. Both TPα and TPβ are rapidly phosphorylated and desensitized following stimulation with U46619 (Habib et al., 1997). However, TPα also undergoes heterologous desensitization in response to prostacyclin and nitric oxide stimulation, a process that involves protein kinase A (PKA)- and protein kinase C (PKC)-mediated phosphorylation of serine residues in the unique TPα C-terminal tail (Walsh et al., 2000b; Reid & Kinsella, 2003). Differences in C-terminal tail sequence also allow for differential agonist-induced phosphorylation leading to internalization. In HEK293 cells, TPβ but not TPα undergoes U46619-induced G protein receptor kinase (GRK) phosphorylation and internalization (Parent et al., 1999), whereas the C-terminus of the TPα is not capable of being phosphorylated by GRKs (Zhou et al., 2001).

Allergic asthma is a Th2-mediated inflammatory airway disease characterized by antigen-induced bronchoconstriction, eosinophilia, and airway remodeling. TXA_2 is increased in the airways of asthmatic patients upon antigen challenge (Murray et al., 1986; Wenzel et al., 1991), and current evidence suggests that it promotes the pathogenesis of asthma at multiple levels. Although the majority of TXA_2 produced in vivo is derived from platelets, inflammatory hematopoetic cells such as monocytes and eosinophils have also been shown to produce TXA_2 (Devillier & Bessard, 1997). Blockade of either thromboxane synthesis or TP receptor activation reduces ovalbumin (OVA)-induced airway cellular infiltration, with splenic mononuclear cells from treated mice exhibiting impaired antigen-induced cytokine production (Shi et al., 1998). These data suggest that TP receptor signaling may promote cytokine production and allergic inflammation, although the exact mechanism and target cell population(s) responsible are not clear.

TXA_2 is a potent bronchoconstrictor, and activation of TP receptors expressed on bronchiole smooth muscle cells leads to intracellular calcium mobilization (Capra et al., 2003), the predominant pathway mediating constriction of airway smooth muscle cells (Hall, 2000). Studies using TP receptor antagonists have suggested that in addition to TXA_2, TP receptors mediate the bronchoconstrictive response to PGD_2 (Coleman & Sheldrick, 1989; Francis et al., 1991; Johnston et al., 1992). However, the recent reports that the TP antagonist ramatroban and TXA_2 metabolite 11-deoxy-TXB_2 are ligands for the recently described PGD_2 receptor CRTH2 (Sugimoto et al., 2003; Bohm et al., 2004) have raised the possibility that activation of the CRTH2 receptor may be responsible for some of the bronchoconstrictive effects attributed to the TP receptor. TXA_2 may also exacerbate airway hyperresponsiveness by potentiating airway smooth muscle contraction elicited by cholinergic parasympathetic neurotransmission (Chung et al., 1985; Munoz et al., 1986). Pretreatment with ipratropium bromide, a muscarinic receptor antagonist, attenuates U46619-induced bronchoconstriction in asthmatic subjects, suggesting that TXA_2-mediated bronchoconstriction may itself be mediated by cholinergic neurons (Saroea et al., 1995).

Bronchiole smooth muscle cells express both TPα and TPβ, and stimulation by U46619 leads to an increase in proliferation that can be blocked with the TP antagonist SQ29548 (Tomlinson et al., 1994; Capra et al., 2003). Thus, TP receptor signaling may contribute to the bronchiole smooth muscle hyperplasia and airway remodeling that occurs in response to chronic airway inflammation in asthma (Vignola et al., 2003). Other prostaglandin receptors such as EP2 and FP have been demonstrated to mediate proliferation via transactivation of the EGF receptor (EGFR; discussed below). However, the observed mitogenic response to TP receptor activation appears to be independent of EGFR transactivation, although TP receptor activation may potentiate the proliferative response to EGF (Capra et al., 2003).

Aside from its role in the pathogenesis of allergic airway disease, TP receptor signaling has been proposed to play a role in modulating T-cell activation, although the current evidence for a pro- versus anti-inflammatory role is conflicting. Inhibition of thromboxane synthesis locally depresses anti-donor cytotoxic T-cell function in the rat renal allograft model, suggesting that the inflammatory cell infiltration associated with immune-mediated acute allograft rejection depends, in part, on TP receptor signaling (Ruiz et al., 1989). TXA_2 is produced by macrophages and dendritic cells and has also been proposed to play a role in regulating T-cell differentiation and proliferation (Kabashima et al., 2003a). Inhibition of thromboxane synthesis or direct TP antagonism decreases the alloproliferative response of total lymphocyte populations in the mixed lymphocyte reaction, a model of the cellular immune response (Ruiz et al., 1992), and lymphocyte populations from mice deficient in the TP receptor exhibit a decreased proliferative response following both mitogen and alloantigen stimulation compared to wild-type controls (Thomas et al., 2003). In contrast, other studies utilizing TP null mice demonstrate an increase in contact hypersensitivity, suggesting an inhibitory role for the TP receptor in dendritic cell-T-cell interaction (Kabashima et al., 2003a). TP signaling may also play an early role in the development of the adaptive immune response by influencing thymic selection of T lymphocytes. Immature thymocytes initially express both CD4 and CD8 coreceptors, but during thymic selection most undergo apoptosis and those that remain express either CD4 or CD8. TXA_2 is produced in the thymic microenvironment (Homo-Delarche et al., 1985), and in vitro stimulation of mixed thymocyte populations with the TP agonist STA_2 leads to increased apoptosis of immature double positive ($CD4^+CD8^+$) thymocytes and an increase in the relative percentage of mature

single positive ($CD4^+CD8^-$ or $CD4^-CD8^+$) cells (Ushikubi et al., 1993). TP receptor stimulation has no apoptotic effect on the latter group, suggesting that TXA_2/TP signaling specifically modulates apoptosis during the selection of developing T lymphocytes. Consistent with TXA_2-mediated apoptosis during thymic selection, TP receptor signaling has been shown to be critical for elimination of reactive T-cell clones in a model of acquired renal allograft tolerance (Remuzzi et al., 1994).

