Polarization of the Innate Immune Response by Prostaglandin E₂: a
Puzzle of Receptors and Signals

Mario Rodríguez, Esther Domingo, Cristina Municio, Yolanda Alvarez, Etzel Hugo, Nieves Fernández, and Mariano Sánchez Crespo

Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas, Valladolid, Spain (E.D., C.M., Y.A., E.H., M.S.C.); and Departamento de Bioquímica y Biología Molecular, Universidad de Valladolid, Valladolid, Spain (M.R., N.F.)
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Address correspondence to: Dr. M. Sánchez Crespo
Instituto de Biología y Genética Molecular
C/ Sanz y Forés s/n
47003-Valladolid, SPAIN
Phone number: 34-983-423273
FAX number: 34-983-184800
e-mail: mscres@ibgm.uva.es

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Abbreviations: AA, arachidonic acid; Ab, antibody; C/EBPβ, CCAAT/enhancer-binding protein β; CBP, CREB-binding protein; cPLA₂, cytosolic phospholipase A₂; COX, cyclooxygenase; CREB, cAMP response element-binding protein; CRTC, CREB-regulated transcriptional coactivator; DC, dendritic cells; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; EP, E prostanoid receptor; EPAC, exchange protein activated by cAMP; ERAD, endoplasmic reticulum-associated degradation; IκB, inhibitory subunit of NF-κB; IKK, IκB kinases; ITAM, immunoreceptor tyrosine-based activation motif; MAPK, mitogen-activated protein kinases; MDSC, myeloid-derived suppressor cells; NF-κB, nuclear factor κB; NOD, nucleotide-binding oligomerization domain family proteins; LT, leukotriene; PAMP, pathogen-associated molecular patterns; PGES, PGE synthase; PL, phospholipase; PRR, pattern recognition receptors; SIK, salt inducible kinase; SYK, spleen tyrosine kinase; TORC, transducer of regulated CREB activity; TLR, Toll-like receptors.
Abstract

Eicosanoids tailor the innate immune response by supporting local inflammation and exhibiting immunomodulatory properties. Prostaglandin E₂ (PGE₂) is the most abundant eicosanoid in the inflammatory milieu due to the robust production elicited by pathogen-associated molecular patterns on cells of the innate immune system. The different functions and cell distribution of E prostanoid (EP) receptors explain the difficulty so far encountered to delineate the actual role of PGE₂ in the immune response. The biosynthesis of eicosanoids includes as the first step the Ca²⁺- and kinase-dependent activation of the cytosolic phospholipase A₂, which releases arachidonic acid from membrane phospholipids, and later events depending on the transcriptional regulation of the enzymes of the cyclooxygenase routes, where PGE₂ is the most relevant product. Acting in an autocrine/paracrine manner in macrophages, PGE₂ induces a regulatory phenotype including the expression of IL-10, sphingosine kinase 1, and the TNF family molecule LIGHT. PGE₂ also stabilizes the suppressive function of myeloid-derived suppressor cells, inhibits the release of IL-12 p70 by macrophages and dendritic cells, and may enhance the production of IL-23. PGE₂ is a central component of the inflammasome-dependent induction of the eicosanoid storm that leads to massive loss of intravascular fluid, increases the mortality rate associated with coinfection by *Candida* ssp. and bacteria, and inhibits fungal phagocytosis. These effects have important consequences for the outcome of infections and the polarization of the immune response into the Th2 and Th17 types and can be a clue to develop pharmacological tools to address infectious, autoimmune, and autoinflammatory diseases.
Introduction

Eicosanoids are molecules derived from the oxidative metabolism of arachidonic acid (AA) that display numerous functions in organ homeostasis, including reproduction, haemostasis, acute inflammation, allergy, and pain. Their roles in inflammation at the site of pathogen entry and in IgE-mediated reactions have been the focus of intensive study. More recently, it has been underscored the involvement of some eicosanoids in the induction of the immune response following immunization, in the control of the activation of cytotoxic T lymphocyte and Th1-mediated responses, and in the polarization of the immune response into Th2 and Th17 types. This diversity of actions makes it necessary to distinguish the effects of the different compounds and the environmental context where they are produced. The first classification of eicosanoids stems from their production in either the lipoxygenase or the cyclooxygenase (COX) routes. Leukotrienes (LT) and lipoxins are the main products of the lipoxygenase route, whereas thromboxanes and prostaglandins, with prostaglandin E2 (PGE2) as the most relevant element, are the main COX products involved in the inflammatory response. The function of LT in the pathogenesis of the immediate hypersensitivity reactions elicited by allergens was most prominent from early studies in sensitized tissues (Kellaway and Trethewie, 1940), although at that time their chemical structure was unknown and more than three decades were needed to characterize unambiguously the activity that had been termed slow-reacting substance of anaphylaxis (SRS-A) with the cysteinyl-leucotrienes C4, D4, and E4 (Hammarström et al., 1979). As regards the eicosanoids released from phagocytes, it was necessary to wait for the characterization of the receptors involved in the innate immune response to ascertain their actual role in inflammation. In fact, microbes have unique molecules termed pathogen-associated molecular patterns (PAMP) that are recognized through pattern-recognition receptors...
(PRR) by the host innate immune system. The Toll-like receptor family (for review, see Casanova et al., 2011) and NOD-like receptors (NLR, for review see, Davis et al., 2011) are representative of what Janeway (1989) first called PRR. More recently, C-lectin type receptors have enlarged the list of PRR and reached centre stage in view of their capacity to interact with carbohydrate-based structural signatures expressed in microbial cell walls (for review see, Geijtenbeek and Gringhuis, 2009). Signaling through TLR leads to the activation of the MAPK cascade, IKK and the NF-κB family of transcription factors, tank-binding kinase-1, and interferon regulatory factors. NLR are associated with IKK and inflammasome activation that allows the caspase-1 mediated processing of pro-IL-1β and pro-IL-18 to fully active proteins. The C-type lectin receptors may induce rapid responses because they contain ITAM motifs that initiate a cascade of protein tyrosine phosphorylation reactions, phospholipase Cγ activation, Ca2+ mobilization, and IKK activation (Fig. 1). These features make signaling through C-type lectin receptors a unique paradigm for a comprehensive study of eicosanoid production in the innate immune response.

