

Therapeutic approaches to asthma–chronic obstructive pulmonary disease overlap syndromes

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The recognition that there are some patients with features of asthma and chronic obstructive pulmonary disease (COPD) has highlighted the need to develop more specific treatments for these clinical phenotypes. Some patients with COPD have predominantly eosinophilic inflammation and might respond to high doses of inhaled corticosteroids and newly developed specific antieosinophil therapies, including blocking antibodies against IL-5, IL-13, IL-33, and thymic stromal lymphopoietin, as well as oral chemoattractant receptor-homologous molecule expressed on T_H2 cells antagonists. Other patients have severe asthma or are asthmatic patients who smoke with features of COPD-induced inflammation and might benefit from treatments targeting neutrophils, including macrolides, CXCR2 antagonists, phosphodiesterase 4 inhibitors, p38 mitogen-activating protein kinase inhibitors, and antibodies against IL-1 and IL-17. Other patients appear to have largely fixed obstruction with little inflammation and might respond to long-acting bronchodilators, including long-acting muscarinic antagonists, to reduce hyperinflation. Highly selected patients with severe asthma might benefit from bronchial thermoplasty. Some patients with overlap syndromes can be conveniently treated with triple fixed-dose combination inhaler therapy with an inhaled corticosteroid, long-acting β_2 -agonist, and long-acting muscarinic antagonist, several of which are now in development. Corticosteroid resistance is a feature of asthma-COPD overlap syndrome, and understanding the various molecular mechanisms of this resistance has identified novel therapeutic targets and presented the

prospect of therapies that can restore corticosteroid responsiveness. (*J Allergy Clin Immunol* 2015;136:531-45.)

Key words: Corticosteroids, bronchodilator, cytokine, chemokine, IgE, kinase, p38 mitogen-activated protein kinase, bronchial thermoplasty, corticosteroid resistance, macrolide

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Asthma and chronic obstructive pulmonary disease (COPD) are distinct clinical entities with different management strategies, although in clinical practice they are often treated with the same medications. However, some patients appear to have features of both diseases, which is termed asthma–chronic obstructive pulmonary disease overlap syndrome (ACOS).^{1,2} ACOS is poorly defined and understood and includes several phenotypes that might necessitate different therapeutic approaches: (1) patients with COPD who have increased eosinophil counts that might respond to corticosteroids or specific antieosinophil therapy, (2) asthmatic patients with severe disease or who are current smokers who have predominantly neutrophilic inflammation, and (3) asthmatic patients who have had largely irreversible airflow obstruction and might or might not have increased inflammation. Examination of sputum cellularity can identify patients with airway disease with eosinophil-predominant, neutrophil-predominant, mixed-pattern, and no inflammation (paucigranulocytic).³ The different therapeutic options are shown in [Fig 1](#) and [Table I](#).

TREATING EOSINOPHILIC COPD

The presence of increased eosinophil counts in sputum (>3% or blood >400/dL) might predict a clinical response to inhaled corticosteroids (ICSs), and these patients can have increased airway reversibility.⁴⁻⁷ These patients can have both asthma and COPD (because these are both common diseases), or they might represent a variant of COPD. It is appropriate to treat these patients with bronchodilators and ICSs. However, even high doses of oral corticosteroids might not suppress eosinophils and eosinophil activation markers in patients with COPD.⁸ This suggests that more specific antieosinophilic therapy might be more effective in the face of such corticosteroid resistance ([Fig 2](#)).

Anti-IL-5

Patients with eosinophilic COPD have increased *IL-5* concentrations in sputum, suggesting that targeting *IL-5* might be beneficial in these selected patients with COPD.⁹ *IL-5* can be targeted by blocking antibodies (mepolizumab and reslizumab) or its

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Terms in boldface and italics are defined in the glossary on page 532.

Abbreviations used

ACOS:	Asthma–chronic obstructive pulmonary disease overlap syndrome
COPD:	Chronic obstructive pulmonary disease
CRTH2:	Chemoattractant receptor-homologous molecule expressed on T _H 2 cells
ERK:	Extracellular signal-regulated kinase
GR:	Glucocorticoid receptor
HDAC2:	Histone deacetylase 2
ICS:	Inhaled corticosteroid
IKK2:	I κ B kinase
ILC2:	Type 2 innate lymphoid cell
JAK:	Janus-activated kinase
JNK:	c-Jun N-terminal kinase
LABA:	Long-acting β_2 -agonist
LAMA:	Long-acting muscarinic antagonist
MAPK:	Mitogen-activated protein kinase
MKP:	Mitogen-activated protein kinase phosphatase
NF- κ B:	Nuclear factor κ B
Nrf2:	Nuclear factor erythroid-derived 2 like 2
PDE:	Phosphodiesterase
PGD ₂ :	Prostaglandin D ₂
PI3K:	Phosphoinositide 3-kinase
ROR γ t:	Retinoic acid–related orphan receptor γ t
Treg:	Regulatory T
TSLP:	Thymic stromal lymphopoietin

receptor (IL-5 receptor α ; benralizumab). Mepolizumab and reslizumab are effective in reducing exacerbations in patients

with severe asthma with increased sputum eosinophil counts not suppressed by high-dose ICSs or oral corticosteroids.^{10–13} In patients with eosinophilic COPD, benralizumab did not significantly reduce exacerbations or improve symptoms or lung function, and therefore patients with even higher degrees of eosinophilia might be selected in future studies.¹⁴

Anti-IL-13

IL-13 also drives eosinophilia in patients with severe asthma, and anti-IL-13 therapies have shown some benefit in reducing exacerbations in these patients.¹⁵ The role of IL-13 in patients with COPD is uncertain, despite the fact that it can stimulate mucus hypersecretion and airway fibrosis, as well as induction of corticosteroid resistance. In unselected patients with COPD, there was no increase in sputum IL-13 concentrations, but this did not specifically include patients with eosinophilia.¹⁶ Bronchoalveolar lavage studies have demonstrated that patients with COPD have a population of **CD8⁺ T cells** (T_C2 cells) that secrete T_H2 cytokines, such as **IL-4** and IL-13.¹⁷ Plasma **periostin** levels might reflect stimulation of airway epithelial cells by IL-13, and high periostin concentrations have been described in patients with severe asthma who have rhinosinusitis with fixed airway narrowing that can be regarded as a subtype of ACOS.¹⁸ There is a correlation between annual decrease in FEV₁ and periostin expression in bronchial biopsy specimens of asthmatic patients, supporting the idea that IL-13 is linked to structural remodeling in the airways and therefore might be relevant to the development of fixed obstruction in asthmatic patients.¹⁹

GLOSSARY

APOPTOSIS SIGNAL-REGULATING KINASE (ASK1): A kinase that activates c-Jun N-terminal kinase and p38 mitogen-activated protein kinases in response to an array of stresses, such as oxidative stress, endoplasmic reticulum stress, and calcium influx.

BARDOXELONE METHYL: An experimental and orally available semi-synthetic molecule related to sulforaphane that has been reported to have antioxidative and anti-inflammatory properties.

CASPASE-1: An enzyme that proteolytically cleaves other proteins, such as the precursor forms of the inflammatory cytokines IL-1 β and IL-18, into active mature peptides.

CD8⁺ T CELLS: Cytotoxic T lymphocytes that kill cancer cells, infected cells (particularly those infected with viruses), or damaged cells. CD8 T cells often express T-cell receptors that can recognize a specific antigen bound to the class I MHC molecule of infected cells and ultimately kill the cell through release of granzymes and perforin.

c-JUN N-TERMINAL KINASE (JNK): A kinase responsive to stress stimuli, such as cytokines, UV irradiation, heat shock, and osmotic shock. It also plays a role in T-cell differentiation and the cellular apoptosis pathway.

CYCLIC NUCLEOTIDES: Cyclic AMP and cyclic GMP have been recognized as important signaling molecules within cells. Under normal physiologic conditions, cyclic nucleotides regulate a myriad of biological processes, acting as second messengers between an extracellular signal, such as a hormone, neurotransmitter, or cytokine, and the elicited intracellular response.

CYSTEINYL LEUKOTRIENES (cys-LTs): A family of inflammatory lipid mediators synthesized from arachidonic acid by a variety of cells, including mast cells, eosinophils, basophils, and macrophages.

