



REVIEW

Endogenous lipid mediators in the resolution of airway inflammation

O. Haworth and B.D. Levy

ABSTRACT: Acute inflammation in the lung is fundamentally important to host defence, but chronic or excessive inflammation leads to several common respiratory diseases, including asthma and acute respiratory distress syndrome.

The resolution of inflammation is an active process. In health, events at the onset of acute inflammation establish biosynthetic circuits for specific chemical mediators that later serve as agonists to orchestrate a return to tissue homeostasis. In addition to an overabundance of pro-inflammatory stimuli, pathological inflammation can also result from defects in resolution signalling.

The understanding of anti-inflammatory, pro-resolution molecules and their counter-regulatory signalling pathways is providing new insights into the molecular pathophysiology of lung disease and opportunities for the design of therapeutic strategies.

In the present review, the growing family of lipid mediators of resolution is examined, including lipoxins, resolvins, protectins, cyclopentenones and presqualene diphosphate. Roles are uncovered for these compounds, or their structural analogues, in regulating airway inflammation.

KEYWORDS: Acute respiratory distress syndrome, asthma, inflammation, lipoxins, mediators, resolution

Inflammation of the lungs and airways has a significant medical and economic burden in both Europe and the USA [1]. Asthma has the highest morbidity amongst inflammatory lung diseases and its incidence has doubled since the 1980s [2]. Asthma is characterised by airway infiltration of eosinophils, T-cells and mast cells with excess mucus, and, in some cases, airway remodelling with changes in smooth muscle, which together contribute to the clinical hallmark of airflow obstruction. Acute respiratory distress syndrome (ARDS) is the inflammatory lung disease with the highest mortality [3]. ARDS is characterised by extensive inflammation with polymorphonuclear leukocyte (PMN) activation in the lungs [3]. The chronic inflammation of asthma and exuberant acute inflammatory response in ARDS represent two different ends of a spectrum of inflammatory lung disease, yet both of these respiratory illnesses are characterised by an inability to limit inflammation.

Inflammation is a physiological response to tissue injury, infection and allergen challenge that evolved to limit damage from noxious agents and the spread of infectious organisms [4]. The

inflammatory response is inherently protective and intimately involved in the restoration of tissue homeostasis. Until recently, the resolution of inflammation has been an under-represented focus of inflammation research. It is now clear that the resolution phase of an inflammatory response is an active and orchestrated process, similar in complexity to the onset and maintenance of inflammation [5]. In restoring tissue homeostasis, resolution not only dampens inflammation, but also promotes immune defence mechanisms. The process of resolution has its origins at the beginning of the inflammatory response, with the establishment of biosynthetic circuits for later production of counter-regulatory chemical mediators. Molecules that promote resolution are inherently different from purely anti-inflammatory compounds, as pro-resolution molecules contribute to tissue catabasis, returning the tissue to normal [6, 7].

Five classes of naturally occurring pro-resolution molecules are reviewed in the present study (fig. 1), in addition to their signalling pathways, role in restoring tissue homeostasis and impact on cellular effectors of lung inflammation (table 1).

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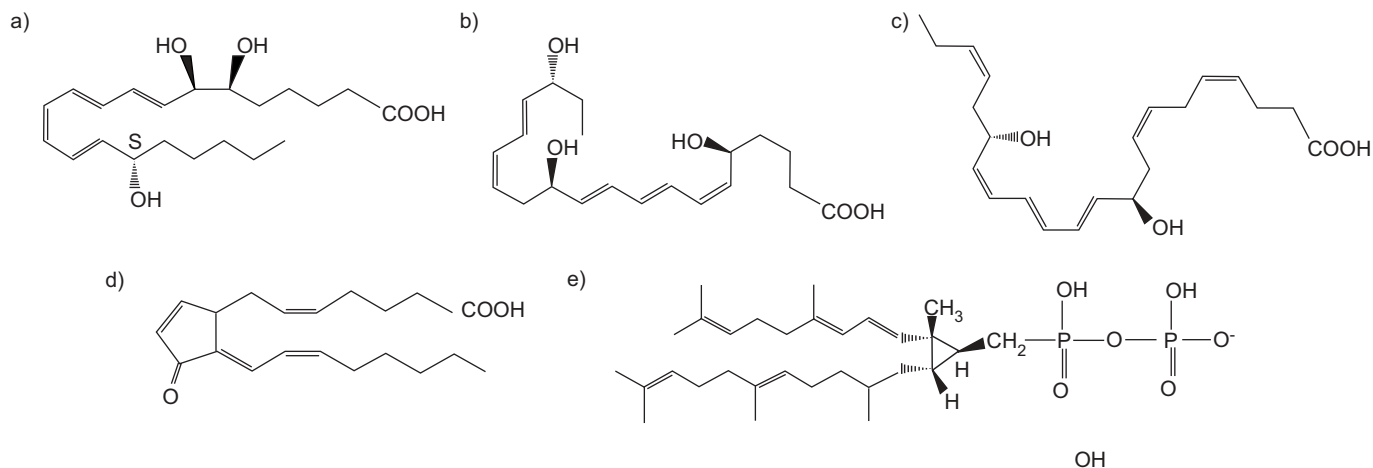


FIGURE 1. Classes of endogenous anti-inflammatory lipids. Representative members of five classes of endogenous anti-inflammatory lipids are shown: a) lipoxins, lipoxin A₄; b) resolvins, resolvin E₁; c) protectins, protectin D₁; d) cyclopentenones, 15deoxy Δ^{12-14} prostaglandin J₂; and e) polyisoprenyl phosphates, presqualene diphosphate.

