Pathophysiology of Bronchiectasis

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Semin Respir Crit Care Med 2021;42:499–512.

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Abstract

Bronchiectasis is a complex, heterogeneous disorder defined by both a radiological abnormality of permanent bronchial dilatation and a clinical syndrome. There are multiple underlying causes including severe infections, mycobacterial disease, autoimmune conditions, hypersensitivity disorders, and genetic conditions. The pathophysiology of disease is understood in terms of interdependent concepts of chronic infection, inflammation, impaired mucociliary clearance, and structural lung damage. Neutrophilic inflammation is characteristic of the disease, with elevated levels of harmful proteases such as neutrophil elastase associated with worse outcomes. Recent data show that neutrophil extracellular trap formation may be the key mechanism leading to protease release and severe bronchiectasis. Despite the dominant of neutrophilic disease, eosinophilic subtypes are recognized and may require specific treatments. Neutrophilic inflammation is associated with elevated bacterial loads and chronic infection with organisms such as *Pseudomonas aeruginosa*. Loss of diversity of the normal lung microbiota and dominance of proteobacteria such as *Pseudomonas* and *Haemophilus* are features of severe bronchiectasis and link to poor outcomes. Ciliary dysfunction is also a key feature, exemplified by the rare genetic syndrome of primary ciliary dyskinesia. Mucus symptoms arise through goblet cell hyperplasia and metaplasia and reduced ciliary function through dyskinesia and loss of ciliated cells. The contribution of chronic inflammation, infection, and mucus obstruction leads to progressive structural lung damage. The heterogeneity of the disease is the most challenging aspect of management. An understanding of the pathophysiology of disease and their biomarkers can help to guide personalized medicine approaches utilizing the concept of "treatable traits."

Keywords
► bronchiectasis
► inflammation
► airway infection
► personalized medicine

Bronchiectasis is characterized by a dysregulated inflammatory response which results in lung damage; abnormal, irreversible dilation of the bronchi; and recurrent bacterial infection. Although once considered an "orphan disease," the incidence and prevalence of bronchiectasis has increased 40% in the past 10 years throughout the world, with rates of up to 566 per 100,000 population being reported.[1,2] Despite this, the pathophysiology and underlying mechanisms of bronchiectasis are still poorly understood.[3,4] The heterogeneity of bronchiectasis coupled with a lack of both experimental and animal models has made research efforts more challenging. Many previous studies have investigated bronchiectasis either in the advanced stages of disease or as part of a specific underlying etiology, such as cystic fibrosis (CF) or primary ciliary dyskinesia (PCD),[5,6] which, although useful, means that our understanding of other stages and etiologies of bronchiectasis are still lacking.

There are many underlying causes of bronchiectasis and in up 50% of patients, no cause is identified (summarized in ►**Table 1**).[7] Causes can include postinfectious, bacterial infections, such as severe pneumonia or tuberculosis; congenital conditions such as CF and PCD; aspiration syndromes; primary or secondary immunodeficiency; hypersensitivity disorders such as allergic bronchopulmonary

Table 1 Etiologies of bronchiectasis

Idiopathic
Postinfective
Bacterial (e.g., pseudomonas, haemophilus)
Viral (e.g., human immunodeficiency virus, influenzae virus, adenovirus)
Mycobacterium tuberculosis *Aspergillus* species
Secondary lung disease
Chronic obstructive pulmonary disease
Asthma
Allergic bronchopulmonary aspergillosis
Interstitial lung disease
Congenital condition
Cystic fibrosis
Primary ciliary dyskinesia
Marfan's syndrome
Williams–Campbell syndrome
Mounier–Kuhn syndrome
Yellow nail syndrome
Young's syndrome
Immunodeficiency
Alpha-1 antitrypsin deficiency
Hypogammaglobulinemia
Secondary to cancer (e.g., chronic lymphatic leukemia, chemotherapy, or immune modulation)
Inflammatory disease
Rheumatoid arthritis
Inflammatory bowel disease
Crohn's disease
Connective tissue disease
Systemic lupus erythematosus
Aspiration/esophageal reflux
Anatomical disruption
Intraluminal airway obstruction (e.g., foreign body)
Intramural obstruction (e.g., complete cartilage rings)
External airway compression (e.g., by tuberculous lymph nodes)

aspergillosis (ABPA); bronchial obstruction; rheumatic conditions; or connective tissue diseases.[7–13] Identifying the underlying cause of bronchiectasis is important, as it guides treatment strategy and management in patients. Although there are guidelines available for the treatment of bronchiectasis,[14] to date no medications have been approved by regulatory bodies in Europe or the United States. This is partly due to the complex, heterogenous nature of the disease. Improving our understanding of the underlying biology and pathophysiology of bronchiectasis is critical to the development of new therapies.

