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Cyclooxygenase 2: its regulation, role and impact in airway inflammation

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COX-2 and respiratory disease

Abstract

Cyclooxygenase 2 (COX-2: official gene symbol – PTGS2) has long been regarded as playing a pivotal role in the pathogenesis of airway inflammation in respiratory diseases including asthma. COX-2 can be rapidly and robustly expressed in response to a diverse range of pro-inflammatory cytokines and mediators. Thus, increased levels of COX-2 protein and prostanoid metabolites serve as key contributors to pathobiology in respiratory diseases

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typified by dysregulated inflammation. But COX-2 products may not be all bad: prostanoids can exert anti-inflammatory/bronchoprotective functions in airways in addition to their pro-inflammatory actions. Herein we outline COX-2 regulation and review the diverse stimuli known to induce COX-2 in the context of airway inflammation. We discuss some of the positive and negative effects that COX-2/prostanoids can exert in *in vitro* and *in vivo* models of airway inflammation, and suggest that inhibiting COX-2 expression to repress airway inflammation may be too blunt an approach; because although it might reduce the unwanted effects of COX-2 activation, it may also negate the positive effects. Evidence suggests that prostanoids produced via COX-2 upregulation show diverse actions (and herein we focus on prostaglandin E₂ as a key example); these can be either beneficial or deleterious and their impact on respiratory disease can be dictated by local concentration and specific interaction with individual receptors. We propose that understanding the regulation of COX-2 expression and associated receptor-mediated functional outcomes may reveal number of critical steps amenable to pharmacological intervention. These may prove invaluable in our quest towards future development of novel anti-inflammatory pharmacotherapeutic strategies for the treatment of airway diseases.

Non-standard abbreviations: ASM, airway smooth muscle; BAL, bronchoalveolar lavage; β_2 -AR, β_2 -adrenergic receptor; ERAD, ER-associated degradation; UTR, untranslated region.

Introduction

Cyclooxygenase (COX), also known as prostaglandin H/G synthase, is the rate-limiting enzyme responsible for the production of prostanoids from arachidonic acid, hydrolyzed from cell membrane phospholipids catalyzed by phospholipase A₂ (Figure 1). Prostanoid is the common term for molecules including prostaglandins, prostacyclins and thromboxanes. Two isoforms of COX have been identified: COX-1 and COX-2, each encoded by different genes

(official gene symbol: PTGS1 and PTGS2, respectively). COX-1 is believed to be expressed constitutively as a “housekeeping” enzyme, and COX-2, can be either inducible or constitutive, depending on the tissue. As an inflammation-associated enzyme, induction of COX-2 is triggered by a number of cytokines and inflammatory mediators present in various inflammatory cells. In acute and chronic inflammatory states, induced COX-2 is primarily responsible for the synthesis of prostanoids and these molecules contribute to the pathological processes of several inflammatory conditions including cancer, rheumatoid arthritis, Alzheimer’s disease, and respiratory disorders.

In this review, we focus on the role and function of COX-2 in respiratory disease and highlight the impact of COX-2/prostanoid induction in airway inflammation and function. We provide an overview of COX-2 regulation, review the diverse stimuli known to induce COX-2 in the context of airway inflammation, demonstrate the positive and negative effects that COX-2/prostanoids (and PGE₂ in particular) can exert in inflammation in order to highlight the promise of more selective manipulation with new pharmacological tools.

COX-2 regulation

COX-2 expression can be regulated at different levels including transcriptional, post-transcriptional or post-translational. In order to better understand the role and function of COX-2 upregulation in respiratory disease, it is necessary to first explore the regulatory mechanisms and molecules that control COX-2 expression more generally.

Transcriptional regulation: The human COX-2 gene is located on chromosome 1, which contains 10 exons and 9 introns and is approximately 8.3 kb long. Structural analysis of human COX-2 gene reveals the presence of 5'-flanking region, 10 exons encoding site, the exon-intron boundaries and the 3'-untranslated region (UTR) in both strands of DNA [1]. The 5'-flanking region consists of ~ 800 bp of nucleotides and this 5'-UTR region contains distinct

combinations of *cis*-regulatory elements including peroxisome proliferator response element (PPRE), two cyclic AMP response elements (CRE), a sterol response element (SRE), two nuclear factor kappa B (NF- κ B) sites, a specificity protein-1 (SP1) site, a CAAT enhancer binding protein (C/EBP), nuclear factor for interleukin-6 expression (NF-IL6) motif, two activator protein-1 (AP-1) sites, an E-box, and a TATA box [1-3]; all are binding sites for a number of *trans*-regulatory transcription factors. The 10 exon region encodes the entire open reading frame of COX-2 gene which is unusually large as it includes not only the final 410 bp of the coding region but also 2550 bp of 3'-UTR [1]. The three potential poly-adenylation sequences of 3'-UTR are approximately 280 bp apart from each other. The 23 copies of the ATTTA RNA instability element of the 3'-UTR of the human COX-2 gene contribute to post-transcriptional regulation of COX-2 expression [4]. Steady state levels of COX-2 mRNA expression are determined by transcriptional mechanisms along with post-transcriptional regulation [5]; these mechanisms will be outlined in the next section.