3. PGD_2 receptors (DP and CRTH2)

PGD_2 has long been associated with inflammatory and atopic conditions. In the early 1980s, PGD_2 was discovered to be the predominant prostanoid produced by activated mast cells, which initiate IgE-mediated Type I acute allergic responses (Roberts et al., 1980; Lewis et al., 1982). PGD_2 is released into the airways following antigen challenge as well as the skin during an acute allergic response (Murray et al., 1986; Barr et al., 1988). PGD_2 challenge elicits several hallmarks of allergic asthma such as bronchoconstriction and airway eosinophil infiltration (Hardy et al., 1984; Emery et al., 1989), and mice that overexpress lipocalin-PGD synthase have elevated PGD_2 levels and an increased allergic response in the OVA-induced model of airway hyperreactivity (Fujitani et al., 2002). Other immune cells such as antigen-presenting dendritic cells and helper Th2-type T-cells also possess the capacity to produce PGD_2, suggesting a modulatory role for PGD_2 in the development of antigen-specific immune system response (Urade et al., 1989; Tanaka et al., 2000). In addition to its role as an inflammatory mediator, PGD_2 mediates a number of other effects including inhibition of platelet aggregation (Whittle et al., 1985), smooth muscle relaxation and contraction (Narumiya & Toda, 1985), vasodilation and vasoconstriction (Giles & Leff, 1988), mucus secretion (Wright et al., 2000), and sleep induction (Mizoguchi et al., 2001).

PGD_2 exerts its effects by binding and activating two distinct GPCRs—the DP receptor and the recently discovered CRTH2 receptor ("DP2"). The DP receptor is a member of the prostanoid GPCR subfamily and shows significant sequence identity with the other members of the family such as the IP and EP2 receptors (Fig. 2; Boie et al., 1995). The human DP receptor binds PGD_2 and PGJ_2 with high affinity, as well as the synthetic agonists BW245C and L-644,698 and the partial agonist BWA868C (Wright et al., 1998). Recently, a class of bicycloheptane derivative DP antagonists including S-5751 has been developed that blocks the actions of PGD_2 in experimental models of inflammation (Tsuri et al., 1997; Arimura et al., 2001; Mitsumori et al., 2003a, 2003b). DP receptor activation leads to G_s-mediated increases in intracellular cAMP; agonist-evoked calcium flux has also been observed (Hirata et al., 1994a; Boie et al., 1995).

Pharmacological studies showing that the effects of PGD_2 are not mimicked by all PGD_2 analogues in all contexts have suggested the presence of more than a single PGD_2 receptor (Narumiya & Toda, 1985; Rangachari et al., 1995). Moreover, despite intense interest in the role of PGD_2 in the inflammatory response, a direct link between DP receptor activation and PGD_2-stimulated eosinophil migration has not been established (Woodward et al., 1990, 1993). The recent discovery of the second PGD_2 receptor CRTH2 has begun to clarify the mechanism of action of PGD_2. The CRTH2 receptor was initially identified as a Th2 cell-specific surface receptor and was subsequently demonstrated to bind PGD_2 with approximately equal affinity as that observed for the DP receptor (Nagata et al., 1999b; Hirai et al., 2001). The CRTH2 receptor shows little similarity with the DP receptor and the other prostanoid receptors despite the fact that it possesses similar affinity for PGD_2; instead, it is most closely related to other chemoattractant receptors (Fig. 2). The CRTH2 receptor binds an overlapping but distinct set of ligands compared with the DP receptor. For example, the PGD_2 metabolite 13,15-dihydro-15-keto-PGD_2 but not the synthetic DP agonist BW245C binds with high affinity to the CRTH2 receptor (Hirai et al., 2001). The CRTH2 receptor also binds the PGD_2 metabolite 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) with equal high affinity to PGD_2 itself (Sawyer et al., 2002), raising the possibility that metabolites of PGD_2 may differentially exert effects through CRTH2 but not the DP receptor. Although 15d-PGJ_2 has been proposed to be a ligand for the peroxisome proliferator-activated receptor γ nuclear receptor, the affinity of 15d-PGJ_2 for CRTH2 is several orders of magnitude greater than that observed for PPARγ (Forman et al., 1995). The NSAID indomethacin has been demonstrated to be a potent agonist at the CRTH2 receptor (Hirai et al., 2002). This effect is independent of inhibition of COX, as demonstrated by the fact that other NSAIDs do not share this property. Recently, it was reported that the TP antagonist ramatroban (BAY u4305) is also a CRTH2 antagonist (Sugimoto et al., 2003).

Similar to many chemoattractant receptors, the CRTH2 receptor couples to a G_i-type G protein leading to inhibition of cAMP and increases in intracellular calcium in a variety of cell types (Hirai et al., 2001; Sawyer et al., 2002). CRTH2-mediated chemotaxis requires G_i signaling but is independent from changes in intracellular Ca^{2+} signaling, with the specific functional response being influenced by cell type (Nagata & Hirai, 2003). In addition, CRTH2 receptor stimulation has been reported to lead to pertussis toxin-insensitive activation of pI 3-kinase, PLC, and MAP kinases in eosinophils (Stubbs et al., 2002). These pathways mediate eosinophil shape change, actin polymerization, and CD11b up-regulation, functions consistent with a role for the CRTH2 receptor in

eosinophil activation (Monneret et al., 2001; Stubbs et al., 2002). CRTH2-induced cell migration is at least partially dependent on PI3K signaling (Hata et al., 2003).

The pro-inflammatory effects of PGD_2 appear to be mediated by both DP and 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 DP or CRTH2 receptors or both are locally expressed. The DP 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 (Kabashima & Narumiya, 2003). DP antagonists exert anti-inflammatory properties in several experimental models including inhibition of antigen-induced conjunctival microvascular permeability in guinea pigs and OVA-induced airway hyperreactivity in mice (Tsuri et al., 1997; Arimura et al., 2001). Mice deficient in the DP receptor exhibit reduced airway hypersensitivity and Th2-mediated lung inflammation in the OVA-induced asthma model, suggesting that the DP receptor plays a key role in mediating the effects of PGD_2 released by mast cells during an asthmatic response (Matsuoka et al., 2000). PGD_2 released from mast cells may also mediate recruitment of Th2 lymphocytes and eosinophils directly via the CRTH2 receptor. In humans, the CRTH2 receptor is expressed on Th2 lymphocytes, eosinophils, and basophils (Nagata et al., 1999a, 1999b; Hirai et al., 2001), and an increase in $CRTH2^+$ T-cells has been positively associated with certain forms of atopic dermatitis (Iwasaki et al., 2002). The CRTH2 receptor has been demonstrated to mediate PGD_2-stimulated chemotaxis of these cells in vitro (Hirai et al., 2001) and leukocyte mobilization in vivo (Shichijo et al., 2003). Antagonism of the CRTH2 receptor may also explain the reduction in eosinophilic infiltration observed following antigen challenge in ramatroban-treated animals (Nagai et al., 1995; Narita et al., 1996), since TP receptor activation has no effect on eosinophil migration (Monneret et al., 2001). Furthermore, PGD_2 may inhibit eosinophil apoptosis via the DP receptor (Gervais et al., 2001). Therefore, PGD_2 acting through both DP and CRTH2 receptors likely contributes to the eosinophilic infiltration that is a hallmark of allergic asthma. Characterization of mice deficient in the CRTH2 receptor will begin to clarify the exact role that each receptor plays in mediating the effects of PGD_2 in allergic inflammation.