DIMETHYL FUMARATE (BG12): The methyl ester of fumaric acid thought to have immunomodulatory properties without significant immunosuppression. It also works on Nrf2.

DNAzymes: DNA molecules that are catalytically active and can cleave complementary RNA molecules after appropriate binding, directly exerting RNA endonuclease activity.

EXTRACELLULAR SIGNAL-REGULATED KINASE (ERK): A widely expressed protein kinase intracellular signaling molecule that transmits signals from many extracellular agents to regulate cellular processes, such as proliferation, differentiation, and cell-cycle progression.

GATA3: A transcription factor that is an important regulator of T-cell development and plays a significant role in endothelial cell biology. GATA3 is required for the T_H2 differentiation process and secretion after immune and inflammatory responses.

GLUCOCORTICOID-INDUCED LEUCINE ZIPPER (GILZ): A protein that plays a key role in the anti-inflammatory action of glucocorticoids.

HISTONE DEACETYLASE 2 (HDAC2): An enzyme responsible for the deacetylation of lysine residues on the N-terminal part of the core histones (H2A, H2B, H3, and H4). Histone deacetylation produces a tag for epigenetic repression, plays an important role in regulation of inflammatory genes, and mediates the anti-inflammatory effects of glucocorticoids.

I κ B KINASE (IKK2): An enzyme that serves as a protein subunit of I κ B kinase and leads to the activation of nuclear factor κ B.

IL-1 β : A member of the IL-1 cytokine family that is produced by activated macrophages as a proprotein and is proteolytically processed to its active form by caspase 1 by the inflammasome. IL-1 β is an important mediator of the inflammatory response and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis.

IL-2: A cytokine produced by T cells in response to antigenic or mitogenic stimulation, which is required for T-cell proliferation and other activities crucial to regulation of the immune response.

IL-4: A pleiotropic cytokine produced by activated T cells, this cytokine is a ligand for IL-4 receptor $\alpha\alpha$, which also binds IL-13, and might contribute its overlapping functions with IL-13.

IL-5: A major maturation and differentiation cytokine expressed mainly by T_H2 cells and eosinophils in mice and human subjects. IL-5 has been shown to play a critical role in eosinophilic inflammation in patients with allergic diseases.

IL-6: Implicated in a wide variety of inflammation-associated disease states, this cytokine is involved in the maturation of B cells and has been shown to be an endogenous pyrogen capable of inducing fever in patients with autoimmune diseases or infections.

IL-10: A cytokine produced primarily by monocytes, macrophages, and, to a lesser extent, lymphocytes that has pleiotropic effects in immunoregulation and inflammation by limiting the immune response to pathogens and thereby preventing damage to the host. It has a broad spectrum of anti-inflammatory effects.

IL-13: A cytokine produced primarily by T_H2 cells that is involved in switching B cells to IgE-producing cells, stimulating mucus secretion, and indirectly increasing eosinophil counts.

IL-18: A proinflammatory cytokine that augments natural killer cell activity in spleen cells and stimulates IFN- γ production in T_H1 cells. It is generated by the inflammasome through caspase-1.

IL-21: A member of the common γ chain family of cytokines with immunoregulatory activity. The protein plays a role in both the innate and adaptive immune responses by inducing the differentiation, proliferation, and activity of multiple target cells, including macrophages, natural killer cells, B cells, and cytotoxic T cells.

IL-22: A cytokine that has important functions in host defense at mucosal surfaces, as well as in tissue repair. It is unique in that it is produced by immune cells, including T_H cell subsets and innate lymphocytes, but acts only on nonhematopoietic stromal cells and epithelial cells.

IL-25: A cytokine that shares sequence similarity with IL-17 and has been shown to be a proinflammatory cytokine favoring the T_H2 -type immune response when released from airway epithelial cells. IL-25 can induce nuclear factor κB activation and stimulate IL-8 production.

IL-33: A member of the IL-1 family that potently drives production of T_H2 -associated cytokines and is released from airway epithelial cells in response to danger signals.

INNATE LYMPHOID CELLS (ILCS): Innate immune cells that belong to the lymphoid lineage but lack a B- or T-cell receptor and thus cannot respond in an antigen-specific manner. ILCs are a recently described group of cells with physiologic functions analogous to T_H cells and cytotoxic natural killer cells. They have a role in protective immunity and regulation of homeostasis and inflammation, and their dysregulation has been shown to lead to immune pathologies, such as allergy and autoimmune disease.

LPS: An endotoxin found in the outer membrane of gram-negative bacteria that elicits a strong inflammatory responses in animals.

MACROPHAGE MIGRATORY INHIBITORY FACTOR (MIF): A proinflammatory cytokine involved in the innate immune response to bacterial pathogens and counteracts the anti-inflammatory activity of glucocorticoids. The expression of MIF at sites of inflammation suggests a role as a mediator in regulating the function of macrophages in host defense.

MAPK-ACTIVATED PROTEIN KINASE 2 (MK2): In conjunction with p38 MAPK, this kinase is known to be involved in many cellular processes including stress and inflammatory responses, nuclear export, gene expression regulation, and cell proliferation.

MUSCARINIC M_3 RECEPTORS: Members of the G protein-coupled receptor family that play several important roles. They mediate many of the effects of acetylcholine in the central and peripheral nervous system to cause bronchoconstriction and mucus secretion.

NATURAL KILLER (NK) CELLS: A cytotoxic lymphocyte of the innate immune system that produces and releases small toxic granules in the

cytoplasm, which contain proteins, such as perforin and proteases, known as granzymes. NK cells are well-known players in the host rejection of both tumors and virally infected cells.

NEUTROPHIL ELASTASE: A serine proteinase secreted by neutrophils during inflammation that breaks down elastin fibers.

NUCLEAR FACTOR κB (NF- κB): A transcription factor that plays a key role in regulating the immune and inflammatory response to infection. NF- κB is found in almost all animal cell types and is involved in cellular responses to a variety of stimuli.

PERIOSTIN: An extracellular matrix protein that is induced by IL-4 and IL-13 in airway epithelial cells and lung fibroblasts. It has proved to be a potential biomarker of T_H2 -associated airway inflammation and a predictor of airway eosinophilia driven by T_H2 cells.

PROSTAGLANDIN (PG) D_2 : A major prostaglandin produced by mast cells that recruits T_H2 cells, eosinophils, and basophils and might play a role in the development of allergic diseases, such as asthma.

P2X7: A purinoceptor for ATP, this receptor functions as a ligand-gated ion channel and is responsible for ATP-dependent lysis of macrophages through the formation of membrane pores permeable to large molecules.

REGULATORY T (Treg) CELL: $CD4^+CD25^+$ T cells under the control of the transcription factor forkhead box protein 3 that develop and emigrate from the thymus to perform their key role in immune homeostasis. Treg cells secrete immunosuppressive soluble factors, such as IL-9, IL-10, and TGF- β .

ROR γ t: A retinoic acid receptor (RAR)-related orphan receptor protein that in human subjects is encoded by the *RORC* (RAR-related orphan receptor C) gene that appears to be highly restricted to the thymus, where it is expressed exclusively in immature $CD4^+/CD8^+$ thymocytes and lymphoid tissue inducer cells.

SULFORAPHANE: A molecule known for its antioxidant properties found in cruciferous vegetables, such as broccoli. Its antioxidant effects might be due to activation of the transcription factor Nrf2, which regulates antioxidant genes.

γ/δ T CELLS: A small subset of T cells comprising the highest abundance of T cells in the gut mucosa that possess a distinct T-cell receptor (TCR) on their surfaces. These T cells have a TCR that is made up of one γ chain and one δ chain unlike most T cells, which are $\alpha\beta$ T cells.

TGF- β : A cytokine secreted by many cell types, including macrophages, that controls proliferation, cellular differentiation, and other functions in most cells. It regulates immune function and stimulates fibroblast and fibrosis.

THEOPHYLLINE: A bronchodilator that is used to relax the muscles in the bronchial tubes of the lungs but was recently discovered to have anti-inflammatory effects and also to increase HDAC2 activity and reverse corticosteroid resistance.

TNF- α : Secreted primarily by several cell types, this cytokine's primary role is the regulation of immune cells to amplify inflammation. Moreover, it is involved in the regulation of a wide spectrum of biological processes, including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation.