Post-transcriptional regulation: COX-2 is regulated at the post-transcriptional level via interaction between *cis*-acting motifs within the COX-2 mRNA and *trans*-acting molecules, such as RNA-binding proteins and microRNAs (miRNAs). *Cis*-acting regions within the COX-2 mRNA include the cap, the poly-A tail and adenylate and uridylylate rich (AU-rich) elements (AREs) in the 3'-UTR. COX-2 mRNA contains multiple copies of adenylate and uridylylate rich (AU-rich) elements (AREs).

Much has been learned over the past decade or so in regards to the *cis*-acting motifs within 3'-UTR and the RNA-binding proteins that interact with AREs to dictate mRNA stability. Mukhopadhyay *et al.* identified a novel RNA binding protein, CUGBP2 (CUG triplet repeat, RNA-binding protein 2), which stabilizes COX-2 mRNA but inhibits its translation [6]. Augmented binding of the stabilizing human antigen R (HuR) to the ARE of the COX-2 3'-UTR is responsible for the COX-2 mRNA stability [7-9] in different cells. Studies performed

by Hall-Pogar *et al.* suggest that alternative polyadenylation of COX-2 gene is an important post-transcriptional regulatory event and some RNA-binding proteins, U1A, PTB (polypyrimidine tract-binding protein), p54^{nrb} and PSF (PTB-associated splicing factor) can regulate the polyadenylation signal upon binding with upstream sequence elements [10, 11]. Time-dependent modulation of transcriptional and post-transcriptional phases of the COX-2 biosynthetic pathway can be achieved by anti-inflammatory corticosteroids and confirmed by actinomycin D analysis [12]. Tristetraprolin (TTP), an mRNA-binding protein that promotes message instability, is shown to bind with the COX-2 mRNA in the region of the 3'-UTR and thus participate in post-transcriptional regulation of COX-2 [4, 13]. Other cytoplasmic proteins with RNA-binding properties can also regulate COX-2 gene expression including: TIA-1 (T-cell intracellular antigen 1; TIA-1) [14, 15], RBM3 (RNA-binding motif protein 3) [16, 17] and Apobec-1 [18]. The stress-inducible 70-kDa heat shock protein, Hsp70, has also been recently identified as a novel post-transcriptional regulator of COX-2 gene that binds and stabilizes COX-2 mRNA [19]. Notably, the p38 MAPK pathway activated by various inflammatory mediators plays an important role in post-transcriptional regulation of COX-2 by influencing mRNA message stability as well as its abundance [20-24].

In addition to regulation by RNA-binding proteins, COX-2 has more recently been found to be a target of various microRNAs. Small non-coding RNAs called microRNAs (miRNAs), composing approximately 21–24 nucleotides, are considered as vital post-transcriptional gene regulators through their ability to control mRNA stability and translation by imperfect base-pairing to the 3'-UTR of their target mRNAs. Binding of two miRNAs, mmu-miR-199a* and mmu-miR-101, results in post-transcriptional regulation of COX-2 gene by affecting mRNA stability and translation [25]. Another microRNA, miR-16, is identified to silence COX-2 gene expression by two mechanisms: directly, by binding to the microRNA response element motif in the COX-2 3'-UTR and indirectly, by reducing the levels of HuR [26]. MicroRNA-

320a is found to be regulating COX-2 expression through ERK/NF- κ B pathways, hence effecting pro-inflammatory cytokine production [27]. MicroRNA-146 and miR-155 have also recently been shown to be relevant in asthmatic inflammatory context [28, 29]

Post-translational regulation: Although most research on COX-2 regulation has focused at the transcriptional and post-transcriptional levels, post-translational control is increasingly recognized as an important contributor to overall COX-2 protein production. These mechanisms will likely reveal novel strategies that could be targeted to modulate COX-2 production in the future. Accordingly, spatial regulation and the control of COX-2 degradative pathways by post-translational modifications will be outlined below.

When COX-2 protein is translated from stable mRNA it then undergoes several post-translational regulatory modifications that dictate cellular location and abundance of active protein. COX-2 protein is found localized in the endoplasmic reticulum (ER) and the nuclear envelope [30]. This spatial regulation of COX-2 protein is regulated by *N*-glycosylation. Among five potential *N*-glycosylation sites on COX-2 protein, three are always glycosylated, one (Asn⁵⁸⁰ in human and mouse) is glycosylated more than 50% of the time, and one is never glycosylated [31]. The variability of glycosylation at Asn⁵⁸⁰ results into the formation of two distinct glycoforms, unmodified and modified, molecular weights 72 and 74 kDa, respectively. The mature monomer of COX-2 consists of three folding domains including an epidermal growth factor-like domain, a membrane binding domain, and a catalytic domain in sequence [32]. A number of reports have identified the mechanistic cascades required for the maturation of COX-2 in the ER lumen including cleavage of the *N*-terminal signal sequence, *N*-glycosylation at multiple sites, disulfide bond formation, membrane insertion, and dimer formation [31, 33-35].

