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Invited Review Article

Non-type 2 inflammation in severe asthma is propelled by neutrophil cytoplasts and maintained by defective resolution

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ABSTRACT

Asthma is a highly prevalent heterogeneous inflammatory disorder of the airways. Not all patients respond to anti-inflammatory treatment with corticosteroids, leading to significant morbidity in severe asthma. Much attention has been paid to defining the cellular and molecular mechanisms of type 2 inflammation that are operative in asthma. Development of targeted therapies for pathologic type 2 inflammation is opening a new approach to asthma treatment; however, not all asthmatics have type 2 airway inflammation, especially those with severe corticosteroid-refractory asthma. Much less is known about non-type 2 immunological mechanisms in asthma. In health, inflammation triggers resolution mechanisms that control immune (type 1 and type 2) responses and enable the restoration of tissue homeostasis. The resolution response is comprised of cellular and molecular events, including production of specialized pro-resolving mediators (SPMs). SPMs halt leukocyte recruitment, promote macrophage efferocytosis, and restore epithelial barrier integrity, all of which are critical to resolution of inflammation in the lungs. Here, we review recent insights into the disruption of these homeostatic mechanisms and their contributions to non-type 2 inflammation in severe asthma immunopathogenesis. Copyright © 2018, Japanese Society of Allergology. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

The resolution of inflammation is an active process comprised of cellular and biochemical processes that promote tissue catabasis.¹ In health, resolution of inflammation is the most common outcome of an acute response, but persistent and non-resolving inflammation can result and contributes to several lung-related pathologies, including severe asthma.² Specialized pro-resolving mediators (SPMs) are pivotal signals for the resolution of tissue inflammation³; however, SPM abundance or signaling receptors are disrupted in chronic inflammatory disease.²

Asthma is a multifactorial, heterogeneous inflammatory disorder of the lower airways resulting from an overzealous immune response to otherwise seemingly innocuous particles and/or microbes to which the lung is constantly exposed. Principally, asthma is most commonly associated with type 2 airway and systemic

inflammation leading to IL-4, IL-5 and IL-13 release that drives IgE production from B cells, airway recruitment of eosinophils, and epithelial mucous cell metaplasia, respectively.⁴ In the recent decade, research involving human patient samples coupled with murine models of allergic inflammation provide concrete evidence that asthma pathogenesis is complex, with a heterogeneous immune profile that is typically eosinophilic or type 2 dominant in patients with the mild-to-moderate form of the disease.^{4,5} Evidence of an exaggerated type 2 immune response in more severe asthma spurred the development of novel therapeutics specifically targeting IgE, type 2 cytokines, and the master regulator of the pathway, GATA-3⁶.

Of note, not all asthmatics have purely type 2 inflammation. Some patients have a mixed population of airway granulocytes, with both eosinophils and neutrophils in sputum and bronchoalveolar lavage and a cytokine signature with low type 2 cytokine expression in combination with elevated non-type 2 cytokines, typically IL-17 or IFN- γ .^{5–7} These patients often suffer from severe asthma, which affects ~10–15% of asthmatics. These patients experience persistent symptoms and frequent exacerbations, which are inadequately controlled by anti-inflammatory treatment with corticosteroids. The stymied response to

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treatment often leads to prolonged courses of systemic corticosteroids that can have immunosuppressive and severe systemic side effects.

This review highlights the rapid strides being made in our understanding of cellular mechanisms that propel chronic non-type 2 inflammation and the dysfunctional lung resolution pathways that together are linked to severe asthma immunopathogenesis.^{7–10}

Decreased levels of specialized pro-resolving mediators in severe asthma

SPMs can be detected in a variety of human biospecimens, including plasma, serum, sputum, bronchoalveolar lavage fluid, sputum, exhaled breath condensates, and body fluids from diverse organs such as breast milk, tears, urine, cerebrospinal fluid and synovial fluid.¹ There are over 25 structurally and functionally distinct SPMs that are enzymatically derived from omega-3 and omega-6 fatty acids and are detectable in the bioactive (pg/mL) range (reviewed in³). In the airway, epithelial cells express 15-lipoxygenase and infiltrating leukocytes express 5-lipoxygenase, so heterotypic cell–cell interactions during airway inflammation can lead to the formation of unique lipoxygenase interaction products (termed “lipoxins” when from arachidonic acid) (Fig. 1). Of note, relative to healthy subjects, patients with severe asthma have decreased levels of both circulating and airway-localized SPMs. Arachidonic acid-derived lipoxin A₄ (LXA₄) levels are lower in induced sputum, BAL fluid, and exhaled breath condensates from patients with severe asthma compared with healthy controls.^{11–15} Importantly, lower levels of LXA₄ are associated with lower lung function, in particular worsened airflow obstruction in severe asthma.^{11,16} Patients with aspirin-exacerbated respiratory disease have impaired LXA₄ biosynthesis and elevated leukotriene levels, an imbalance in pro-resolving and pro-phlogistic mediators that may contribute to the