TYPE 2 INNATE LYMPHOID CELLS (ILC2s): ILCs capable of producing the T_H2 cytokines IL-5, IL-9, and IL-13 in response to helminth infection that have been implicated in the development of allergic lung inflammation. They require IL-7 for their development, which activates 2 transcription factors required by these cells: ROR α and GATA3.

TYPE 3 INNATE LYMPHOID CELLS (ILC3s): ILCs found mainly in mucosal tissue that express the retinoic acid receptor (RAR)-related orphan receptor ROR γ t and the signature cytokines IL-17 and IL-22.

TYPE 17 INNATE LYMPHOID CELLS (ILC17s): A subset of ILCs restricted to the mucosal tissue that secrete IL-17, which was shown to be induced by IL-6, TGF- β , and IL-23.

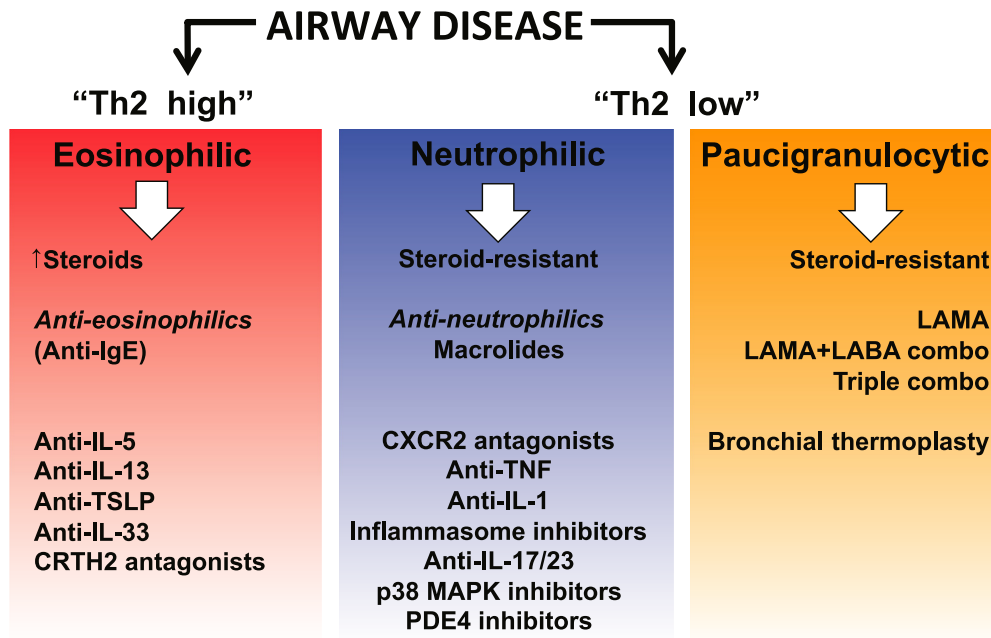


FIG 1. Airway diseases can show different patterns of inflammation in sputum and can be classified as “Th2 high” or “Th2 low.” Th2-high disease is associated with eosinophilic inflammation and usually indicates steroid responsiveness. If there is a poor response to steroids, there might be an indication for antieosinophilic therapies, including mAbs to block IgE, IL-5, IL-13, TSLP, and IL-33 or oral CRTH2 (DP2) antagonists. Th2-low or non-Th2 disease can be neutrophilic and targeted by macrolides; CXCR2 antagonists; antibodies to block IL-1, TNF, IL-17, or IL-23; inflammasome inhibitors; p38 MAPK; or PDE4 inhibitors. Paucigranulocytic disease results in no increase in inflammatory cell counts and might respond to long-acting bronchodilators, which reduce hyperinflation, including LABAs, LAMAs, or their combination or triple combination with an ICS. Selected asthmatic patients might respond to bronchial thermoplasty.

TABLE I. Targeting different inflammatory phenotypes of ACOS

Inflammatory phenotype	Current therapies	New therapies in development
Eosinophilic	ICSs Anti-IgE (omalizumab)	Anti-IL-5/IL-5Rα (mepolizumab, reslizumab, benralizumab)
		Anti-IL-13/IL-4Rα (lebrizumab, tralokinumab, dupilumab)
		GATA3 inhibitors (SB010)
		Anti-IL-33/ST2
		Anti-IL-25
		Anti-TSLP
Neutrophilic	Macrolides	CRTH2 antagonists
		Anti-IL-1, inflammasome inhibitors
		Anti-IL-17/IL-17Rα (ixekizumab, brodalumab)
		Anti-IL-23, anti-p40 (guselkumab)
		CXCR2 antagonists
		p38 MAPK inhibitors/JAK inhibitors
Paucigranulocytic	LAMA (tiotropium) LAMA+LABA (indacaterol/glycopyrronium, vilanterol/umeclidinium) Bronchial thermoplasty	PDE4 inhibitors (roflumilast)
		Triple inhalers (corticosteroid/LABA/LAMA)

IL-4Rα, IL-4 receptor α; IL-5Rα, IL-5 receptor α; IL-17Rα, IL-17 receptor α.

Both IL-5 and IL-13 are regulated by the transcription factor *GATA3*, which is a potential target for therapy.²⁰ An antisense oligonucleotide that cleaves *GATA3* RNA is effective in experimental models of allergic disease and currently in clinical trials in asthmatic patients.²¹ A recent study showed that an inhaled *GATA3 DNzyme* (SB010) significantly reduces the early and late response to inhaled allergen in patients with mild asthma.²²

Anti-thymic stromal lymphopoietin

Thymic stromal lymphopoietin (TSLP) is an upstream cytokine that drives dendritic cells to attract Th2 cells in asthmatic patients and is highly expressed in airway epithelial cells of these patients.²³ A blocking antibody protects against allergen challenge and reduces eosinophilia in asthmatic patients.²⁴ There is a high degree of TSLP expression in airway epithelial cells of

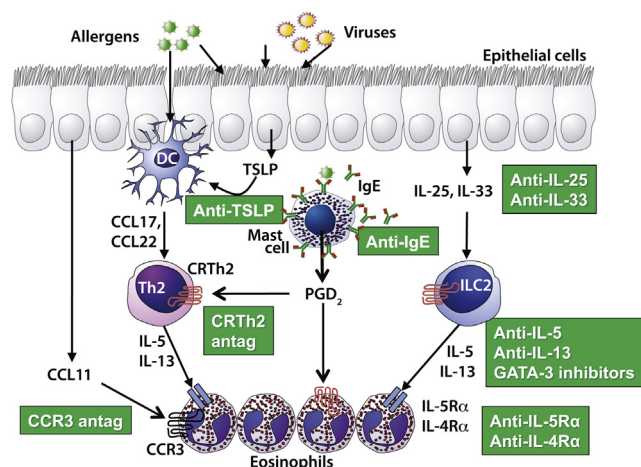


FIG 2. Targeting eosinophilic inflammation in the airways. Eosinophilic inflammation in allergic patients is regulated by T_H2 lymphocytes through allergen-induced activation of dendritic cells (DC) and release of TSLP. Innate type 2 lymphoid cells (ILC2) might be important in patients with nonallergic asthma and are regulated by the alarmins IL-25 and IL-33, which can be blocked by antibodies. Both types of T cells release IL-5 and IL-13, which can be blocked by mAbs or inhibiting GATA-3 or by blocking their receptors (IL-5 receptor α and IL-4 receptor α). Epithelial cells release CCL11 (eotaxin), which acts on CXCR3 expressed on eosinophils that can be blocked by small-molecule antagonists. Mast cells release PGD_2 , which acts on a chemotactic receptor (CRTH2, also known as DP₂ receptor) for which small-molecule antagonists have been developed. Anti-IgE molecules can also reduce eosinophilic inflammation by blocking mast cell activation.

patients with COPD, but it is not clear how this relates to eosinophilic inflammation.²⁵

Chemoattractant receptor-homologous molecule expressed on T_H2 cells antagonists

Chemoattractant receptor-homologous molecule expressed on T_H2 cells (CRTH2) are expressed on T_H2 cells and eosinophils and mediate the chemotactic response to *prostaglandin D₂* (PGD_2), which is secreted predominantly by mast cells.²⁶ Several small-molecule antagonists of CRTH2 (or DP₂ receptors) have been developed, although clinical studies in asthmatic patients have thus far proved disappointing. This might be because the most suitable phenotype of patients has not been selected and better outcomes are seen in patients with eosinophilia (T_H2 high).²⁷ A CRTH2 antagonist provided no clinical benefit (lung function or symptoms) in patients with moderate-to-severe COPD over a 4-week period, but unfortunately, eosinophilic patients were not selected for this study.²⁸

