Innate immunity is a key factor for the resolution of inflammation in asthma

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ABSTRACT The resolution of inflammation is an integral and natural part of the physiological response to tissue injury, infection and allergens or other noxious stimuli. Resolution is now recognised as an active process with highly regulated cellular and biochemical events. Recent discoveries have highlighted that innate inflammatory cells have bimodal effector functions during the inflammatory response, including active roles during the resolution process. Several mediators displaying potent pro-resolving actions have recently been uncovered. Lipoxin A_4, the lead member of this new class of pro-resolving mediators, has anti-inflammatory actions on type 2 innate lymphoid cells and pro-resolving actions through natural killer cells in asthma immunobiology. Eosinophils are also able to control crucial aspects of resolution through the generation of pro-resolving mediators. Uncontrolled asthma has been associated with a defect in the generation of specialised pro-resolving mediators, including lipoxin A_4 and protectin D1. Thus, bioactive stable analogue mimetics of these mediators that can harness endogenous resolution mechanisms for inflammation may offer new therapeutic strategies for asthma and airway inflammation associated diseases.

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Introduction

Asthma is characterised by increased and chronic airway inflammation, with mucosal infiltration of inflammatory cells and release of pro-inflammatory cytokines and lipid mediators [1]. The airway inflammation of asthma, which is often allergic by nature, has been attributed to ongoing adaptive helper T-cell type-2-mediated inflammation [2]. There is increasing evidence that innate immunity plays critical roles in the pathobiology of asthma, in chronic stable inflammation and during episodes of exacerbated acute inflammation in response to a variety of stimuli, such as allergen inhalation, exposure to environmental pollutants or microbial infection [3].

Most studies have focused on the role of innate inflammatory cells (*i.e.* eosinophils, mast cells, basophils, neutrophils, macrophages, several different subsets of dendritic cells, and newly described innate lymphoid

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cells (ILCs)) along with activated resident structural cells (epithelial cells, fibroblasts and airway smooth muscle cells) to accentuate and perpetuate the airway inflammation in asthma. Indeed, these cells release a vast array of pro-inflammatory and potentially tissue destructive compounds (eicosanoids, reactive oxygen species, cytokines, chemokines, growth factors and proteases) into the extracellular space [4]. Recent discoveries have highlighted that many innate inflammatory cells have bimodal effector functions during the inflammatory response, with some having active roles during the resolution process.

Resolution of inflammation in asthma is characterised by clearance of inflammatory leukocytes from the lung, restoration of epithelial barrier function and dampening of airway hyperreactivity [5]. During resolution, multiple specialised mediators and cellular mechanisms are enlisted to generate endogenous "braking signals" to restore tissue homeostasis [6]. Several classes of counter-regulatory lipid mediators have been recently discovered that are generated from polyunsaturated fatty acids (PUFAs) during inflammation to promote resolution [7]. These specific pro-resolving lipid mediators are produced *via* biosynthetic circuits engaged during cell–cell interactions between different innate immune cells and structural cells at sites of inflammation in the lung and have a large array of anti-inflammatory and pro-resolving actions, including on the newly described ILCs [8].

In this article, we discuss recent studies on the role of pro-resolving lipid mediators in asthma inflammation with a focus on ILCs and eosinophils.

Inflammatory responses and the resolution of inflammation

Acute inflammation is an indispensable host response to insult or tissue injury and is initiated within minutes of recognition of a danger signal [9]. The acute inflammatory process is characterised by rapid recruitment of granulocytes (*i.e.* neutrophils, eosinophils and basophils) to the inflammatory site, the relative contributions of these cell types are dependent on the nature and the location of the inflammatory response. The initial events of acute inflammation are coordinated by many pro-inflammatory mediators (*i.e.* lipid mediators such as prostaglandins and leukotrienes, cytokines, and chemokines) that regulate vascular permeability and initial recruitment of leukocytes [10].

In health, the acute inflammatory response is generally self-limited, resolving within hours or days; however, in many human diseases, including asthma, resolution fails and inflammation stalls for a prolonged period. Therefore, failure to adequately resolve acute inflammation in asthma may contribute to chronic changes in airway structure and function causing clinical expression of asthma symptoms (reviewed in [11]).