Table 1
Decreased Production of SPMs in uncontrolled and severe asthma.²

SPM	Disease	Human Findings	Biospecimen
LXA ₄	Severe asthma	Diminished LXA ₄ biosynthesis correlates with worse lung function and disease severity	Blood, sputum, BAL, exhaled breath condensates
	Exercise-induced asthma	Decreased LXA ₄ in children triggered by exercise induced bronchospasm	Plasma
	Aspirin-related asthma	Decreased lipoxins in aspirin-intolerant asthmatics compared to aspirin-tolerant asthmatics	Blood, BAL
Protectin D1	Severe asthma	Impaired PD1 production in eosinophils from subjects with severe asthma compared to healthy control	Blood
	Asthma exacerbation	PD1 is diminished during asthma exacerbation compared to healthy control	Exhaled breath condensates

frequent symptoms and increased severity in this asthma sub-phenotype.^{16–18} Airway mucosal levels of DHA are reduced in asthma,¹⁹ limiting the fatty acid precursors available locally for conversion to DHA-derived SPMs including protectin D1, maresins, and the D-series resolvins. Several studies report an association of lower levels of SPMs during acute exacerbations of asthma. DHA-derived protectin D1 (Fig. 1) levels are significantly lower in exhaled breath condensates in asthma patients during acute exacerbations relative to healthy subjects.²⁰ Eosinophils from severe