**Arachidonic acid release is the initial step for eicosanoid production**

Phospholipases A2 (PLA$_2$) catalyze the release of fatty acids from the $sn$-2 position of phospholipids. There are 22 genes encoding PLA$_2$ in mammals that are usually grouped in three main types: the secreted PLA$_2$, the intracellular group VI calcium-independent PLA$_2$, and the group IV cytosolic PLA$_2$. These structurally diverse enzymes carry out distinct functional roles (Dennis et al., 2011). The group IV cPLA$_2$ family includes the cPLA$_2$$^\alpha$, PLA$_{2}$$^\beta$, and cPLA$_2$$^\gamma$ isoforms. However, cPLA$_2$$^\alpha$ is the only PLA$_2$ with specificity of AA and its primary function is to mediate agonist-induced release of AA. Because of its position as the upstream regulatory enzyme for initiating production of
bioactive lipid mediators, cPLA<sub>α</sub> has been considered a potentially important pharmacological target for the control of inflammatory responses. cPLA<sub>α</sub> is subject to post-translational mechanisms of regulation to ensure that the levels of free AA are tightly controlled. These mechanisms include: i) the phosphorylation of Ser505-cPLA<sub>2</sub> by MAPK in the catalytic domain, which explains the increase of catalytic activity elicited by cell agonists, and ii) Ca<sup>2+</sup>-induced translocation from the cytosol to membranes to allow cPLA<sub>α</sub> interaction with phospholipid substrate. cPLA<sub>2</sub> phosphorylation was initially related to MAPK of the ERK group (Lin et al., 1993), but the involvement of p38 MAPK was reported subsequently (Kramer et al., 1996). The agonist-stimulated translocation is explained by a N-terminal domain containing a 45 amino acid region with high homology with the Ca<sup>2+</sup>-dependent phospholipid binding motif expressed in PKC and PLC (Clark et al., 1991). Due to this structural feature, Ca<sup>2+</sup> induces the translocation of cPLA<sub>2</sub> from the cytoplasm to Golgi, endoplasmic reticulum, and nuclear membrane. In keeping with this mechanism, only agonists creating a sustained Ca<sup>2+</sup> influx are capable of activating some elements of the AA cascade, for instance the 5-lipoxygenase pathway, which are critically dependent on Ca<sup>2+</sup> (Buczynski et al., 2007). Since activation of TLR4 by LPS fails to induce Ca<sup>2+</sup> transients directly, but elicits prostaglandin production in the absence of lipoxygenase activation, a corollary to these findings is that acute release of AA and long-term production of COX-2 metabolites are under the control of different signaling pathways. Notably, PAMP derived from the cellular wall of fungi, the main component of which are β(1,3)-glucans, have stood out as a class of stimuli able to activate both pathways and this has pathophysiological relevance to understand the response of the host to infection by fungi such as *Candida*, *Cryptococcus*, *Aspergillus*, and *Pneumocystis jirovecii*. The recognition of β-glucans is carried out by the C-type lectin receptor
dectin-1 (clec7a gene, Brown et al., 2002), which contains an ITAM in its intracellular portion. Cross-linking of this receptor allows activation of tyrosine kinases, including spleen tyrosine kinase (SYK), tyrosine-phosphorylation of phospholipases Cγ, and the elevation of the intracellular levels of Ca²⁺. In an elegant study stemming from the observation that cPLA₂α exhibits calcium-dependent targeting to the endoplasmic reticulum, the site of COX localization, cPLA₂α and COX-2 were found to colocalize to the forming phagosome in mouse peritoneal macrophages uptaking the fungal mimic zymosan (Girotti et al., 2004), thus fitting well with an efficient substrate channeling model yielding AA for PGE₂ synthesis at the early stages of microbial ingestion and explaining the strong ability of β-glucans to fulfill the conditions associated with acute release of AA and production of COX products, in contrast to the effect of the TLR4 ligand LPS that only elicits robust PGE₂ production after COX-2 induction. These findings also help explain the adjuvanticity associated with β-glucans that is being used for vaccine technology (Huang et al., 2010), since COX-2 activity is crucial for optimal antibody response, especially when vaccines are poorly immunogenic (Ryan et al., 2006).