The Vicious Cycle and Vicious Vortex

The hypothesis of a "vicious cycle" of bronchiectasis was first proposed by Cole in 1986.[15] In this model, impaired mucociliary clearance results in an accumulation of airway secretions that disrupt normal host defenses, leaving the patient more vulnerable to infection. Persistent infection triggers an inflammation response which causes abnormal airway remodeling and structural damage. This cycle of events results in a persistent and progressive process with an "entry point" that can be related to the underlying etiology of bronchiectasis. For example, inflammatory bowel disease induces localized inflammation and structural damage; patients with CF and PCD have impaired mucociliary clearance resulting in increased susceptibility to infections[5]; and immunodeficiency can result in chronic infections. It is important to note that the interactions within the cycle are highly complex and will not always take place in a stepwise fashion; it is likely that each component of the pathophysiology contributes to all others. This model was therefore further revised by Flume et al to describe a vicious vortex, where airway dysfunction, airway inflammation, infection, and structural damage are linked.[1] There is convincing evidence for this interconnected concept since, for example, *Pseudomonas aeruginosa* infection induces neutrophilic inflammation by promoting the release of chemotactic factors such as CXCL-8 (chemokine [C-X-C motif] ligand 8), interleukin (IL)-1β, and others, but also directly affects mucociliary clearance through the action of ciliotoxins such as pyocyanin which slows down ciliary beat frequency. *Pseudomonas* elastase may direct damage lung structure. *P. aeruginosa* infection is therefore only one example of why an interdependent vortex rather than cycle model is a more accurate representation of bronchiectasis pathophysiology (►**Fig. 1**).

This may explain why "breaking the cycle" with treatments for bronchiectasis has not consistently demonstrated clinical benefits. Inhaled antibiotics and anti-inflammatory therapies are among the principle therapies for bronchiectasis, but when used individually only target one aspect of the vicious vortex.[16,17] Although inhaled antibiotic treatments may reduce bacterial infection, other components of the vortex can maintain inflammation and structural damage. Antibiotics do not appear to be disease modifying, as bacterial loads return to baseline after discontinuation of antibiotics. This would support the need for multimodality treatment, as targeting only one part of the vortex is not enough to disrupt the cycle and halt the progression of lung damage.

This review will discuss the components of the vicious vortex and consider how they contribute to the disease progression of bronchiectasis.