Natural resolution of inflammation is now recognised an active host response. While it is driven, in part, by decrements in pro-inflammatory mediators, the promotion of resolution involves early signalling pathways engaging biosynthetic circuits for the later formation of counter-regulatory mediators [12]. For effective resolution of inflamed tissues to occur cessation of the recruitment of granulocytes is required, followed by the recruitment of monocytes that differentiate into macrophages, which clear inflammatory cells and tissue debris, leading ultimately to the restoration of tissue structure and function [13]. During this process, tissue granulocytes undergo apoptosis, a highly regulated cell death mechanism that prevents the release of histotoxic cellular contents [14]. Clearance of apoptotic neutrophils prompts a switch from a pro-inflammatory to an anti-inflammatory macrophage phenotype, which is a prerequisite for macrophage egress *via* the lymphatic vessels favouring a return to tissue homeostasis [15]. Clearance of apoptotic neutrophils also leads to the production of additional mediators that suppress the progression of inflammation and promote repair of damaged tissues [16, 17].

While several classes of mediators participate in resolution, the enzymatic transformations of PUFAs to specific pro-resolving agonists are of particular interest. These PUFA-derived mediators display cell-type selective anti-inflammatory, pro-resolving, anti-fibrotic, anti-angiogenic and anti-infective actions [7, 18].

PUFAs derived pro-resolving mediators

The use of experimental models of acute inflammation that naturally resolve (*i.e.* self-limited return to homeostasis) has led to the identification of a novel family of lipid mediators generated from PUFAs, named lipoxins, resolvins, protectins and maresins. These endogenous counter-regulatory mediators actively stimulate cardinal signs of resolution, namely, cessation of leukocytic infiltration, counter-regulation of pro-inflammatory mediators, and the uptake of apoptotic neutrophils and cellular debris (reviewed in [7]).

The omega-6 PUFA, arachidonic acid (20:4n-6) is incorporated into cellular phospholipids, and upon cell activation, specific phospholipase A_2 enzymes release arachidonic acid from the sn-2 fatty acyl bond of phospholipids. Arachidonic acid can then be converted enzymatically by cyclooxygenase (COX) to prostaglandins, by 5-lipoxygenase (LOX) to leukotrienes, or by 5-LOX in collaboration with 12-LOX or 15-LOX to lipoxins [19]. The omega-3 PUFAs eicosapentaenoic acid (EPA) (20:5n−3) and docosahexaenoic acid (DHA) (22:6n−3) can be enzymatically transformed *via* LOX pathways to resolvins,

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protectins and maresins (fig. 1) [20–22]. The enzymatic generation of these families occurs primarily *via* transcellular biosynthesis, and in some cases within a single cell-type.

During the acute inflammatory response, the biosynthesis of these resolution-phase mediators is initiated during lipid-mediator class switching, in which production of the classic initiators of acute inflammation, prostaglandins and leukotrienes, switches to specialised pro-resolving mediators as inflammation resolves [23].

These mediators display receptor-mediated cell type specific actions and display potencies in the low nanomolar range (table 1).

Lipoxins

Arachidonic acid-derived lipoxin A_4 (LXA_4) was the first PUFA-derived mediator found to have anti-inflammatory and pro-resolving activities [59]. Lipoxins are derived from the sequential actions of LOXs and are principally generated *via* biosynthetic circuits engaged during cell–cell interactions at sites of inflammation. Although lipoxins are present in low abundance during the initiation of acute inflammation, their levels increase substantially during resolution [23, 60]. In the lung, 15-LOX is a key enzyme for lipoxin generation and is expressed by many cells in the inflamed lung, including bronchial epithelial cells, macrophages and eosinophils [61–64].

Lipoxins act locally and then are rapidly inactivated by metabolic enzymes *via* pathways shared with other eicosanoids [18, 65]. In addition, lipoxin epimers can be generated in the presence of aspirin (acetylsalicylic acid) that are longer acting because of a reduced rate of inactivation [26, 66].

LXA_4 is an agonist for ALX/FPR2 receptors, which are expressed on both human airway epithelial cells and leukocytes [67]. In addition, ALX/FPR2 receptors can be induced by specific inflammatory mediators [68]. ALX/FPR2 receptors are also expressed on natural killer (NK) cells and type 2 ILCs [31]. In addition to lipoxins signalling through ALX/FPR2, these small molecules can act as antagonists at cysteinyl leukotriene receptors and can also signal *via* the aryl hydrocarbon receptor (AHR) [69].

Lipoxins demonstrate cell type-specific actions *in vitro* relevant to asthma immunobiology (table 1). These actions include inhibition of granulocyte locomotion, shape change and transmigration, and degranulation and stimulation of monocyte chemotaxis and macrophage engulfment of apoptotic neutrophils. In addition to this leukocyte-specific activity, lipoxins promote restoration of injured airway epithelium by indirectly blocking the release of the pro-inflammatory cytokines interleukin (IL)-6 and IL-8 by the epithelium [39].

