Targeting pro-resolution pathways to combat chronic inflammation in COPD

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Abstract: Chronic obstructive pulmonary disease (COPD) is an inflammatory lung condition that is associated with irreversible airflow obstruction as a consequence of small airways disease, excessive mucus production and emphysema. Paradoxically, excessive inflammation fails to control microbial pathogens that not only colonise COPD airways, but also trigger acute exacerbations, which markedly increase inflammation underlying host tissue damage. Excessive production of leukocyte mobilising cytokines such as CXCL8 (IL-8) and leukotriene B4 (LTB4) in response to environmental stimuli (cigarette smoke and microbial products) are thought to maintain chronic inflammation, in conjunction with inefficient macrophage clearance of microbes and apoptotic neutrophils. In this perspective, we discuss an alternative view on why inflammation persists with a focus on why pro-resolution mediators such as lipoxin A4 (LXA4), D-series resolving and Annexin A1 fail to effectively switch off inflammation in COPD. These pro-resolving mediators converge on the G-protein coupled receptor, ALX/FPR2. This receptor is particularly relevant to COPD as the complex milieu of exogenous and host-derived mediators within the inflamed airways include agonists that potently activate ALX/FPR2, including Serum Amyloid A (SAA) and the cathelicidin, LL-37. There is emerging evidence to suggest that ALX/FPR2 can exist in alternative receptor conformations in an agonist-biased manner, which facilitates alternate functional receptor behaviors. Hence, the development of more stable pro-resolving analogs provides therapeutic opportunities to address ALX/FPR2 conformations to counteract pathogenic signaling and promote non-phlogistic clearance pathways essential for resolution of inflammation.

Keywords: ALX/FPR2; chronic obstructive pulmonary disease (COPD); inflammation; catabasis; resolution; Serum Amyloid A (SAA)

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Introduction

Chronic obstructive pulmonary disease (COPD) is a major global health issue and is a leading cause of morbidity and mortality worldwide (1). Cigarette smoke exposure is a primary cause of COPD and as smoking rates continue to rise in South-East Asia, the long disease latency of COPD will impact dramatically on global health care resources for many decades to come. It is also well established that environmental influences such as indoor and outdoor air pollutants contribute to the development of chronic lung disease in individuals with no smoking history (2). Pathologically, COPD manifests into impaired lung function and irreversible airflow obstruction as a consequence of small airways disease, excessive mucus production and the development of emphysema. The mechanisms that drive such deleterious remodelling processes are heterogeneous in nature; however it is evident that chronic inflammation and excessive oxidative stress become central to the progression of COPD (3).

Nature of persistent inflammation in COPD

Innate and adaptive immune cells accumulate with
progression of COPD. CD8+ T cells increase with disease severity and can release proteolytic enzymes that contribute to apoptosis of structural cells (4). CD4+ T cells, B cells and dendritic cells also aggregate into organised tertiary lymphoid organs (TLOs), which are found in COPD lungs and experimental models of chronic cigarette smoke exposure (5). The significance of these lymphoid follicles has yet to be established, where B cell accumulation may prove to be beneficial in terms of antibody production to colonising and invading pathogens, or detrimental through autoantibody production against lung tissue antigens (6).

Of note, targeted disruption of TLO formation through neutralisation of CXCL13 does not reduce lung inflammation and alveolar enlargement in a chronic smoke exposure model (7), suggesting that immune cells of the innate system are sufficient to drive pathological changes that occur in COPD. In addition, inflammation does not fully resolve even when individuals with COPD stop smoking. Inflammation persists in bronchial biopsies and in sputum samples from COPD patients when compared to asymptomatic smokers (8-10). In particular, neutrophilic inflammation remained elevated in COPD subjects who had ceased smoking for at least one year (8-10). Given that neutrophils are a relatively short-lived immune cell; their persistence in COPD indicates that there is continual recruitment of leukocytes into the airways even when the primary insult of smoke exposure is removed.

Smoke exposure is particularly deleterious to host immunity against respiratory pathogens. Macrophage function is compromised in a microenvironment where there is excessive oxidative stress, leading to a deficiency in phagocytosis of bacteria (11) and efferocytosis of apoptotic cells (12). Free radicals have been shown to impair clearance mechanisms by directly causing cytoskeletal instability and carbonyl modification of pseudopodia (13-15). Macrophages also interact with carbonyl-adduct modified extracellular matrix proteins, which impair their ability to clear apoptotic neutrophils (16). Reduced macrophage function is consistent with high colonisation rates in COPD, where up to 50% of COPD patients are chronically colonized with microorganisms including Haemophilus influenzae, Streptococcus pneumoniae and Moraxella catarrhalis (17,18). Impaired immunity to respiratory pathogens also contributes to susceptibility to acute exacerbations of COPD (AECOPD), which are predominately caused by a new respiratory pathogen that is different to the colonized pathogen (19). AECOPDs result in a more rapid decline in lung function (20), which adversely affects mortality rates (21) and health related quality of life (22). Viral infections are a common cause of AECOPDs (23,24), as are bacterial infections, which are associated with a marked increase in neutrophilic inflammation (25).