CHEMICAL MEDIATORS OF RESOLUTION

Lipoxins

Lipoxins (LXs) are lipoxygenase (LO) interaction products of arachidonic acid (C_{20:4}) metabolism with structures and biological properties distinct from other eicosanoids [35, 36]. LXs have potent anti-inflammatory properties with cell type-specific actions on leukocytes, endothelium, epithelia and other stromal cells (fig. 2; table 1). Of particular relevance for the resolution of inflammation in asthma and ARDS, LXs inhibit eosinophil trafficking [12, 13, 37] and PMN chemotaxis, trans-migration across post-capillary venules, generation of superoxide anions and degranulation of azurophilic granules [6]. In addition, LXs stimulate clearance of apoptotic PMNs by macrophages [18], block natural killer cell cytotoxicity and tumour necrosis factor (TNF)- α release from T-cells [14, 15].

LXs are formed *via* transcellular biosynthesis with intermediates transferred in a bi-directional manner between cells [38]. LXs can be generated *via* at least three distinct pathways. One pathway involves leukocyte 5-LO-catalysed conversion of C_{20:4} to leukotriene (LT)A₄, which, in the vasculature, is subsequently taken up by platelets and converted into LXA₄ by 12-LO [39]. A second pathway involves the conversion of epithelial cell-, eosinophil- or monocyte-derived C_{20:4} by 15-LO, producing 15(S)-hydroperoxyeicosatetraenoic acid, which can also serve as a substrate for leukocyte 5-LO. This reaction generates an unstable epoxytetraene intermediate that is converted to LXs by hydrolases [35, 40]. 5-LO derived LTA₄ can also be converted by 15-LO to LXs. Although these three pathways are the principal means of LX generation, additional 5-LO-independent pathways probably exist.

Interestingly, aspirin, the lead nonsteroidal anti-inflammatory drug, inhibits prostaglandin (PG) synthesis, but at doses much lower than the dose needed to exert its anti-inflammatory effects [41]. This paradox was recently addressed by the identification of the aspirin-triggered 15-epimer-LXs (ATLs) [42]. Aspirin acetylates the active site of cyclooxygenase (COX)-2 to inhibit production of PGs, but the enzyme is still

able to convert C_{20:4} to 15(R)-hydroxyeicosatetraenoic acid (15R-HETE). This compound can serve as a substrate for 5-LO for further conversion to ATLs [42]. 15-epimer-LXs increase nitric oxide synthesis *via* constitutive or inflammatory nitric oxide synthase, and nitric oxide decreases leukocyte-endothelial cell interactions inhibiting leukocyte accumulation within inflamed tissues [43]. Thus, aspirin can exert anti-inflammatory effects by both inhibiting pro-inflammatory PG biosynthesis and promoting the formation of anti-inflammatory 15-epimer-LXs. In the absence of aspirin, 15R-HETE can also be produced by cytochrome p450 enzymes to act as a substrate for 15-epimer-LX transcellular biosynthesis [44, 45].

LXs are metabolically inactivated by the actions of 15-hydroxyprostaglandin dehydrogenase and PG reductase to form 13,14-dihydro-15-hydroxy-LXA₄ [9, 46]. LX and 15-epimer-LX metabolism is stereospecific, with 15-epimer-LXs metabolised less efficiently, thereby increasing the biological half-life of these ATLs approximately two-fold [46]. LX analogues that resist inactivation have been generated [9]. These modifications enhance the biological activity of LXs and have proved to be useful tools in the study of the biological functions of LXs *in vitro* and *in vivo*.

LXs not only play a role directing leukocyte function towards resolution, but can also signal the local stromal micro-environment towards resolution (table 1). Normal human bronchial epithelial cells (NHBE) exposed to hydrochloric acid increase expression of COX-2 and the high-affinity LXA₄ receptor ALX. LXA₄ promotes restitution from acid injury by increasing basal NHBE proliferation and inhibiting pro-inflammatory events in differentiated NHBE, such as cytokine release and PMN trans-migration [26]. Despite potent regulation of epithelial cell and leukocyte function, LX bioactions are distinct from immunosuppressive compounds, in that LX signalling regulates pathogen-mediated inflammation [20, 47] and promotes mucosal bacterial killing *via* expression of bacterial/permeability inducing protein (BPI) in epithelial cells [22]. Thus, in addition to anti-inflammation, LXs are also host protective.

TABLE 1 Cell type-specific counter-regulatory actions for chemical mediators of resolution

