Prostaglandins as modulators of immunity

Sarah G. Harris, Josue Padilla, Laura Koumas, Denise Ray and Richard P. Phipps

Prostaglandins are potent lipid molecules that affect key aspects of immunity. The original view of prostaglandins was that they were simply immunoinhibitory. This review focuses on recent findings concerning prostaglandin E₂ (PGE₂) and the PGD₂ metabolite 15-deoxy-Δ₁₂,14-PGJ₂, and their divergent roles in immune regulation. We will highlight how these two seminal prostaglandins regulate immunity and inflammation, and play an emerging role in cancer progression. Understanding the diverse activities of these prostaglandins is crucial for the development of new therapies aimed at immune modulation.

Interest in the ability of prostaglandin E₂ (PGE₂) to regulate the immune system has exploded in the past decade. Many new and exciting data have emerged concerning the role of PGE₂ in cells of the immune system and disease states. In this review, we will provide highlights of that research, focusing first on the interactions of PGE₂ with T cells, B cells and antigen-presenting cells (APCs). Second, the role of PGE₂ in two disease states that have strong immune components – periodontal disease and cancer – will be discussed. Finally, we will summarize the effects of a newly discovered and prominent prostaglandin, 15-deoxy-Δ₁₂,14-PGJ₂, on the immune system. The volume of literature describing the roles of this lipid has increased exponentially in recent years. We will review the actions of 15-d-PGJ₂ on cells and specifically, its effects on inflammation and cancer.

Prostaglandins

Prostaglandins are small lipid molecules that regulate numerous processes in the body, including kidney function, platelet aggregation, neurotransmitter release and modulation of immune function [1,2]. The production of prostaglandins begins with the liberation of arachidonic acid from membrane phospholipids by phospholipase A₂ in response to inflammatory stimuli. Arachidonic acid is converted to PGH₂ by the cyclooxygenase enzymes COX-1 and COX-2 (known formally as prostaglandin endoperoxide H synthases). Generally, it is thought that COX-1 is expressed constitutively in most tissues of the body and acts to maintain homeostatic processes, such as mucus secretion. COX-2, by contrast, is mainly an inducible enzyme and is involved primarily in the regulation of inflammation [3]. The recent development of COX-1- and COX-2-knockout mice has provided much information concerning the roles of these enzymes in development, inflammation and carcinogenesis [4,5].

Cell-specific prostaglandin synthases convert PGH₂ into a series of prostaglandins, including PGI₂, PGF₂α, PGD₂ and PGE₂ (Fig. 1). The recent discovery that the expression of PGE synthases can be induced by proinflammatory stimuli provides another layer of regulation and complexity in the production of prostaglandins [6]. Upon production, prostaglandins are released rapidly from cells and act near their site of production by binding to specific, high-affinity receptors on plasma membranes [7].

PGE₂

One of the best known and most well-studied prostaglandins is PGE₂. PGE₂ is produced by many cells of the body – including fibroblasts, macrophages and some types of malignant cells – and exerts its actions by binding to one (or a combination) of its four subtypes of receptor (EP₁, EP₂, EP₃ and EP₄). In the mouse, the EP₂ subtype consists of three different isoforms, termed α, β and γ, and there are seven EP₃ splice variants in humans identified to date. The receptors are rhodopsin-type receptors containing...
seven transmembrane-spanning domains. The four main subtypes of EP-R (EP1, EP2, EP4) are coupled to Gs proteins and induce the expression of cAMP, which leads to gene regulation. The three isoforms of EP1 (α, β, and γ) are coupled primarily to Gs and are most often inhibitory to cAMP. (There is some evidence that additional signaling cascades may be activated by EP1, binding.)

**Fig. 2.** Prostaglandin E receptor (EP-R) signaling pathways. EP-Rs are rhodopsin-type receptors with seven transmembrane-spanning domains, coupled through their intracellular sequences to specific G proteins and use different second messenger signaling pathways. EP1 is coupled to Gs, and ligand binding results in an increase in the level of intracellular calcium. EP2 and EP4 are coupled to Gi and Go proteins and induce the expression of cAMP, which leads to gene regulation. The three isoforms of EP1 (α, β, and γ) are coupled primarily to Gs and are most often inhibitory to cAMP. (There is some evidence that additional signaling cascades may be activated by EP1, binding.)