Bacterial colonization and AECOPDs contribute to persistent inflammation of the airways, as neutrophils and monocytes are recruited from the circulation in an attempt to clear the invading pathogens. Since resident airway macrophages in COPD have a reduced capacity to clear efferocytic neutrophils, the emergence of necrotic neutrophils may further facilitate a vicious cycle of inflammation through release of endogenous damage associated molecular patterns (DAMPs). For example, degranulating necrotic bodies release neutrophil elastase, which localize to lung elastic fibers in emphysematic patients and degrades extracellular matrix components (26). Excessive neutrophil elastase activity can also promote the release of mucus through Epidermal Growth Factor Receptor (EGFR)-dependent mechanisms (27).

Furthermore, increased EGFR transactivation augments inflammatory responses initiated by rhinovirus infection in bronchial epithelial cells (28). Necrotic neutrophils also release High-Mobility Group Box-1 (HMGB1), which is a DNA binding protein that is elevated in COPD airways (29). HMGB1 can synergize with microbial products and endogenous cytokines to enhance inflammation through Toll Like Receptor (TLR) (30,31) and Receptor for Advanced Glycosylation End (RAGE) (29) dependent mechanisms. Hence, COPD airways represent a highly complex milieu consisting of inhaled irritants, respiratory pathogens and endogenous mediators released from damaged tissue, which collectively drive excessive host immunity and sterile inflammation.

**Pro-resolution mediators promote catabasis in the inflamed airway**

The ongoing recruitment of leukocytes from the circulation is consistent with increased local production of CXCL chemokines in COPD including IL-8 (CXCL8), ENA78 (CXCL5), GCP-2 (CXCL6) and GRO isoforms (CXCL1-3) (32,33). The CXCR family of G coupled protein receptors (GPCR) binds to these endogenous chemokines, where CXCR2 is the cognate receptor for this family (34). There is also a high degree of redundancy in leukocyte mobilization as alternate mediators such as leukotriene B4 (LTB4) are associated with neutrophilic inflammation in colonized COPD patients (35). These
pathways are essential to lung host immunity and are normally self-limiting through the activation of pro-resolution pathways. Pro-resolving mediators actively counterbalance inflammation by effectively switching off mechanisms that maintain leukocyte recruitment and survival. Eicosanoids such as lipoxins, resolvins and protectins and the protein termed Annexin A1 are integral to resolution of inflammation, [reviewed in (36-38)]; where there is a class switch of eicosanoid production from chemoattractants (such as the leukotriene LTβ4) to pro-resolving mediators (such as lipoxin A4, LXA4) (39).

Lipoxins are synthesized in response to cell-cell interactions [reviewed in (40,41)]. In the lung, 15-lipoxygenase is expressed in epithelial cells and converts arachidonic acid to 15S-hydroxyeicosatetraenoic acid (15S-HETE). This metabolite is then taken up by neutrophils and converted to LXA4 by the enzyme 5-lipoxygenase (42). LXA4 opposes leukocyte migration and activation through multiple mechanisms including suppression of transendothelial (43) and transepithelial (44) migration and azurophilic degranulation (45). LXA4 can also directly target activated mucosal epithelial cells by opposing production of inflammatory cytokines (46). In vivo, administration of a stable analog of LXA4 reduced pulmonary inflammation and airway hyperreactivity in a murine model of asthma (47). Furthermore, this eicosanoid can directly contribute to tissue repair through the promotion of basal cell proliferation required for wound healing following mucosal injury (46). Another important anti-inflammatory role for LXA4 is its influence on macrophage function. Treatment with nanomolar concentrations of LXA4 stimulated more efficient efferocytosis of apoptotic neutrophils in human monocyte derived macrophages without provoking release of inflammatory mediators (48,49). LXA4-induced uptake of apoptotic neutrophils was reduced in macrophages from Fpr2 deficient mice, which is the mouse ortholog of the human ALX/FPR2 G-protein coupled receptor (GPCR) that potently interacts with lipoxins (50).