EPA and DHA derived pro-resolving mediators

Population surveys report that diets rich in omega-3 fatty acids are associated with lower asthma prevalence [70]. Recent studies have identified a new family of pro-resolving lipid mediators generated from the omega-3 fatty acids, EPA (20:5n-3) and DHA (22:6n-3) [20]. These include the EPA-derived E-series resolvins (RvE1 and RvE2), the DHA-derived D-series resolvins (RvD1–D6), neuroprotectin/protectin, and maresin (fig. 1) [71].

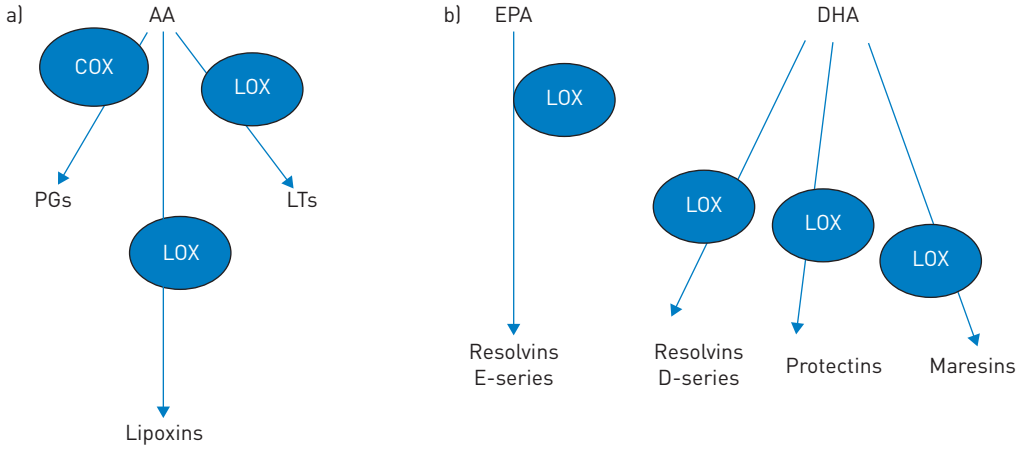

FIGURE 1 Formation of bioactive lipid mediators. Specific pro-resolving lipid mediators are enzymatically derived from host essential polyunsaturated fatty acids (PUFAs), including a) the omega-6 PUFA arachidonic acid (AA) (C20:4n-6), and b) the omega-3 PUFAs eicosapentaenoic acid (EPA) (C20:5n-3) and docosahexaenoic acid (DHA) (C22:6n-3) *via* lipoxygenase (LOX) pathways. AA also forms a range of pro-inflammatory mediators, such as prostaglandins (PGs) *via* cyclooxygenase (COX)-2, and the leukotrienes (LTs) *via* multiple LOX actions.

TABLE 1 Cellular actions of specialised pro-resolving lipid mediators in innate immunity relevant to asthma

Mediator	Target cell	Action(s)	References
Lipoxin A_4	Eosinophil	Inhibits migration and chemotaxis	[24, 25]
		Inhibits generation of eotaxin and IL-5	
	Neutrophil	Inhibit chemotaxis	[26–30]
		Inhibit trans-endothelial and trans-epithelial migration	
		Inhibit neutrophil–epithelial cell interactions	
		Inhibit superoxide anion generation	
		Inhibit degranulation	
	NK cell	Inhibits NK cell cytotoxicity	[31, 32]
		Increases granulocyte induced apoptosis	
	ILC2	Inhibits IL-13 release	[31]
	Monocyte	Stimulates chemotaxis and adhesion	[33–35]
		Inhibits peroxynitrite generation	
		Reduces IL-8 release by cells from individuals with asthma	
	Macrophage	Increases engulfment of apoptotic neutrophils	[36, 37]
	Dendritic cell	Inhibits IL-12 production	[38]
	Epithelial cell	Increases proliferation after acid injury	[39, 40]
		Inhibits cytokine release	
		Increases intracellular Ca^{2+}	
	Endothelial cell	Stimulates protein kinase-dependent prostacyclin formation	[41–43]
		Blocks the generation of reactive oxygen species	
		Inhibits VEGF-induced endothelial-cell migration	
	Fibroblast	Inhibits IL-1β-induced IL-6, IL-8 and MMP3 production	[44, 45]
		Inhibits CTGF-induced proliferation	
	Smooth muscle	Inhibits LTC4-initiated migration	[46]
	Bronchial epithelial cell	Stimulates basal cell proliferation after acid injury	[39]
		Blocks IL-6 and IL-8 release	
Resolvin E1	Neutrophil	Inhibits trans-epithelial and trans-endothelial migration	[47, 48]
		Inhibition of superoxide generation	
	Macrophage	Stimulates nonphlogistic phagocytosis of apoptotic neutrophils	[49]
	Dendritic cell	Inhibits IL-12 production	[50, 51]
		Inhibits migration	
Resolvin E3	Neutrophil	Inhibits infiltration	[52]
Resolvin D1	Neutrophil	Inhibits transmigration	[53]
	Macrophage	Inhibits LPS-induced TNF release	[54, 55]
		Promotes phagocytosis of antigen	
Protectin D1	Neutrophil	Inhibits TNF-α and IFN-γ release	[56–58]
		Inhibits PMN transmigration	
		Upregulates CCR5 expression	
	Macrophage	Stimulates nonphlogistic phagocytosis of apoptotic neutrophils	[49]