COX enzymes and COX/lipoxygenase cross-talk

**COX enzymes.** PGE₂ is the predominant eicosanoid generated in response to TLR ligands, proinflammatory cytokines, and fungal-derived PAMP. Central to PGE₂ production are the COX enzymes, of which two isoforms exist that are similar in structure and catalytic activity. COX-1 is a constitutive enzyme that is coupled to the cPLA₂α and paves the way for the production of PGE₂ shortly after AA release. In contrast, COX-2 is an inducible enzyme regulated at the transcriptional level by an array of latent transcription factors that are activated downstream PRR engagement, namely
NF-κB, CREB, C/EBPβ, and interferon regulatory factors. The relevance of posttranscriptional mechanisms that regulate mRNA stability and protein translation have been disclosed in the last years, and include several proteins and miRNA that modulate the decay rate of \textit{ptgs2} (the gene encoding COX-2) mRNA as well as two mechanisms of protein degradation. Identification of multiple mRNA regulatory elements present within the \textit{ptgs2} 3'-UTR (untranslated sequence) was the first evidence suggesting that COX-2 might be regulated at a post-transcriptional level. This is explained by the presence of adenylate- and uridylate (AU)-rich elements (ARE) that interact with ARE-binding proteins, for instance, HuR (Hu antigen R), CUGBP2 (CUG triplet repeat–binding protein 2), TIA-1 (T-cell intracellular antigen 1), tristetraprolin, RBM3 (RNA-binding motif protein 3), and most recently Hsp70 (Kishor et al., 2013).

As regards miRNA, miR-16, miR-101, miR-199, miR-143, and miR-542-3p have shown to exhibit complementarity to AU-rich regions of \textit{ptgs2} that makes them suitable to alter their mRNA stability (Dixon et al., 2013). COX-2 protein has two independent routes of degradation: the endoplasmic reticulum-associated degradation (ERAD) and substrate-dependent degradation. N-glycosylation of Asn594 leads to entry of COX-2 into the ERAD pathway. The second COX-2 degradation process is substrate turnover-dependent and is independent of N-glycosylation at Asn594 (Wada et al., 2009). Notably, COX-2 protein stability has been found to be enhanced by atorvastatin, which may influence DC function and counteract some of the untoward effects associated with sustained inhibition of COX-2 (Alvarez et al., 2009b). COX enzymes control PGE$_2$ production by catalyzing the conversion of AA to PG in a two-step process. First, hydrogen is abstracted from carbon 13 of AA. Then a molecule of oxygen is added to carbons 11 and 9, thus provoking the cyclization and the addition of a second molecule of oxygen to carbon 15 to generate PGG$_2$. Second, the hydroperoxide at carbon 15 of
PGG₂ is reduced to hydroxide by the peroxidase active site of COX to produce PGH₂. PGH₂ can be converted to PGD₂, PGE₂, PGF₂α, prostacyclin, or thromboxane A₂ by specific isomerases. PGE₂ can be produced by three types of prostaglandin E synthases (PGES): one cytosolic (cPGES) and two membrane-associated (microsomal PGES-1 and -2). mPGES-1 is induced by the same stimuli that induce COX-2. On this basis, the combined induction of both COX-2 and mPGES-1 by pro-inflammatory stimuli explains the efficient conversion of AA into PGE₂.

**COX/5-lipoxygenase cross-talk.** Cross-talk between the 5-lipoxygenase route and the COX pathways was observed in early studies by showing a widespread inhibitory effect of PG on LT production. In addition to the marked effect of PGE₂ in dendritic cells (DC) by inhibiting the expression of the protein FLAP, which is required for the activity of 5-lipoxygenase (Harizi et al., 2003), recent studies have disclosed new molecular targets by showing that whereas phosphorylation of cPLA₂ enhances its catalytic activity, phosphorylation of LTC₄ synthase is inhibitory. In a detailed study using zymosan as a stimulus, inhibition of LTC₄ synthase was preceded by the production of PGE₂ and abolished by aspirin treatment. This effect was associated with activation of several types of receptors for PGE₂, the major downstream signaling of which were PKA-dependent phosphorylation reactions (Esser et al., 2011). A corollary to these findings is that when COX routes are operative, tissues possess a negative feedback mechanism to control the 5-lipoxygenase route. In the particular case of the lung, where PGE₂ exerts anti-inflammatory effects and LT drive inflammation, these findings provide a mechanistic explanation for two relevant clinical situations in asthma patients: the exacerbation of symptoms associated with aspirin intolerance and the risks conferred by fungal exposure in patients treated with high doses of non-steroidal anti-inflammatory drugs.
The plasticity of mononuclear phagocytes explains distinct patterns of PGE$_2$ production

**Opsonic and non opsonic phagocytosis.** Uptake of phagocytosable particles and signaling from PRR depend on both receptors that bind opsonic proteins and non-opsonic receptors. This is relevant to the engulfment of bacteria and fungi since these microbes can be coated by the complement factor 3 derived protein iC3b and by opsonic IgG class antibodies, which bind the integrin $\alpha_m\beta_2$/Mac-1 and Fc$\gamma$R receptors, respectively. Among non-opsonic receptors, scavenger receptors and C-type lectin receptors are of special relevance to recognize directly PAMP expressed on the microbe surface. Whereas the C-type lectin receptor dectin-1 recognizes $\beta$-glucans (Brown et al., 2002), dectin-2 recognizes $\alpha$-mannans (Sato et al, 2006) and DC-SIGN recognizes N-linked mannans (Cambi et al., 2008). The mannose receptor recognizes terminal mannose and fucose residues, but its ability to modulate cellular activation has not been characterized completely (Martinez-Pomares, 2012). The combinatorial usage of receptors for $\beta$-glucans and $\alpha$-mannans makes it possible an efficient recognition of pathogen dimorphic fungi since their cell wall contains an external layer made up of mannose polymers and an underlying layer of $\beta$-glucans. Cross-linking of opsonic and the non-opsonic receptors dectin-1 and dectin-2 allows the recruitment of non-receptor tyrosine kinases to the ITAM expressed either in the intracellular portion of the receptor or in adaptor proteins, thus activating a cell signalling cascade involving protein tyrosine phosphorylation reactions, phospholipase C$\gamma$ activation, Ca$^{2+}$ mobilization, and IKK activation. This diversity of receptors explains why signals elicited by a receptor may be balanced by concomitant signals induced by associated PAMP on the same microbe or from the environment. In fact, the formation of the so-called phagocytic
Synapse is a pivotal element to define the degree of response of the host to the invading microbe, because recognition of PAMP in a particulate state is a critical checkpoint for scaling the microbial threat versus the single recognition of soluble PAMP (Blander and Sander, 2012). Notably, the construction of this general paradigm for tailoring the host responses stems from studies carried out with large β-glucan complexes, where the exclusion of inhibitory phosphatases from the phagocytic synapse was a key element to start dectin-1-coupled signalling (Goodridge et al., 2011).