Targeting innate T cells

It has recently been recognized that *innate lymphoid cells* might play an important role in host defense and that *type 2 innate lymphoid cells* (ILC2s) might play a role in orchestrating eosinophilia in response to parasites but could also be important in patients with nonallergic (intrinsic) asthma.²⁹ ILC2s express the transcription factor GATA3 (as do T_H2 cells) and secrete IL-5 and IL-13. They are regulated by *IL-25* and *IL-33* (alarmins) from epithelial cells but not by allergen and might lead to activation of T_H2 cells.³⁰ They could play a role in driving eosinophilia in patients with COPD. ILC2s are relatively corticosteroid

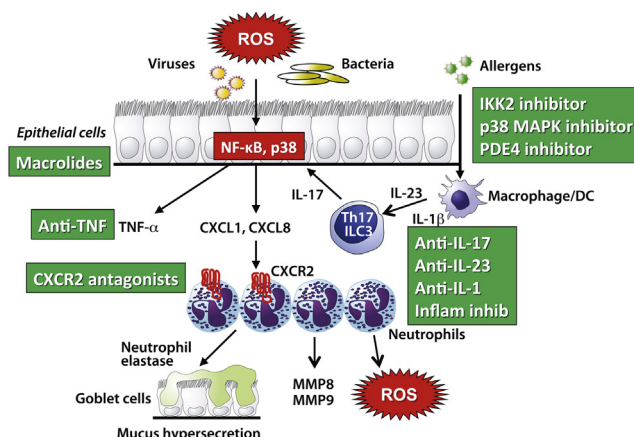


FIG 3. Targeting neutrophilic inflammation in patients with airway disease. Airway epithelial cells and macrophages can be activated by infections and reactive oxygen species (ROS) through activation of NF- κ B and p38 MAPK to release chemokines (CXCL1 and CXCL8), which attract neutrophils through CXCR2 that can be blocked by small-molecule antagonists. Inhibitors of IKK2, p38 MAPK, and PDE4 also inhibit neutrophilic inflammation. Epithelial cells and macrophages can also release TNF- α and IL-1 β , which can be blocked by mAbs. IL-1 release can be blocked by inflammasome inhibitors. Macrophages release IL-23, which acts on T_H17 cells that release IL-17, and can be blocked by mAbs against IL-17, as well as against IL-17 receptor α , which releases chemokines from epithelial cells. Neutrophils can amplify inflammation through releasing ROS, as well as neutrophil elastase and the elastolytic enzymes matrix metalloproteinase (MMP) 8 and 9.

resistant. CRTH2 is expressed on ILC2s, suggesting that CRTH2 antagonist might be beneficial when ILC2s are predominant.

Anti-IL-33

The alarmin IL-33 shows increased expression in airway epithelial cells and circulating T lymphocytes of patients with COPD, with increased circulating concentrations of not only IL-33 but also its soluble receptor, ST2.³¹ IL-33 is expressed in airway epithelial stem cells in patients with COPD and is released by ATP.³² In mice exposed to cigarette smoke, IL-33 expression is increased in airway epithelial cells, and ST2 receptors are suppressed on ILC2s but increased on macrophages and *natural killer cells*, so that there is an enhanced inflammatory response to viral infection that is abolished in IL-33 knockout animals.³³ This suggests that blocking antibodies to IL-33 and ST2 might be beneficial in patients with COPD, particularly in preventing infective exacerbations of the disease.

TARGETING NEUTROPHILIC INFLAMMATION

Increased airway neutrophils counts are found in patients with COPD, some patients with severe asthma, and smoking asthmatic patients, but it is not clear to what extent this inflammation contributes to disease expression. Neutrophils release reactive oxygen species, proteases, and inflammatory mediators, which might exacerbate the disease and are poorly responsive to corticosteroids. This suggests that reducing neutrophilic inflammation might provide clinical benefit, but this must be balanced against the important protective role of neutrophils in innate immune defense and an increased risk of lung infection. This suggests that the optimal strategy might be to reduce airway neutrophil counts to normal levels that are able to provide

sufficient immune defense. There are several mechanisms for reducing airway neutrophilia (Fig 3), although thus far, none have been found to be useful in treating asthma, COPD, or ACOS.³⁴

CXCR2 antagonists

Levels of several chemokines, including CXCL1 (GRO- α), CXCL5 (ENA-78), and CXCL8 (IL-8), are increased in patients with COPD, severe asthma, and smoking asthma and attract neutrophils through the common chemokine receptor CXCR2, which is expressed on both neutrophils and monocytes.³⁵ Because chemokine receptors are G protein–coupled receptors, this has made it possible to develop small-molecule antagonists that can be administered orally. CXCR2 antagonists are effective in inhibiting neutrophilic airway inflammation in response to ozone or endotoxin.^{36–38} In a preliminary report patients with COPD had a significant reduction in sputum neutrophil counts (approximately 50%) and a small improvement in FEV₁, although these were not significant, over 3 months of study with the oral CXCR2 antagonist navarixin.³⁹ However, there was significant neutropenia, with some patients having marked neutropenia. In a larger trial of the same drug over 6 months, there was a small but significant improvement in FEV₁ and a reduction in exacerbations in active smokers only.⁴⁰ There was also a dose-related reduction in circulating neutrophil counts, which led to drug discontinuation in some patients. In a small study of patients with severe asthma with sputum neutrophilia, oral navarixin reduced sputum neutrophil counts by approximately 40%, with some reduction in mild exacerbations but no improvement in FEV₁, symptoms, or asthma control and no reduction in sputum neutrophil activation markers.⁴¹ These studies all suggest that CXCR2 antagonists reduce sputum neutrophilia, although with little or no clinical benefit. This might be because the reduction in sputum neutrophil counts is insufficient or that sputum neutrophil counts are not closely related to disease mechanisms. Increasing the dose of CXCR2 antagonists might be a problem caused by neutropenia that could increase susceptibility to infections.

Blocking proinflammatory cytokines

Levels of proinflammatory cytokines, such as *TNF- α* , *IL-1 β* , and *IL-6*, are increased in patients with COPD and severe asthma and are targets for anti-inflammatory treatments. Anti-TNF blocking antibodies have proved to be unsuccessful in patients with COPD and also in patients with severe asthma, and there appears to be a high risk of adverse effects.^{42–44} *IL-1 β* levels are also increased in sputum of patients with COPD and might increase during exacerbations. The blocking *IL-1* antibody canakinumab, which has been shown to be effective in the rare *IL-1*–overproducing Muckle-Wells syndrome, does not appear to be clinically effective in patients with COPD.⁴⁵ *IL-1 β* can be produced by activation of the NLRP3 inflammasome, which is a multiprotein cytosolic assembly that generates *caspase-1*, which in turn releases *IL-1* and *IL-18* from their precursor proteins. The NLRP3 inflammasome is activated by proinflammatory cytokines through the transcription factor *nuclear factor κ B* (NF- κ B), together with other signals, such as ATP, through *P2X7* receptors and by mitochondrial oxidative stress.^{46–49} The role of inflammasome activation in chronic inflammation in patients with severe asthma and COPD is uncertain, and a recent study showed no NLRP activation in patients with stable COPD. Patients with predominantly neutrophilic asthma also have evidence of NLRP3 activation.⁵⁰