Protein degradation is also an important point of post-translational regulatory control and properly folded COX-2 has been reported to be degraded via two independent pathways [36, 37]. The first degradative pathway is called substrate-induced COX-2 degradation and it is considered to be proteasome independent [36, 38]. Arachidonic acid is the endogenous fatty acid substrate of COX-2 which is subject to oxygenation as a consequence of suicide inactivation of COX-2 catalytic activity [39, 40]. Substrate-dependent degradation of COX-2 occurs as a result of structural damage to the enzyme that occurs by fatty acid substrate oxygenation.

The second pathway involves ER-associated degradation (ERAD), a process by which ER-associated *N*-glycoproteins are selectively removed for degradation to subsequently undergo proteolysis by 26 S proteasome. Because COX-2 is a luminal integral membrane protein, it needs to be transported out of the ER for degradation; this characteristic feature makes COX-2 an exclusive ERAD substrate [38]. Several studies have described the ERAD process for COX-2 protein [2, 32, 38, 41] in different cells. This process occurs at a relatively constant rate and it is believed that glycosylation of Asn⁵⁹⁴ of COX-2 is critical for initiating the entry of the enzyme into the ERAD system and most importantly, any mutation of this site will prevent glycosylation as well as make COX-2 more stable [36, 41]. COX-2 protein ubiquitination and degradation through the 26 S proteasome in the cytoplasm was also shown in several other reports [42-45] and Sing *et al.* revealed the importance of the ER-mannosidase I enzyme in facilitating the ERAD-mediated ubiquitin-proteasomal degradation of COX-2 in human uterine stromal cells [46]. The membrane protein caveolin-1 has been implicated in the post-translational regulation of COX-2 via ERAD-mediated ubiquitination and proteasome degradation pathways [45, 47]. Further investigations are warranted to fully understand the contribution of this pathway to COX-2 degradation.

COX-2 induction in airway inflammation

A diverse range of mediators present in the airway microenvironment have been demonstrated to induce COX-2 and increase COX-2-mediated prostanoid products in cells implicated in airway inflammation. The key molecules and their impact on signalling cascades responsible for COX-2 expression are tabulated (Table 1) and outlined in the following sections.

Cytokines: The level of a number of pro-inflammatory cytokines, including interleukin 1 β (IL-1 β), tumour necrosis factor α (TNF α) and interferon- γ (IFN γ) are found to be increased in bronchoalveolar lavage (BAL) fluid from asthmatic patients [48-50]. These cytokines play a significant role in the pathogenesis of inflammatory diseases in the airways and many investigators have examined COX-2 regulation in response to these cytokines alone, or in combination (referred to as “cytomix”)[28, 51-56]. Much investigation has centered on COX-2 upregulation in human airway structural cells, including epithelial and airway smooth muscle (ASM) cells. Mitchell *et al.* [51] were the first to describe induction of COX-2 by cytomix in primary airway epithelial cells; prostanoid products were produced and PGE₂ was shown to be the predominant metabolite. They also utilized the human epithelial cell line (A549) and showed that cytokine-induced COX-2 upregulation was repressed by corticosteroids in both primary cell cultures and A549 cells [51]. In 1997, ASM cells were first demonstrated to be an important source of prostanoids in the human airways, with Belvisi *et al.* reporting COX-2 induction/prostanoid production in cells stimulated with pro-inflammatory cytokines [52]. The relative contribution of each cytokine to cytomix-induced COX-2 was delineated, with IL-1 β being the greatest contributor. Pang and Knox [53] also demonstrated a major role for stimulation by IL-1 β and showed that IL-1 β -induced prostanoid production (6-keto-PGF_{1 α} , PGF_{2 α} , thromboxane B₂ (TxB₂) and PGD₂), with PGE₂ and 6-keto-PGF_{1 α} as the principal products. These publications were also first to show that

COX-2/prostanoid production in ASM cells could be repressed by the corticosteroid dexamethasone or by non-selective or selective COX-2 inhibitors [52, 53]. Notably, in Belvisi *et al.* [52] the authors remarked that “it is still unknown whether the increased expression of COX-2 in human airway smooth muscle cells and the exaggerated production of PGE₂ and prostacyclin would be deleterious or beneficial for airway function in inflammatory diseases such as asthma”. This question remains incompletely understood to date.