**The different macrophage populations.** Classical distinction between macrophage types includes the type M1 inflammatory macrophage and the M2 regulatory macrophage (for review, see Sica and Mantovani, 2012). Peripheral blood monocytes cultured in the presence of serum, a source of M-CSF but not GM-CSF (Vogt and Nathan, 2012), are considered non-polarized macrophages (M0 type), whereas addition of different cytokine cocktails elicits polarization versus the M1 or M2 type. For instance, the M1 type is induced by IFN-γ and LPS, and the M2 type is induced by IL-4 and IL-13. The ability of macrophages to produce eicosanoids in response to some PAMP is strongly dependent on the type of polarization achieved and the ensuing pattern of receptor expression. A central role for macrophage-derived PGE$_2$ in the induction of Th2 type immune responses has been reported during the processing of particulate crystal-like materials like alum and silica through a mechanism regulated by SYK and p38 MAPK. Under these conditions, the production of PGE$_2$ depends on receptor-independent particle uptake and signaling from phagolysosome, rather than on the engagement of cell membrane receptors and may occur in the absence of inflammasome activation and IL-1β production (Kuroda et al., 2011). These findings are reminiscent of the response to other crystals such as sodium monourate particles (Kool et al., 2011) and hemozoin (Shio et al., 2009), and also agree with the reported
activation of SYK by receptor-independent, direct membrane binding of monosodium urate crystals in dendritic cells (Ng et al., 2008). These results are in keeping with the notion that particulate PAMP are more active than soluble PAMP for scaling microbial threat and are relevant to understand the inflammation induced by microcrystals. In addition, they underscore the complexity of the process of recognition of particles bearing PAMP, which may include receptor-dependent and independent mechanisms, and is influenced by receptor expression, the state of differentiation of the cell, and the presence of molecules that affect receptor function. Attempts to correlate the state of macrophage differentiation with eicosanoid release have steadily shown a predominant production of PGE\(_2\) and PGD\(_2\) and a varying pattern of response dependent on the presence of co-stimulatory substances. For instance, growth factors such as M-CSF and GM-CSF elicit profound changes on the expression of receptors such as DC-SIGN and the dectin-1 B isoform, i.e., the isoform displaying the most efficient productive binding to \(\beta\)-glucans (Rosas et al., 2008; Municio et al., 2013). Macrophages cultured in the presence of human serum show a high sensitivity to LPS as judged from a rapid induction of COX-2 and a high production of PGE\(_2\). In contrast, particulate zymosan, an extract of \textit{Saccharomyces cerevisiae} cell wall mainly composed of \(\beta\)-glucans, only induces COX-2 expression several hours after particle uptake (Municio et al., 2013). This putative phagolysosomal route of PGE\(_2\) production is synergistically enhanced by low concentrations of LPS, which agrees with the results observed with crystal-like materials (Kuroda et al., 2011). These findings are relevant to vaccine technology, since the release of PGE\(_2\) under these conditions help explain the adjuvant effect of alum, one of the few approved adjuvants for use in humans, and maybe of \(\beta\)-glucans as well (Huang et al., 2010). The response of the macrophages is also influenced by their origin. For instance, peritoneal macrophages readily respond to bacterial products, whereas this
response is less pronounced or even absent in bone marrow-derived macrophages. From a pathophysiological point of view, this is most evident in the response termed “eicosanoid storm” observed in response to bacterial toxins, where systemic inflammasome-dependent activation of cPLA2 and COX-1 in peritoneal macrophages by i.p. injection of flagellin leads to a rapid loss of intravascular fluid, hemoconcentration, hypothermia, and death, which is not observed in mice deficient in COX-1 (von Moltke et al., 2013).