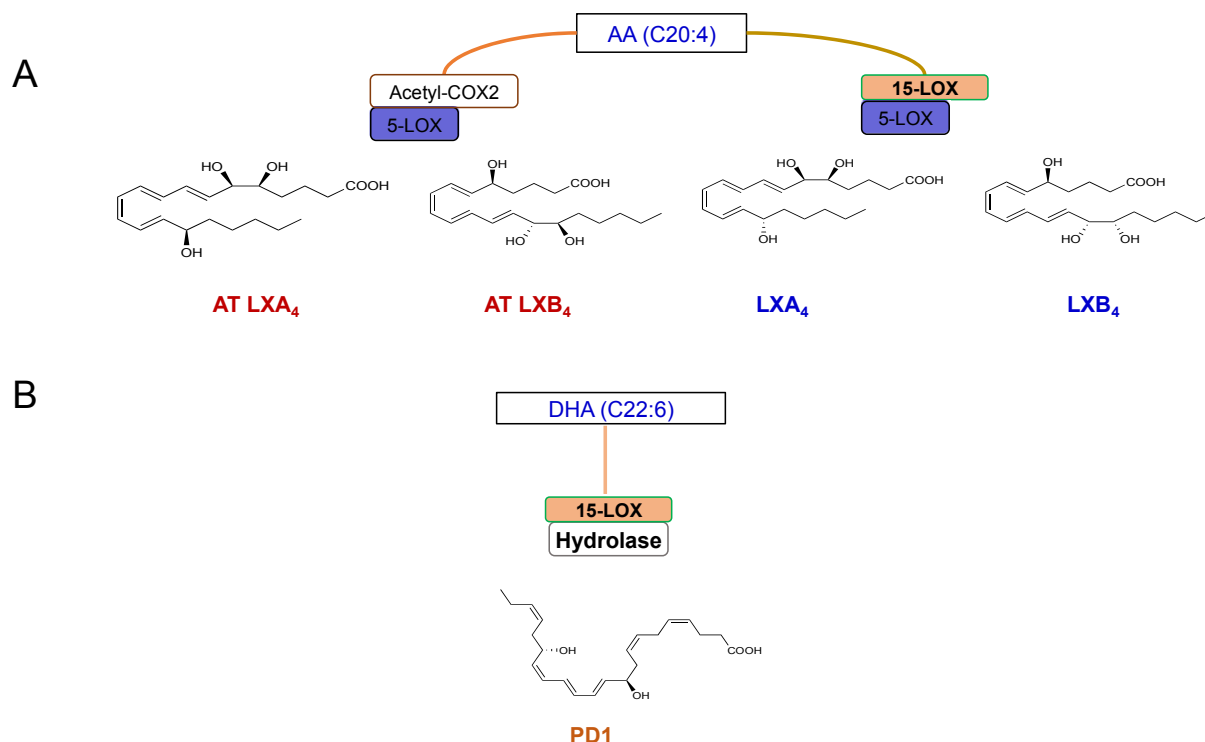


Fig. 1. Biochemical Pathway for SPM generation. **(A)** Biosynthesis of Lipoxin family. Arachidonic acid (AA; C20:4n-6) conversion to LXA₄ and LXB₄ utilizes the enzymes 15-lipoxygenase (15-LOX) and 5-lipoxygenase (5-LOX). The aspirin triggered lipoxins are generated through enzymatic conversion by aspirin acetylated COX2 and/or cytochrome P450 mechanisms (not shown) independent of aspirin. **(B)** Biosynthesis of protectins. Docosahexaenoic acid (DHA; C22:6n-3) can also serve as a substrate precursor for 15-LOX derived bioactive SPMs. For example, DHA is converted via 15-LOX and hydrolases to protectin D1 (PD1).

asthma patients display impaired protectin D1 biosynthesis.²¹ Similarly, LXA₄ levels are lower in exhaled breath condensates in severe asthma relative to healthy controls¹⁵ and plasma levels of LXA₄ after exercise challenge are lower in children who are prone to exercise-induced bronchoconstriction.²² The lower bioavailability of these important endogenous SPMs in asthmatic patients at baseline and during clinical exacerbations may reflect an insufficiency of pro-resolving signals that fail to mitigate acute inflammation and can underlie a conversion of the acute response to chronic unresolved inflammation. The decreased production of SPMs in severe asthma has been confirmed by multiple investigators across a wide variation in patient demographics (Table 1).

Leukocyte SPM receptor expression is altered in severe asthma

SPMs signal via receptors expressed on a number of leukocyte effector cells. To date, the molecular identity of five SPM receptors have been described (reviewed in²). Primarily, SPMs act as agonists at 7-transmembrane G protein coupled receptors for downstream anti-inflammatory and pro-resolution cell-type specific actions. Additionally, the E-series resolvins can also antagonize leukotriene B₄ at BLT1 receptors to impede NF- κ B activation and neutrophil chemotaxis.²³ The most comprehensively studied receptor is ALX/FPR2, the cognate high affinity receptor for LXA₄ and a number of additional SPMs including 15-epi-LXA₄, resolvin D1 (RvD1), and aspirin-triggered resolvin D1 (AT-RvD1).^{24–28} ALX/FPR2 is expressed on a number of airway leukocytes including macrophages, neutrophils, eosinophils, lymphocytes, natural killer cells, and innate lymphoid cells as well as airway epithelial cells.^{8,24,25,29–31} ALX/FPR2 expression on effector leukocytes is altered in severe asthma. Peripheral blood neutrophil and eosinophil surface expression of ALX/FPR2 is lower in subjects with asthma, in particular severe asthma, relative to healthy controls.¹² In contrast, ALX/FPR2 expression on BAL neutrophils and macrophages is higher in subjects with severe asthma^{7,9} and peripheral blood NK cell ALX/FPR2 expression is also increased in severe asthma.⁸ These studies highlight cell-type specific and anatomic compartment-specific alterations in ALX/FPR2 expression in severe asthma that in combination with deficient levels of LXA₄ may be linked with the phenotype of chronic airway inflammation characteristic of severe asthma.

ALX/FPR2 engages both lipid and protein ligands with distinct downstream actions

With an interesting twist on evolutionary convergence, ALX/FPR2 can interact with a wide variety of lipid and peptide ligands with various affinities.²⁸ Ligand recognition sites on ALX/FPR2 differ in the extracellular and transmembrane domains and trigger divergent downstream signaling cascades that can result in a range of bioactions from counter-regulatory to pro-inflammatory effects³² (Fig. 2). LXA₄ binds ALX/FPR2 with a K_d of approximately 1nM²⁸ at the third extracellular loop and seventh transmembrane domain²⁴ to trigger downstream pro-resolving actions, including inhibition of neutrophil chemotaxis and activation and induction of a switch from proinflammatory to anti-inflammatory cytokine production (Fig. 2). Annexin A1, a glucocorticoid inducible protein, can bind ALX/FPR2 receptors at the N-terminus and second extracellular loop with downstream pro-resolving effects similar to LXA₄.^{28,33} In contrast, serum amyloid A (SAA), an acute phase inflammatory protein, engages the first and second extracellular domain of ALX/FPR2 to transmit pro-inflammatory signals, including neutrophil transmigration and survival and pro-inflammatory cytokine production^{34,35} (Fig. 2). Thus, distinct ALX/FPR2 agonists can engage the same receptor to transmit directly opposing downstream signals and actions.