Chemical mediator	Cell type/molecule	Response	Species	Ref.
Lipoxin A₄	PMN	Inhibit chemotaxis	Human	[8]
		Inhibit trans-endothelial PMN migration	Human	[9]
		Inhibit trans-epithelial migration of PMN	Human	[8]
		Inhibition of superoxide anion generation	Human	[10]
		Inhibition of azurophilic granule release	Human	[11]
	Eosinophil	Inhibition of eosinophil migration	Human	[12, 13]
		T-cells	Inhibition of TNF release	Human
	NK cells	NK cell cytotoxicity	Human	[15]
	Monocytes/macrophages	Adhesion to laminin	Human	[16]
		Chemotaxis	Human	[17]
	Dendritic cells	Augment engulfment of apoptotic PMN	Human	[18]
		Block IL-12 production	Mouse	[19]
		Prevent toxoplasmosis morbidity	Mouse	[19]
	Enterocytes	Prevent tuberculosis morbidity	Mouse	[20]
		Inhibit TNF induced IL-8 release	Human	[21]
	Synovial fibroblasts	Increase BPI expression	Human	[22]
		Killing of Salmonella	Human	[22]
	Endothelium	Inhibits IL-1β induced IL-6, IL-8 and MMP3 release	Human	[23]
Stimulates prostacyclin release		Human	[24]	
Bronchial epithelial cells	Block CysLT-initiated Weibel–Palade body expression	Human	[25]	
	Basal cell proliferation after acid injury	Human	[26]	
CyPGs Annexin-1 Resolvin D1 Resolvin E1	Macrophages	Blocks IL-6 and IL-8 release	Human	[26]
		Inhibits adhesion and trans-endothelial migration	Human	[27]
	PMN	Inhibition of PMN migration	Human	[28]
	Macrophages	Inhibit LPS-induced TNF release	Mouse	[29]
	Dendritic cells	Inhibition of IL-12 release	Mouse	[30]
		Inhibition of migration	Mouse	[31]
	PMN	Inhibition of superoxide generation	Human	[32]
	Apoptotic T-cells, PMN	Upregulation of CCR5	Human	[33]
	Macrophages	Inhibit LPS-induced TNF release	Mouse	[29]
	PMN	Inhibition of superoxide anion generation	Human	[34]

PMN: polymorphonuclear leukocyte; TNF: tumour necrosis factor; NK: natural killer; IL: interleukin; BPI: bactericidal/permeability increasing protein; MMP: matrix metalloproteinase; CysLT: cysteinyl leukotriene; CyPG: cyclopentenone prostaglandins; LPS: lipopolysaccharide; CCR: CC chemokine receptor; PSDP: presqualene diphosphate.

LXA₄ receptors

LXs interact with one or more specific receptors, including their own specific receptor, a subclass of LTD₄ receptors (*i.e.* cysteinyl (Cys)LT1), and additional intracellular recognition sites [48, 49]. The LXA₄ receptor ALX is a G-protein-coupled protein that binds LXA₄ with high affinity (K_D=1.7 nM) [48]. ALX was the initial receptor identified to bind both lipid and peptide ligands [48, 50]. In PMNs, signalling by ALX occurs, in part, *via* polyisoprenyl phosphate (PIPP) remodelling (*vide infra*) [10] and inhibition of leukocyte-specific protein-1 phosphorylation, which is a downstream regulator of the p38-mitogen-activated protein kinase cascade (table 2) [51].

ALX mediates annexin-1 anti-inflammatory signals

Glucocorticoids are potent anti-inflammatory molecules that play a major role in resolving inflammation by inhibiting the production of pro-inflammatory mediators [58, 59] and decreasing the expression of leukocyte adhesion molecules [60]. Dexamethasone also promotes monocyte and T-cell

emigration out of inflamed tissues [61, 62]. Glucocorticoids act *via* cognate receptors within the cell cytoplasm that, upon ligand binding, move to the nucleus to regulate transcription [63]. Of interest for asthma therapy, corticosteroids induce expression of ALX [64] and annexin-1, which can also interact with ALX to initiate anti-inflammatory signals [65].

Annexin-1 is a potent anti-inflammatory molecule that is abundantly expressed by PMNs [66]. Most of the annexin-1 is within the cytoplasm. Upon PMN activation and adhesion to inflamed vascular endothelium, annexin-1 is rapidly externalised [67], leading to cell detachment from inflamed blood vessels [68] and decreased PMN recruitment. The addition of antisera to annexin-1 in a model of acute inflammation results in the persistence of PMNs in the inflammatory exudates [69]. In annexin-1 knockout mice, PMNs are more easily activated [28, 69]. Some of the anti-inflammatory effects of annexin-1 and its N-terminal peptide, Ac2-26, are mediated by direct interactions with ALX, but at a lower affinity (K_D=900 nM) than LXA₄ [70].