The regulation of expression of the various subtypes of EP receptors on cells by inflammatory agents, or even PGE2 itself, enables PGE2 to affect tissues in a very specific manner [7,9]. Although knockout mice for all four EP receptors have been produced, no functional analysis of the effects on lymphocytes has been performed [10]. Recently, several reports have documented the expression of functional EP receptors (EP1, EP2, and EP4) on the nuclear membranes of cells [11]. For example, EP2- and EP4 have been localized to the nuclear envelope of endothelial cells. The receptors are functional, because they can modulate transcription, including that of the gene encoding inducible nitric oxide synthase (iNOS) [11]. The discovery of nuclear EP receptors provides an additional level to the regulation of expression of Fas ligand (FasL) on the T-cell surface. PGE2 decreases FasL mRNA and protein expression, which inhibits apoptosis [18]. Therefore, the induction of apoptosis by PGE2 is involved in the selection of immature thymocytes, thus shaping the T-cell repertoire. In addition, PGE2 regulates differentially the activities of mature resting and activated T cells by inducing and inhibiting apoptosis, respectively.

PGE2 has a profound effect on the production of cytokines by T cells. Recently, PGE2 has been implicated in the enhancement of Th helper 2 (Th2)-type responses. For example, PGE2 has no effect on or enhances the production of Th2 cytokines, such as IL-4, IL-5 and IL-10, by Th2 cells, but inhibits drastically the production of Th1 cytokines, such as interferon-γ (IFN-γ) and IL-2, by Th1 cells [19]. The induction of the Th2 response by PGE2 is mediated most probably by cAMP, because elements that increase the level of cAMP mimic the effects of PGE2 [2]. Although a few reports suggest that PGE2 inhibits Th2 responses [20], the vast majority of reports shows that primarily, PGE2 has a Th2-inducing activity on T cells. Harnessing the ability of PGE2 to modulate Th responses and thus, immunity in general could be extremely beneficial for treating the numerous disorders in which there is a dysregulated Th response.

Although less is known about the effects of PGE2 on CD8 T cells, it has been shown that, as for CD4 T cells, PGE2 can inhibit CD8 T-cell proliferation [21]. In terms of regulating cytokine production, PGE2 decreases the production of IFN-γ by CD8 T-cell clones through a cAMP-dependent pathway [22]. Such inhibition of IFN-γ production is consistent with the dogma that PGE2 favors type-2 responses in general. Therefore, PGE2 plays a variety of crucial roles throughout the life of T cells, from the regulation of positive and negative selection in the thymus to the
PGE2 acts on T cells to enhance their production of type-2 cytokines and antibodies. Acting on B cells, PGE2 suppresses the production of IL-2 and IL-10, but inhibits the expression of IL-4, IL-5, IL-10, and IL-12. In addition, B cells isolated from neonatal mice are susceptible to PGE2-induced apoptosis, whereas B cells from mature mice are unaffected. Further evidence from the studies of Shimozato and Kincade confirms the suppressive effect of PGE2 on immature B cells [23]. In addition, B cells isolated from neonatal mice are susceptible to PGE2-induced apoptosis, whereas B cells from mature mice are unaffected [24]. Furthermore, BM-derived DCs can produce PGE2 themselves [28] and thus, autoregulate their functions in the immune system.

Another established function of PGE2 is its regulation of cytokine production by activated macrophages. The effects of PGE2 on macrophages are suppressive for type-1 immune responses. PGE2 down-regulates the expression of the IL-12 receptor and inhibits the production of tumor necrosis factor-α (TNF-α), IL-12, IL-8 and IL-12 by macrophages [30]. Similarly, PGE2 reduces the production of TNF-α by LPS-treated peritoneal macrophages [31]. Studies of zymosan-treated mouse peritoneal macrophages show that PGE2 causes a down-regulation of TNF-α production and an up-regulation of IL-10 production, through the EP2 and EP4 receptors [32]. Therefore, PGE2 acts to up-regulate type-2 responses in macrophages (Fig. 3). As for some DCs, PGE2 is produced in large quantities by macrophages, primarily in response to proinflammatory mediators, such as LPS and IL-1 [33]. Therefore, PGE2 might act as an autocrine feedback regulator, because it has been shown to positively regulate its own expression by up-regulating the expression of COX-2.