Importantly, despite their different binding sites, peptide ligands including SAA can allosterically inhibit LXA₄ binding at ALX/FPR2. For example, during acute exacerbations of chronic obstructive pulmonary disease (COPD), plasma levels of SAA far outweigh LXA₄ by a magnitude of several thousand-fold.³⁴ This disproportionate abundance of pro-inflammatory SAA overwhelms LXA₄ signaling at ALX/FPR2 and is associated with increased IL-8 levels in BAL fluid and neutrophil recruitment in acute COPD exacerbation.³⁴ *In vitro*, LXA₄ partially abrogates SAA-mediated IL-8 release from airway epithelial cells further demonstrating that each of these ALX ligands can allosterically inhibit the effect of the other.³⁴

ALX/FPR2 receptor ligand levels are linked to severe asthma

In view of the divergent bioactions that ligands can evoke via the pivotal ALX/FPR2 receptors, select candidate ALX/FPR2 receptor

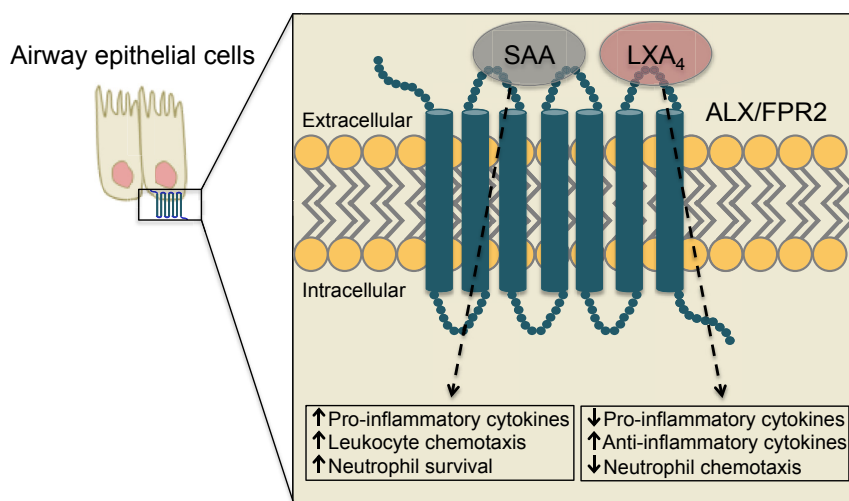


Fig. 2. ALX/FPR2 receptor signaling. ALX/FPR2 is a 7-transmembrane G-protein coupled receptor expressed on a variety of cell types related to asthma pathobiology. ALX/FPR2 is expressed on airway epithelial cells primarily at the basolateral membrane and can engage both lipid and protein ligands to trigger distinct downstream actions. The acute phase inflammatory peptide SAA binds the first and second extracellular domain of ALX/FPR2 to transmit pro-inflammatory signals. In contrast, LXA₄ binds the third extracellular loop and seventh transmembrane domain to initiate pro-resolving actions. Through these distinct binding sites, peptide and lipid ligands can allosterically inhibit the other's interaction with ALX/FPR2 and antagonize downstream signaling pathways.