Airway structural cells have been widely utilized in a number of studies to explore the molecular mechanism of COX-2 regulation. Newton *et al.* reported that increased PGE₂ release in response to IL-1 β requires *de novo* transcription and translation [57] and in further studies showed that IL-1 β stimulates prostanoid production via NF- κ B-mediated transcriptional activation of COX-2 [58, 59]. Post-transcriptional regulation of COX-2 also plays an important role [60] and explains the mechanisms responsible for repression by corticosteroids [61]. MAPK kinase signalling pathways are involved; in ASM cells, IL-1 β -induced PGE₂ synthesis occurs via ERK and p38 MAPK pathways [62, 63] and in A549 epithelial cells, MAPK kinase inhibitors can repress PGE₂ secretion via mechanistically distinct processes [64]. Nie *et al.* demonstrated COX-2 transcription by bradykinin and IL-1 β involves different regulatory elements, transcription factors, and histone H4 acetylation [56]. MicroRNAs also play an important role, with two recent studies by Comer *et al.* [28, 29] linking miRNAs and COX-2 in ASM cells. Interestingly, cytomix itself can induce miR-146a and mi-146b and miRNA-146a serves as an endogenous negative regulator of COX-2 expression in ASM cells. Moreover, both COX-2 and miR-155 expression are found elevated in ASM cells from people with asthma [29]. Our recent work in ASM cells has confirmed earlier studies [52, 53] to show that TNF α is a relatively modest inducer of COX-2 mRNA expression and PGE₂ secretion, but importantly this expression can be robustly upregulated in

a synergistic or additive manner under pro-inflammatory or exacerbating conditions modelled *in vitro* [65, 66]. COX-2 in ASM cells can be repressed by celecoxib [65] or dexamethasone [66] and in our unpublished studies (Rumzhum and Ammit} we have learned that TNF α -induced COX-2 is subject to proteasomal degradation, the first evidence demonstrating post-translational regulation of COX-2 protein stability.

Taken together, these studies elucidating the molecular mechanisms of cytokine-induced COX-2 expression have highlighted that multiple levels of COX-2 regulation can (co)-exist in airway cells implicated in respiratory disease.

TLR ligands: Toll-like receptors (TLRs) are a class of pathogen-recognition receptors that can recognize specific molecular structures found in microbes and are important for the innate and adaptive immune responses to pathogen- and damage-associated molecular patterns. Stimulation of TLR4 by lipopolysaccharide is the prototypical pathogen mimic approach that has been long recognised to induce COX-2 in lung models *in vitro* and *in vivo* [67]. More recently, rhinoviral infection of epithelial cells *in vitro* have been shown to increase viral genetic material and activation of TLRs on ASM cells. This cross-talk activates TLR3 and TLR 7/8-induced COX-2 expression and results in prostanoid production by ASM cells [68]. Stimulation of TLR receptors can also augment COX-2 expression; in this way infectious stimuli can greatly enhance COX-2 upregulation induced by inflammatory mediators. We showed this in experiments where we mimicked bacterial infection by activating TLR2 with Pam3CSK4 (a synthetic bacterial lipoprotein) and observed that TLR2 ligand engagement upregulates TNF α -induced COX-2 mRNA expression and increases PGE₂ secretion from ASM cells *in vitro* [65]. Mouse models with COX isoform deletions have also revealed differential COX-2 induction by viral and bacterial stimuli with implications for anti-viral interferon responses [69]. Further investigation into the interaction between inflammatory mediators and TLR pathways are warranted.

Bradykinin: Bradykinin (BK) is a potent pro-inflammatory mediator and it is found that asthmatic patients have elevated levels of kinin concentrations in BAL fluid [70]. Pang and Knox first identified the role of BK in COX-2 induced PGE₂ release in human airway structural cells [54, 71, 72]. This group also investigated the molecular mechanisms by which BK regulates COX-2 transcription in human ASM cells and found that BK upregulates COX-2 transcription mainly through CREB-1 binding to the CRE in the COX-2 promoter via a PGE₂/cAMP dependent mechanism [56]. BK sets in train activation of phospholipase A₂ to promote release of the COX-2 substrate - arachidonic acid [73]. In this way, BK has dual effects on PGs by 1) COX-2 induction and 2) increasing arachidonic acid production.

Oxidative Stress: Oxidative stress plays an important role in the pathogenesis of airway inflammatory diseases such as asthma and COPD. Oxidative stress is the result of the flux of reactive oxygen species (ROS) or free radical generation that exceeds available antioxidant defenses of the body [74]. Exposure to cigarette smoke rapidly induces production of ROS, causing airway inflammation [75]. This is particularly relevant in COPD. Cigarette smoke can induce COX-2/PGE₂ in human lung fibroblasts by increasing steady-state COX-2 mRNA expression in an ERK/NF- κ B dependent manner [76]. Cigarette smoke extract induced ROS production can also activate TLR4/MyD88/TRAF6/c-Src/NADPH oxidase signalling that leads to the activation of MAPKs and NF- κ B which is recruited to the promoter region of COX-2 leading to an increase in COX-2 expression [77]. This research team also showed that ATP-induced ROS production led to an increase of COX-2 expression associated with PGE₂ release via P2 purinoceptors/PKC/NADPH oxidase-mediated activation of Jak2 and STAT3 pathway [78]. In airway epithelium nitric oxide-mediated COX-2 upregulation is cyclic GMP-mediated [79].