Like TXA_2, PGD_2 may also exacerbate nonimmune events associated with asthma, such as airway remodeling and bronchoconstriction. During airway remodeling, there is proliferation of airway epithelial goblet cells leading to mucus hypersecretion. The DP receptor is expressed on bronchiole epithelial cells in antigen-challenged mice (Matsuoka et al., 2000) as well as nasal epithelial goblet cells in normal human volunteers (Nantel et al., 2004). The DP receptor has been linked to mucus secretion by colonic goblet cells (Wright et al., 2000). Taken together, these observations suggest that the DP receptor may evoke mucus secretion in response to PGD_2 in asthma and allergic rhinitis. Asthma is also characterized by severe but reversible antigen-induced bronchoconstriction. PGD_2 is a potent bronchoconstrictor (Hardy et al., 1984); however, these effects are unlikely to be mediated by the DP receptor since an increase in intracellular cAMP is typically associated with smooth muscle relaxation (Hall, 2000). Studies using TP receptor antagonists have suggested that TP receptors mediate the bronchoconstrictive response to PGD_2 (Francis et al., 1991; Johnston et al., 1992) despite the fact that PGD_2 has relatively low affinity for the TP receptor (Abramovitz et al., 2000). It was recently reported that the TP antagonist ramatroban is a CRTH2 antagonist (Sugimoto et al., 2003). Additionally, the TXA_2 metabolite 11-dehydro-TXB_2 has been demonstrated to be a CRTH2 agonist (Bohm et al., 2004). These observations raise the possibility that activation of the CRTH2 receptor may contribute to bronchoconstriction in asthma, although this remains to be established.

In contrast to the pro-inflammatory role of PGD_2 in allergic inflammation, PGD_2 may act to inhibit inflammation in other contexts. The DP receptor is expressed on dendritic cells that play a key role in initiating an adaptive immune response to foreign antigens. PGD_2 activation of the DP receptor inhibits dendritic cell migration from lung to lymph nodes following OVA challenge, leading to reduced proliferation and cytokine production by antigen specific T cells (Hammad et al., 2003). The DP receptor also mediates inhibition of antigen-presenting Langerhans cell function by PGD_2. PGD_2 released during cutaneous infection by *Schistosoma mansoni* inhibits migration of Langerhans cells via the DP receptor, thereby protecting the parasite from the host immune response (Angeli et al., 2001). Similarly, DP receptor activation during antigen sensitization leads to a reduced antigen-specific contact hypersensitivity response.

4. PGE_2 receptors (EP1–4)

PGE_2 is a major cyclooxygenase product in a number of physiological settings. In the gastrointestinal tract, COX-1-derived PGE_2 plays a protective role in maintaining the integrity of the gastric mucosa, and significant gastrointestinal adverse events are associated with prostaglandin inhibition by nonselective NSAIDs (Woo et al., 1986; Warner et al., 1999). Reduced incidence of these complications is observed upon treatment with the PGE_2 analogue misoprostol (Silverstein et al., 1995) or use of COX-2-selective inhibitors (FitzGerald & Patrono, 2001). PGE_2 production in the kidney is critical for normal renal function by preserving renal blood flow and glomerular filtration rate in settings of physiological stress, modulating

salt and water transport in the distal tubule, and stimulating renin release from the juxtaglomerular apparatus (for a review, see Breyer & Breyer, 2001). PGE_2 has also been shown to play a role in the maintenance of blood pressure, particularly in the setting of salt overload (Kennedy et al., 1999). In certain instances, PGE_2 has been observed to have multiple and apparently opposing functional effects. For example, PGE_2 elicits both smooth muscle relaxation and constriction. In cat trachea, PGE_2 acts as a dilator, but it is a constrictor in guinea pig ileum (Gardiner, 1986). Similarly, PGE_2 may have either dilator or constrictor effects on vascular smooth muscle (Walch et al., 2001; Davis et al., 2004). Complexity is also observed in modulation of the immune response by PGE_2, whereby activation of specific EP receptors has been shown to regulate the function of many cell types including macrophages, dendritic cells, T and B lymphocytes leading to both pro- and anti-inflammatory effects.

The diverse effects of PGE_2 may be accounted for in part by the existence of four receptors, designated EP1, EP2, EP3, and EP4, and heterogeneity in the coupling of these receptors to intracellular signal transduction pathways. Prior to cloning, the EP receptors were numerically designated based on pharmacological profiles and physiologic effects. Of the four EP receptors, the EP3 receptor was the first to be cloned (Sugimoto et al., 1992), followed by the EP1 and EP4 receptors (Funk et al., 1993; Honda et al., 1993). Originally, the cloned EP4 receptor was misdesignated EP2, but subsequent cloning of a fourth PGE_2 receptor and further pharmacological characterization revealed the former to be the EP4 receptor and the latter the pharmacologically defined EP2 receptor (Regan et al., 1994). As discussed below, the EP3 receptor is unique in that multiple splice variants that arise from alternative splicing of the C-terminal tail have been identified in several species.

Of the four EP receptors, the EP3 and EP4 receptors bind PGE_2 with highest affinity (K_d < 1 nM), whereas the EP1 and EP2 receptors bind with lower affinity (K_d > 10 nM; Abramovitz et al., 2000). Pharmacological agents with varying selectivities for the individual EP receptors have been used extensively to investigate which EP receptors mediate the differential effects of PGE_2. Commonly used selective EP receptor agonists include butaprost (EP2), sulprostone (EP3—also has low affinity for EP1 and FP), and PGE_1-OH (EP4; Breyer et al., 2001). In contrast, most EP1 agonists have rather poor subtype selectivity, with sulprostone, iloprost, and 17-phenyl-PGE_2 exhibiting even higher binding affinities for EP3. A novel series of selective EP receptor ligands have been developed including the agonists ONO-DI-004, ONO-AE1-259, ONO-AE-248, and ONO-AE1-329 (EP1, EP2, EP3, and EP4, respectively) and antagonists ONO-8713, ONO-AE3-240, and ONO-AE208 (EP1,3,4, respectively; Suzawa et al., 2000; Watanabe et al., 2000; Kabashima et al., 2002; Amano et al., 2003). In addition, a family of acylsulfonamide EP3 antagonists, which includes L-826266, has been described (Juteau et al., 2001; Gallant et al., 2002). Recently, it has been shown that the E-ring 8-isoprostanes (8-iso-PGE_2) are capable of binding and activating EP1 and EP3 receptors (Sametz et al., 2000; Clarke et al., 2004). As with the TP receptor, isoprostane metabolites may play an important role in activating prostaglandin receptors in settings of oxidative stress.