ligand levels were recently determined in severe asthma subjects to assess whether relative levels of these ligands could provide a biomarker to define sub-populations of asthma patients.⁹ BAL fluid samples were collected from asthmatic and healthy subjects participating in the National Heart, Lung, and Blood Institute's Severe Asthma Research Program-3 (SARP-3) and levels of ALX/FPR2 ligands were measured, including ligands with pro-resolving properties (i.e. LXA₄, 15-epi LXA₄, ANXA1) or pro-inflammatory actions (i.e. SAA). Subjects with severe asthma had significantly lower levels of airway LXA₄ and 15-epi LXA₄ and higher levels of SAA.⁹ Levels of BAL fluid LXA₄ were inversely correlated with asthma symptoms and subjects with lower LXA₄ levels also had lower lung function. Notably, SAA levels were strongly correlated with airway neutrophilia, in distinct contrast with LXA₄ levels that were inversely correlated with BAL neutrophil number. When considered individually, SAA and LXA₄ levels were both associated with asthma severity albeit in opposing directions. More severe asthma patients had low LXA₄ levels and high SAA levels. Conversely, more non-severe asthma subjects had relatively high LXA₄ levels and low SAA levels. Notably, when the levels of both LXA₄ and SAA were combined, there were distinct groups of asthma patients, some with SAA^{hi}LXA₄^{lo} levels and others with SAA^{lo}LXA₄^{hi} levels. The group of asthma patients characterized by SAA^{hi}LXA₄^{lo} levels in BAL fluid had more asthma exacerbations and more asthma-associated comorbidities including sinusitis and gastroesophageal reflux disease and were more likely to be obese. Further, the SAA^{hi}LXA₄^{lo} group of asthma patients had more airway neutrophils, described more asthma symptoms at baseline, and had worse lung function than the SAA^{lo}LXA₄^{hi} group of asthmatics. Defining groups of asthmatic patients by relative levels of the ALX/FPR2 ligands SAA and LXA₄ also aligned with the clinical clusters of asthma subjects defined by the Severe Asthma Research Program.⁵ 71% of subjects with SAA^{hi}LXA₄^{lo} levels in BAL fluid were assigned to one of the severe asthma phenotypic clusters and 64% of subjects with SAA^{lo}LXA₄^{hi} levels were assigned to non-severe asthma clusters.

These findings suggest a potential new asthma biochemical endotype, namely the relative abundance of ALX/FPR2 receptor ligands LXA₄ and SAA. The SAA^{hi}LXA₄^{lo} subset of asthmatics had neutrophilic (i.e. non-type 2) inflammation, poor lung function, and increased symptoms.

Natural killer cell function is impaired in severe asthma

While most asthmatic patients have eosinophilic type 2 airway inflammation that responds to glucocorticoid treatment,⁶ 10–15% of subjects have a mixed inflammatory response marked by severe symptoms that are refractory to anti-inflammatory treatment with corticosteroids.⁴ Recently, immunophenotyping of BAL leukocytes in subjects with asthma characterized natural killer (NK) cell and CD4⁺ T cell subsets and found that the percent of BAL NK cells was significantly lower in subjects with asthma relative to healthy controls.⁷ Further, the ratio of BAL neutrophils and CD4⁺ Th1 and Th17 cells to NK cells was much higher in asthmatics, particularly those with severe asthma, than in health suggesting an association between low numbers of airway NK cells and non-type 2 inflammation with abundant effector T cells and neutrophils.⁷ NK cells are innate lymphocytes that are vitally important for swift host defense against viral pathogens.³⁶ NK cells can also serve as important effectors for the resolution of inflammation by promoting apoptosis of activated granulocytes and T cells to facilitate their clearance by macrophage efferocytosis.^{8,37} A deficiency of lung NK cell numbers or function could predispose to viral exacerbation and defective clearance of tissue leukocytes.

The asthma BAL NK cells were further characterized by surface expression of CD56 and CD16 into CD56^{bright} and CD56^{dim} subsets (Fig. 3A).⁷ CD56^{bright} NK cells are a less mature subset with modest

to no cytotoxic capacity but robust cytokine secretion (Fig. 3B). CD56^{bright} NK cells are most abundant in secondary tissues, including the airway, but comprise only 10–20% of circulating NK cells. CD56^{dim} NK cells are the cytotoxic subset primarily responsible for inducing apoptosis of abnormal or injured cells through targeted delivery of cytotoxic mediators, including perforin, granzymes and granulysin (Fig. 3B). In health, ≥ 75% of circulating NK cells are CD56^{dim} but this subset is less abundant in the airway (Fig. 3A). The BAL NK cell pool in asthmatic patients has fewer CD56^{bright} and more CD56^{dim} NK cells than in healthy individuals. The ratio of CD56^{bright} to CD56^{dim} BAL NK cells in healthy subjects is about 7:1 respectively, which is significantly higher than the ratio of about 4:1 in severe asthma patients, highlighting a relative accumulation of CD56^{dim} BAL NK cells in severe asthma. Of interest, in asthma, lung function was inversely correlated with the number of BAL CD56^{dim} NK cells. Increased CD56^{dim} NK cells in severe asthma BAL was also associated with increased BAL fluid levels of granzyme A, a protease that can degrade matrix proteins when released into the extracellular space and has been associated with chronic inflammatory diseases.³⁸ Together, these findings suggest that a skewed repertoire of NK cell subsets in the asthmatic airway may promote excess extracellular release of cytotoxic mediators promoting chronic airway non-type 2 inflammation and remodeling that may contribute to compromised lung function and increased symptoms.