IL: interleukin; NK: natural killer; ILC2: type 2 innate lymphoid cell; VEGF: vascular endothelial growth factor; MMP3: matrix metalloproteinase 3; CTGF: connective tissue growth factor; LTC4: leukotriene C4; LPS: lipopolysaccharide; TNF: tumor necrosis factor; IFN-γ: interferon-γ; PMN: polymorphonuclear leukocyte; CCR5: CC-chemokine receptor 5.

Resolvins and protectins bear similaritiy to lipoxins in that their epimers can also be generated by the "alternative" acetylated COX-2 pathway in the presence of aspirin, thus producing "aspirin-triggered" forms [21].

These mediators, like lipoxins, act as potent anti-inflammatory lipid mediators limiting neutrophil influx, and also promote resolution of inflammation by stimulating the clearance of apoptotic cells and inflammatory debris by macrophages (table 1) [72]. RvE1 serves as an agonist at CMKLR1 receptors [50]. CMKLR1 is expressed on monocytes/macrophages and plasmacytoid dendritic cells [73–75]. CMKLR1 is also expressed on NK cells and type 2 ILCs [31].

PUFA derived pro-resolving mediators in allergic airway inflammation and asthma

The biological activity of the lipoxins in asthma and allergic disease has been defined over the past two decades (table 2). Bioactive, LXA_4 stable analogues have been prepared that block airway hyperresponsiveness and allergic inflammation in animal models, including eosinophil trafficking and tissue accumulation [68, 76]. They also block oedema formation and reduce the levels of the pro-inflammatory mediators IL-5, IL-13, CCL11, prostanoids and cysteinyl leukotrienes [68]. In humans,

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TABLE 2 Biological activities of pro-resolving lipid mediators in asthma and allergic disease models

Mediator	Action	References
Lipoxins	Inhibits airway hyperresponsiveness and pulmonary inflammation	[68, 76]
Resolvin E1	Reduces airway inflammation; stimulates LXA_4 production; and reduces SRS-A	[60]
Resolvin D1	Inhibits airway hyperresponsiveness and pulmonary inflammation	[54]
Protectin D1	Protects from lung damage, airway inflammation and airway hyperresponsiveness	[77]

LXA_4: lipoxin A_4; SRS-A: slow-reacting substance of anaphylaxis.

LXA_4 is generated during asthmatic responses [78–80] and, when administered to asthmatic subjects *via* nebulisation, LXA_4 attenuates leukotriene C_4-induced bronchoconstriction [81].

More severe variants of asthma are associated with diminished lipoxin biosynthesis compared with milder asthma [79, 82], suggesting that the chronic inflammatory response in asthma may be due, in part, to defective generation of pro-resolving mediators leading to inadequate counter-regulation (table 3). Decreased formation of lipoxins in uncontrolled asthma has now been identified in distinct populations of adults and children from several countries [33, 86, 87, 89]. There is also a decrease in lipoxins in the lungs of patients with aspirin-intolerant asthma [83], and exercise-induced asthma compared with the lungs of healthy persons [87].

RvE_1 is present in the lung [95]. In an experimental model of asthma, RvE_1 dampens the development and promotes the resolution of allergic airway responses [60, 96, 97]. RvD_1 and aspirin-triggered RvD_1 also promote resolution of allergic airways responses [54]. Protectin D1 (PD1) mediates bronchoprotective actions in a murine experimental model of allergic lung inflammation [77] and is decreased in exhaled breath condensates during acute exacerbations of asthma [77].