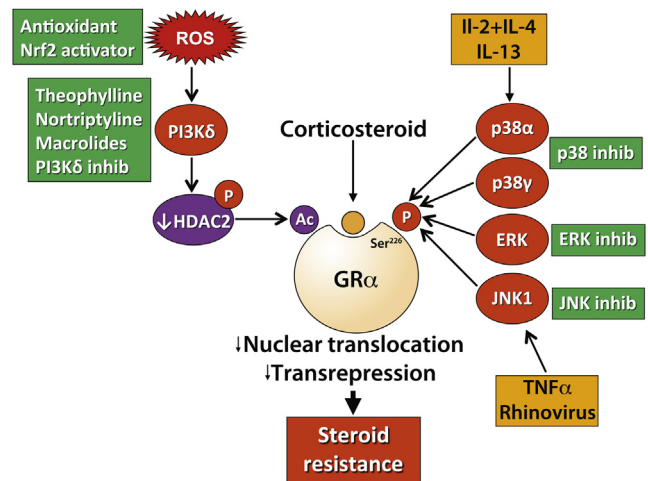


FIG 4. Mechanisms of corticosteroid resistance in asthmatic patients and patients with COPD. In patients with COPD, asthmatic smoking patients, and patients with severe asthma, oxidative stress activates PI3K δ , leading to phosphorylation and reduction in HDAC2 activity, which increases acetylation of GR α , resulting in reduced ability to transrepress inflammatory genes activated by proinflammatory transcription factors. The reduction in HDAC2 activity can be reversed by antioxidants (including Nrf2 activators) and PI3K δ inhibitors, as well as by low-dose theophylline, nortriptyline, and macrolides, which also inhibit this enzyme. GR α can be phosphorylated at serine²²⁶ by MAPKs, including p38 α , p38 γ , JNK, and ERK, which are activated by inflammatory cytokines and reduce translocation of GR α from the cytoplasm to the nucleus. Rhinovirus infection also induces corticosteroid resistance through activation of JNK. There are several selective MAPK inhibitors that can increase GR translocation. ROS, Reactive oxygen species.

However, experimental studies suggest that inflammasome activation might be important in acute exacerbations.⁵¹ There are several approaches to inhibiting the inflammasome, including blocking *IL-1*, *IL-18*, and *caspase-1* but also blocking activating signals with *P2X7* antagonists. Recently, small-molecule inhibitors of NLRP3 itself have been developed that inhibit inflammasome activation in animal models.⁵²

Blocking *IL-17/T_H17*

IL-17A and *IL-17F* can orchestrate neutrophilic inflammation in the lungs through stimulating the release of CXCL1 and CXCL8 from airway epithelial cells.⁵³ A major source of *IL-17* is the CD4⁺ T_H17 cell, which also releases *IL-21* and *IL-22*, expresses the transcription factor retinoic acid–related orphan receptor γ t (*ROR γ t*), and is regulated by *IL-1 β* , *IL-6*, *IL-23*, and *TGF- β* . However, *IL-17* is also released from other cell types, including innate T cells (*type 3 innate lymphoid cells* [ILC3s] or *type 17 innate lymphoid cells* [ILC17] cells), CD8⁺ cells, γ/δ T cells, and B cells.⁵⁴ *IL-17* is an important mediator in autoimmunity and can be involved in patients with severe COPD and severe asthma. *IL-17* is increased in sputum and bronchial biopsy specimens of patients with severe asthma and COPD, suggesting that it might drive neutrophilic inflammation in these patients.^{55–60} *IL-17* and T_H17 cells are relatively insensitive to corticosteroids, and therefore there might be clinical benefit of blocking this axis with other drugs. Blocking *IL-17* and its receptors with antibodies has been effective in the treatment of rheumatoid arthritis and psoriasis, suggesting that it might be of value in treating COPD and severe asthma.⁶¹ Brodalumab, an mAb blocking *IL-17* receptor α , has been tested in patients with severe asthma with no

improvement in asthma control or symptoms, although patients with neutrophilia were unfortunately not selected for this study.⁶² Anti-IL-17 therapies are also being studied in clinical trials in patients with COPD, but no results have been reported thus far. IL-23 is a key regulator of T_H17 cells and can be inhibited by specific antibodies (guselkumab and tildrakizumab) or ustekinumab, which blocks the shared p40 component of IL-12 and IL-23, all of which are effective in the treatment of psoriasis.⁶³ However, the downside of blocking IL-17 and related cytokines might be reduced immunity to infection because the IL-17 axis plays an important role in defending the lungs against bacterial infections through the release of antimicrobial peptides from airway epithelial cells. This could be of concern when there is bacterial colonization of the lower airways in patients with COPD but should be of less concern in asthmatic patients.

There appears to be an imbalance between T_H17 and **regulatory T (Treg) cells** in patients with severe asthma and COPD, which can be demonstrated by measurement of IL-17A and **IL-10** in sputum of these patients.^{64,65} This imbalance can be switched back to normal after treatment with a statin.^{66,67} Statins inhibit T_H17 secretion by inhibiting ROR γ ⁶⁸ through protein geranylgeranylation, which at the same time increases differentiation of forkhead box protein 3-expressing Treg cells.⁶⁹ Further studies on the clinical use of statins in carefully selected patients with asthma or COPD in whom there is evidence for T_H17/Treg cell imbalance are warranted because these drugs are well tolerated and relatively inexpensive.

Kinase inhibitors

Several kinase pathways are activated in patients with COPD and severe asthma and activate transcription factors that result in steroid-insensitive neutrophilic inflammation and are therefore targets for inhibition by small-molecule inhibitors. It has proved to be challenging to develop drugs that are orally available, penetrate cells, and then selectively inhibit specific activated kinases. Many of the inhibitors developed have poor selectivity because they target the ATP binding site that is common to all kinases, resulting in many off-target effects and often resulting in high toxicity, precluding their use in the treatment of chronic inflammation. To overcome these problems, inhaled formulations have been developed that are retained within the lungs to reduce systemic exposure, but thus far, inhaled kinase inhibitors have not been shown to be effective. Sometimes the lack of specificity can be turned to an advantage because blocking several related kinases can provide a greater benefit and might block escape pathways that lead to drug resistance.

Mitogen-activated protein kinases (MAPKs) regulate the expression of many inflammatory genes. The p38 MAPK pathway is activated by cellular stress and regulates the expression of many inflammatory genes that are involved in COPD and severe asthma, including CXCL8, IL-1 β , TNF- α , and MMP-9.⁷⁰ p38 MAPK is activated in alveolar macrophages of patients with COPD and those with severe asthma.^{71,72} More than 20 oral p38 α MAPK inhibitors have entered clinical trials for rheumatoid arthritis and inflammatory bowel disease, but none have reached phase III studies because of side effects and toxicity, as well as poor or transient efficacy.⁷³ Inhibition of p38 α MAPK can lead to activation of compensatory pathways, such as **c-Jun N-terminal kinase** (JNK) and inhibitor of **I κ B kinase** (IKK2), which can be avoided when the downstream kinase **MAPK-activated protein kinase 2** (MK2) is targeted. In patients with COPD, oral p38 α MAPK

inhibitors have provided few clinical benefits, but this might be because doses are limited by side effects, including liver toxicity, skin rashes, and cardiovascular abnormalities.^{74,75} An inhaled p38 α antisense oligonucleotide is effective in suppressing allergic inflammation in mice, indicating the feasibility of local p38 MAPK inhibition.⁷⁶ Several potent and selective inhaled p38 MAPK inhibitors, such as PF-03715455 and GSK610677, have been in clinical development for COPD.⁷⁷ **Apoptosis signal-regulating kinase** is upstream of p38 MAPK and JNK and can be activated by cigarette smoke and oxidative stress and therefore is a potential target for inhibition.⁷⁸ Other MAPKs, including JNK and **extracellular signal-regulated kinase** (ERK), have also been implicated in the inflammation involved in patients with severe asthma and COPD, including responses to growth factors, mucus secretion, and secretion of inflammatory mediators, but thus far, no selective JNK or ERK inhibitors have been tested in patients with severe asthma and COPD.

NF- κ B is activated in patients with asthma and COPD and orchestrates the expression of multiple inflammatory proteins, particularly in patients with severe disease.^{79,80} Inhibitors of IKK2 are effective in some animal models of COPD (**LPS** exposure) but not in others (**neutrophil elastase** instillation), indicating that the effects might be complex.⁸¹ Although several IKK2 inhibitors have been in development,⁸² thus far, none have been clinically developed because of side effects.⁸³ A major concern about long-term inhibition of NF- κ B is that effective inhibitors can result in immune suppression and impair host defenses because mice that lack NF- κ B genes die from overwhelming septicemia.