Sphingosine 1-phosphate: Sphingosine 1-phosphate (S1P) is a bioactive sphingolipid found elevated in BAL fluid from individuals with allergic asthma and was associated with inflammatory cell influx into the airways [80]. Nodia *et al.* suggests that S1P stimulates COX-2 induction in rat vascular smooth muscle cells through mechanisms involving Ca^{2+} -dependent PKC and Src activation via S1P3 receptor coupled to pertussis toxin-sensitive and -insensitive G proteins [81]. Fuerst *et al.* reported that S1P potently regulates calcium mobilization and gene expression (including COX-2) in ASM grown from bronchial biopsies from healthy and asthma individuals [82]. S1P was recently shown to increase COX-2/PGE₂ in tracheal ASM cells *in vitro* through S1PR1/3/c-Src/PYK2/p42/p44 MAPK- or JNK1/2- and S1PR1/3/c-Src/p38 MAPK-dependent AP-1 activation [83]. We have recently shown that S1P increases COX-2 mediated PGE₂ in human ASM cells, both alone and in combination with TNF α [66].

Other stimuli: Peroxisome proliferator-activated receptor- γ (PPAR γ)-dependent COX-2 expression by non-steroidal anti-inflammatory drugs (NSAIDs) enhances IL-1 β -induced COX-2 expression in human ASM cells [84]. This report showed that PGE₂, the major COX product of these cells, induces COX-2 in a positive feedback loop. Bonazzi *et al.* suggested that metabolites of COX-2, and specifically PGE₂, positively modulate the expression of COX-2 in human ASM cells [85]. Chambers *et al.* suggested that trypsin increased PGE₂ release and COX-2 mRNA and protein levels via a protease-activated receptor-2 (PAR-2) independent mechanism [86].

COX-2 upregulation in airway inflammation: functional outcomes

COX-2 immunoreactivity is found to be increased in asthmatic airways [87] and levels of PGE₂ are increased in the lungs of people with COPD [88] in a manner correlated with airflow obstruction severity [89]. Thus, it could be considered that a feasible

pharmacotherapeutic strategy to combat respiratory disease is to inhibit COX-2 and/or repress PGE₂-mediated effects. However this approach is an oversimplification and does not take into account the complexity of COX-2/prostanoid pathway. Additionally, COX-1 and the other biochemical pathways responsible for the breakdown of arachidonic acid (such as the lipoxygenase enzymes, and 5-lipoxygenase in particular: Figure 1) can also have significant impact (as demonstrated in aspirin-exacerbated respiratory disease (AERD) – outlined in later sections). Complex interactions exist as COX-2 regulation is also a function of the interaction between inducers (Table 1) and repressors such as Th2-derived cytokines IL-4 and IL-13 (recently reviewed by Claar *et al.* [90]). This is supported by the observation that COX-2 expression may be inhibited in allergic inflammatory diseases [91] due to the biological activity of Th2-derived cytokines [92]. Moreover, COX-2 has multiple prostanoids products (Figure 1); and although studies have shown that PGE₂ predominates in some inflammatory contexts [52, 68, 93], other COX metabolites can also play important roles. For example, PGD₂ exerts important deleterious effects as it can act as a bronchoconstrictor, induce vascular leakage, selectively recruit and activate dendritic cells via DP₁/DP₂ (reviewed in [90]). Thromboxanes and PGF_{2α} are also potent prostanoid modulators of disease pathogenesis. However, in order to underscore the importance of both positive and negative effects of prostanoids we have focused predominately on PGE₂ in this review. This focus is not to diminish the importance of other prostanoids (see recent review by Claar *et al.* [90]), but serves to highlight the multiple physiological and pathophysiological effects exerted by PGE₂ in airways. In this way we hope to show that it is an oversimplification to block COX-2 or target PGE₂, because in our quest to remove the unwanted effects, we may also negate the positive effects. This is particularly relevant when we consider the role of COX and PGE₂ in aspirin-exacerbated respiratory disease (AERD). A greater knowledge of receptor involvement may also hold the key, as some of the studies outlined below show.