Peripheral blood NK cells from asthma patients are less effective *ex vivo* at inducing apoptosis of myeloid target cells than NK cells from healthy donors.⁷ Corticosteroids are commonly used as anti-inflammatory agents in the treatment of asthma and *in vitro* exposure to corticosteroids significantly impairs the ability of NK cells to induce target cell apoptosis in both asthma and health.⁷ Peripheral blood NK cells express ALX/FPR2 and exposure to LXA₄ enhances NK cell-mediated apoptosis of autologous granulocytes in asthma.⁸ Exposure of healthy donor NK cells to LXA₄ and a corticosteroid partially blunts the corticosteroid's suppressive effects on NK cell cytotoxicity; however, asthmatic NK cells were not similarly rescued from the suppressive effects of corticosteroids by LXA₄. The inability of LXA₄ to blunt steroid-mediated suppression of NK cell function in asthma may reflect a lower availability of ALX/FPR2 on CD56^{dim} cytotoxic NK cells in asthma. Thus, asthma BAL CD56^{dim} NK cells with lower expression of ALX/FPR2 and low airway levels of LXA₄^{11,12} may be predisposed to immunosuppression by chronic corticosteroid exposure. Together, several aspects of NK cell dysfunction appear to contribute to the pathogenesis of severe asthma and highlight a potential unintended consequence of steroid administration that may perpetuate chronic lung inflammation by increasing host susceptibility to viral infection and disabling NK cell-mediated leukocyte apoptosis for clearance in catabasis.

Role for NETs and cytoplasts in non-type 2 inflammation in severe asthma

Efforts to determine the cellular and molecular profile in asthmatics have involved a combinatorial approach of genetic analysis and incorporation of clinical data to identify distinct phenotypes that correlate with disease severity.⁴ Of interest, many adults with severe asthma have increased sputum neutrophilia either alone or in conjunction with sputum eosinophilia.³⁹ This neutrophilic response suggests distinct immuno-pathogenetic roles for non-type 2 mechanisms in adult asthma. These patients also exhibited diminished lung function, were on high-dose inhaled corticosteroids, and had increased frequency of comorbidities, consistent with severe disease. Identifying the immunophenotype and spectrum of disease severity in asthma has been an important stepping-stone in closely examining the associated molecular immune mechanisms that are evoked in these patients.

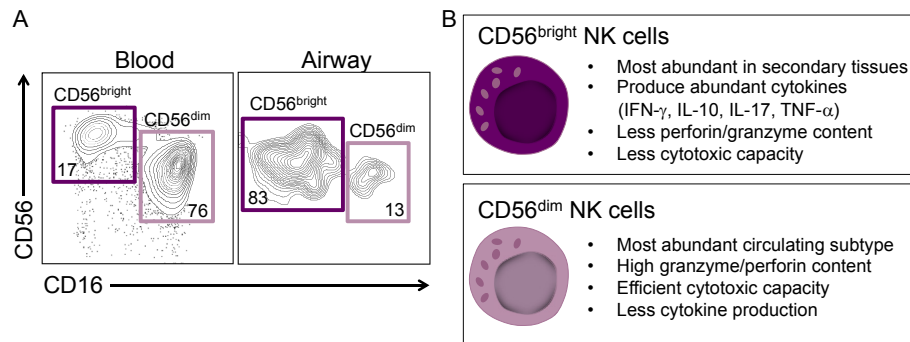


Fig. 3. NK cell subsets have distinct functions. Human NK cell subsets can be defined by surface expression of CD56 and CD16. (A) CD56^{bright}CD16⁻ NK cells (dark purple) and CD56^{dim}CD16⁺ NK cells (lavender) have differential distribution in the peripheral blood relative to airway (bronchoalveolar lavage). Numbers denote a representative percent of total CD3⁺NKp46⁺ NK cells for each subset in BAL from a healthy human subject. (B) CD56^{bright} and CD56^{dim} NK cells have distinct functional profiles.