Phosphoinositide 3-kinases (PI3Ks) are a family of enzymes that lead to the generation of lipid second messengers that regulate a number of cellular events, including innate and adaptive immune responses.⁸⁴ PI3K- γ is involved in neutrophil recruitment and activation. Knockout of the PI3K- γ gene results in inhibition of neutrophil migration and activation, as well as impaired T-lymphocyte and macrophage function, and therefore PI3K- γ inhibitors might be potential anti-inflammatory therapy for patients with COPD. PI3K- δ is also involved in expression of inflammatory genes, and several PI3K- δ or mixed PI3K- γ/δ inhibitors are now in development. Pan-isoform inhibitors of PI3K are likely to be associated with side effects because these enzymes appear to serve a number of key cell functions, but the PI3K- γ and PI3K- δ isoforms have a distribution more restricted to leukocytes and can therefore be better tolerated, especially if delivered by means of inhalation. PI3K- δ inhibitors also have the potential to reverse corticosteroid resistance in patients with COPD.⁸⁵ Several companies are developing inhaled PI3K- δ or PI3K- γ/δ inhibitors for the treatment of severe asthma and COPD.⁸⁶

Janus-activated kinases (JAKs), which activate signal-activated transcription factors, play an important role in regulation of the inflammatory cytokines involved in asthma and COPD, and several selective JAK inhibitors have now been tested clinically.⁸⁷ JAK inhibitors are now approved for the treatment of arthritis, although side effects are dose limiting and there is concern about hematopoietic side effects. This has led to the development of inhaled JAK inhibitors for the treatment of severe asthma and COPD, but thus far, there are no clinical studies reported.

Phosphodiesterase inhibitors

Phosphodiesterases (PDEs) break down **cyclic nucleotides** (cyclic AMP and cyclic GMP) in cells and thereby activate them, so

that PDE inhibition is generally anti-inflammatory. PDE4 is the predominant PDE in neutrophils, T cells, and macrophages, suggesting that PDE4 inhibitors would be effective in controlling inflammation in patients with COPD and severe asthma.^{88,89} A selective PDE4 inhibitor, roflumilast, reduces sputum neutrophil and eosinophil counts in patients with COPD, suggesting that it might be suitable for treating different forms of ACOS.⁹⁰ Roflumilast provides only modest clinical benefit in patients with COPD, even when the most favorable phenotype of patient is selected with severe disease, chronic bronchitis, and a history of exacerbations.^{91,92} Roflumilast also provides some clinical benefit to asthmatic patients but has not been studied in those with severe disease.⁹³ Side effects, including nausea/vomiting, diarrhea, headaches, and weight loss, are a problem and limit the dose that can be administered chronically. This problem could be overcome by inhaled delivery, but to date, 3 inhaled PDE4 inhibitors have been found to be ineffective, although well tolerated. Another approach is to develop isoenzyme-selective inhibitors. PDE4D inhibition appears to account for nausea and vomiting, whereas PDE4B inhibition might account for anti-inflammatory effects, so that PDE4B-selective inhibitors might be better tolerated. PDE4B inhibitors have been developed, but clinical data have not yet been reported.⁹⁴ PDE7A is also expressed in the same inflammatory cells as PDE4, and therefore inhibition of PDE7 is potentially beneficial. However, a selective PDE7 inhibitor had only a small anti-inflammatory effect alone but potentiated the anti-inflammatory effects of a PDE4 inhibitor, suggesting that a combined inhibitor might be useful because it should not increase side effects. PDE3 inhibitors can produce bronchodilatation so that dual PDE3/4 inhibitors potentially combine bronchodilatation with anti-inflammatory activity in patients with asthma and COPD.⁹⁵ A nebulized PDE3/4 inhibitor (RPL554) provides bronchodilatation and possible anti-inflammatory effects.⁹⁶ However, there are concerns about the potential cardiovascular toxicity of PDE3 inhibitors, even those administered by means of inhalation, and therefore there remain long-term concerns about this approach.

Macrolides

Some patients with severe asthma are chronically infected with atypical bacteria, such as *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. In patients with infection confirmed by means of PCR and culture, there was a significant improvement in FEV₁ after a 6-week course of clarithromycin.⁹⁷ However, in a larger trial of patients with poorly controlled asthma, treatment with clarithromycin over a 16-week period did not produce any clinically meaningful improvement in asthma control, even in the patients who were PCR positive for atypical bacteria, although there was a significant reduction in airway hyperresponsiveness.⁹⁸ In patients with COPD, long-term treatment with erythromycin and azithromycin significantly reduces exacerbations, particularly in patients with frequent exacerbations.⁹⁹⁻¹⁰²

The beneficial effect of macrolide antibiotics might be explained by their antibiotic actions but could also be due to their anti-inflammatory or immunomodulatory effects. Macrolide antibiotics have long been recognized to have anti-inflammatory effects in addition to their broad-spectrum antibacterial effects, although the molecular mechanism of this effect is still unclear.^{103,104} In mice with steroid-insensitive inflammation induced by *Chlamydia* species infection during allergic

inflammation, clarithromycin was more effective than the nonmacrolide antibiotic amoxicillin, suggesting that the anti-inflammatory action of macrolides is important.¹⁰⁵ There is some evidence that macrolides inhibit NF- κ B, with reduced CXCL8 release and reduced neutrophilic inflammation.¹⁰⁶ They also might increase phagocytosis and efferocytosis, which is impaired in patients with COPD and severe asthma.¹⁰⁷ In addition, macrolides can reverse corticosteroid resistance by inhibiting PI3K.¹⁰⁸ Most of the studies of these nonantibiotic effects of macrolides have been with cultured cell lines and primary cells *in vitro*, and therefore there is a need to study the anti-inflammatory and immunomodulatory effects in clinical studies in patients with asthma and COPD. In patients with severe neutrophilic asthma, a course of azithromycin significantly reduced sputum neutrophil counts and CXCL8 concentrations, with some improvement in symptoms.¹⁰⁹ In patients with severe asthma, although there was no overall benefit of azithromycin over 6 months, there was a significant reduction in exacerbations and improvement in asthma symptoms in the patients with noneosinophilic sputum inflammation.¹¹⁰ This suggests that it might be worth using a therapeutic trial of macrolide antibiotics in patients with severe asthma who have predominantly neutrophilic inflammation. For patients with COPD, it is not clear whether reduction of exacerbations is due to an antibiotic or anti-inflammatory effect, and further studies are warranted. Macrolides have adverse effects, including gastrointestinal disturbance, deafness with azithromycin, and increased cardiovascular risks (cardiac arrhythmias) but particularly the likelihood of infection with macrolide resistant bacterial infection. The patients with COPD most likely to benefit are those with chronic bacterial colonization or who have concomitant bronchiectasis, but further studies are needed in more carefully phenotyped patients. Older patients with mild disease who are exsmokers appear to benefit more from prophylactic azithromycin.¹¹¹

The anti-inflammatory effects of macrolides are mediated through different mechanisms than their antibiotic effects and can be dissociated in macrolide derivatives. Several nonantibiotic macrolides that retain their anti-inflammatory/immunomodulatory effects are now in development as anti-inflammatory therapies.^{112,113} Macrolides could be delivered by means of inhalation to reduce any systemic effects. Novel macrolides, such as solithromycin, are less likely to lead to microbial resistance because they interact at different sites in the bacterial ribosomal binding sites.¹¹⁴

REVERSING CORTICOSTEROID RESISTANCE

Resistance to the anti-inflammatory effects of corticosteroids might be an important factor in determining asthma severity and is a major barrier to effective therapy in patients with severe asthma, smoking asthmatic patients, and patients with COPD. Several molecular mechanisms have now been described to account for corticosteroid resistance in asthmatic patients, including activation of p38 MAPK, JNK and ERK activity, increased expression of an alternatively spliced variant of glucocorticoid receptor (GR) β , increased production of *macrophage migratory inhibitory factor* (MIF), and reduced expression of *histone deacetylase 2* (HDAC2; Fig 4).^{115,116} This suggests that there might be therapies that could reverse corticosteroid resistance and that there might be different phenotypes of

corticosteroid resistance in asthmatic patients and patients with COPD that could require different therapeutic approaches.