β₂-adrenergic receptor desensitization: It is well-recognized that the β₂-adrenergic receptor (β₂-AR) on human ASM cells is subject to heterologous desensitization induced by PGE₂. This was first demonstrated in 1993 when Hall *et al.* [94] showed that pretreatment of cells with PGE₂ induced a dose-dependent reduction in cAMP release in response to isoprenaline. Further mechanistic insights into the underlying mechanisms and direct links to COX-2-mediated prostanoid production followed [95-99]; with many of these studies demonstrating that PGE₂ directly induces β₂-AR desensitization and thus causes tachyphylaxis to the first line bronchodilators in respiratory disease - β₂-agonists (reviewed in [99, 100]). Thus, the clinical consequence of COX-2-mediated upregulation and PGE₂ secretion in this context may prove detrimental and perhaps particularly so in infectious exacerbation. Our recent studies modelling bacterial exacerbation *in vitro* and *ex vivo* have shown that bacterial mimics induce PGE₂-dependent heterologous desensitization of the β₂-AR to severely curtail the beneficial bronchodilatory and anti-inflammatory actions of short-acting (salbutamol) and long-acting (formoterol) β₂-agonists [65]. Rhinoviral infection has also been shown to desensitize the β₂-AR and reduce cAMP generation in response to β₂-agonists [101] in a prostanoid-dependent manner [68]. Taken together, these studies show that infectious agents known to induce infectious exacerbation and β₂-agonist hyporesponsiveness clinically induce prostanoid-dependent β₂-AR desensitization on ASM cells. A number of possible molecular mechanisms underlie β₂-AR desensitization [100, 102]. Triantafyllidis *et al.* revealed that membrane levels of β₂-AR on ASM cells were reduced by rhinoviral infection [101] and we have demonstrated that heterologous β₂-AR desensitization induced by COX-2 dependent PGE₂ occurs in a manner upstream of adenylate cyclase [66]. EP receptors [102] have been shown to form heterodimeric signalling complexes with β₂-AR on ASM cells and contribute to β₂-agonist resistance. Further investigation of the impact of EP/β₂-AR heterodimeric complexes in infectious exacerbation is warranted.

Mucus secretion: Increased production of mucus is a common feature of airway inflammatory diseases resulting in airflow limitation. Both COX-2 substrate (arachidonic acid) and product (prostaglandins) are considered responsible for mucus glycoprotein production in human airways [103]. IL-1 β can trigger MUC2 and MUC5AC mucin expression via either the ERK or the p38 MAPK-mediated COX-2/PGE₂ pathway in airway epithelial cells [104]. Expression of the MUC5AC mucin gene has been further linked to COX-2 induction and PGE₂ release via EP₂/EP₄ receptor activated cAMP-dependent PKA-mediated signalling pathways in response to diverse pro-inflammatory stimuli [105].

Matrix metalloprotease production: COX-2 and PGE₂ have also been linked to production of matrix metalloproteases (MMPs); MMP-2 in particular. MMP-2 is a protease that plays a significant role in airway remodelling in asthma and COPD [106, 107]. Chen *et al.* [89] reported that PGE₂ was positively correlated with MMP-2 levels in sputum and that both were inversely related to airflow limitation in COPD. These data suggest that targeting COX-2, or more specifically its product PGE₂, may modulate MMP-2-mediated progression of COPD pathogenesis.

Cough reflex: Cough is a common manifestation in patients with respiratory infections. PGE₂ has long been recognised as an airway irritant that can induce cough [108] and the role of prostanoids in the cough reflex has been reviewed [109-111]. Notably, Maher *et al.* identified the receptor involved in PGE₂-mediated cough as the EP₃ receptor [112] and recently revealed the prostanoid receptor-mediated actions of PGD₂ on cough [113].

Cell proliferation and migration: Airway remodelling is a key characteristic of respiratory diseases, especially chronic asthma, and COX-2/PGE₂ has been implicated in the cellular responses that drive remodelling. In general, the effect of PGE₂ has been beneficial. Exogenous PGE₂ was found to control human ASM cell growth by inhibiting DNA synthesis and inhibiting fibroblast collagen formation via decreased synthesis and increased

degradation [114, 115]. A key study performed by Belvisi *et al.* showed that COX-2 exert a powerful anti-proliferative effect that was likely mediated by a mixture of PGE₂ and prostacyclin production in ASM cells [116]. COX-2-dependence of ASM DNA synthesis was demonstrated with non-selective and selective COX inhibitors [117]. Migration of ASM cells could also be repressed by exogenous PGE₂ [118] and COX-2-dependent PGE₂ secretion [119].

Bronchoprotection and anti-inflammatory effects: PGE₂ can also have bronchoprotective effects and anti-inflammatory impact in human airways [108, 120]. Several early studies showed that inhaled PGE₂ relaxes ASM in animal and human airways [121-124] and PGE₂ is effective against allergen- and exercise-induced bronchoconstriction [125, 126]. This makes sense given the known ability of PGE₂ to induce cAMP; the mediator responsible for ASM bronchodilation. Additionally, like other cAMP-inducing agents, PGE₂ can have a significant impact as a repressor of cytokine production [100, 127-129]. Moreover, PGE₂ can promote production of IL-10, an anti-inflammatory protein that plays an important role in resolution of inflammation [130]. But it is important to note that the modulation of cytokines may not always be beneficial, as studies in ASM cells have shown that cytokines containing cAMP-responsive CRE in their 5'-UTR, or regulatory elements, may be upregulated by PGE₂ [72, 128, 131-133].