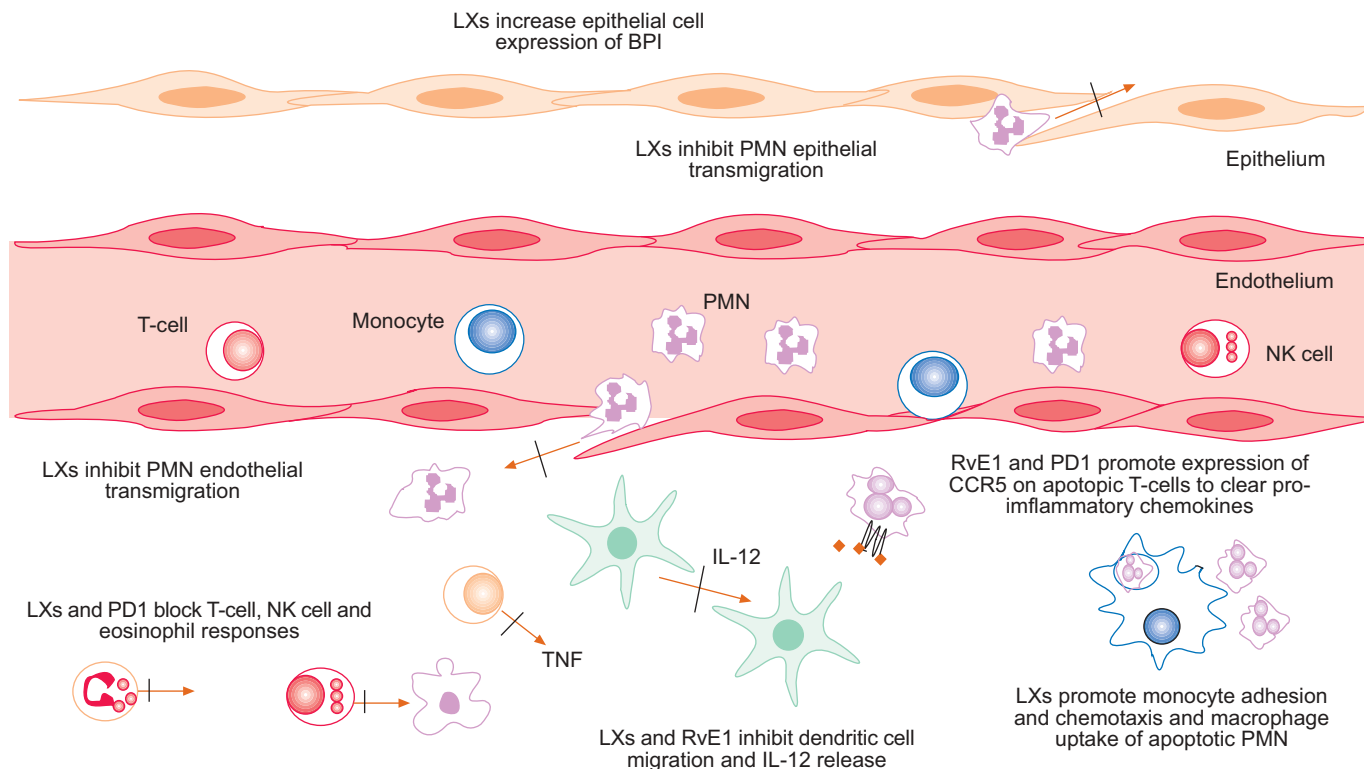


FIGURE 2. Cell-type specific counter-regulatory actions of some lipid mediators. Endogenous autacoids display cell type-specific actions to promote inflammation. Lipoxins (LXs) engage specific receptors (e.g. ALX) to inhibit polymorphonuclear leukocyte (PMN) transmigration through endothelial and epithelial cells, and can inhibit pro-inflammatory responses of innate immune effectors, including PMNs, T-cells, eosinophils and natural killer (NK) cells. Clearance of apoptotic PMN is also enhanced by LXs. Of interest, these compounds increase mucosal epithelial cell expression of bactericidal/permeability increasing protein (BPI) to protect against pathogens. Resolvins (e.g. resolvin E (RvE)1) and protectins (e.g. protectin D (PD)1) also display cell type-specific counter-regulatory responses to promote resolution. CCR: CC chemokine receptor; TNF: tumour necrosis factor; IL: interleukin.

Role of LXs in lung disease

LXs are generated in the lung during a range of respiratory illnesses [71]. Of interest is the fact that a low biosynthetic

capacity for LXs is associated with severe airway inflammation. Aspirin-exacerbated respiratory disease is a more severe and protracted form of asthma and, in whole blood, these

TABLE 2 Counter-regulatory lipid mediator signalling mechanisms

Chemical mediator	Signalling molecule	Action	Species	Ref.
Lipoxin A₄	PIPP remodelling	Inhibits LTB ₄ -stimulated PSDP conversion to PSMP	Human	[10]
	LSP-1	Blocked phosphorylation of LSP-1	Human	[51]
	SOCS-2	Induces the expression of SOCS-2	Mouse	[47]
	Akt/PKB	Induces phosphorylation of Myc9 and Cdc42 polarisation	Human	[52]
	PPAR-γ and CINC-1	Reduced PPAR-γ and CINC-1 levels in hepatocytes	Rat	[53]
CyPGs	PPAR-γ	Ligand for PPAR-γ	Mouse	[54]
	NF-κB	Suppression of NF-κB binding	Mouse	[54]
	AP1 and STAT activity	Inhibition of AP1 binding to DNA	Mouse	[55]
Resolvin E1	NF-κB	Inhibition of NF-κB signalling	Mouse	[31]
PSDP	PLD	Blocks PLD activation in PMN	Human	[10]
	SH ₂ domains	Interacts with SH ₂ domains	Human	[56]
	PI3K	Inhibits PI3K activity in PMN	Human	[57]

PIPP: polyisoprenyl phosphate; LT: leukotriene; PSDP: presqualene diphosphate; PSMP: presqualene monophosphate; LSP: leukocyte-specific protein; SOCS: suppressor of cytokine signalling; PKB: protein kinase B; PPAR: peroxisome proliferator-activated receptor; CINC: cytokine-induced neutrophil chemoattractant; CyPG: cyclopentenone prostaglandins; NF: nuclear factor; AP: activator protein; STAT: signal transducers and activators of transcription; PLD: phospholipase D; PMN: polymorphonuclear leukocyte; SH₂: Src homology 2 domain; PI3K: phosphatidylinositol 3-kinase.

asthmatic individuals have a decreased ability to produce LXs compared with aspirin-tolerant asthmatics [72]. In addition, lower levels of LXA₄ are present in supernatants of induced sputum in severe, compared with mild, asthma [73]. Moreover, individuals with severe asthma have a reduced capacity, in whole blood, to convert C20:4 to 15-LO-catalysed products, including both 15-HETE and LXA₄ [74]. In sharp contrast to this decrement in 15-LO activity, 5-LO-derived products, including 5-HETE, LTB₄ and CysLTs, are all increased in severe asthma [74]. The forced expiratory volume in one second percent predicted values and circulating levels of LXA₄ and CysLTs are correlated, suggesting a link between biosynthetic capacity for these bioactive lipid mediators and airflow obstruction in asthma. Thus, decrements in LX generation and increases in LT production in severe asthma create an imbalance that maintains the persistent airway inflammation and airflow obstruction typical of this condition.