TABLE 3 Defects of pro-resolving mediators in asthma

Mediator		Population	References
Lipoxin A4 (LXA_4)	Aspirin-tolerant asthmatics generate more lipoxins than aspirin-intolerant asthmatics	Adults	[83]
	Higher urinary aspirin-triggered lipoxin levels in aspirin-tolerant asthma than in aspirin-intolerant asthma	Adults	[84]
	Diminished lipoxin biosynthesis in severe asthma	Adults	[82]
	LXA_4 defect in induced sputum in severe asthma	Adults	[33]
	Severe asthma is associated with a loss of LXA_4 in induced sputum	Adults	[80]
	LXA_4 generation is decreased in aspirin-sensitive patients in nasal lavage after aspirin nasal challenge	Adults	[85]
	LXA_4 levels in asthma show a relationship with disease severity and aspirin sensitivity	Adults	[86]
	Airway LXA_4 generation and LXA_4 receptor expression are decreased in severe asthma	Adults	[79]
	The role of LXA_4 in exercise-induced bronchoconstriction in asthma	Adults	[87]
	LXA_4 biosynthesis is decreased in severe asthma alveolar macrophages	Adults	[88]
	Reversed changes of LXA_4 and leukotrienes in children with asthma of different severity degree	Children	[89]
	LXA_4 is decreased in the EBC of children recovering from status asthmaticus	Children	[90]
	LXA_4 levels are lower in severe asthma and correlate negatively to lung function	Adults	[91]
	LXA_4 levels in EBC are lower in moderate-to-severe asthma than in mild asthma	Adults	[92]
	Decreased levels of LXA_4 in wheezy infants (blood)	Children	[93]
Protectin D1 (PD1)	PD1 is diminished in EBC from subjects with asthma exacerbation	Adults	[77]
	Impaired PD1 production in eosinophils from subjects with severe asthma	Adults	[94]

EBC: exhaled breath condensate.

ILCs are newly identified players in asthmatic inflammation and targets for specialised pro-resolving mediators

ILCs comprise a newly described family of haematopoietic effectors that play protective roles in innate immune responses to infectious microorganisms, lymphoid tissue formation, tissue remodelling after damage inflicted by injury or infection, and the homeostasis of tissue stromal cells (reviewed in [98]).

NK cells

NK cells are prototypical members of the ILC family. NK cells serve essential roles in host defence, including cytokine secretion, contact-dependent cell–cell signalling and direct killing of other immune cells, and are involved in combating tumours, viral infections, parasites and bacteria [99]. Roles for NK cells in asthma and allergic diseases are undefined and both disease-promoting and disease-controlling functions have been suggested [100].

Potential roles for NK cells in resolution of allergic airway responses have been recently defined. In a murine model of allergic lung inflammation, NK cells accumulate during resolution in the lung draining lymph nodes [97]. Depleting NK cells at the peak of the inflammatory response after allergen challenge delays clearance of airway eosinophils and antigen-specific $CD4^+$ T lymphocytes [97]; however, depletion of NK cells before allergen challenge has been shown to inhibit airway eosinophilia [101, 102]. Therefore, the timing of depletion of NK cells during the allergic inflammatory response may reveal different functions of NK cells during inflammation.

Co-culture experiments revealed that human NK cells could induce neutrophil [103] and eosinophil apoptosis [31, 104]. Moreover, NK cells from severe asthmatic subjects have a reduced capacity to augment eosinophil apoptosis [31]. Apoptosis of inflammatory cells is a non-inflammatory mechanism of cell removal and plays a critical role in successful resolution of the inflammatory response. Neutrophils and eosinophils can release toxic substances and enzymes harmful not only to pathogens, but also to surrounding tissue. In asthma, timely regulation of eosinophil activation and apoptosis is probably crucial to avoid tissue damage and induce resolution of inflammation [105]. Apoptotic granulocytes can subsequently be removed by tissue macrophages before their toxic contents leak out into the tissue and result in extensive damage. By accelerating granulocyte apoptosis, NK cells may play a role in limiting the inflammatory response and may be involved in the resolution of acute inflammation.

It is of interest that NK cells express pro-resolving receptors and that binding to pro-resolving mediators can modulate NK cell effector functions (fig. 2). NK cells were identified to express ALX/FPR2, a receptor for the pro-resolving mediator LXA_4, and NK cells from subjects with severe asthma have increased ALX/FPR2 expression [31]. LXA_4 can inhibit in a dose-dependent fashion the cytotoxic activity of human NK cells against K562 target cells assayed *in vitro* [106]. Moreover, when NK cells are exposed to LXA_4, the cells display an increase in NK cell-mediated apoptosis of both eosinophils and neutrophils [31]. NK cells also express CMKLR1 (Chemokine-like receptor 1), also known as ChemR23 (Chemerin Receptor 23) [31, 107], a receptor for the pro-resolving mediator RvE1. RvE1 has been shown to be a potent pro-resolving mediator for allergic airway inflammation [60]. In a murine model of allergic lung inflammation, NK cell depletion markedly impaired RvE1's protective actions [97]. Moreover, RvE1 regulates NK cell cytotoxicity *in vitro* [97].