**PGE2 in disease**

PGE2 plays an integral role in a myriad of infections and diseases. We have chosen to highlight two diseases for which PGE2 has been shown to be central to disease progression - periodontal disease and certain cancers.
Periodontal disease

Periodontitis is a chronic inflammatory process that degrades the tooth support structures (i.e. gingiva, periodontal ligament, cementum and alveolar bone). Periodontal disease is thought to be induced by the accumulation of bacterial plaque at the gum line. The presence of bacterial plaque in the gingival crevice elicits a complex bacteria–host interaction, which leads to the degradation of connective tissue, alveolar bone destruction and eventually, tooth loss. The host responds to bacterial endotoxins (e.g. LPS) by stimulating cells present in the periodontal tissues to release proinflammatory mediators, such as IL-1β, TNF-α and PGE2 (Fig. 4). It is the local action of prostaglandins and cytokines, which play crucial roles in the inflammatory process, that leads to the pathology associated with periodontal disease.

Since the early 1970s, PGE2 has been used as a biochemical marker for periodontitis, because inflamed periodontal tissues have high levels of PGE2 [34]. PGE2 causes increased vasopermeability and vasodilation, leading to redness and edema. Also, PGE2 induces the synthesis of MMPS by infiltrating resident cells, such as monocytes and fibroblasts, respectively. MMPS cause connective tissue degradation and osteoclastic bone destruction, both of which are hallmark signs of periodontal disease [35].

Owing to the high-level expression of PGE2 and its detrimental effects in the periodontium, there are numerous models of periodontal disease in animals and humans evaluating the effectiveness of cyclooxygenase inhibitors. The suppression of PGE2 synthesis by these drugs diminishes greatly the loss of periodontal connective tissue [34,36]. Thus, these data indicate not only an association between disease activity and levels of PGE2 within tissues, but that eliminating PGE2 with cyclooxygenase inhibitors (e.g. the COX-2-selective inhibitor Vioxx) leads to a concomitant reduction of periodontal disease progression (Fig. 4).

Cancer

The relationship between enhanced cyclooxygenase expression and selected cancers is becoming well established (for review, see Ref. [37]). Over-expression of COX-2 has been noted in many cancers, including, but not limited to, cancers of the breast, colon and prostate [37]. The discovery that the inhibition of COX activity inhibits cancerous tumor growth in many systems has further solidified our knowledge of the integral role that cyclooxygenases play in tumor progression. Key epidemiological studies reveal that inhibiting the cyclooxygenase enzymes reduces the incidence of certain cancers. For example, individuals taking cyclooxygenase inhibitors have a 40–50% reduction in the incidence of colorectal cancer [37]. Because the COX enzymes produce prostaglandins, the roles that these mediators play in tumorigenesis is under intense investigation also.

PGE2, which promotes tumor-cell survival, has been found at higher concentrations in tumor tissues than normal tissues [38]. PGE2 mediates tumor survival by several mechanisms. It inhibits tumor-cell apoptosis and induces tumor-cell proliferation [39]. Also, PGE2 increases tumor progression by altering cell morphology, and increasing cell motility and migration [40]. In addition to the direct effects of PGE2 on tumor cells, this lipid mediator induces the production of metastasis-promoting MMPS and stimulates angiogenesis [40] (Fig. 5). PGE2 acts also as an immunomodulator (as described earlier), to promote humoral and Th2-type immune responses that do not favor tumor destruction, and inhibit Th1-type responses that do favor tumor destruction. Therefore, the relationship between PGE2 and tumor progression is quite important, and further study in the field is warranted to understand the additional roles of this lipid. In the future, specific inhibition of PGE2 by the inhibition of PGE synthases, for example, might prove extremely beneficial for the abrogation of tumor progression.

15-deoxy-Δ12,14-PGJ2

The J series of prostaglandins, once thought to comprise inactive degradation products of PGD2, is now well established as regulating diverse processes, such as adipogenesis, inflammation and tumorigenesis. 15-d-PGJ2 is the end-product metabolite of PGD2 and is produced by a variety of cells, including mast cells, T cells, platelets and alveolar macrophages. The exact mechanism of entry of 15-d-PGJ2 into cells is unknown, but it is possible that 15-d-PGJ2 enters by an active transport system similar to those described by Narumiya’s group for other cyclopentanone prostaglandins (e.g. Δ12-PGJ2) [41]. Once inside the cell, an additional cryptic mechanism allows transport of the cyclopentanone prostaglandins into the nucleus, where they affect...
Proliferation and 15-deoxy-Δ12,14-PGJ2 (15-d-PGJ2) on tumor cells. PGE2 and 15-d-PGJ2 have opposite effects on malignant-cell development. PGE2 promotes some types of cancers by inducing the production of matrix metalloproteinases (MMPs), leading to enhanced metastasis. PGE2 also induces cancer-cell proliferation and inhibits apoptosis. By contrast, 15-d-PGJ2 inhibits tumor formation. 15-d-PGJ2 decreases MMP production, inhibits malignant-cell proliferation and stimulates apoptosis.