Kinase inhibitors

In patients with severe asthma, there is phosphorylation of the GR, which reduces translocation into the nucleus and therefore impairs corticosteroid responsiveness.^{117,118} Several kinases might be involved in this phosphorylation, including p38 MAPK- α , p38 MAPK- γ , and JNK1. A p38 MAPK inhibitor that inhibits the α - and β -isoforms of the enzyme increases the responsiveness to corticosteroids of alveolar macrophages and PBMCs from patients with severe asthma.^{72,119} The effect of a p38 MAPK inhibitor is most marked in the PBMCs from patients with severe asthma, which show the greatest defect in GR translocation.¹¹⁹ Furthermore, the patients with severe asthma with the greatest defect in GR translocation are those with the lowest FEV₁ and the highest use of oral corticosteroids. The p38 MAPK inhibitor was shown to inhibit phosphorylation of serine 226 (Ser²²⁶) on the GR, which is induced by exposure to IL-2 plus IL-4 or IL-13, which are known to induce corticosteroid resistance in PBMCs. A MAPK inhibitor also increases sensitivity to corticosteroids in PBMCs from patients with COPD.¹²⁰ The currently available p38 MAPK inhibitors selectively inhibit the α - and β -isoforms, but the p38 MAPK- γ isoform might also play a role in phosphorylation of GR and steroid resistance.¹²¹ p38 MAPK- γ levels are increased in PBMCs from patients with severe asthma, and knockdown of p38 MAPK- γ by using RNA interference prevents the development of steroid resistance in response to IL-2 plus IL-4. This suggests that pan-p38 MAPK inhibitors might be more effective than the current inhibitors that target p38 MAPK- α/β .

JNK, which is activated by proinflammatory cytokines, also directly phosphorylates the GR at Ser²²⁶ and inhibits glucocorticoid response element binding.¹²² JNK1 is also involved in GR phosphorylation in PBMCs from patients with severe asthma.^{121,123} Rhinovirus infection of human airway epithelial cells induces corticosteroid resistance through phosphorylation of the GR as a result of JNK activation and is completely reversed by a JNK inhibitor.¹²⁴ Microbial superantigens, such as staphylococcal enterotoxin B, have been implicated in patients with intrinsic asthma¹²⁵ and induce steroid resistance in T cells through activation of ERK, which also results in GR phosphorylation.¹²⁶

Mitogen-activated protein kinase phosphatase (MKP) 1 is an endogenous inhibitor of the p38 MAPK and JNK pathways and is activated by corticosteroids.¹²⁷ Macrophages from MKP-1 gene knockout mice show reduced anti-inflammatory responses to corticosteroids *in vitro*.¹²⁸ In alveolar macrophages from patients with severe asthma with reduced steroid responsiveness, there is a significant reduction in MKP-1 expression after corticosteroid treatment, which is correlated with increased p38 MAPK activity.¹²⁹ The serine/threonine phosphatase PP2A is also involved in dephosphorylation of phosphorylated GR.¹²³ PP2A expression and activity are reduced in PBMCs from asthmatic patients with steroid resistance, and knockdown of PP2A or the phosphatase inhibitor okadaic acid with increases in GR Ser²²⁶ phosphorylation reduces nuclear translocation and thereby reduces steroid responsiveness. Coprecipitation studies show that GR, PP2A, and JNK1 are all physically associated. Furthermore, overexpression of PP2A in a macrophage cell line (U937) results in increased steroid responsiveness, indicating the important role of this phosphatase in maintaining steroid responsiveness.

MIF levels are reported to be increased in patients with severe asthma and block the anti-inflammatory effects of corticosteroids, but the molecular mechanisms are poorly understood, and it has been difficult to find drugs that block its actions.¹³⁰ MIF might signal and cause corticosteroid resistance through the activation of p38 MAPK via inhibition of MKP-1 mediated through an effect on the protein *glucocorticoid-induced leucine zipper* (GILZ).¹³¹

HDAC2 activation

There is growing evidence that corticosteroid resistance in patients with COPD is due to a reduction in HDAC2 activity and expression as a result of oxidative and nitrative stress.¹³² This results in increased acetylation of the GR, which prevents it from inhibiting NF- κ B-driven inflammation.¹³³ A similar mechanism might underlie corticosteroid resistance in smoking asthmatic patients and even in those with severe asthma because of increased oxidative stress from endogenously generated oxidants.^{134,135} In children exposed to passive cigarette smoke at home, there is also evidence for reduced HDAC2 activity and increased PI3K activity in alveolar macrophages with reduced corticosteroid responsiveness.¹³⁶ Exacerbations of COPD induced by experimental rhinovirus infection result in a further reduction in HDAC2 activity, which might contribute to amplified inflammation during viral exacerbations of COPD.¹³⁷

Therefore increasing HDAC2 activity might be an attractive approach for reversing corticosteroid resistance in patients with severe asthma, smoking asthmatic patients, and patients with COPD. Restoration of HDAC2 activity with a plasmid vector fully restores steroid responsiveness in macrophages from patients with COPD that are normally resistant, providing important evidence that increasing HDAC2 activity is a potentially useful therapeutic strategy.¹³³ There are several drugs that can increase HDAC2 activity that has been reduced as a consequence of oxidative stress, suggesting that these therapies might reverse the steroid resistance associated with oxidative stress in patients with COPD, patients with severe asthma, and smoking asthmatic patients.

Selective activation of HDAC2 can be achieved with low therapeutic concentrations of *theophylline*, which restores HDAC2 activity in macrophages from patients with COPD back to normal and thereby restores corticosteroid responsiveness.^{138,139} In cigarette smoke-exposed mice, which have steroid-resistant inflammation, oral theophylline is also effective in reversing steroid resistance.⁸⁵ In patients with COPD, a low dose of oral theophylline combined with an ICS is more effective in reducing inflammation in sputum than either drug alone.¹⁴⁰ This action of theophylline is independent of PDE inhibition and mediated by direct inhibition of oxidant stress-activated PI3K δ .^{85,141} Larger clinical trials with low-dose theophylline combined with oral corticosteroids or ICSs are now underway in patients with COPD. Low-dose theophylline is also effective in improving responses to ICSs in active smokers with asthma who have a poor response to low-dose ICSs.¹⁴² Previous studies have shown that controlled withdrawal of theophylline therapy causes worsening of both severe asthma and COPD, indicating that this is likely to be an effective therapeutic approach.^{143,144}

The tricyclic antidepressant nortriptyline also increases HDAC2 activity and reverses steroid resistance by directly inhibiting PI3K δ .¹⁴⁵ Selective PI3K δ inhibitors, such as IC87114, are similarly effective.^{85,141} As discussed above,

inhaled PI3K δ inhibitors are now in clinical development for COPD and severe asthma.

Oxidative stress is a major mechanism leading to steroid resistance in patients with COPD, patients with severe asthma, and smoking asthmatic patients through reduced HDAC2 activity, suggesting that antioxidants should also reverse steroid resistance. Currently, available antioxidants are poorly effective because they are inactivated by oxidative stress, but there is now considerable interest in activators of the transcription factor nuclear factor erythroid-derived 2 like 2 (Nrf2), which regulates the expression of multiple antioxidant genes. Nrf2 activity is markedly reduced in cells from patients with COPD and appears to be a consequence of reduced HDAC2 activity, which normally keeps this protein deacetylated and active.¹⁴⁶⁻¹⁴⁸ **Sulforaphane** is an activator of Nrf2 and has been shown to increase HDAC2 levels and reverse steroid resistance in mice exposed to cigarette smoke and in macrophages from patients with COPD.¹⁴⁸ **Sulforaphane** is nonselective and toxic in high concentrations, leading to a search for safer and more selective Nrf2 activators. **Bardoxelone methyl** is more potent than sulforaphane but appears to be toxic in clinical studies.¹⁴⁹ **Dimethyl fumarate** (BG12) is also an Nrf2 activator that is better tolerated and has been approved for the treatment of multiple sclerosis but is also nonspecific.¹⁵⁰ There is now a search for more specific Nrf2 activators.¹⁵¹

Curcumin (diferuloylmethane), which is found in the turmeric spice used in curry, also increases HDAC2 activity after it has been reduced by oxidative stress and at concentrations lower than required for its antioxidant effects.¹⁵² Macrolides also restore HDAC2 activity through inhibition of PI3K pathways, providing another therapeutic option.¹⁵³

Overall, the novel strategy of reducing corticosteroid resistance in patients with severe asthma, smoking asthmatic patients, and patients with COPD looks promising and can be achieved with existing, well-tolerated drugs, such as low-dose theophylline, nortriptyline, and macrolides, or with novel therapies, such as inhaled PI3K δ or p38 MAPK inhibitors. Appropriate clinical studies are now needed to confirm these experimental studies.