Mucociliary Function

The conducting airways are predominantly lined by ciliated epithelial cells, followed by secretory and goblet cells and finally a small number of "brush" and neuroendocrine
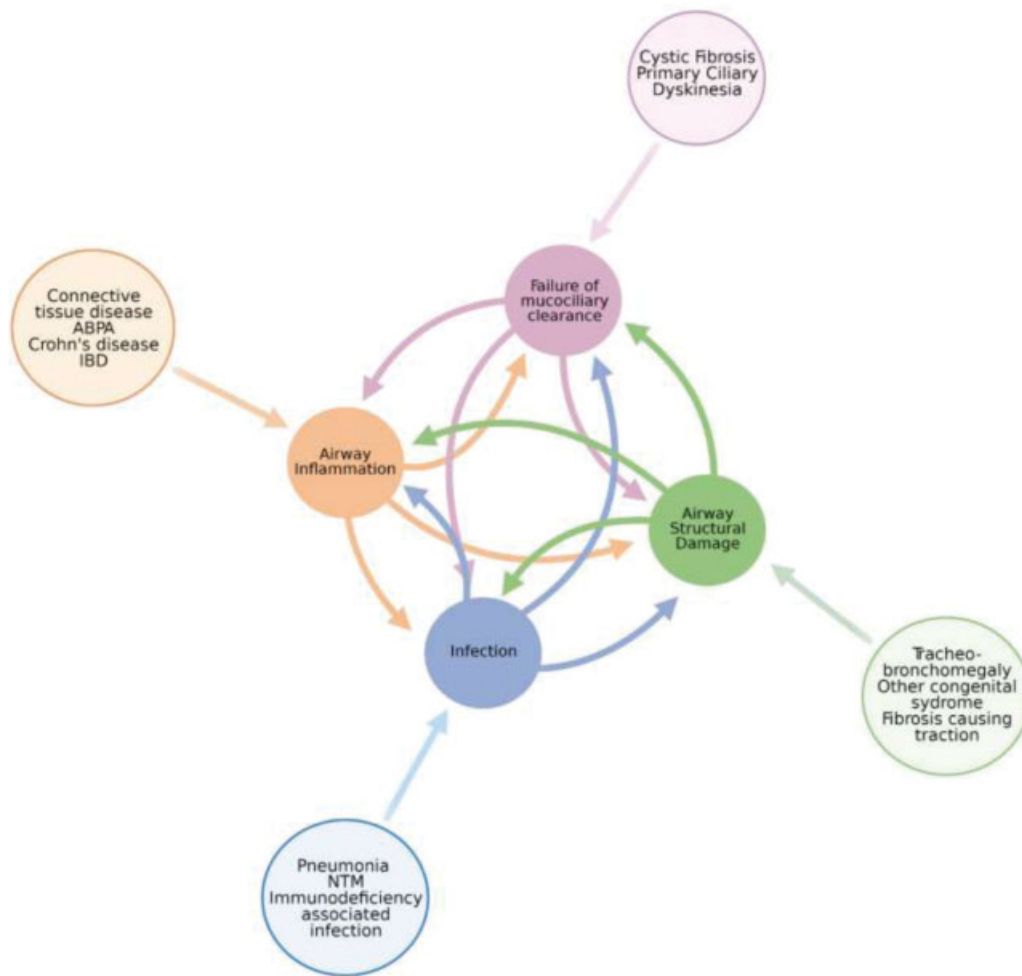
Fig. 1 The vicious vortex of bronchiectasis with examples of etiology "entry points" which can lead to the development of bronchiectasis. ABPA, allergic bronchopulmonary aspergillosis; IBD, inflammatory bowel disease; NTM, nontuberculosis mycobacterium. (Created with BioRender.com.)

cells.[18] Motile cilia beat in a coordinated, continuous fashion to clear overlying mucus from the airways. The mucociliary escalator acts as one of the first lines of defense against infection, as bacteria, viruses, and particles are trapped in the mucus layer and transported by cilia to be expectorated or swallowed. Dysfunctional mucociliary clearance can lead to sputum retention in the airways, creating a harbor for infection and inflammation.

A known etiology of bronchiectasis is PCD, an autosomal recessive genetic disorder. Mutations in genes encoding proteins involved in ciliary biogenesis, structure, and function result in a range of defects in which cilia are absent, immotile or dysmotile, and leave the epithelium vulnerable to infection.[19–21] Mutations in more than 40 genes have been identified as pathogenic in PCD; however, genetic testing cannot confirm PCD in 20 to 25% of cases, indicating more genes are likely to be discovered.[22] Due to suffering from recurrent infections, most patients with PCD develop bronchiectasis by adulthood.[9,23]

Ciliary dyskinesia can occur in bronchiectasis patients without known PCD. Both environmental and physiological factors are believed to reduce ciliary clearance. Neutrophil elastase (NE) is a serine protease released by neutrophils in response to bacterial infection but can also have detrimental effects on the airway epithelium. NE is a biomarker of disease severity and exacerbation in bronchiectasis and increases in severe disease.[24–26] NE can damage the extracellular matrix and reduce ciliary beat frequency, reducing effective clearance.[27–29] In vitro, NE increases the expression of MUC5AC from bronchial epithelial cells and studies in murine models have demonstrated that NE increases mucus hypersecretion and goblet cell metaplasia, hampering effective mucociliary clearance.[30,31] Mucus cell hyperplasia and metaplasia is likely to be a key mechanism leading to mucus hypersecretion in bronchiectasis, as a study of bronchial biopsies by Gaga et al found up to 40% of tissue in bronchiectasis biopsies were composed of mucus glands with no hyperplasia identified in control samples.[32] Bacterial infection has also been associated with reduced mucociliary clearance in patients with bronchiectasis. In vitro, high levels of Pseudomonas elastase have been demonstrated to be cytotoxic and cause the detachment of epithelial cells from neighboring cells and the basement membrane.[28] Bacterial products such as pyocyanin, a product of P. aeruginosa, reduce ciliary beat frequency, impacting effective clearance.[28,33]