Dendritic cells. DC are professional antigen-presenting cells that bridge the innate and the adaptive immune response due to their ability to stimulate naive T cells and initiate immune responses through the activation of T-helper cells. PGE2 is a key factor for the surface expression of CCR7 (C-C chemokine receptor type 7) and the ensuing acquisition of responsiveness to CCL21 and CCL19, which are expressed in lymphatic vessels and the T-cell areas of lymph nodes (Kabashima et al., 2003; Scandella et al., 2002). Mice lacking CCR7 fail to migrate into draining lymph nodes and congruent with this pivotal function of PGE2, failure of DC to produce PGE2 has been considered a major obstacle for the successful application of DC in therapy (Thurnher et al., 2001; Zelle-Rieser et al., 2002). Several reports have stressed the role of 5-lipoxygenase products in DC function: i) deficient extracellular export of LTC4 is associated with a decreased migratory response of DC (Robbiani et al., 2004), ii) cysteinyl-LT increase IL-10 production by myeloid DC (Machida et al., 2004), and iii) the dectin-2/cysteinyl-LT pathway is critical for the induction of Th2 immunity to major allergens (Barrett et al., 2011). Similar to the distinct responses observed in the different types of differentiated macrophages, AA metabolism in DC shows distinct patterns in mature and immature DC. Whereas the release of AA elicited by some ligands showed no difference between immature and TNFα-mature cells, an enhanced expression of COX-
2 was best observed in immature DC (Valera et al., 2008), thus highlighting the distinct patterns of response associated with environmental cues. In the case of LPS, DC express the LPS co-receptor CD14 at lower levels than macrophages (Segura et al., 2013) and this may explain the different intensity of the response. As regards C-type lectin receptors, the engagement of dectin-1 has been the object of close attention since it is expressed on the surface of polymorphonuclears, monocytes, and DC, but DC show the most robust responses. These differential responses might be explained by several mechanisms: i) expression in some myeloid cell types of an inhibitor, e.g. tetraspanin CD37, that restricts dectin-1-CARD9 signaling (Goodridge et al., 2009), ii) gain of function of DC by differentiation-induced expression of a receptor cooperating with dectin-1, and iii) induction along the process of differentiation of C-lectin receptor isoforms supporting productive binding. The last two mechanisms seem most likely in view of the high level of alternative exon splicing observed in dectin-1 and the current notion that zymosan-induced AA requires cooperation with other C-type lectin receptors, for instance DC-SIGN, the expression of which is enhanced during the differentiation of monocytes into DC (Domínguez-Soto et al., 2011). Altogether, these data indicate that cooperative binding of particulate β-glucans and mannans to C-type lectin receptors explains the activation of a cPLA_2α-dependent route for AA release in DC. Likewise, two different routes are involved in the κB-driven induction of COX-2 and the delayed production of PGE_2 produced by zymosan, the CARD9/MALT/BCL10/TRAF6 complex triggered by dectin-1 (Hara et al., 2007; LeibundGut-Landmann et al., 2007) and TLR2-dependent activation of IκB kinases (Gantner et al., 2003; Brown et al., 2003).

The Candida paradigm for AA release. The fungal mimic zymosan has been used for many decades as a relevant stimulus to study complement activation, phagocytosis, and
local inflammatory responses. On this basis, the results observed using it as a stimulus can be translated into the context of clinical infection by fungi. This has been confirmed in models using Candida and macrophages from engineered mice to address AA release and PGE₂ production. Reports from Leslie lab (Suram et al., 2006 & 2010; Parti et al., 2011) have demonstrated that deletion of dectin-1 decreases by ~ 60% the release of AA elicited by Candida albicans. MyD88⁻/⁻ macrophages stimulated for 1 h with C. albicans released ~ 50% less AA than wild type cells. In contrast, wild type and MyD88⁻/⁻ macrophages release similar amounts of AA in response to particulate β-glucan. The up-regulation of COX-2 6 h after adding C. albicans was almost completely ablated in MyD88⁻/⁻ peritoneal macrophages, and the production of PGE₂, PGI₂, and LTC₄ at 6 h was reduced by 80–90% (Suram et al., 2006). These results suggest a major role for dectin-1 in the early release of AA and of TLR2, which uses the MyD88 adaptor, in the activation of κB-dependent transcription and COX-2 expression in the late phase of eicosanoid production (Fig. 1). The translation of this paradigm to clinical settings is specially relevant to understand the outcome of polymicrobial infections, for instance peritonitis, where Candida spp. accompanies either Gram + or Gram – bacteria. Whereas monomicrobial infection is nonlethal, coinfection with the same doses leads to a 40% mortality and increases microbial burden in kidney and spleen. Notably, coinfection is associated with a synergistic production of PGE₂ and blockade of the COX routes prevents mortality (Peters and Noverr, 2013). A sound explanation for the molecular mechanism whereby PGE₂ leads to an immunocompromised state during Candida spp. infection has been reported recently by showing that PGE₂ promotes the formation of a complex between the actin depolymerization factor cofilin-1 and PTEN (phosphatase and tensin homolog deleted on chromosome 10) that decreases polymerization of F-actin and increases monomeric G-actin (Serezani et al., 2012). A
recent study has disclosed how the timing of eicosanoid production may be coupled to either pro- and anti-inflammatory responses during Candida infection (Suram et al., 2013). G-CSF (csf3 gene), a cytokine that regulates the production and function of polymorphonuclear leukocytes, was the gene showing the highest degree of induction in response to Candida and its expression was reduced in cPLA2–/– animals. The expression of il10 increased 78-fold in cPLA2α+/+ mice as compared to only 7-fold in the cPLA2–/– mice. Conversely, TNFα expression was increased in cPLA2α−/− mice treated with Candida as compared to wild type animals. These finding are in keeping with the reported role of PGE2 in the induction of the Th17 response and the central role of IL-17 in host defense in candidiasis (Ferwerda et al., 2009; Conti et al., 2009; Puel et al., 2011), and also show that Candida stimulates an autocrine loop in macrophages involving cPLA2α, PG, and cAMP that globally effects expression of genes involved in host defense and the control of inflammation.