BRONCHODILATATION

Long-acting inhaled bronchodilators, including long-acting β_2 -agonists (LABAs) and long-acting muscarinic antagonists/anticholinergics (LAMAs), are the mainstay of treatment in patients with COPD and are equally effective because they reduce air trapping by relaxing airway smooth muscle as a result of reducing the effects of intrinsic cholinergic tone.¹⁵⁴ Air trapping increases with exertion (dynamic hyperinflation), leading to exertional dyspnea, which is the major symptom of COPD.¹⁵⁵ In asthmatic patients LABAs are generally more effective bronchodilators than LAMAs because they act as functional antagonists to inhibit not only cholinergic tone but also mainly the bronchoconstrictor effects of mast cell–derived mediators, such as **cysteinyl leukotrienes** (cys-LTs) and PGD₂. In patients with ACOS with eosinophilic COPD, bronchodilators generally have a greater effect than in the usual patients with COPD,⁴ and this could reflect the effects of bronchoconstrictors derived from eosinophils, including cys-LTs and PGD₂, but there might also be mast cell activation, which is not a feature of COPD. Asthmatic patients with neutrophilic inflammation might have less reversible disease and have structural changes leading to irreversible airway obstruction. These patients might benefit particularly from

LAMAs, which might not only reduce cholinergic tone but also have potentially important nonbronchodilator effects. The LABAs salmeterol and formoterol are administered twice daily, but more recently, LABAs with a 24-hour duration of action have been developed, including indacaterol, vilanterol, and olodaterol. Tiotropium is a once-daily LAMA mainly used in the treatment of COPD, but recently, several other LAMAs have been developed, including once-daily glycopyrrolate and umeclidinium and twice-daily aclidinium.¹⁵⁶

LAMAs in asthmatic patients

Several studies have now shown that tiotropium bromide is an effective add-on bronchodilator in patients with severe asthma whose symptoms are not controlled by maximal doses of ICSs and LABAs.^{157,158} Patients with severe asthma have increased air trapping because of small-airway obstruction, as seen in patients with COPD.¹⁵⁹ It is likely that LAMAs can reduce this air trapping through reducing cholinergic tone, although it is surprising that LABA treatment does not achieve this through functional antagonism. However, addition of tiotropium does not improve asthma control, although there is a small reduction in exacerbations.¹⁵⁸ Addition of tiotropium in patients with moderate asthma not controlled by ICSs is similar to the add-on effect of a LABA.^{160,161} This is difficult to understand because the functional antagonism of the LABA should be effective against the bronchoconstrictor effects of mast cell mediators, as well as cholinergic tone. It is possible that there are nonbronchodilator effects of LAMAs mediated through antagonism of **muscarinic M₃ receptors** on inflammatory cells, such as macrophages, T lymphocytes, and neutrophils.^{162,163} In murine models tiotropium suppresses allergen-induced eosinophilia in the lungs through suppression of IL-5 and IL-13 secretion from T_H2 cells,¹⁶⁴ and exposure to inhaled endotoxin tiotropium reduces lung neutrophilia in mice.¹⁶⁵ Thus far, these anti-inflammatory effects of LAMAs have not been demonstrated in either patients with asthma or those with COPD, however.

LABA-LAMA combination inhalers

The bronchodilator response to LABAs and LAMAs might be additive in patients with COPD, suggesting that LABA-LAMA combination inhalers might be more effective than single bronchodilators. In patients with COPD treated with a maximally effective dose of indacaterol, addition of glycopyrrolate approximately doubles the increase in FEV₁.¹⁶⁶ This is difficult to understand because the maximal dose of LABA should inhibit any cholinergic tone and suggests that there might be crosstalk between the M₃ receptor and β_2 -receptor signaling pathways, resulting in greater bronchodilatation. This also produces a greater improvement in symptoms and a small additional effect in preventing exacerbations.^{167,168}

Several LABA-LAMA combinations are now in development for COPD, including once-daily indacaterol plus glycopyrrolate (Ultibro; Novartis, Basel, Switzerland), vilanterol plus umeclidinium (Anoro; GSK, Brentford, United Kingdom), and olodaterol plus tiotropium, as well as twice-daily formoterol and glycopyrrolate and formoterol and aclidinium.¹⁶⁹ Although these LABA-LAMA combinations might be suitable for patients with COPD, they are unlikely to be useful in patients with ACOS or severe asthma because there would be a risk that patients might not

take concomitant ICSs. This has led to the development of triple inhalers.

Triple inhalers

Combination inhalers containing an ICS, LABA, and LAMA are now in clinical development, and one (budesonide, formoterol, and tiotropium) is already on the market in some countries.^{170,171} Many patients with severe COPD are already treated with these therapies (usually in the form of an ICS-LABA combination and a separate LAMA inhaler), with some evidence of additional benefit.^{172,173} Triple inhalers might be suitable for patients with ACOS who need an ICS plus LABA combination with the additional bronchodilation of a LAMA or in patients with severe asthma when a LAMA might provide additional bronchodilation and asthma control. Although triple inhalers might be more convenient for patients, there can be problems in their formulation because the components can react chemically and there could be regulatory requirements to show added benefit. Several triple inhalers are already in clinical trials, including budesonide/formoterol/glycopyrrolate, mometasone/indacaterol/glycopyrrolate and fluticasone furoate/vilanterol/umeclidinium.

Bronchial thermoplasty

Bronchial thermoplasty involves the application of high radio-frequency energy through fiberoptic bronchoscopy to selectively ablate airway smooth muscle in the proximal airways (down to the subsegmental level). This technique was approved by the US Food and Drug Administration for the treatment of patients with severe asthma based on a single controlled clinical trial with a sham control treatment, which showed a reduction in severe exacerbations.¹⁷⁴ However, the primary outcome measure of this trial was quality of life, which showed no clinically significant difference between treatments because there was a large placebo effect. Furthermore, many of the patients included in the study did not fulfill the normally accepted definition of severe asthma, and patients with steroid-dependent asthma were excluded. Subsequent studies have been criticized for a lack of sham procedure control data, making any positive effects difficult to interpret.¹⁷⁵ Although thus far there are no long-term adverse effects (up to 5 years), patients can have exacerbations after the procedure. Another problem is that the procedure does not treat the peripheral airways, which are known to be obstructed in patients with severe asthma. It seems likely that bronchial thermoplasty might benefit those patients with severe asthma in whom there is marked hypertrophy of airway smooth muscle that might be contributing to fixed narrowing of the airways, but thus far, it has been difficult to identify such patients. Patients with COPD would not benefit from the procedure because airway smooth muscle hypertrophy is generally not a feature of this disease.

FUTURE DIRECTIONS

The recognition that there are several phenotypes of asthma, COPD, and ACOS suggest that more specific treatments might be beneficial, particularly in patients whose symptoms are not controlled by maximal doses of currently recommended treatments, such as ICSs and bronchodilators. To select appropriate therapy will require biomarkers that are predictive of response to

specific therapy. These include blood biomarkers, such as eosinophil counts and levels of plasma periostin and fraction of exhaled nitric oxide, which reflects eosinophilic inflammation of the airways; these measures are easily performed in routine clinical practice. In specialized centers sputum cell counts might be useful to distinguish eosinophilic, neutrophilic, mixed, and paucigranulocytic patterns of inflammation.³ Currently, there are no reliable noninvasive biomarkers for neutrophilic inflammation in the airways, either in blood or breath, but there are several approaches in development, including blood proteomics and measurement of volatile organic compounds in the breath.

New treatments are urgently needed for COPD¹⁷⁶ and severe asthma,¹⁷⁷ and several of these therapies are discussed above. It is likely that these new therapies will be effective only in small subpopulations of patients, and therefore it will be important to identify responsive phenotypes, as already well shown for specific antieosinophilic therapies.

It might also be important to treat the disease phenotypes early in their natural history because this will increase the chances of long-term success by preventing long-term structural changes that might be irreversible. There is little information about the long-term stability of inflammatory phenotypes of airway disease and some evidence that the pattern of inflammation in sputum can vary from time to time in asthmatic patients and patients with COPD, which further complicates the selection of treatment.¹⁷⁸

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