The EP receptors were originally classified by their function on smooth muscle–relaxant versus constrictor—as well as distinct pharmacology (Gardiner, 1986). The "relaxant" EP2 and EP4 receptors couple to a G_s-type G protein leading to stimulation of cAMP, whereas the "constrictor" EP1 receptor couples to increases in intracellular calcium. Additionally, the EP3 receptor, which couples to a G_i-type G protein has been termed "inhibitory." This functional differentiation of the EP receptors is reflected in amino acid homology (Fig. 2). The EP2 receptor is more closely related to other relaxant prostanoid receptors such as the IP and DP receptors and the constrictor EP1 receptor is more closely related to other Ca^{2+}-coupled prostanoid receptors such as the TP and FP receptors (Toh et al., 1995). However, this simple dichotomy based on classic signal transduction coupling belies the complexity in the signaling repertoire of the EP receptors.

The EP1 receptor was originally characterized as coupling to stimulation of intracellular calcium (Funk et al., 1993; Watabe et al., 1993), and little is known about other signal transduction pathways. Interestingly, EP1-induced calcium flux correlates with only a small increase in IP_3 generation and is dependent on the presence of extracellular calcium, leading some to suggest that the EP1 receptor couples to calcium through a mechanism independent of the G_q-type G protein (Narumiya et al., 1999).

Although both EP2 and EP4 receptors couple to G_s resulting in stimulation of adenylyl cyclase and increased cAMP levels (Honda et al., 1993; Regan et al., 1994), direct comparison of their relative abilities to increase cAMP demonstrate much weaker G_s coupling by EP4 compared with the EP2 receptor (Fujino et al., 2002b). Recent studies have also revealed important functional differences that suggest unique roles for each receptor. For instance, PGE_2 stimulation of the EP4 but not EP2 receptor leads to PI 3-kinase-dependent phosphorylation of extracellular signal-related kinase 1/2 (ERK 1/2) and expression of early growth response factor-1 (Fujino et al., 2003). PI 3-kinase has also been reported to play a role in EP4-induced activation of the GSK-3/β-catenin signaling pathway (Fujino et al., 2002b). β-Catenin is a transcriptional activator and has been implicated in embryonic development and cancer (for a review, see Moon et al., 2002). GSK-3 phosphorylates β-catenin leading to cytosolic sequestration and degradation; phosphorylation of GSK-3 inhibits its activity and allows β-catenin to translocate to the nucleus. Stimulation of the EP2 receptor also leads to GSK-3 phosphorylation and subsequent activation of β-catenin, but in a PKA-dependent, P13-

kinase-independent manner (Fujino et al., 2002b). Taken together, these studies raise the possibility that G_s-mediated increases in cAMP play a less important role for EP4 receptor signaling compared with the EP2 receptor, a possibility further supported by observations that the EP4 receptor mediates PGE_2-stimulated proliferation of colon carcinoma cells in the absence of detectable increases in cAMP (Pozzi et al., 2004). Recently, a novel EP2 receptor signaling pathway has been described in which PGE_2 simulation leads to transactivation of the EGF receptor leading to increased migration and invasion of colon cancer cells (Pai et al., 2002; Buchanan et al., 2003).

Functional differences in EP2 versus EP4 receptor signaling may also arise from differential agonist-induced desensitization and internalization. Compared with EP2, the EP4 receptor has a much longer C-terminal tail that is required for rapid agonist-induced desensitization (Bastepe & Ashby, 1999). In contrast, the EP2 receptor does not undergo rapid agonist-induced desensitization (Nishigaki et al., 1996). Furthermore, the difference in C-terminal tail length has been shown to be responsible for the observation that the EP4 but not EP2 receptor undergoes agonist-induced internalization (Desai et al., 2000).

A striking feature of the EP3 receptor that sets it apart from the other EP receptors is the existence of multiple splice variants generated by alternative splicing of the C-terminal tail. In humans, at least eight EP3 receptor splice variants have been identified, and multiple splice variants exist for other species including mouse, rabbit, and cow (for a review, see Breyer et al., 2001). A number of functional differences between the splice variants have been reported including differential activation of signal transduction pathways, constitutive activity, agonist-induced desensitization, and intracellular trafficking. Originally, the EP3 receptor was described to couple to a G_i-type G protein leading to inhibition of intracellular cAMP (Sugimoto et al., 1992), but subsequently, it was shown that individual splice variants could also couple to stimulation of cAMP and IP_3 generation (Irie et al., 1993; Namba et al., 1993). The mouse EP3γ splice variant is capable of coupling to G_s in addition to G_i, a property not shared by EP3α and EP3β, whereas EP3α and EP3γ but not EP3β exhibit agonist-independent constitutive inhibition of cAMP (Hasegawa et al., 1996; Negishi et al., 1996). In contrast, G_s-mediated stimulation of cAMP by EP3γ is agonist dependent. Differences in EP3 receptor splice variant signal transduction may also result from differential receptor desensitization. When expressed in CHO cells, EP3α undergoes rapid agonist-induced desensitization and sequestration followed by long-term down-regulation, whereas no changes in EP3β signaling occur (Negishi et al., 1993). Although these observations clearly demonstrate that important functional differences exist between the EP3 receptor splice variants in cell culture systems, the physiological significance of these different C-terminal splice variants remains uncertain. EP3 receptor-deficient mice have been reported, but these lack all EP receptor splice variants (Ushikubi et al., 1998). Generation of mice deficient in specific EP3 receptor splice variants, on the other hand, may begin to clarify the functional roles of the individual splice variants in vivo.