**AA metabolites are major modulators of the release of cytokines from DC**

*IL-10, PGE2, and the down-regulation of the inflammatory response.* The induction of cytokines by fungal PAMP has been the object of detailed analysis. In the case of β-glucans, the pattern of cytokines includes a high production of TNFα, IL-6, IL-10 and IL-23, and a low secretion of IL-12 p70 (Rogers et al., 2005; Dillon et al., 2006; Gerosa et al., 2006). Since these cytokines are produced concomitantly with the release of eicosanoids, the question arises as to the role played by eicosanoids in the induction of those cytokines. The induction of the expression of TNFα and IL-6 can be explained on the basis of the activation of NF-κB, but the expression of IL-10 requires close attention due to its complex mechanism of regulation and its role in the down-regulation of the
inflammatory response. Most recent views have focused on STAT3 (Benkhart et al., 2000; Chang et al., 2007; Weichhart et al. 2008) and CREB (Platzer et al., 1999; Hu et al., 2006; Alvarez et al., 2009a). The central role of CRE-dependent transcription on \( il10 \) regulation explains its strong PGE2-dependency, since one of the best effects of PGE2 is the activation of the system E prostanooid receptor (EP)/cAMP/protein kinase A, which phosphorylates S133-CREB and creates a docking site for the coactivator CREB-binding protein (CBP) and the initiation of \( il10 \) transactivation. In addition, PKA activity inhibits salt induced kinases (SIK), the function of which is allowing the cytoplasmic retention of the CREB coactivators TORC/CRTC2 and TORC/CRTC3 through their cytoplasmic docking to 14-3-3 proteins. In keeping with this mechanism, inhibition of SIK activity by the kinase inhibitor MRT67307 elevates IL-10 production by inducing the dephosphorylation of TORC/CRTC3, its dissociation from 14-3-3 proteins, and its translocation to the nucleus where it enhances the gene transcription program controlled by CREB. Proof of concept of the involvement of PGE2 in the PKA/SIK/CRTC3 pathway has been provided recently (Clark et al., 2012; Mackenzie et al., 2013a). Taken together, these data show that PGE2 acting through EP receptors modulates two upstream elements of the EP/PKA/SIK/CRTC-CREB pathway that reprograms macrophages to an anti-inflammatory phenotype (Fig. 2). A corollary to these findings is that inhibition of SIK can be used as a molecular target to switch macrophage into an anti-inflammatory phenotype. In addition to the robust connection between PGE2 and IL-10 that explains a portion of the anti-inflammatory phenotypes in macrophages and DC, PGE2 can still repress TLR-induced cytokine induction in the absence of IL-10 (van der Pouw Kraan et al., 1995; Mackenzie et al., 2013a). Several studies have disclosed the distinct mechanisms involved in the modulation of cytokine production elicited by PGE2. Whereas the inhibitory effect of PGE2 on IL-6 induction
was dependent on IL-10 as judged from its reversal by anti-IL-10 Ab, the production of IL-12 p70 was not influenced by this treatment (van der Pouw Kraan et al., 1995). The inhibitory effect on the induction of TNFα has been explained through PKA-anchoring protein 95, which positions the kinase in close proximity to its substrates, and interference with the phosphorylation of Ser536 in the C-terminal transactivation domain of the NF-κB family protein p105 (Wall et al., 2009). The inhibition of the inflammatory chemokines CCL3 and CCL4 has been explained through a signaling route involving EP/exchange protein activated by cAMP (EPAC)/phosphatidylinositol 3-kinase/protein kinase B/glycogen synthase kinase 3, which results in an increased binding of the CCAAT displacement protein to the regulatory regions of a variety of genes, where it behaves as a potent transcriptional repressor (Jing et al., 2004). Another way of cross-talk between IL-10 and PGE2 production has been disclosed by showing the inhibitory effect of IL-10 on ptgs2 mRNA expression. This has observed in different systems (Niiro et al., 1995; Berg et al., 2001; Shibata et al., 2005) and a mechanism explanation has been provided in a recent study by showing that IL-10 regulates ptgs2 mRNA via tristetraproline-dependent degradation (Mackenzie et al., 2013b).

**PGE2 and the IL-12 p70/IL-23 balance.** Some studies have shown the involvement of PGE2 in the production of Th1 responses (Nagamachi et al., 2007; Yao et al., 2009), but most work has stressed its ability to suppress the differentiation of functionally competent Th1-inducing DC (Kaliński et al., 1997). The most accepted mechanism is that PGE2 weakens the Th1 response by inhibiting the production of the Th1-polarizing cytokine IL-12 p70, because the expression of the IL-12 p40 chain is not accompanied by the parallel expression of the IL-12 p35 chain, which is necessary to form the bioactive IL-12 p70 heterodimer. This effect is so strong that combination of PGE2 with
other stimuli such as LPS and CD40 ligand also results in failure to induce IL-12 p70 (Kaliński et al., 2001). Most recent studies have identified the DC resulting from PGE$_2$ exposure as myeloid-derived suppressor cells (MDSC) capable of suppressing cytotoxic T lymphocyte responses (Obermaier et al., 2011).