Reduced levels of LXs have also been found in the airways of some patients suffering from cystic fibrosis [75]. Because similar results have been obtained with chronic bronchitis, altered LX formation in the airway may represent a more generalised consequence of chronic PMN-enriched airway inflammation [76]. In support of the notion that LXs might provide a viable therapeutic strategy in the treatment of respiratory inflammation, mice treated with a stable LXA₄ mimetic have reduced airway inflammation, and transgenic mice for human ALX coupled to a component of the CD11b promoter have reduced leukocyte infiltration in murine models of allergic airway inflammation and acute lung injury (ALI) [37, 77].

PGs and LTs

Like LXs, PGs and LTs are also enzymatically derived from C20:4 and serve as potent lipid mediators [78]. They perform crucial functions in normal physiology and play important roles early in acute inflammation. At the onset of inflammation, C20:4 is metabolised by COXs to PGs, such as PGD₂, PGE₂, PGF_{2 α} , PGI₂ and thromboxane A₂, that are well known to have potent biological effects on inflammatory cells and lung tissues. In addition to the well-described roles of PGs in promoting inflammatory responses, recent studies have also highlighted a role for COX-2-derived PGs serving anti-inflammatory and anti-fibrotic roles in the resolution of inflammation [77, 79, 80]. In a model of acute resolving pleural inflammation, COX-2 levels transiently increased early (2 h) and late (48 h) after the onset of inflammation to generate COX-2-derived PGD₂ and 15deoxy Δ^{12-14} PGJ₂ (15d-PGJ₂) [79]. COX-2-derived PGE₂ can also resolve allergic pleural inflammation [79, 80] and TNF-initiated PMN activation in exudates [81]. Of interest, COX-2 derived PGD₂ and PGE₂ can induce 15-LO expression to promote LX biosynthesis [81]. In a model of spontaneously resolving ALI, selective COX-2 inhibition or deficiency results in prolonged inflammation, in part, by decreasing production of PGE₂ and pro-resolving mediators, including LXA₄ and 15-epimer-LXA₄ [77, 79]. In addition to PGs, LTs also carry pro-phlogistic properties important for leukocytes and respiratory tissues [82]. Metabolism of C20:4 by 5-LO leads to the formation of LTs. LTA₄ is an unstable, yet pivotal intermediate that can be converted to LTB₄ by LTA₄ hydrolase, LTC₄ by LTC₄ synthase or to LXs by 12- or 15-LO

[6]. In particular, LTB₄ is a potent PMN chemoattractant and secretagogue, and LTC₄ and LTD₄ are potent bronchoconstrictors [82]. Of interest, LTB₄ can be important for host defence, promoting PMN phagocytosis of pathogenic bacteria and antiviral mechanisms [83, 84]. These same properties are not shared by CysLTs, as mice deficient in the multidrug resistance protein 1, which is involved in cellular extrusion of LTC₄, are resistant to *Streptococcus pneumoniae*-induced pneumonia [85], and a CysLT1-receptor selective antagonist provides a survival advantage in animal models of sepsis [86].

Cyclopentenones

The COX-derived intermediate PGG₂ can be converted by PGD₂ synthase to PGD₂, which displays pro-inflammatory and provocative actions on leukocytes and airway tissues [87]. However, PGD₂ is further dehydrated to produce PGs of the J series, including Δ^{12-14} -PGJ₂ (PGJ₂) and 15d-PGJ₂. It has been proposed that the induction of COX-2 during resolution of inflammation is necessary for the formation of cyclopentenone PGs (cyPGs) that can transduce counter-regulatory effects *via* activation of peroxisome proliferator-activated receptor (PPAR)- γ (table 2) [54, 79]. For example, the cyclopentenone 15d-PGJ₂ inhibits the expression of vascular cell adhesion molecule (CD106) and intercellular adhesion molecule (CD54) on human umbilical vein endothelial cells [88]. The actions of 15d-PGJ₂ contrast to those of LXs in that they inhibit, rather than augment, macrophage activity while having little effect on PMNs (table 1) [89]. In addition, pharmacological concentrations of 15d-PGJ₂ can dampen lymphocyte proliferation and interleukin (IL)-2 production [90, 91]. These cell type-specific actions are highlighted by the evidence that 15d-PGJ₂ blocks monocyte adhesion to human aortic vessels while having no effect on PMN. 15d-PGJ₂ also inhibits CC chemokine ligand (CCL)2 (monocyte chemoattractant protein-1) expression on endothelial cells, but not CCL8 (IL-8) [27].

Role of cyclopentenones in lung disease

Cyclopentenones can promote resolution of ALI. 15d-PGJ₂ protects mice from ALI by activating PPAR- γ and the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2) that in turn transduces a number of genes to protect against oxidative damage [92, 93]. 15d-PGJ₂ can serve as a ligand for PPAR- α and PPAR- γ , and display protective actions in a murine model of allergic airway inflammation [94].

Resolvins

Resolvins (resolution phase interaction products) are omega-3 fatty acid derived anti-inflammatory lipids that were originally identified in spontaneously resolving exudates [95, 96]. Resolvins are organised into different series, depending upon the origin of the lipid. Resolvins of the D series (*e.g.* RvD1) are derived from docosahexaenoic acid (DHA; C22:6) and resolvins of the E series (*e.g.* RvE1) are derived from eicosapentaenoic acid (EPA; C20:5) [6].