**ALX/FPR2 is central to resolution of inflammation**

In addition to lipoxins, the alternate anti-inflammatory mediators Annexin A1 and Resolvin D1 also interact with ALX/FPR2, implicating this receptor as an integral component of pro-resolution pathways. Accordingly, they display overlapping functions, where both LXA4 (51) and Annexin A1 (52) promote apoptosis of neutrophils as a mechanism of resolving acute inflammatory responses. Unlike lipid mediators derived from cell-cell interactions, Annexin A1 is highly abundant in neutrophils where the 37kDa protein is localized to the cytoplasm and in gelatinase/azurophilic granules. Annexin A1 is a glucocorticoid-induced lipocortin that can be rapidly mobilized through degranulation of neutrophils. Cytoplasmic Annexin A1 is also subjected to post translational modifications such as phosphorylation, which facilitate translocation to the cellular membrane [reviewed in (52)]. In addition to promoting neutrophil apoptosis, Annexin A1 reduces neutrophil endothelial attachment and subsequent transmigration. Like LXA4, Annexin A1 has also been shown to enhance efferocytosis of apoptotic neutrophils by macrophages [reviewed in (52)], thereby facilitating catabasis of the inflamed tissue to homeostasis.

Another family of mediators that target ALX/FPR2 is the D series resolvins derived from the omega-3 fatty acid, docosahexaenoic acid (DHA) [reviewed in (53)]. Although there is relatively less information on the actions of D-series resolvins in lung diseases, there is emerging evidence to support an important role in resolution of lung inflammation. In a murine model of acute lung injury, treatment with stable derivatives of Resolin D1 improved epithelial and endothelial integrity and reduced neutrophilic inflammation concurrently with reduced inflammatory cytokine secretion in the airways (54). In addition, aspirin-triggered Resolvin D1 (AT-RvD1) significantly reduced airway inflammation and enhanced macrophage phagocytosis in a murine asthma model (55). Furthermore, Resolvin D1 reduced neutrophilic lung inflammation, inflammatory cytokine production and phagocytosis in an acute cigarette smoke exposure model (56).

**Is there an imbalance between inflammatory and pro-resolving mediators in COPD?**

Given their important role in resolution of acute inflammation, any perturbation in the production of pro-resolving mediators can lead to the generation of pathogenic inflammatory conditions. Indeed, it has been shown that reduced production of LXA4 relative to cysteinyl leukotrienes is associated with the persistence of inflammation in severe asthma (57,58). Reduced levels of LXA4 are also observed in exhaled breath condensate during asthma exacerbations (59) and in the airway fluid of patients with cystic fibrosis (60). Since multiple mediators converge on ALX/FPR2, their relative abundance within the airway milieu of the chronically inflamed lung can profoundly...
ALX/FPR2 belongs to the N-formyl peptide receptor (FPR) family, of which there are three human members (FPR1, ALX/FPR2 and FPR3). FPRs demonstrate wide tissue distribution, although ALX/FPR2 expression is particularly prominent on myeloid cells including neutrophils and monocytes (61). Airway mucosal epithelial cells also express ALX/FPR2 in a manner that is increased via COX-2 dependent mechanisms in response to injury (46). Prominent staining is also observed on the apical and basolateral side of the epithelium of COPD airways (62).

ALX/FPR2 is a GPCR superfamily member characterized by seven putative TM domains that displays diverse ligand affinities that extend beyond interactions with lipoxins, series D-resolvins and Annexin A1. Accordingly, ALX/FPR2 can interact with over 30 ligands that can exert opposing biological actions (63). Hence, selective agonists are likely to bind with different affinities and given the diverse conformation of endogenous and synthetic ligands, are likely to bind to alternate regions of the receptor. Numerous receptor conformations can exist where ligand binding alters receptor formation, which facilitates alternate functional behaviors that control multiple downstream signaling pathways (64). This is true for ALX/FPR2, where agonist biased signaling can either promote inflammation or resolution contingent on alternate receptor conformations [reviewed in (65)]. Serum Amyloid A (SAA) and the anti-microbial peptide LL-37 both interact with ALX/FPR2 and in complete contrast to pro-resolving mediators, promote neutrophil transmigration, activation and survival (summarized in Figure 1). Another high affinity ligand for ALX/FPR2 includes mitochondrial N-formylated hexapeptides derived from NADH dehydrogenase and cytochrome c oxidase subunits (66). These formylated peptides are released from damaged cells and can interact with FPR1 and ALX/FPR2 (66). Although it is not known whether these ligands are elevated in COPD, there is increased mitochondrial dysfunction and cytochrome c oxidase levels in the skeletal muscle of COPD patients (67-69).

There are multiple mechanisms that may address why ALX/FPR2 receptors modulate cell responsiveness in a ligand-biased fashion. This includes the formation of homologous and/or heterologous receptor dimers and differential conformational ligand activation of specific receptor domains. The molecular nature of how ALX/FPR2 downstream signaling pathways are activated in a ligand-specific manner has yet to be fully characterized. However, there is evidence for conformational ligand activation. LXA4 has been shown to activate ALX/FPR2 by interacting with extracellular loop III and the associated transmembrane region (70), whereas SAA initiates extracellular loops I and II dependent signaling (71). In addition, SAA has recently been shown to promote ALX/FPR2 homodimerisation and activation of pro-survival pathways, which can be counteracted by Annexin A1 that promotes an alternative receptor conformation that engages pro-apoptotic pathways (72).