IL-23 plays a central role in the expansion and maintenance of the Th17 phenotype, which agrees with the enhancing role of PGE$_2$ on IL-23 production and Th17 polarization (Khayrullina et al., 2008; Boniface et al., 2009). This effect seems restricted to DC and has not been observed in macrophages (Kalim and Groettrup, 2013). A recent study has deciphered the PGE$_2$-triggered signaling pathway from the most proximal EP receptors to transcription factor activation and binding to the promoter of $il23a$, i.e. the gene encoding the IL-23 p19 chain that dimerizes with IL-12 p40 to form the IL-23 heterodimer (Kocieda et al., 2012). This mechanism is consistent with the aforementioned general route including EP receptors/cAMP/PKA/P-CREB, but a portion of the effect depends on the activation of EPAC by cAMP and is related to an effect on C/EBP$\beta$ transcriptional activity rather than on CREB-mediated trans-activation. The response is mediated primarily through EP4 and to a lesser extent via EP2. The effect of PGE$_2$ was only observed when it was used in combination with LPS, which is consistent with the need for cooperation with other transcription factors activated by LPS, for instance NF-κB and/or C/EBP$\beta$. Further analysis showed that two putative CREB binding sites in the $il23a$ promoter were involved in transcriptional regulation and that the CREB distal site is an important regulator of EP4-induced $il23a$ transcription (Fig. 3). A thorough scrutiny of the distinct role of the different EP receptors in human DC has been reported recently (Poloso et al., 2013). By using selective EP receptor antagonists it was observed that PGE$_2$ controls IL-23 release in a dose-dependent manner by differential use of EP2 and EP4. Low concentrations of
PGE2 trans-activate il23a via EP4, whereas concentrations above 50 nM suppress IL-23 by an EP2-dependent mechanism.

The contribution of animal models to decipher the function of PGE2 in the innate immune response

The different EP receptor subtypes. The apparently opposing biological effects of PGE2 can be explained by its distinct actions on many cell types. On the one hand, PGE2 promotes local vasodilatation, inflammatory edema, and local attraction and activation of leukocytes. On the other hand, its prominent effects on the induction of suppressive IL-10 and blunting of IL-12 p70 production account for its immunomodulatory properties. These actions explain that the most outstanding effect of PGE2 in in vivo models is to keep at bay excessive inflammatory responses. A risk derived from this action is that it may contribute to the immune suppression associated with chronic inflammation and cancer. Notably, PGE2 on its own induces high levels of COX-2 in differentiating MDSC and initiates a positive feedback loop that stabilizes the suppressive functions of MDSC (Obermaier et al. 2011). The distinct effects of PGE2 are best explained not only by the existence of four different EP receptors, but also by the diversity resulting from multiple variants of EP3 due to alternative splicing of its C-terminal tail. Although EP receptors are G-protein coupled, they are structurally and functionally distinct, have limited amino acid identity, and show variable levels of expression among the different tissues. EP3 and EP4 represent high-affinity receptors, whereas EP1 and EP2 require significantly higher concentrations of PGE2 for effective signaling (for review, see Sugimoto and Narumiya, 2007). EP2 and EP4 signaling mainly depends on the cAMP/PKA/CREB pathway (Gαs-coupling), and they mediate the most relevant anti-inflammatory and suppressive activity of PGE2. However, a
detailed scrutiny of the function of EP2 and EP4 has disclosed that EP4 ligation induces a weaker stimulation of intracellular cAMP when compared to the ligation of EP2 expressed at similar levels. Unlike EP2, EP4 can also be coupled to β-arrestin and β-catenin (Yokoyama et al., 2013). This explains the formation of an EP4/β-arrestin 1/c-Src signaling complex that plays an important role in cancer cell migration (Kim et al., 2010) and in DC function (De Keijzer et al., 2013). EP1 is coupled to a yet unknown G-protein, but binding of PGE₂ to this receptor type leads to a transient increase in intracellular Ca²⁺ concentration and to activation of phospholipase Cβ, presumably by coupling to a Gα₅ protein. The G-protein coupling of EP3 is more promiscuous in view of the different C-terminal splice variants that signal via Gα₅ and Gαᵢ proteins and induce an increase of inositoltrisphosphate and Ca²⁺ (Gα₅-coupling) or a decrease of cAMP (Gαᵢ-coupling). This array of differences in sensitivity, susceptibility to desensitization, and ability to activate different signaling pathways among the different EP receptors allows for adaptable patterns of responses at different stages of the immune response.

**Animal studies as a proof of concept.** Studies in animal models of infection have shown that PGE₂ differentially affects cytokine production depending on the developmental state of the cell. In contrast to *in vitro* studies where PGE₂ has been found to enhance the Th17 immune response (Khayrullina et al., 2008), PGE₂ has been reported to suppress antifungal immunity by inhibiting IRF4 function and IL-17 expression in T cells in a mouse model of *Cryptococcus neoformans* infection (Valdez et al., 2012). Likewise, EP2 and EP4 activation suppresses pulmonary host defenses through increases in cAMP (Aronoff et al., 2004; Ballinger et al., 2006; Sadikot et al., 2007). EP3 is also involved in the suppression of host defenses, since EP3−/− are protected from pneumococcal mortality (Aronoff et al., 2009). Studies addressing the
role of PGE2 in the development of immunosuppression secondary to sepsis in a model of cecal ligation and puncture complemented with secondary infection with *Aspergillus fumigatus* in sepsis survivors, disclosed that inhibition of COX was associated with improved survival to secondary infection and enhancement of macrophage phagocytosis and neutrophil recruitment to the lungs. EP4 receptors were identified as the main type involved in modulating cytokine production (Brogliato et al., 2012). Altogether, these data indicate that the predominant function of PGE2 *in vivo* consists in the down-regulation of the phagocytic activity and the immune response. Consistent with PGE2 being the most abundant eicosanoid at sites of inflammation, there is not a definite notion of the function of the distinct LT during infections; however, from pharmacological and genetic models in mice deficient in 5-lipoxygenase, it has emerged the notion that the cAMP-reducing action of LTB4 carried out on the Gαi-coupled, high affinity BLT1 receptor enhances microbial phagocytosis during *Streptococcus pyogenes* infection and may exert a function opposed to that of PGE2 (Soares et al., 2013).