Fig. 5. Opposing effects of prostaglandin E2 (PGE2) and 15-deoxy-Δ12,14-PGJ2 (15-d-PGJ2) on tumor cells. PGE2 and 15-d-PGJ2 have opposite effects on malignant-cell development. PGE2 promotes some types of cancers by inducing the production of matrix metalloproteinases (MMPs), leading to enhanced metastasis. PGE2 also induces cancer-cell proliferation and inhibits apoptosis. By contrast, 15-d-PGJ2 inhibits tumor formation. 15-d-PGJ2 decreases MMP production, inhibits malignant-cell proliferation and stimulates apoptosis. PGE2 and 15-d-PGJ2 inhibit apoptosis. By cell proliferation and 15-d-PGJ2 decreases inhibits tumor formation. PGE2 also induces cancer-enhanced metastasis. MMPs, leading to metallocproteinases production of matrix promotes some types of development. PGE2 malignant-cell have opposite effects on (15-d-PGJ2) on tumor of prostaglandin E2 (PGE2) 15-d-PGJ2 action in these systems is unknown, it has been postulated that 15-d-PGJ2 could be acting through another cytoplasmic prostaglandin receptor. For example, the chemotactic receptor on Th2 cells (CRTH2) – a recently discovered G-protein-coupled receptor expressed on Th2 cells, basophils and eosinophils – binds both PGD2 and 15-d-PGJ2.

Other mechanisms to regulate transcription. There is also an unidentified mechanism by which 15-d-PGJ2 mediates transcription in a PPAR-γ-independent manner.

15-deoxy-Δ12,14-PGJ2 in disease
Although 15-d-PGJ2 is implicated in the regulation of many immune functions and disorders, here, we focus on the role of this lipid in inflammation and cancer.

Inflammation
15-d-PGJ2 is emerging as a key anti-inflammatory mediator. For example, 15-d-PGJ2 inhibits the production of iNOS, TNF-α and IL-1β by mouse and human macrophages [49,51]. The mechanism involves the inhibition of mitogen-activated protein (MAP) kinases, NF-κB and IkB kinase. Also, 15-d-PGJ2 can induce the apoptosis of mouse T and B cells, a potential mechanism to down-regulate an inflammatory immune response [45,52]. In addition, many in vivo studies support a role for 15-d-PGJ2 as an anti-inflammatory agent. 15-d-PGJ2 and PPAR-γ

Fig. 6. Possible modes of action of 15-deoxy-Δ12,14-PGJ2 (15-d-PGJ2) in the cell. Prostaglandin D2 (PGD2) is broken down into 15-d-PGJ2. 15-d-PGJ2 could gain entry to the cell by an active transport system, and then enter the nucleus by a nuclear transporter, as described for other cyclopentanone prostaglandins. Also, 15-d-PGJ2 could bind to an undiscovered cytoplasmic receptor. Alternatively, PGD2 could gain entry to the cell by means of an anionic carrier protein, and then be metabolized in the cytoplasm to 15-d-PGJ2. 15-d-PGJ2 could then gain entry to the nucleus by a nuclear transporter protein. Once inside the nucleus, 15-d-PGJ2 can bind to and activate peroxisome proliferator-activated receptor γ (PPAR-γ) [43]. PPARs are a family of ligand-activated nuclear transcription factors, which, upon the binding of ligand, form a heterodimer with the retinoid X receptor. This complex then binds to PPAR-responsive elements (PPREs) in the promoter regions of target genes [43]. PPAR-γ was found initially in adipose tissue, where it plays a key role in the regulation of adipogenesis. More recently, the receptor has been found also in many immune cells, including neutrophils, macrophages, T cells and B cells [44].