Type 2 ILCs

In addition to NK cells, the ILC family also includes type 2 ILCs (ILC2s). ILCs have similar morphologies to T-cells but do not express T- or B-cell antigen receptors or markers of other lineages. They are involved in responses to helminth infection and murine models of allergic lung inflammation, and can respond to the epithelial-derived cytokines IL-25 (also known as IL-17E), IL-33 and thymic stromal lymphopoietin. In an antigen-independent manner, ILC2s can generate the cytokines IL-5 and IL-13 that were previously linked to T-helper type 2 (Th2) lymphocytes. ILC2s were recently identified in humans as a population of Lin^- cells expressing IL-7R, CRTH2 (a chemoattractant receptor for prostaglandin D_2 that is also expressed on Th2 cells), and the common NK cell marker CD161 [108].

ILC2s are instrumental in several models of experimental asthma where they significantly contribute to the production of IL-5 and IL-13, and to AHR development (fig. 2) [109–114]. ILC2s are present in the blood of asthmatic patients [31]. Human ILC2s express the pro-resolving receptors ALX/FPR2, and LXA_4 prevents prostaglandin D_2-stimulated release of IL-13 from ILC2s.

In some cases, ILC2 can help restore lung-tissue homeostasis after influenza infection in a murine model. In a study by Monticelli *et al.* [115] following influenza virus infection, ILC2 depletion led to impaired lung function and tissue repair, along with increased permeability of the lung epithelial barrier. It was shown that ILC2s produce a factor from the epidermal growth factor family linked to tissue remodelling and repair in asthma, known as amphiregulin. Administration of amphiregulin restored epithelial cell

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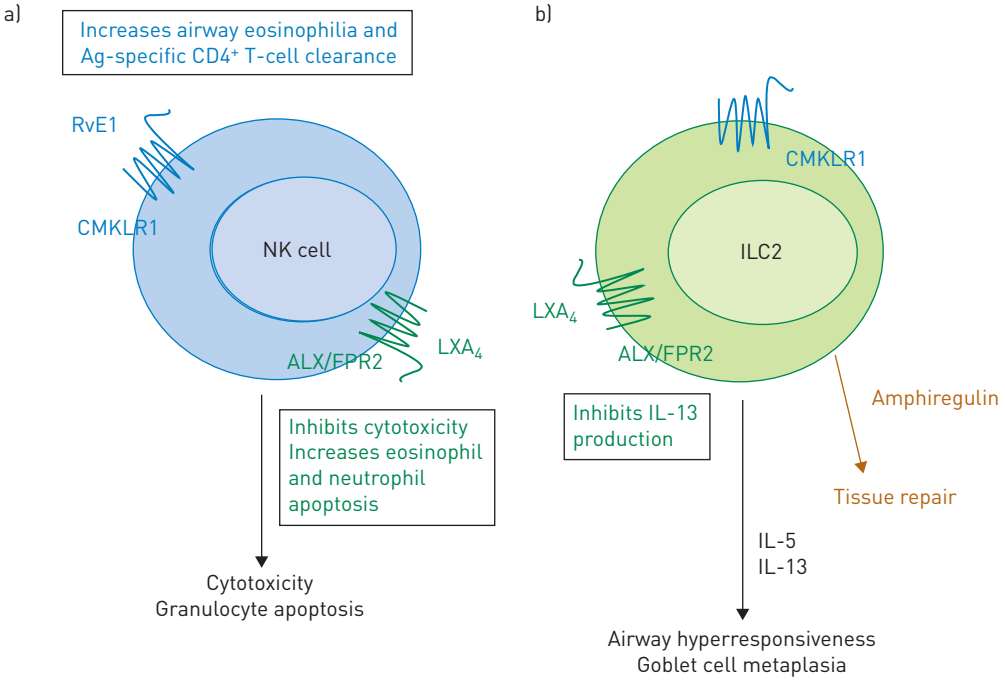

FIGURE 2 Key functions of innate lymphoid cells in resolution of inflammation in asthma. a) Natural killer (NK) cells and b) type 2 innate lymphoid cells (ILC2s) express the pro-resolving receptors ALX/FPR2 for lipoxin A_4 (LXA_4) and CMKLR1 (Chemokine-like receptor 1) for resolvin E1 (RvE1). LXA_4 inhibits NK cell cytotoxicity and increases eosinophil-induced apoptosis by NK cells, and inhibits interleukin (IL)-13 release by ILC2s. RvE1 increases airway eosinophilia and antigen (Ag)-specific $CD4^+$ T-cell clearance.

integrity, airway remodelling, and lung function [115]. In line with these findings, the IL-33–ILC2–IL-13 axis has been reported to mediate tissue repair functions in a mouse model of biliary injury by promoting epithelial restoration [116].