PGE_2 is generated in settings of inflammation, such as activation of macrophages by LPS (Nishijima et al., 1985), and exerts potent immunomodulatory effects. Intracerebroventricular injection of LPS activates the innate immune response and results in oxidative damage that can be suppressed by systemic treatment with NSAIDs (Montine et al., 2002). The suppressed oxidative damage is also observed in EP2 receptor null mice, suggesting that EP2 receptor-evoked pathways are important in the generation of the innate immune response. These results reveal that the EP2 receptor is pro-inflammatory in this model and suggest that this receptor would be a potential therapeutic target for the suppression of oxidative damage associated with the activation of the innate immune response. In contrast, many studies on macrophages in vitro suggest that PGE_2 suppresses macrophage function, most likely through the EP4 receptor, and thus would be anti-inflammatory (Snyder et al., 1982; Nataraj et al., 2001; Takayama et al., 2002; Akaogi et al., 2004). Thus, the net result of PGE_2 on macrophage function and the innate immune response is dependent on the precise stimulus and context of cellular stimulation.

PGE_2 synthesis during an inflammatory insult has been shown to play a role in inducing symptoms such as tissue edema and hyperalgesia in animal models (Portanova et al., 1996; Trebino et al., 2003). At the local site of injury, PGE_2 causes hyperalgesia by modulating voltage-gated sodium currents in nociceptive primary afferent nerve terminals (Khasar et al., 1998). PGE_2 is also generated in the central nervous system during a peripheral inflammatory response and further contributes to inflammatory pain hypersensitivity (Samad et al., 2001). Intrathecal administration of an EP1 receptor antagonist reduces hyperalgesia associated with carrageenan-induced paw inflammation in rats (Nakayama et al., 2002), and mice deficient in the EP1 receptor have a reduced stretching response following administration of acetic acid or 2-phenyl-1 benzyoquinone (PBQ; Stock et al., 2001), suggesting that the central hyperalgesic effects of PGE_2 are mediated by spinal EP1 receptors. Other studies have also implicated the EP3 receptor in the inflammatory pain response (Minami et al., 2001).

Analyses of expression patterns of the four EP receptors have revealed their presence on most major subsets of cells involved in the adaptive immune response including T, B, and dendritic cells (for a review, see Tilley et al., 2001), suggesting that PGE_2 may play a role at multiple levels within the immune system. The adaptive immune response is initiated when antigen-presenting cells such as dendritic cells encounter antigen and become activated. Activated dendritic cells produce pro-inflammatory cytokines that attract other inflammatory cells to the local environment or undergo a process of maturation and migration to lymph

nodes where they stimulate proliferation of antigen-specific T-cell populations. Dendritic cells express all four EP receptor subtypes (Harizi et al., 2003; Kabashima et al., 2003b) and PGE_2 has been shown to modulate dendritic cell function. When activated in the presence of PGE_2, dendritic cells lose their ability to secrete cytokines such as IL-12, CCL3, and CCL4, but up-regulate expression of chemokine receptors necessary for migration to lymph nodes (Luft et al., 2002; Scandella et al., 2002; Jing et al., 2003). Pharmacological analysis using selective EP receptor agonists has implicated EP2 and EP4 receptors, and EP4 receptor-deficient mice exhibit reduced migration of Langerhans cells to draining lymph nodes following cutaneous antigen exposure (Kabashima et al., 2003b). Furthermore, EP4 receptor signaling may also enhance the T-cell stimulatory capacity of Langerhans cells. These results suggest that PGE_2 signaling through the EP4 receptor facilitates immune activation by enhancing migration of antigen-stimulated Langerhans cells to lymph nodes and subsequent T-cell activation during the contact hypersensitivity response. In contrast, other studies have revealed that tumor-derived PGE_2 exerts inhibitory effects on the host immune response (Stolina et al., 2000). These effects include inhibition of dendritic cell differentiation and T-cell stimulatory capacity via the EP2 receptor, leading to cancer-associated immunodeficiency, which allows tumors to escape immune surveillance (Yang et al., 2003).

In addition to indirect effects on T-cell priming and proliferation via modulation of antigen presenting cell function, PGE_2 is capable of binding EP receptors expressed on T cells and directly modulating T-cell proliferation. PGE_2 has long been known to suppress T-cell mitogenesis (Goodwin et al., 1977), possibly by inhibiting IL-2 synthesis (Minakuchi et al., 1990). PGE_2 is produced by dendritic cells (Harizi et al., 2001; Fogel-Petrovic et al., 2004), and mixed lymphocyte reactions utilizing lymphocyte populations derived from mice deficient in specific EP receptors have revealed that the anti-proliferative effect is primarily mediated by the EP2 receptor (Nataraj et al., 2001), although the EP4 receptor may play a role in some contexts (Kabashima et al., 2003b). Activation of the EP2 receptor may also play a role in early thymic T-cell development by promoting the transition of immature T cells from the double negative ($CD4^-CD8^-$) to double positive ($CD4^+CD8^+$) stage (Rocca et al., 1999) and suppressing apoptosis of double positive cells (Goetzl et al., 1995). Thus, EP2 receptor signaling in immature thymocytes may oppose the pro-apoptotic effects of thromboxane A_2 mediated by the TP receptor, although the relative contribution of EP2 versus TP receptor signaling toward shaping the functional T-cell repertoire remains to be determined. PGE_2 has been shown to further influence thymic T-cell development by playing a role in positive selection of single positive $CD4^+$ T cells (Rocca et al., 1999).

The presence of PGE_2 during T-cell activation and maturation following priming by antigen presenting cells modulates the nature of the cellular immune response. During this process, $CD4^+$ helper cells differentiate into different functional classes based on cytokine expression—Th1 (IL-2 and IFN-γ), Th2 (IL-4 and IL-5), or Th0, which secrete both Th1- and Th2-type cytokines. Selective generation of Th1 cells favors a cellular immune response whereas production of Th2 cells leads to a humoral immune response. The regulation that governs the balance between a Th1 versus Th2 response is influenced by a number of factors including the specific dendritic cell type providing the stimulus and the local presence of specific cytokines. The presence of IL-12 during T-cell activation is critical for induction of a polarized Th1 response (Hsieh et al., 1993). PGE_2 is thought to shift the balance in favor of a Th2 response in part by EP4 receptor-mediated inhibition of IL-12 production by monocytes and macrophages (van der Pouw Kraan et al., 1995; Nataraj et al., 2001) as well as by acting directly on T-cells to suppress production of IL-2 and IFN-γ (Katamura et al., 1995; Kabashima et al., 2003b). Macrophages from BALB/c mice, which are commonly used in models of Th2-mediated inflammation, produce greater levels of PGE_2 and lower levels of Th1 cytokines upon LPS stimulation (Kuroda & Yamashita, 2003). PGE_2 may further influence Th2-mediated humoral immune responses by EP2/4 receptor-driven cytokine-induced class switching to IgE in antibody-producing B cells (Fedyk & Phipps, 1996) and EP3 receptor-mediated potentiation of mast cell degranulation (Nguyen et al., 2002).