Neutrophils are important immune effectors for host defense against pathogens, particularly bacteria and viruses. However, in the setting of non-infectious disease, an increased presence of neutrophils in the setting of autoimmune and sterile inflammation, highlights a potential Janus role for neutrophils in bystander tissue injury and chronic inflammation.

The production of neutrophil extracellular traps (NETs) in NETosis produces DNA structures from nuclear contents that can interact with pathogens. When neutrophils encounter specific, particularly threatening stimuli, they degranulate and undergo NETosis. The most common NETosis pathway triggers cell death resembling the process of apoptosis with nuclear delobulation, followed by rapid disassembly of the nuclear envelope and chromatin decondensation resulting in plasma membrane rupture.⁴⁰ Upon membrane rupture, DNA is ejected in a fascinating process resembling “cell-suicide”.^{40,41} A second pathway is non-lytic and sometimes termed “vital” NETosis, which involves the expulsion of nuclear chromatin accompanied by resealing of the plasma membrane to form enucleated cytoplasts (Fig. 4).^{42,43} Vital NETosis occurs *in vivo* in response to bacterial infection (e.g. *Staphylococcus aureus*)⁴² and endotoxin exposure.¹⁰

Recently, NETs have been identified in several lung pathologies. For example, NETs have been detected in the airways of subjects with asthma and COPD, linking NETosis and innate immune activation to the pathogenesis of chronic airway disease.⁴⁴ In patients suffering from COPD, NETosis occurs during acute exacerbations, although NETs are also detectable in stable phase COPD, and

correlate significantly with the severity of airflow obstruction.⁴⁵ Increased NET production occurs in asthma compared to healthy controls following exposure to *Rhinovirus*.⁴⁶ Viral infections are a common trigger for asthma exacerbation, underscoring a link between NETs as a host defense response to viral infection and the pathogenesis of airway inflammation in asthma exacerbation. NET production is also increased in allergen-challenged mice infected with *Rhinovirus* and of interest, disrupting NETs by instilling DNase protects the mice from an exuberant type 2 inflammatory response.⁴⁶

Most recently, BAL samples from a subset of patients with severe asthma and high neutrophil counts were discovered to have detectable NETs.¹⁰ Importantly, in addition to the NETs, enucleated neutrophil cytoplasts were also detected in these patients, indicative of vital NETosis. Of note, cytoplast numbers were positively correlated with IL-17 levels and BAL neutrophilia in severe asthma.¹⁰ To better understand the molecular mechanisms linking cytoplasts to non-type 2 inflammation in severe asthma, a pre-clinical murine model was designed using concomitant exposure to allergen and endotoxin during the sensitization phase, which resulted in the production of NETs and intact cytoplasts. Interestingly, the exposure to allergen and endotoxin solely during the sensitization phase changed the immune response to later airway allergen challenge. Early allergen and endotoxin exposure led to allergen-initiated neutrophilia and elevated IL-17 levels compared to allergen-induced eosinophilia in mice that were exposed to allergen alone during sensitization. Administration of DNase to

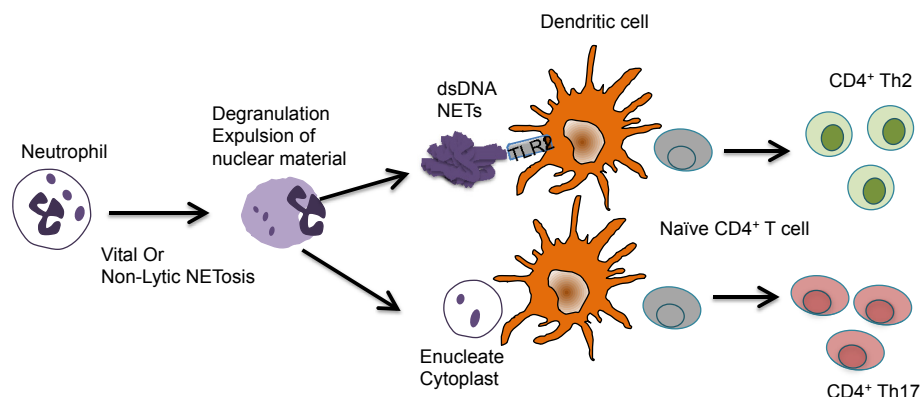


Fig. 4. Vital NETosis leads to neutrophil extracellular traps (NETs) and cytoplasts. NETosis can proceed in a non-lytic manner (aka “vital” NETosis). This process involves the expulsion of nuclear chromatin accompanied by formation of enucleated neutrophil cytoplasts. These cells exhibit distinct functional responses compared to the parent neutrophils. The ejected double-stranded DNA (dsDNA) can interact with dendritic cells via TLR2 receptors promoting the generation of CD4 Th2 cells. In contrast, cytoplasts can influence dendritic cells to evoke allergen-driven Th17 cells and non-type 2 inflammation with neutrophilia.