Thus, ILC2 may both promote inflammatory lung disease and also restore airway epithelial cell integrity after injury. While these two functions of ILC2s may appear contradictory, the homeostatic *versus* the pathological role of ILCs may be similar to the contrasting roles of several other immune cell types. The context in which ILC2s function may determine whether the cells are beneficial (enhancing epithelial cell integrity) or detrimental (causing airway inflammation and airway hyperresponsiveness). ILCs may have evolved to respond rapidly during viral infections and when activated in the absence of appropriate regulation, ILC2s may cause disease, such as airway inflammation and AHR.

Emerging roles of eosinophils and eosinophil-derived pro-resolving lipid mediators in asthma

Eosinophils are associated with the pathogenesis of asthma, and their accumulation in the lungs is often regarded as a defining feature of allergic asthma in humans and in animal models [117]. It is assumed that eosinophils are recruited to the lungs by Th2 cells as end-stage effector cells, because of their ability to secrete a wide array of cytotoxic and pro-inflammatory mediators. For example, eosinophils can serve as major effector cells inducing tissue damage and dysfunction by releasing an array of cytotoxic granule cationic proteins including major basic protein, eosinophil cationic protein and eosinophil-derived neurotoxin (fig. 3) [118]. Nevertheless, the role of eosinophils in specific features of asthma has been controversial in several experimental and clinical studies [119–121]. Recent work showed that eosinophils are also able to contribute to the resolution of acute inflammation. In a murine model of self-limited zymosan-induced peritonitis, 12/15-LOX-expressing eosinophils were recruited to the inflamed loci during the resolution phase of the acute inflammatory response and were shown to generate pro-resolving lipid mediators, including PD1 [122]. In this nonallergen model, eosinophil-derived PD1 was shown to induce macrophage activity to clear apoptotic neutrophils from the site of inflammation. Eosinophils can also promote resolution in murine zymosan-induced inflammation by regulating the expression of macrophage CXCL13 through the control of the 12/15-LOX-derived mediator, LXA_4 [123].

Interestingly, in a murine model of allergic lung inflammation, PD1, administered before aeroallergen challenge, reduced airway inflammation and dampened airway hyperresponsiveness [77]. In addition, in

this study levels of PD1 were significantly lower in exhaled breath condensates from subjects with asthma exacerbations when compared with healthy subjects. PD1 has been confirmed as one of the main anti-inflammatory and pro-resolving molecules synthesised by human eosinophils [94]. PD1 is an autacoid regulator of eosinophils, and suppresses in nanomolar concentrations eosinophil chemotaxis induced by CCL11/eotaxin-1 or 5-oxo-eicosatetraenoic acid and modulates the expression of the adhesion molecules CD11b and L-selectin; although it has no significant effects on eosinophil degranulation, superoxide anion generation or survival (fig. 3). When compared with the cells harvested from healthy subjects, biosynthesis of PD1 is decreased in severe asthma [94].

Eosinophils are also able to convert 18-hydroperoxyeicosapentaenoic acid (HEPE) into 17,18-diHEPE, known as RvE3, *via* the 12/15-LOX pathway [52]. RvE3 displays potent anti-inflammatory activity by blocking PMN infiltration in acute peritonitis (fig. 3) [52].

The role of eosinophils in the resolution of inflammation probably extends beyond production of pro-resolving lipid mediators and includes pathways resulting from interactions with other pro-inflammatory and resident cells in the lung. Recent work showed that eosinophils are able to contribute to the resolution of lung-allergic responses following repeated allergen challenge in a murine model by producing IL-10, a potent anti-inflammatory cytokine [124]. *In vitro,* eosinophils have the potential to polarise macrophages through IL-4/13 release to an M2 phenotype, the precursor to resolution macrophages (*i.e.* increased phagocytic activity and production of resolving lipid mediators) [125, 126]. Eosinophils were also shown to promote alternatively activated macrophages in other disease models (fig. 3) [127, 128].

Therapeutic implications

For asthma, currently available anti-inflammatory agents or therapies under development (*e.g.* glucocorticoids and the biological therapeutics anti-IgE, IL-5 or IL-13) target pro-inflammatory mediator pathways [129]. While this strategy has been beneficial in some clinical conditions, long-term use of corticosteroids can be associated with significant side-effects and there remain substantial unmet clinical needs [130].