**Concluding remarks**

Eicosanoids are generated as a result of the signaling cascades triggered by the binding of PAMP to their cognate PRR. This makes the innate immune response one of the most relevant settings where eicosanoids play a pathophysiological role. Signaling cascades based on phosphorylation and Ca\(^{2+}\)-driven translocation of cPLA2α are involved in the rapid generation of eicosanoids, whereas induction of the expression of COX-2 by activation of transcription factors such as NF-κB, CREB, and C/EBPβ explains the delayed and high-rate production of PGE2 and PGD2 associated with processing the AA available as a result of the basal acylation/deacylation cycle. The distinct ligand-binding properties of the PRR, the different signaling mechanisms
associated, and the cooperation of receptors in the binding of microbial-derived particles explain the different patterns of responses that can be observed. Another layer of complexity is provided by the role of opsonic proteins. In this connection, complement coating of PAMP seems essential for the activation of polymorphonuclears and monocytes by particles mimicking the fungal cell wall, whereas monocyte-derived DC are a cell type specially endowed to respond to fungal patterns in the absence of opsonins. The analysis of PGE₂ effects has disclosed its capacity: i) to inhibit phagocytosis and to enhance mortality in models of fungal and polymicrobial infection, ii) to stabilize the suppressive functions of MDSC, iii) its capacity to promote an anti-inflammatory phenotype in macrophages associated with a high production of IL-10, and iv) its ability to inhibit IL-12 p70 and to enhance the production of IL-23. The application of mechanistic data on the role of kinases such as SYK and SIK to modulate PGE₂ production, and the selective modulation of EP receptor signaling can be valuable clues to develop useful pharmacological tools to address infectious, autoimmune, and autoinflammatory diseases.
Authorship Contributions

Wrote or contributed to the writing of the manuscript: Rodriguez, Domingo, Municio, Alvarez, Hugo, Fernández, and Sánchez Crespo.
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Footnotes

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Legend to the figures

Fig. 1. The *Candida* paradigm for AA metabolism in DC. The mannan and β-glucan components of the fungal cell wall are recognized by at least DC-SIGN, TLR2, dectin-1, the mannose receptor, and dectin-2. This gives rise to a series of signalling events implicating activation of SYK and Src families of tyrosine kinases. Both routes converge to activate phospholipases Cγ and through the generation of diacylglycerol activate protein kinase C and MAPK cascades. Phosphorylation by MAPK and Ca2+-driven translocation of cPLA2α explain AA release from cell phospholipids. Activation of IκB kinases via MyD88 is a major factor explaining COX-2 and PGES induction. The CARD9/MALT/BCL10/TRAF6 complex is also involved in the activation of IκB kinases. DAG, diacylglycerol; IP₃, inositol 1,4,5-trisphosphate; IκB, inhibitory subunit of NF-κB; IRAK, interleukin-1 receptor-associated kinase; MAL, MyD88 adaptor-like; MAPK, mitogen-activated protein kinases; MyD88, myeloid differentiation primary response gene 88; PLCγ, phospholipase Cγ; P-Y, phosphotyrosine; *ptgs2*, COX-2 gene; *mpges1*, microsomal prostaglandin E synthase-1 gene; TAK1, transforming growth factor-β-activated kinase-1; TRAF6, TNF receptor-associated factor-6. This scheme has been constructed from the results reported by Valera et al. (2008), Suram et al. (2006&2010), and Parti et al. (2011).

Fig. 2. Mechanism whereby PGE₂ promotes the transcription of *il10* in LPS- and zymosan-induced macrophages and DC. Binding of PGE₂ to the EP2 and EP4 receptors triggers the production of cAMP and the activation of PKA. PKA induces the phosphorylation of S133-CREB and the phosphorylation/inactivation of salt inducible kinases (SIK). P-S133-CREB binds to the CRE site in the *il10* promoter, whereas
dephosphorylation of TORC/CRTC2 and TORC/CRTC3 by the serine-threonine phosphatase calcineurin releases the cytoplasmic retention of these coactivators by binding to 14-3-3 proteins and allows their nuclear translocation and interaction with the complex P-CREB/CBP to initiate the transcriptional response. The scheme has been elaborated from results reported by Alvarez et al. (2009a), Clark et al. (2012), and Mackenzie et al. (2013a). The indication of acetylated lysines is explained by the relevance of these post-translational modifications related to the lysine acetyltransferase activity of CBP.

**Fig. 3.** Cooperation of PGE$_2$ and TLR4 signaling in the induction of $il23a$ transcriptional activation in murine bone-marrow derived DC. PGE$_2$ activates transcription factors that cooperate with NF-κB proteins. A part of the signals are conveyed via cAMP and protein kinase A and allows the activation of CREB. In addition, EPAC contributes to the activation of C/EBP$\beta$ that binds to a site in the proximal promoter. P, phosphate. This diagram has been constructed from data by Kocieda et al. (2012).
Figure 1
Figure 3