Despite evidence suggesting that PGE_2 influences $CD4^+$ T-cell maturation in favor of a Th2-dominant immune response, in vivo studies suggest that PGE_2 suppresses Th2-mediated allergic inflammation. Acute administration of PGE_2 prior to aerosolized antigen challenge reduces pulmonary resistance in humans (Pavord et al., 1993; Gauvreau et al., 1999) and decreases airway eosinophilia and Th2 cytokine production in the OVA-induced murine asthma model (Martin et al., 2002). Similarly, abrogation of PGE_2 synthesis in COX-deficient mice correlates with an increase in allergic airway inflammation, although the exacerbation of the allergic response may be in part a result of increased leukotriene B_4 synthesis (Gavett et al., 1999). The exact mechanism of the anti-inflammatory effect of PGE_2 in these models in not clear, but may involve suppression of PGD_2 production (Hartert et al., 2000).

Thus, the emerging picture reveals that PGE_2 exerts both pro- and anti-inflammatory effects depending on receptor subtype, cell population, and context of activation (Table 2). This complexity is highlighted by a comparison of the effects of EP4 receptor activation during specific inflammatory responses. As discussed above, EP4 receptor signaling promotes the initiation of the contact hypersensitivity immune response. The EP4 receptor also appears to play a pro-inflammatory role in the pathogenesis of rheumatoid arthritis. PGE_2 produced by rheumatoid synovium has been implicated in IL-6 production (Portanova et

Table 2
Prostaglandin exert both pro- and anti-inflammatory effects

Class	Subtype	Pro-inflammatory	Anti-inflammatory
TXA_2	TP	Increased cytokine production by splenic mononuclear cells and OVA-induced airway inflammation (Shi et al., 1998)	Inhibition of dendritic cell-T-cell interaction (Kabashima et al., 2003a)
		Bronchiole smooth muscle hyperplasia and proliferation (Tomlinson et al., 1994; Capra et al., 2003)	Elimination of self-reactive T-cells (Remuzzi et al., 1994)
		Increased T-cell proliferation (Thomas et al., 2003)	
		Enhanced local cytotoxic T-cell function (Ruiz et al., 1989)	
PGD_2	DP	Vasodilatation	Inhibition of dendritic cell migration following OVA challenge (Hammad et al., 2003)
		Increased OVA-induced airway inflammation (Matsuoka et al., 2000)	Inhibition of Langerhans cell migration during *S. mansoni* infection and cutaneous hypersensitivity reaction (Angeli et al., 2001)
		Inhibition of eosinophil apoptosis (Gervais et al., 2001)	
	CRTH2	Stimulation of Th2 cell and eosinophil chemotaxis (Hirai et al., 2001)	
		Activation of eosinophils (Monneret et al., 2001; Stubbs et al., 2002)	
PGE_2	EP1	Inflammatory hyperalgesia (Stock et al., 2001; Nakayama et al., 2002)	
	EP2	LPS-stimulated oxidative damage (Montine et al., 2002)	Inhibition of T-cell proliferation (Nataraj et al., 2001)
		IgE class switching by B-cells (Fedyk & Phipps, 1996)	Inhibition of dendritic cell function in cancer-associated immunodeficiency (Yang et al., 2003)
	EP3	Inflammatory hyperalgesia (Minami et al., 2001)	
		Enhancement of antigen-stimulated mast cell degranulation (Nguyen et al., 2002)	
	EP4	Enhanced migration, maturation, and T-cell stimulatory capacity of Langerhans cells (Kabashima et al., 2003a)	Suppression of macrophage cytokine production (Nataraj et al., 2001; Takayama et al., 2002)
		Inhibition of IL-12 production by macrophages leading to Th2 polarized immune response (van der Pouw Kraan et al., 1995; Nataraj et al., 2001)	Inhibition of T-cell proliferation (Kabashima et al., 2003b)
			Inhibition of OVA-induced airway inflammation (Martin et al., 2002)
		IgE class switching by B-cells (Fedyk & Phipps, 1996)	Inhibition of dextran-sulfate induced experimental colitis (Kabashima et al., 2002)
		Increased Th1-mediated inflammation in arthritis model (McCoy et al., 2002)	
PGI_2	IP	Increase in microvascular permeability (Murata et al., 1997)	Stimulation of T-cell IL-10 production and inhibition of OVA-induced airway inflammation (Takahashi et al., 2002)
$PGF_{2\alpha}$	FP	Facilitation of inflammatory pain transmission (Murata et al., 1997)	Inhibition of lymphocyte-endothelial cell interactions (Della Bella et al., 2001)

al., 1996) and joint destruction (Dayer et al., 1976). Mice deficient in the EP4 but not the EP1, EP2, or EP3 receptor exhibit an attenuated response in the collagen antibody-induced arthritis model, with significantly lower levels of the inflammatory cytokines IL-6 and IL-1β and a dramatic reduction in the clinical signs of disease (McCoy et al., 2002). In contrast, EP4 receptor signaling appears to play an anti-inflammatory role in the dextran sodium sulfate-induced colitis model—an animal model of inflammatory bowel disease—by protecting the intestinal mucosal integrity function and inhibiting $CD4^+$ T-cell proliferation and cytokine production (Kabashima et al., 2002).

5. Prostacyclin receptor (IP)

Prostacyclin (PGI_2) is the primary prostaglandin produced by endothelial cells and plays an important role in

vascular homeostasis as a result of its potent vasodilatory and antithrombotic effects (Vane & Botting, 1995). Thus, prostacyclin functionally opposes the effects of TXA_2 and has been shown to specifically inhibit platelet activation and TXA_2-induced vascular proliferation following vascular injury (Cheng et al., 2002). The vasodilatory actions of prostacyclin have enabled its clinical use for reducing pulmonary vascular resistance in individuals suffering from primary pulmonary hypertension (McLaughlin et al., 1998). Prostacyclin has also been demonstrated to have cardioprotective effects during ischemia/reperfusion injury independent of its effects on platelets (Xiao et al., 2001). In addition to its cardiovascular effects, prostacyclin is an important mediator of acute inflammation and inflammatory pain transmission (Murata et al., 1997; Doi et al., 2002).