Aspirin-exacerbated respiratory disease: PGE₂ plays a critical role in aspirin-exacerbated respiratory disease (AERD), a condition characterized by intense inflammation in the airways typified by mast cell degranulation, eosinophil influx and bronchoconstriction (reviewed in [134]). A recent meta-analysis revealed that 7-10% of people with asthma have AERD and notably ~15% of people with severe asthma are affected [135]. Aspirin hypersensitivity in asthma was first identified over a century ago (reviewed in [136]) and although why some people with asthma are aspirin-intolerant or -insensitive is incompletely understood.

However, we are beginning to understand the biochemical mechanisms and molecular pathways that are perturbed in this condition. The prevailing view is that arachidonic acid metabolism is dysregulated in people with AERD and this is exacerbated by NSAIDs. One of the key features of AERD is the overproduction of leukotrienes (LTs), with increased leukotriene E₄ being a consistent feature [137, 138]. Leukotrienes are produced by the action of lipoxygenase enzymes on arachidonic acid (as highlighted in Figure 1) and 5-lipoxygenase is responsible for the production of LTE₄ (see [139] for an overview of the eicosanoid pathway in AERD). Reduced PGE₂ is a major contributor [134] as this prostanoid plays an important homeostatic role by inhibiting activation and translocation of the 5-lipoxygenase enzymes necessary for leukotriene production [140, 141]. PGE₂ also stabilizes mast cells and prevents degranulation [142] and inhibit eosinophil trafficking [143], both in an EP₂-dependent manner. PGE₂ is generated from the COX-derived precursor PGH₂ (Figure 1) through the action of the terminal synthetic enzyme, microsomal PGE₂ synthase 1 (mPGES-1). Accumulating evidence demonstrates that the COX-2/mPGES-1/PGE₂ production pathway may be impaired in AERD: COX-2 mRNA expression is reduced in nasal polyps from people with aspirin-sensitive asthmatics, compared to healthy nasal mucosa [144]; and the gene for mPGES-1 - *PTGES* – is hypermethylated and thus silenced in people with AERD, compared to aspirin-tolerant individuals [145]. Notably, inhaled PGE₂ was protective in individuals with AERD [138, 146] and prevented LTE₄ production in aspirin-sensitive asthma [138]. More recently, a causal link for PGE₂ with AERD phenotype has been established by knocking out the gene responsible for PGE₂ production (microsomal PGE₂ synthase-1: mPGES-1) [147]. Mice lacking mPGES-1 (*ptges*^{-/-}) recapitulate hallmark features of AERD when challenged with NSAIDs after exposure to allergen (*Dermatophagoides farinae*) to elicit airway inflammation [148]. These mice have been utilized in a series of recent studies, coupling clinical data with mouse models (some utilizing

intercrosses with knockouts of interest with *ptges*^{-/-} to create double-knockouts), to advance knowledge on the cellular and molecular basis of AERD pathogenesis [149, 150]. Recent work *in vivo* also highlights the contribution of PGD₂ to the acute respiratory and extra-respiratory symptoms during an aspirin-induced reaction [151]. Platelets also play a role [148] and the thromboxane A₂ receptor (TP) has been shown to be regulated by PGE₂ in an EP₁/EP₂-dependent manner [148].

By examining the complex interplay between eicosanoids we have achieved more than improved understanding of molecular basis of AERD. This knowledge supports our earlier assertion that it is an oversimplification to block COX-2 and inhibit PGE₂ and there maybe risks in depletion of PGE₂. PGE₂ resistance may also exist as reduced levels of EP₂ have been found in bronchial leukocytes [152] and nasal polyp fibroblasts [153]. Thus, in AERD, expression of COX-2/PGEs is critically low. This leaves COX-1 to be solely responsible for production of homeostatic PGE₂; hence the catastrophic sensitivity to non-selective COX-1 inhibitors. Resistance to homeostatic PGs may also exist. Intriguingly, a recent meta-analysis has shown that selective COX-2 inhibitors can be safely used (at the lowest effective dose) in patients with stable mild-to-moderate asthma and AERD [154]; consistent with the notion that the homeostatic prostaglandins are principally COX-1-derived. Leukotriene-modifying agents can also be used in this patient population [155].

Targeting COX-2 in airway inflammation: what is best approach?

There are multiple ways to target COX-2 and prostanoid metabolites (Figure 1). In this review we have outlined some of the known and novel approaches, including: inhibiting enzymatic activity with non-selective (e.g. NSAIDs) or selective inhibitors (such as coxibs); repressing COX-2 mRNA expression by targeting transcriptional or post-transcriptional mechanisms (RNA-binding proteins or miRNAs); enhancing COX-2 protein stability by

affecting *N*-glycosylation or ERAD-mediated proteasomal degradation. These molecular mechanisms represent feasible options for targeting COX-2 as they are amenable to manipulation and some are controlled by spatiotemporal regulation so that beneficial effects within the context of respiratory disease remain a distinct possibility.