There has been recent controversy over the link between the action of 15-d-PGJ2 and PPAR-γ binding. Initial reports focused on the ability of 15-d-PGJ2 to bind to and activate PPAR-γ. For example, 15-d-PGJ2 inhibits T-cell proliferation and induces apoptosis of T cells by a PPAR-γ-dependent mechanism [45,46]. More recently, however, evidence has shown that there are also effects of 15-d-PGJ2 that are independent of PPAR-γ activation (Fig. 6). For example, 15-d-PGJ2 can down-regulate the production of iNOS by microglial cells through PPAR-γ-independent mechanisms [47]. Vaidya and colleagues have shown that 15-d-PGJ2 can inhibit the production of oxygen free radicals by neutrophils in a PPAR-γ-independent manner [48]. Additionally, the inhibition of IκB kinase can occur through PPAR-γ-independent means [49]. Although the exact mechanism of 15-d-PGJ2 action in these systems is unknown, it has been postulated that 15-d-PGJ2 could be acting...
ligands inhibit inflammation in models of ischemia–reperfusion injury [53], colitis [54] and adjuvant-induced arthritis [55], to name a few. There is some evidence, however, that 15-d-PGJ2 can promote inflammation also. 15-d-PGJ2 can induce expression of the proinflammatory mediators type II secreted phospholipase A(2) and cyclooxygenase 2 in smooth muscle and epithelial cells, respectively [56,57]. Also, 15-d-PGJ2 can stimulate the production of proinflammatory mediators, such as IL-8 and mitogen-activated protein kinases, in some systems [58–60]. In vivo evidence from Thieringer et al. [61] showed that 15-d-PGJ2 and PPAR-γ agonists induce the production of TNF-α and IL-6 in LPS-treated db/db mice. Therefore, the role of 15-d-PGJ2 in the regulation of inflammation is complex and remains under intense investigation.

Cancer
Unlike PGE2, which is involved clearly in the promotion and persistence of carcinogenesis, 15-d-PGJ2 is emerging as a potent anti-tumor agent. There are reports of the inhibition of tumor-cell growth both in vitro and in vivo by 15-d-PGJ2 in a variety of tissues, including breast, prostate, colon, lung, bladder and esophagus [62,63]. 15-d-PGJ2 acts in a myriad of ways to inhibit tumorigenesis. In most types of cancer, for example, 15-d-PGJ2 acts on tumor cells by inhibiting proliferation and stimulating apoptosis. The mechanism in several cases, such as in bladder and esophageal cancers, seems to be by the inhibition of cyclin D1, which results in cell-cycle arrest [62,64,65] and ultimately, apoptotic cell death (Fig. 5). The induction of tumor-cell apoptosis by 15-d-PGJ2 occurs generally by a PPAR-γ-mediated pathway, because agonists for this nuclear receptor also inhibit carcinogenesis. In addition, Sarraf et al. [66] found that colon cancer patients have ‘loss of function’ mutations in PPAR-γ, further supporting a role for this receptor in the inhibition of tumor growth. The over-expression of PPAR-γ in many cancers suggests a possible therapeutic use for 15-d-PGJ2 and other PPAR-γ ligands [67].

Aside from inducing apoptosis in tumor cells, 15-d-PGJ2 and PPAR-γ ligands can also inhibit tumorigenesis by inducing the differentiation of tumor cells [68]. The anti-tumor activity of 15-d-PGJ2 is, however, not restricted to the direct inhibition of tumor cells. 15-d-PGJ2 acts also on surrounding cells, such as endothelial cells, inhibiting their expression of the vascular endothelial growth factor receptor (VEGFR), which results in the inhibition of angiogenesis in vivo [69]. Also, 15-d-PGJ2 can down-regulate the expression of MMP-9 in vascular smooth muscle cells, which results in the inhibition of tissue destruction and a subsequent inhibitory effect on the migration of cells [70]. Therefore, 15-d-PGJ2, in contrast to PGE2, inhibits tumor formation by inducing the apoptosis of tumor cells and inhibiting tumor-cell migration and angiogenesis.

Conclusions
The roles of PGE2 and 15-d-PGJ2 in the immune system are diverse and complex. Both mediators have profound, and opposing, effects on tumorigenesis and are key regulators of inflammation. Owing to the fact that these two lipids are produced from the same precursor (arachidonic acid) by the same cyclooxygenase enzymes, additional means to regulate the production of one or the other prostaglandin must exist. Prime candidates are the prostaglandin synthases, which synthesize specific prostaglandins from the PGH2 precursor. Therefore, future study into the function of these synthases and regulation of the synthase-encoding genes will lead probably to new approaches for the treatment and, perhaps, prevention of immune disorders, ranging from inflammation to cancer.

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