**Conclusions**

Since the accurate detection of lipid pro-resolution mediators in human tissue requires LC-LC-MS approaches that have only recently become available, there are currently relatively few studies demonstrating their presence in human tissue (73). Hence, there is a current knowledge gap in terms of whether there is a deficiency in production of pro-resolving mediators in COPD that facilitates a chronic inflammatory state, and advances in detection technology will reveal new insight here. Importantly, this should inform on the development of new classes of drugs to treat COPD away from conventional steroid-like anti-inflammatory approaches that can detrimentally dampen immunity leading to immunosuppression. Specialized pro-resolving mediators offer an alternative approach to switching off chronic inflammation and may concurrently boost beneficial host immunity and tissue repair mechanisms. Whilst the organic synthesis of pro-resolving lipid mediators can be challenging, chemically stable E-class resolvin analogs have been successfully synthesized and are currently in Phase III clinical trial for chronic inflammatory dry eye syndrome (Resolvyx Pharmaceuticals in partnership with Celtic Therapeutics).

Of interest, two human phenotypes have been described in response to cantharidin-induced skin blisters in male healthy volunteers involving distinct production of LXA4, which controlled the longevity and severity of the inflammation (74). The relative abundance of alternative ALX/FPR2 ligands may also directly influence the functional behavior of this complex receptor in COPD airways, where there is a rich milieu of microbial derived products and endogenous mediators. LL-37 (or hCAP-18) is a breakdown product of cathelicidin that promotes microbial killing through pore formation. LL-37 is also a chemoattractant for neutrophils, monocytes and lymphocytes via its actions on ALX/FPR2 (75). Since LL-37 levels are elevated in sputum samples from stable COPD patients (76) and are further increased during
bacterial AECOPDs (77), this normally protective molecule may contribute to persistent inflammation. Likewise, SAA, which has previously been characterized as a systemic biomarker for AECOPD severity (78), is prominently expressed in lung resection tissue from COPD patients (62). Using the same lung resection tissue, SAA transcript expression was also detected and found to be positively associated with the number of tissue neutrophils (79).

In addition to promoting neutrophil survival via ALX/FPR2, SAA is a potent chemotactic factor that mediates migration of leukocytes (80) and can also stimulate expression of pro-inflammatory mediators under in vitro (81) and in vivo conditions (62). More recently, SAA was shown to promote the differentiation of monocyte derived macrophages into a pro-inflammatory phenotype that expresses higher levels of the TH17 polarizing cytokines, IL-6 and IL-1β in a manner that was dependent on CSF-1R signaling (82). Hence, the relative abundance and persistence of pro-inflammatory agonists such as SAA and LL-37 in COPD airways is likely to facilitate agonist biased signaling that favors leukocyte recruitment, activation and survival. The nature of this receptor also provides therapeutic opportunities to address ALX/FPR2 conformations that may lead to pathogenic functions through the development of more stable analogs of lipoxins, D-series resolvins and cleavage resistant Annexin A1 mimetics, which can override pro-inflammatory signals to initiate resolution of inflammation.

Figure 1 ALX/FPR2 and chronic inflammation in COPD. The ALX/FPR2 complex is expressed on leukocytes and the respiratory mucosa, where expression is particularly prominent on injured epithelial cells. The expression of two known ligands for ALX/FPR2, Serum Amyloid A (SAA) and LL-37 (hCAP-18) are known to be elevated in COPD and acute exacerbations of COPD. Mitochondrial hexapeptides released from damaged tissue also activate ALX/FPR2. SAA and LL-37 have been shown to initiate a pro-inflammatory response involving recruitment of neutrophils and monocytes. SAA can also promote neutrophil survival and release of inflammatory mediators from epithelial cells. In contrast, alternate ligands can bind to a different receptor region and promote distinct receptor conformations that translate to an opposing biological action. When the pro-resolving mediators LipoxinA4, Resolvin D1 and Annexin A1 interact with this receptor, they can actively initiate processes to reduce the influx of leukocytes and stimulate macrophages to clear exhausted immune cells and damaged tissue. SAA is disproportionally expressed relative to LXA4 in circulation during exacerbations and with the recent advance in technology to detect pro-resolving mediators in human tissue; it will be possible to explore this putative imbalance in COPD airways. With the ongoing development of more stable pro-resolving analogs, their therapeutic application may provide an opportunity to suppress chronic inflammation and clear damaged tissue to promote catabasis in the lung.
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