Perhaps at this stage the greatest potential is offered by our advancing knowledge of the role of prostaglandins in allergy and asthma (see recent review [90]) and our greater understanding of the prostanoid receptor family. Prostanoids exert their diverse functions by binding with several receptor subtypes, including: the PGD receptor (DP₁ and DP₂); four subtypes of the PGE receptor (EP₁, EP₂, EP₃, and EP₄), the PGF receptor (FP), the PGI receptor (IP), and the TxA receptor (TP). Development of selective agonists and antagonists are valuable pharmacological tools and exciting lead compounds for future drug discovery in respiratory conditions [113, 156-158]. Whether further studies will reveal that receptor redundancy exists, and that being more specific by targeting individual prostanoids receptors does not offer any added benefit over global approaches will emerge. But coupled with mouse models where individual prostanoid receptors are knocked-out, these approaches offer an elegant means to address unanswered questions regarding the impact of COX-2 products in clinically relevant *in vivo* models of respiratory disease. This was recently shown by Birrell *et al.* where the EP₄ receptor was shown to be responsible for anti-inflammatory effects of PGE₂ in the lung [158]. We can also apply a knowledge of arachidonic metabolism (gained from investigations in AERD) to respiratory disease more broadly to develop targeted strategies; for example, EP₂ receptor signalling has been shown to be essential to repress airway inflammation and hyperactivity in AERD [134] and recently activation of the EP₂ receptor was shown to prevent airway hyperresponsiveness and inflammation in a mouse model of allergic airway disease [159].

Summary and future directions

In their 1979 review [160], Vane *et al.* commented “The years since the identification of COX-2 have been exciting and intriguing as well as frustrating and disappointing”. Since that time we have learnt much about the complexity of COX-2 regulation and the function of prostanoids. With the development of targeted approaches to modulate COX-2 (and associated enzymes e.g. mPGES-1) and molecular tools to selectively modulate prostanoid receptors we are now well-positioned to unravel these complexities and hopefully develop efficacious pharmacotherapeutic strategies to treat respiratory disease without unwanted side effects in the near future.

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Inducer(s)	Cell Type(s)	Involved Pathway(s)	Reference(s)
IL- β	human epithelial cell line (A549)	NF- κ B and post-transcriptional regulation	[58-60]
	human airway smooth muscle (ASM) cells	ERK and p38 MAPK pathways	[62, 63]
	ASM	different regulatory elements, transcription factors, and histone H4 acetylation	[56]
Bradykinin	ASM	CREB-1 binding to the CRE in the COX-2 promoter	[56]
	pulmonary artery smooth muscle cells	activation of phospholipase A ₂ to promote release of the COX-2 substrate - arachidonic acid	[73]
Oxidative stress	human lung fibroblasts	ERK and NF- κ B	[76]
	ASM	TLR4/MyD88/TRAF6/c-Src/NADPH oxidase signalling that leads to the activation of MAPKs and NF- κ B	[77]
	A549	P2 purinoceptors/PKC/NADPH oxidase-mediated activation of Jak2 and STAT3 pathway	[78]
	human airway epithelial cells	cyclic GMP-mediated pathways	[79]
Sphingosine 1-phosphate (S1P)	rat vascular smooth muscle	Ca ²⁺ -dependent PKC and Src activation via S1P3 receptor coupled to pertussis toxin-sensitive and -insensitive G proteins	[81]
	ASM	S1PR1/3/c-Src/PYK2/p42/p44 MAPK- or JNK1/2- and S1PR1/3/c-Src/p38 MAPK-dependent AP-1 activation	[83]
trypsin	ASM	PAR-2-independent mechanism	[86]

Table 1: Key molecules that induce COX-2 and their impact on signaling cascades

Please note that this table only includes reference to inducers signalling pathways have been reported. For further details on the molecules that induce COX-2 please see text.

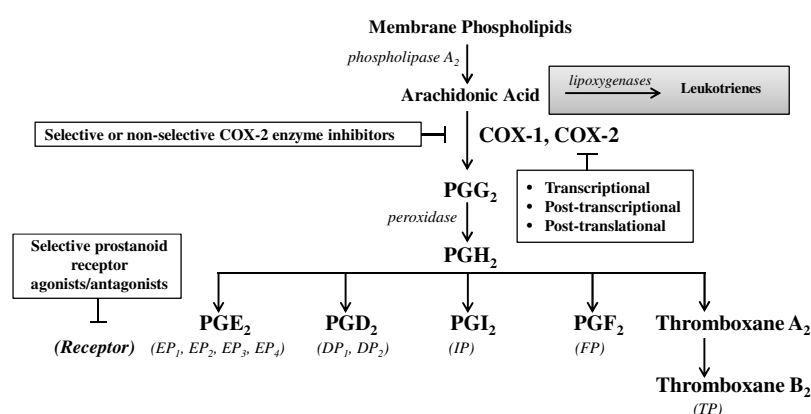


Figure 1: COX/prostanoid pathway - multiple ways to target COX-2 expression/activity or prostanoid receptors. See text for abbreviations and further detail (lipoxygenase pathway leading to leukotriene production is indicated in greyed area).