Resolvins are generated in human whole blood with enzymatic conversion of DHA to 17S-hydroxy-containing D-series resolvins. Generation of these compounds is markedly increased in the presence of aspirin [95, 96]. Endothelial cells grown under hypoxic conditions and treated with aspirin convert DHA into 17R-hydroxy-DHA by aspirin acetylated COX-2 to give rise to

the 17R-Resolvin D series. 17R-hydroxy-DHA is also a substrate for human PMN in the formation of two sets of di- and tri-hydroxy products. Enzymatic oxygenation of 17R-hydroxy-DHA at carbon 7 leads to aspirin-triggered RvD1 (AT-RvD1) and AT-RvD2. When 17R-hydroxy-DHA is oxygenated at carbon 4, AT-RvD3 and AT-RvD4 are generated [6]. *In vitro*, endothelial cells and brain-derived microglial cells treated with aspirin convert EPA to 18R-hydroxyeicosapentaenoic acid (18R-HEPE) and 15R-HEPE. Both 18R- and 15R-HEPE can be rapidly converted by activated PMN to a 5(6)-epoxide-containing molecule, which is then converted to the bioactive 5,12,18R-trihydroxyeicosapentaenoic acid (RvE1) [95].

Resolvins of both the D and E series exert potent anti-inflammatory properties, such as inhibiting PMN migration and shortening the resolution phase of acute inflammation [97] (table 1). The first receptor for RvE1 was identified as ChemR23 [31]. ChemR23 is expressed on monocytes, macrophages and dendritic cells (DCs). Treatment with RvE1 inhibits inflammatory colitis, DC migration and IL-12 production, and attenuates nuclear factor- κ B activation (table 2) [30, 31]. Intriguingly, ChemR23 was originally discovered as a receptor for a chemotactic protein found in inflammatory exudates called Chemerin [98]. Recently, a second receptor for RvE1 was identified, namely the LTB₄ receptor BLT1, which is expressed on PMNs, eosinophils, monocytes and T-cells [99, 100].

Of interest, mice have been developed that are transgenic for an omega-3 fatty acid desaturase thereby producing more omega-3 fatty acids from omega-6 fatty acids [101]. These transgenic animals are protected from colitis in a model of gastrointestinal inflammation [102]. This protective effect was not secondary to a decrease in the pro-inflammatory lipids PGE₂ and LTB₄. Rather, levels of resolvins were significantly increased [102]. Omega-3 fatty acids are concentrated in fish oils and diets rich in omega-3 fatty acids can protect against asthma, cystic fibrosis, heart disease and cancer [103–105]. These newly identified omega-3 resolvins provide a potential molecular rationale for these beneficial effects.

Resolvins in lung disease

Airway epithelial cells in cystic fibrosis and asthma display abnormalities in fatty acid metabolism with low levels of omega-3 fatty acids [106]. Enteric feeding of supplements enriched with omega-3 fatty acids improves clinical outcomes in ARDS, including time to liberation from mechanical ventilation and discharge from the intensive care unit [107]. Generation of resolvins has not yet been reported in ARDS or other respiratory illnesses. Little is known about the role of resolvins in lung disease, but as resolvins have been shown to exert anti-inflammatory effects in models of peritonitis and renal ischaemia-perfusion injury [29, 97], it is likely that resolvins would also be lung protective and promote the resolution of airway injury and inflammation.

Neuroprotectin D1/protectin D1

Protectin (P)D1 is 10R,17S-docosatriene that is generated from DHA in a 15-LO-catalysed reaction [108, 109]. In acute, spontaneously resolving murine peritonitis, new indices of resolution have been defined, including ψ_{\max} , (the maximal number of PMNs present), t_{\max} , (the time when ψ_{\max} occurs)

and R_i , (the resolution interval (t_{\max} to t_{50}) for PMN numbers to reach half the ψ_{\max}) [97]. Using this approach, PD1 was increased in the resolution exudates and had the most potent reduction in R_i when compared with LXs, 15-epimer-LXs and E-series resolvins [97]. This docosatriene was subsequently named neuroprotectin D1/protectin D1 after identification of its generation by glial cells in the brain where DHA is abundantly found and its protection against ischaemic brain injury and Alzheimer's disease [108, 110, 111]. PD1 also modulates macrophage and structural cell responses to protect against renal and hepatic injury (table 1) [29, 112].

Role in lung disease

During asthma exacerbations, PD1 and its biosynthetic intermediate 17S-hydroxy-DHA are present in exhaled breath condensates, but are significantly reduced compared with amounts in exhaled breath condensates from healthy subjects [113]. In addition, PD1 is also present in inflamed murine lung homogenates. When exogenous PD1 is administered to allergen-sensitised mice prior to aerosol allergen challenge, PD1 significantly blocks leukocyte infiltration and airway hyperresponsiveness. When administered after allergic airway inflammation has been established, PD1 also accelerates the clearance of eosinophils. Thus, PD1 has intriguing properties that suggest exciting new therapeutic strategies for the treatment of asthma.

Polyisoprenyl phosphates

Many inflammatory diseases of the airways are associated with an abundance of PMNs, including ARDS, pneumonia and severe asthma [3, 114, 115]. Tissue-infiltrating PMNs can cause extensive damage to the surrounding tissue and perpetuate inflammation *via* the inadvertent release of noxious products, such as superoxide anions and proteases [4]. It is important that natural mechanisms exist to control PMN activation. PIPPs serve unique roles as "stop" signals for PMN. PIPPs are present in PMN membranes. Activation of PMNs initiates PIPP remodelling with presqualene diphosphate (PSDP) converted rapidly into its monophosphate form presqualene monophosphate (PSMP) [34]. PSDP, but not PSMP, dramatically reduces PMN release of superoxide anion generation (table 1) [10, 34].