The human prostacyclin receptor (IP) was first cloned from lung and megakaryocyte cDNA libraries (Boie et al., 1994; Katsuyama et al., 1994; Nakagawa et al., 1994) and has been shown to be expressed in many tissues including kidney, liver, lung, platelets, heart, and aorta. Due to its unstable nature, prostacyclin has limited experimental use. Therefore, synthetic agonists such as iloprost, cicaprost, and carbacyclin are commonly used to study IP receptor function. Of these, iloprost is most widely used, and the IP receptor has been shown to possess both high- and low-affinity binding sites (Boie et al., 1994). Cicaprost has high affinity and selectivity, while other agonists have poor selectivity. Iloprost has equivalent affinities for binding to both IP and EP1 receptors and only slightly lower affinity at EP3. Similarly, carbacyclin has comparable binding affinity at the IP, DP, FP, and EP receptors (Abramovitz et al., 2000).

The IP receptor predominantly couples to a G_s-type G protein leading to an increase in cAMP (Boie et al., 1994; Katsuyama et al., 1994; Nakagawa et al., 1994), and this pathway is responsible for the vasodilatory and anti-aggregatory effects of prostacyclin. Additionally, the IP receptor is capable of coupling to other signal transduction pathways such as G_q-dependent phosphoinositide turnover (Katsuyama et al., 1994; Namba et al., 1994) and G_i-dependent inhibition of cAMP (Hebert et al., 1998). Differential coupling of the IP receptor to multiple signaling pathways is modulated by C-terminal modification. Isoprenylation of the C-terminal tail is required for efficient coupling of the IP receptor to both G_s- and G_q-type G proteins (Hayes et al., 1999; Miggin et al., 2002). The IP receptor also contains multiple cysteine residues in the C-terminal tail that are capable of being palmitoylated. Differing patterns of palmitoylation have been demonstrated to be required for coupling to G_s- versus G_q-mediated signal transduction pathways (Miggin et al., 2003). Phosphorylation of the C-terminal tail also plays a modulatory role in IP receptor signaling, though species differences do exist. The mouse IP receptor is capable of coupling to G_s, G_q, and G_i, with the latter two dependent on agonist-induced, G_s-dependent phosphorylation by PKA (Lawler et al., 2001). Instead of a PKA site, the human IP receptor contains a C-terminal PKC phosphorylation site and independently couples to G_s and G_q but not G_i, with PKC phosphorylation playing very little role in the selectivity of G protein coupling (Miggin & Kinsella, 2002).

The IP receptor is thought to undergo agonist-induced desensitization in vivo, as evidenced by the occurrence of tachyphylaxis during treatment of idiopathic primary pulmonary hypertension with inhaled prostacyclin (McLaughlin et al., 1998). PKC phosphorylation of the C-terminal tail has been shown to play a role in agonist-induced desensitization (Smyth et al., 1998). The IP receptor also undergoes agonist-induced internalization and sequestration independently from desensitization, via a PKC-, GRK-, and arrestin-independent mechanism (Smyth et al., 2000). Isoprenylation of the C-terminal tail, however, is required for agonist-induced internalization (Miggin et al., 2002).

Prostacyclin is an important mediator of the edema and pain that accompany acute inflammation. In IP receptor-deficient mice, potentiation of bradykinin-induced microvascular permeability by prostacyclin is abolished; in addition, these mice have substantially reduced carrageenan-induced paw edema (Murata et al., 1997). The level of paw edema observed in IP-deficient mice was equivalent to that of indomethacin treated controls, indicating that prostacyclin-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 prostacyclin formation leading to enhancement of microvascular permeability and edema (Ueno et al., 2000). Apart from its vascular effects, 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 (Oida et al., 1995). IP receptor-deficient mice have an attenuated writhing response following intraperitoneal injection of either acetic acid or prostacyclin, indicating that the IP receptor plays a role in mediating peripheral nociceptive sensitization to inflammatory stimuli (Murata et al., 1997). The IP receptor is also expressed in the spinal cord and has been implicated in spinal pain transmission in response to peripheral inflammation (Doi et al., 2002).

In contrast to the pro-inflammatory effects of IP receptor activation in nonallergic acute inflammation, recent studies have suggested that IP receptor signaling suppresses Th2-mediated allergic inflammatory responses. IP receptor mRNA is up-regulated in $CD4^+$ Th2 cells and inhibition of prostacyclin formation by the COX-2 inhibitor NS-398 during antigen-induced airway inflammation results in greater lung Th2-mediated lung inflammation (Jaffar et al., 2002). Prostacyclin has been suggested to exert this effect in part by enhancing Th2 cell production of the anti-

inflammatory cytokine IL-10. An immunosuppressive role for the IP receptor in Th2-mediated inflammation is supported by the observation that in the OVA-induced asthma model, IP receptor-deficient mice have increased antigen-induced leukocyte accumulation in bronchoalveolar lavage fluid and peribronchiolar and perivascular inflammatory infiltration (Takahashi et al., 2002). The increase in lung inflammation correlates with dramatically increased Th2 cytokines IL-4 and IL-5 in bronchiolar alveolar lavage fluid, and splenic T-cells obtained from nonsensitized IP null mice showed reduced ability to produce the Th1 cytokine IFN-γ. These mice also experienced greater antigen-stimulated airway, goblet cell hyperplasia, and subepithelial fibrosis compared with wild-type animals, consistent with an inhibitory role for the IP receptor in the chronic airway inflammation of allergic asthma (Nagao et al., 2003). Thus, prostacyclin may shift the balance within the immune system away from a Th2 dominant response and inhibit allergic inflammation.

Prostacyclin has been observed to exhibit cardioprotective effects during ischemia/reperfusion injury (Xiao et al., 2001), a neutrophil mediated inflammatory response leading to irreversible cardiac tissue damage. Following ischemia and reperfusion, neutrophilic infiltration of the affected tissue results in local myocardial inflammation, and inhibition of neutrophil adhesive function reduces myocardial infarct size (Simpson et al., 1990). Migration of neutrophils requires up-regulation of endothelial surface adhesion molecules such as ICAMs, VCAMs, and ELAMs. In vitro experiments suggest that iloprost inhibits ICAM-1, ELAM-1, and VCAM-1 on endothelial cells as well lymphocyte adhesive function (Della Bella et al., 2001). In other studies, iloprost inhibition of lymphocyte adhesion to endothelial cells was not related to changes in expression of endothelial cell adhesion molecules, but instead due to inhibition the platelet activating factor release (Lindemann et al., 2003). The modulation of lymphocyte- and leukocyte-endothelial cell interactions by prostacyclin may also reduce the inflammatory component of peripheral vascular diseases such as systemic sclerosis, as evidenced by the clinical use of iloprost to treat systemic sclerosis and the resulting suppression of inflammatory cytokine levels (Della Bella et al., 1997).