Anti-inflammation and pro-resolution are not synonymous. The definitions of these terms have important differences. Although anti-leukocyte actions are commonly considered anti-inflammatory, it is important to view the role of each cell type in this dynamic process of catabasis. Inhibition of neutrophil transmigration and activation is anti-inflammatory but can lead to immunosuppression that increases the host's susceptibility to infection. By contrast, restitution of barrier integrity (endothelial, epithelial, or

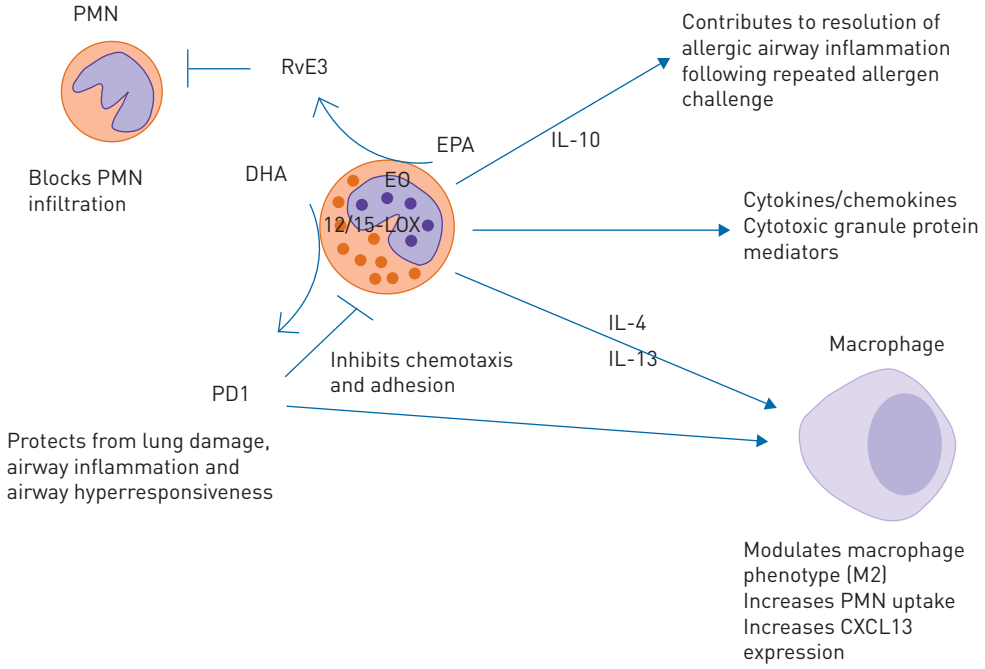

FIGURE 3 Key functions of eosinophils (Eo) in resolution of inflammation in asthma. Eosinophils secrete a wide array of cytotoxic and pro-inflammatory mediators. In addition, eosinophils may contribute to resolution of inflammation in asthma by producing pro-resolving lipid mediators (PD1 and RvE3), by secreting interleukin (IL)-10 and by promoting alternative macrophage activation in an IL-4 and IL-13 dependent manner. PMN: polymorphonuclear leukocyte; PD1: protectin D1; RvE3: resolvin E3; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; LOX: lipoxygenase.

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both); recruitment of monocytoid cells; and promotion of macrophage clearance of apoptotic cells, microbes and tissue debris are all pro-resolving responses, increasing host defence [8, 22]. Therefore, the identification of such endogenous anti-inflammatory and/or pro-resolution mechanisms is of wide interest.

Rather than blocking early or select pro-inflammatory mediators, an alternative therapeutic strategy might emphasise mimetics of lipoxins, resolvins, protectins, maresins 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 asthma model systems (table 2). Moreover, more severe variants of asthma are associated with diminished lipoxin biosynthesis compared with milder asthma (table 3) [79, 82], suggesting that the chronic inflammatory response in asthma may be due, in part, to defective generation of pro-resolving mediators leading to inadequate counter-regulation. Exogenous administration of this mediator may benefit these patients by inhibiting and resolving inflammation.

In a recent clinical study, a LXA_4-based compound was tested for the topical treatment of infantile eczema [131]. Albeit in a small number of patients, the drug reduced the severity of eczema to a similar extent as steroid therapy in a double-blind placebo-controlled setting. No studies, to date, have been published examining the therapeutic effect of LXA_4 in asthma.

Conclusion

The resolution of inflammation is integral to the physiological response to tissue injury, infection and allergens or other noxious stimuli. Resolution is an active process with highly regulated cellular and biochemical events that are engaged to restore tissue function in health. Recent discoveries have highlighted that innate inflammatory cells have bimodal effector functions during the inflammatory response, including active roles during the resolution process. Several mediators displaying potent pro-resolving actions have recently been uncovered. LXA_4, the lead member of this new class of pro-resolving mediators, has anti-inflammatory actions on ILC2 and pro-resolving actions through NK cells in asthma immunobiology. Eosinophils are also able to control crucial aspects of resolution through the generation of pro-resolving mediators. Uncontrolled asthma has been linked to a defect in the generation of specialised pro-resolving mediators, including LXA_4 and PD1. Thus, bioactive stable analogue mimetics of these mediators that can harness endogenous resolution mechanisms for inflammation may offer new therapeutic strategies for asthma and airway inflammation associated diseases.

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