Several regulatory sites of action have been uncovered for PIPPs. PSDP inhibits plant, microbial and mammalian phospholipase (PL)D (table 2) [34, 116]. This enzyme converts phosphatidylcholine to phosphatidic acid (PA) [117], which carries important intracellular signals in PMN that lead to a wide range of functional responses, including actin remodelling, granule release and activation of nicotinamide adenine dinucleotide phosphate-oxidase. Phosphatidylinositol 3-kinase (PI3K) activity is important for early cell activation [118], and PSDP has recently been identified as a regulator of PI3K [57]. LTB₄ stimulates a rapid decrease in PMN PSDP levels and increase in PI3K activity. PSDP interacts with p110 γ -PI3K as it deactivates, and *in vitro*, PSDP displays concentration-dependent inhibition of recombinant p110 γ -PI3K. PSDP structural mimetics have been prepared and can inhibit PLD, PI3K, PMN responses and lung inflammation *in vivo* [57, 116].

PSMP does not share PSDP's potent inhibitory properties. Upon PMN activation, PSDP is converted to PSMP by PA phosphatase domain containing 2 [119]. Functionally characterised as a

phosphatase, this enzyme converts PSDP and farnesyl diphosphate to their monophosphates, and so was recently renamed polyisoprenyl diphosphate phosphatase (PDP)-1 (fig. 3). Expressed in PMNs and numerous human tissues, PDP-1 is a member of the lipid phosphatase/phosphotransferase (LPT) family [120]. There are five sub-groups of LPTs, including lipid phosphate phosphatases, sphingosine-1-phosphate (S1P) phosphatases, sphingomyelin synthases, lipid phosphatase-related proteins/plasticity-related genes and a group without any previously assigned function, which was called candidate sphingomyelin synthases type 2 (CSS2). PDP-1 is a member of the CSS2 family of LPTs [119].

In addition to PMNs, PDP-1 is also highly expressed in major immune organs (lung, spleen, thymus, liver and gut). This suggests that PDP-1 could play important roles in regulating cells not only of the myeloid lineage but also structural cells, macrophages, DCs and lymphocytes. S1P has recently been shown to play a crucial role in controlling the egress of lymphocytes from the thymus, lymph nodes and spleen [121]. S1P is metabolised by S1P phosphatases, suggesting that LPTs are likely to play many important roles in controlling immune responses.

PSDP in lung disease

During an experimental model of lung injury and inflammation from acid aspiration, a reciprocal relationship was present *in vivo* for lung PSDP and PI3K activity [57]. A new

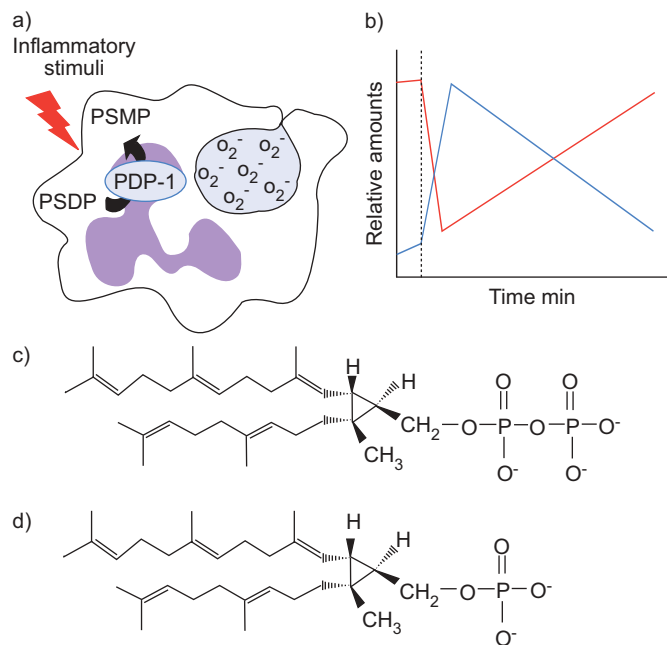


FIGURE 3. a) Illustration and b) time course of presqualene diphosphate (PSDP) remodelling in human polymorphonuclear leukocytes (PMNs). PSDP is an intracellular counter-regulatory molecule present in nanomolar quantities in freshly isolated PMNs. Activation by inflammatory stimuli (.....) results in the rapid and transient conversion of PSDP (red) to presqualene monophosphate (PSMP; blue) concurrent with functional responses, such as superoxide anion (O_2^-) generation. PSDP remodelling is mediated by polyisoprenyl diphosphate phosphatase-1 (PDP-1). The pool of PSDP is restored within a few minutes as the cells deactivate. c) Chemical structure of PSDP; d) chemical structure of PSMP.

diphosphonate PSDP structural mimetic was developed to resist phosphatase-based inactivation, and this PSDP analogue blocks human PMN activation by LTB_4 and murine lung PI3K activity and inflammation. These findings indicate that PSDP is an endogenous PI3K inhibitor, and suggest that in inflammatory diseases characterised by excessive PMN activation, PIPPs can serve as structural templates for an anti-PMN therapeutic strategy to limit tissue injury associated with ARDS.