6. $PGF_{2\alpha}$ receptor (FP)

$PGF_{2\alpha}$ is produced during the menstrual cycle by secretory endometrium (Abel & Baird, 1980) and plays a critical role in mammalian reproduction. Mice deficient in the FP receptor exhibit persistently high progesterone levels during late pregnancy leading to reduced oxytocin receptor expression and impaired parturition (Sugimoto et al., 1997). Fluctuations in $PGF_{2\alpha}$ production have been linked to a number of reproductive abnormalities including prolonged and painful menstrual bleeding (Smith et al., 1981; Rees et al., 1984), and increased FP receptor expression and $PGF_{2\alpha}$ signaling has been implicated in endometrial adenocarcinoma growth (Sales et al., 2004). $PGF_{2\alpha}$ also plays a role in renal function (reviewed by Breyer & Breyer, 2001), cardiac hypertrophy (Lai et al., 1996), and regulation of intraocular pressure (Lee et al., 1988; Camras et al., 1989). Clinically, $PGF_{2\alpha}$ analogues are used to treat ocular hypertension and glaucoma (Alexander et al., 2002).

$PGF_{2\alpha}$ binds a single receptor (FP), which was originally cloned from human kidney, uterus, and placental cDNA libraries (Abramovitz et al., 1994). Two differentially spliced variants of the sheep FP receptor orthologue have been reported (FP_A and FP_B, which differ from each other in C-terminal tail length; Pierce et al., 1997). The FP receptor is the least selective of the prostanoid receptors in binding the principal endogenous prostaglandins, binding both PGD_2 and PGE_2 at nanomolar concentrations (K_i values for the FP receptor expressed in HEK293 cells are 3.2, 6.7, and 119 nM for $PGF_{2\alpha}$, PGD_2, and PGE_2, respectively; Abramovitz et al., 2000). Selective FP agonists such as fluprostenol and latanoprost have been developed, and several are used clinically due to their ocular hypotensive properties (Alexander et al., 2002). These drugs are delivered in the form of an isopropyl ester prodrug, which is hydrolyzed in vivo to the active free acid form.

Activation of the FP receptor leads to G_q-mediated IP_3 generation and increases in intracellular calcium (Abramovitz et al., 1994; Sugimoto et al., 1994; Watanabe et al., 1994). In addition to signaling through G_q, the FP receptor couples to other signal transduction systems. In FP receptor-transfected HEK293 cells, $PGF_{2\alpha}$ stimulation leads to activation of the small G-protein Rho via a G_q-independent mechanism, resulting in cytoskeleton rearrangement, actin stress fiber formation, and phosphorylation of the p125 focal adhesion kinase (Pierce et al., 1999). Similar to the EP2 receptor, cross-talk has been reported between FP and EGF receptor signaling, whereby FP receptor activation leads to PLCβ-dependent transactivation of EGFR, ERK phosphorylation, and increased proliferation of endometrial adenocarcinoma cells (Sales et al., 2004). Recently, a novel link between the FP receptor and the β-catenin pathway has been reported (Fujino & Regan, 2001). As discussed above, PGE_2-induced transactivation of EGFR leading to β-catenin phosphorylation has been implicated in the invasion of colon cancer cells; however, the potential significance of $PGF_{2\alpha}$-EGFR-β-catenin signaling in carcinogenesis remains unclear.

Although the FP_A and FP_B splice variants exhibit similar ligand binding profiles and G-protein coupling preferences, differences have been reported in the regulation of receptor function. The C-terminal tail of the FP_A, but not the truncated FP_B, can be phosphorylated by PKC, leading to agonist-induced desensitization and internalization (Fujino et al., 2000). In contrast, FP_B does not undergo agonist-induced internalization but instead experiences constitutive internalization (Srinivasan et al., 2002). Constitutive internalization of FP_B has been linked to $PGF_{2\alpha}$-induced

activation of the Tcf/β-catenin signaling pathway. A mechanism for GPCR regulation has been suggested in which PI 3-kinase associates with the unstimulated FP_B and β-catenin, forming a complex that undergoes internalization in the absence of agonist. Upon agonist activation, the β-catenin complex is released, increasing cytosolic β-catenin levels and Tcf transcriptional activation (Fujino et al., 2002a). Although the significance remains to be established, this represents a potentially novel mechanism for the regulation of GPCR signaling. Splice variants analogous to the ovine FP_A and FP_B have not been reported for humans or other species, although a truncated variant of the human FP receptor has been identified (Vielhauer et al., 2004). Therefore, the relevance of differential signaling between the splice variants is unclear.

$PGF_{2\alpha}$ plays a major role in reproduction, renal function, and regulation of intraocular pressure and has been implicated in proliferative states such as endometrial carcinoma and cardiac hypertrophy. Consistent with these functions, FP receptor expression has been demonstrated in the corpus luteum, kidney, ocular tissues, and ventricular myocytes (Sakamoto et al., 1994; Sugimoto et al., 1994; Adams et al., 1996; Mukhopadhyay et al., 2001). No expression has been observed in the spleen or thymus, nor has FP receptor expression by immune cell populations been reported (Tilley et al., 2001). Therefore, unlike the other prostaglandins, there is very little evidence to support a role for $PGF_{2\alpha}$-FP receptor signaling in inflammatory and immunological processes.

7. Summary

The diverse actions of prostaglandins are mediated by nine prostanoid-binding GPCRs, which exhibit distinct pharmacology and signaling profiles. These receptors are widely expressed throughout the immune system and function at multiple levels in both the adaptive and innate immune responses. Recent advances in our understanding of the function of the individual receptors have revealed both pro- and anti-inflammatory pathways activated by prostaglandins in physiologic and disease states. Inhibition of prostaglandin production by NSAIDs is widely used clinically to alleviate inflammation but may abolish desirable anti-inflammatory effects as well. Therefore, the development of receptor specific antagonists should offer significant advantages and flexibility over traditional and COX-2-specific NSAIDs that nonselectively inhibit the synthesis of all prostaglandins.

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