assess the effect on vital NETosis only reduced eosinophilia, implicating a critical role for cytoplasts in the allergen-mediated neutrophilic responses. Peptidyl arginine deiminase 4-deficient mice displayed defective NETosis, and allergen-driven airway challenge resulted in significantly reduced lung neutrophils and cytoplasts, with a concomitant decrease in IL-17 and IFN- γ , implicating a role for cytoplasts rather than the NETs in driving the non-type 2 immune responses to allergen.¹⁰ The cytoplasts clearly displayed chemokinesis in microfluidic chambers and migratory capacity as they were also detected in the lung-draining mediastinal lymph nodes. Further, the cytoplasts but not intact neutrophils instructed lung dendritic cells to educate naïve CD4⁺ T cell differentiation into antigen-specific Th17 effector cells. Cytoplasts also exhibited distinct cellular cues compared to neutrophils, such as increased expression of MHCII, indicating a potential role for these cells to directly instruct CD4⁺ T cell differentiation. Taken together these studies highlight a pathogenic mechanism for NETs and cytoplasts in airway disease, in particular non-type 2 immune responses. Detection of cytoplasts in airway samples may provide a biomarker for IL-17 mediated disease.

Conclusion

The lung is frequently exposed to airborne particles and potential pathogens, highlighting the imperative to maintain strong mucosal border regulatory mechanisms for airway homeostasis. The exaggerated and unrestrained immune response triggered in asthmatics indicates that these counter-regulatory mechanisms for resolution are insufficient or defective. Translational research has uncovered molecular and cellular heterogeneity in asthma pathogenesis. Syndromic asthma that is severe is most commonly associated with a mixed granulocytic inflammatory response that includes non-type 2 mechanisms. SPM production and their signaling pathways that are central to the pro-resolving circuitry for inflammation are defective in severe asthma, further contributing to the exuberant airway inflammation in this disease.^{2,47}

Because severe asthma patients do not respond sufficiently to corticosteroids, there is an important unmet need for discovering novel therapeutics. NK cell cytotoxic function is disabled in part by steroids in severe asthma.⁷ These NK cells express SPM receptors and SPMs can in some instances counter the deleterious effects of corticosteroids on the effector function of NK cells.⁷ Together, these findings suggest that some severe asthma patients already refractory to the beneficial actions of corticosteroids may be harmed by the steroids, increasing susceptibility to viral infection and asthma exacerbations. SPMs offer the potential to control inflammation and augment host defense in these patients.

The severe asthma BAL neutrophil to NK cell ratio was markedly increased relative to non-severe asthma,⁷ as was the SAA to LXA₄ ratio that was strongly associated with BAL neutrophilia as well as asthma symptoms.⁹ An increased SAA/LXA₄ ratio may be an indicator of inadequate resolution responses and has the potential to be used to target patients for SPM pharmacology.

The recognition that a subset of severe asthma patients with exaggerated neutrophilic responses have increased vital NETosis has provided a cellular (i.e. cytoplasts) and molecular (i.e. IL-17) mechanism that could be leveraged as biomarkers for targeted therapy rather than steroids.¹⁰ The increased levels of cytoplasts seen in these patients correlated significantly with IL-17 levels in BAL fluid, raising the possibility for anti-IL-17 therapy. A recent randomized, double-blind study of brodalumab, a human anti-IL-17 receptor targeted monoclonal antibody, reported little success in alleviating clinical symptoms in severe asthma patients⁴⁸; however, patients were not selected based on IL-17 levels or neutrophilia. More translational research with targeted SPM or anti-IL-17

approaches are needed to determine the potential benefit of these alternate therapeutic strategies to corticosteroids.

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Conflict of interest

BDL is a co-inventor on patents related to SPMs in asthma that are owned by Brigham and Women's Hospital and licensed for commercial development. The rest of the authors have no conflict of interest.

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