CELLULAR EVENTS IN RESOLUTION

Apoptosis and phagocytosis

During an inflammatory response, there is a substantial increase in the number of immune cells that accumulate in a tissue. In addition to blocking further leukocyte recruitment, leukocytes already present can either exit the inflamed tissue *via* draining lymphatics or undergo programmed cell death for nonphlogistic clearance by phagocytes [122]. Apoptosis and resolution of inflammation are intrinsically linked, as impairment of either apoptosis or clearance of apoptotic cells leads to chronic inflammation and auto-immunity [123]. Similarly, enhancement of apoptosis can accelerate resolution of an inflammatory response [124]. LXs promote phagocytosis of apoptotic PMNs by macrophages [18]. This process further modulates the inflammatory milieu by releasing anti-inflammatory transforming growth factor- β that can convert naïve precursors to regulatory T-cells [125]. After ingestion of apoptotic cells, macrophages also release counter-regulatory lipid mediators, including PGE_2 , $PGF_1-\alpha$ and LXs [126, 127].

Egress of leukocytes during resolution

At the start of an inflammatory response, cells are recruited to an inflammatory focus by specific chemoattractants that orchestrate the recruitment of leukocyte subsets, beginning with PMNs, followed by eosinophils, monocytes/macrophages and lymphocytes [128, 129]. Regulated chemokine production is essential for the normal, physiological migration of leukocytes and for the recruitment of leukocytes during an inflammatory response [130]. Altered chemokine production is often a feature of chronic inflammation, with increased leukocyte recruitment and retention within involved tissues [131]. Inflammatory microenvironments modulate egress of leukocytes by specific chemokine receptor expression, *e.g.* T-cells can utilise CC chemokine receptor 7 to exit *via* afferent lymphatics [132, 133]. Similarly, PMN can be cleared from inflamed tissue by increasing expression of the chemokine receptor CXCR4 to return to bone marrow in a stromal-derived factor-1-directed manner [134]. Pro-resolving lipid mediators are potent regulators of chemokine expression [33]. Modifications of tissue stroma with select chemokines can retain specific leukocytes and compromise resolution [135, 136].

As inflammation resolves, chemokines need to be removed from the microenvironment to halt further leukocyte recruitment. Towards this end, the "silent" chemokine receptor D6 plays an important role in scavenging inflammatory, but not constitutive chemokines [137]. D6 knockout mice fail to resolve acute inflammatory responses, suggesting that D6 has important roles in resolution [138]. Infiltrating leukocytes can also upregulate select chemokine receptors as they undergo apoptosis, in order to sequester chemokines and so prevent further leukocyte recruitment [33]. LXs are potent stimuli for

increased clearance of apoptotic PMNs, but little is known regarding the impact of LXs or pro-resolution lipids on leukocyte egress [18].

Resolution of inflammation and host defence

Resolution of inflammation differs from immunosuppression, partly by the promotion of host defence. Of interest, LXs enhance mucosal host defence by controlling pathogen-induced inflammatory responses [20, 47] and inducing BPI expression [22]. BPI is a 55 kDa protein released from PMN and epithelial mucosa. BPI disrupts the inner and outer lipid membranes of Gram-negative bacteria, enhances phagocytosis of bacteria and sequesters lipopolysaccharides [139]. Exposure of epithelial cells to LXA₄ or ATL upregulates BPI and increases gastrointestinal epithelial killing of salmonella [22]. LXA₄ and RvE1 also protect rabbits from *Porphyromonas gingivalis*-induced periodontal disease, demonstrating that pro-resolution molecules enhance, rather than impair, host defence *in vivo* [140].

Therapeutic implications

Many of the current therapeutic targets of inflammatory diseases focus on blocking the initiating or amplifying mediators of inflammation. While this strategy has been beneficial in some clinical conditions, there remain substantial unmet clinical needs for common inflammatory lung diseases, including asthma and ARDS. Rather than blocking early or select pro-inflammatory mediators, an alternative therapeutic strategy might emphasise mimetics of LXs, cyPGs, resolvins, protectins, PSDP or other natural counter-regulatory molecules that accelerate resolution of inflammation. Metabolically stable analogues of some of these compounds have been developed and display potent *in vivo* protective actions in several model systems [116, 141].

Of interest, anti-inflammatory therapies can impact the formation or actions of pro-resolution compounds. For example, aspirin promotes formation of 15-epimer-LXs and resolvins [17, 96]. Because of concerns for precipitating asthma, this agent is rarely used, despite its potential therapeutic benefits [142]. Glucocorticoids are commonly used to control asthma and, in addition to increasing the ALX ligand annexin-1 (*vide supra*), can increase PMN expression of the ALX receptors [64]. Moreover, some anti-inflammatory experimental agents, including a 5-LO-interacting protein (FLAP) inhibitor (BayX-1005), can both decrease LT and increase LX formation *in vivo* [143].

CONCLUSION

The resolution of inflammation is an integral part of the physiological response to tissue injury and infection. Elucidation of resolution mechanisms has led to its recognition as a fundamental homeostatic process. In health, establishment of resolution signalling pathways is initiated early, at the very start of acute inflammatory responses. The kinetics for the formation of pro-resolving mediators is highly regulated and linked to cellular trafficking events in inflammation. Discovery of endogenous chemical agonists for resolution and their signalling pathways provides opportunities for the development of new therapeutic strategies and to gain further insights into the pathophysiology of chronic and severe inflammatory lung diseases, such as asthma and acute respiratory distress syndrome. A better understanding of the mechanisms of

resolution in airway inflammation may provide new treatment options for the excess morbidity and mortality associated with these and other common respiratory conditions.

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