It is unlikely that bacteria and NE are the only modulators of cilia function in bronchiectasis; although secondary ciliary dyskinesia has not been extensively studied in this disease, there is emerging evidence of an association with other lung conditions. Studies utilizing exome sequencing have identified that genes associated with PCD further disease progression in CF and that abnormal cilia genes contribute to idiopathic nontuberculosis mycobacterium (NTM) infection.[34,35] It is possible that future studies will identify that both primary and secondary cilia dysfunctions are involved in bronchiectasis.

The efficiency of mucociliary clearance is determined by both the action of ciliated epithelium and the characteristics of the overlying mucus. There is evidence of abnormal mucus hydration and rheology in bronchiectasis. Ramsey et al studied a cohort of patients with the BLESS randomized controlled trial. Sputum from patients with bronchiectasis on average had 20-fold higher DNA concentrations than control subjects, and elevated levels of total mucins, composed primarily of MUC5AC and MUC5B. Mucus from bronchiectasis patients was dehydrated and more viscous.[36]

Mucus hydration and viscosity are regulated by epithelial ion channels. The epithelial sodium channel (ENaC) regulates composition of airway surface liquid via sodium reabsorption on the epithelial surface.[37] Functional CF transmembrane conductance regulator (CFTR) downregulates ENaC; in CF, upregulation contributes to excess mucus which is central to the pathophysiology of the disease. Transgenic mice that overexpress ENaC develop CF-like lung disease[38] and it has been proposed that hyperactive ENaC may contribute to the development of bronchiectasis.[39] Patients who carry the p.W493R-SCNN1A, a variant that encodes for a hyperactive ENaC channel, have been identified as being at a higher risk of developing bronchiectasis.[40] In 2008 and 2009, Fajac and colleagues investigated whether a defective ENaC protein could be involved in the development of bronchiectasis. They analyzed ENaC-beta and -gamma genes in 55 subjects who had idiopathic bronchiectasis without two mutations in the coding regions of CFTR. Of the 10 patients identified to have an ENaC mutation, 6 had functional abnormalities suggesting impaired sodium transport.[41,42] These studies suggest that mutations in ENaC-beta and -gamma genes may disrupt ENaC function and lead to bronchiectasis. Downregulating ENaC has been suggested as a potential therapeutic strategy in both CF and PCD to reduce mucus viscosity and improve airway clearance.[43–45]

The role of CFTR and CFTR mutations in bronchiectasis remains under debate in the literature, with conflicting studies being published. A study of 100 patients with idiopathic bronchiectasis in an Australian cohort found that the rate of classical CFTR mutations was 1:25, the same frequency as found in the general population.[46] These results were supported in a study of 19 Serbian patients with disseminated bronchiectasis, published shortly after.[47] Analysis of the whole coding region of the CFTR gene, its flanking regions, and the promoter in 47 patients with diffuse bronchiectasis and 47 controls identified CFTR variants in 24 bronchiectasis subjects and 27 in control subjects. This study suggested that there is no relationship between mutations of CFTR and bronchiectasis.[48] However, conflicting studies have reported that CFTR mutations can occur in 36% of non-CF bronchiectasis patients.[49] Bienvenu and colleagues evaluated the association between CFTR heterozygosity and CFTR protein dysfunction in 122 patients with diffuse bronchiectasis. They found that bronchiectasis patients had a high rate of CFTR mutations and abnormal nasal potential difference measurements compared with healthy control individuals.[50] It is notable that the average age of patients in the study by Bienvenu et al was 45 years, approximately 20 years younger than most published bronchiectasis series suggesting a degree of selection bias. These studies highlight that the role of CFTR in bronchiectasis remains contentious and that further multicenter studies on larger cohorts of patients are needed to resolve this debate.

Defective mucus clearance can also occur due to anatomical changes to the airway such as congenital tracheobronchomegaly, tracheomalacia, and bronchomalacia.[51,52]

Inflammation

Chronic inflammation is a key component of bronchiectasis pathophysiology. Patients have extensive infiltration of the airways by inflammatory cells, particularly in severe disease. Sputum and bronchoalveolar lavage (BAL) samples from bronchiectasis patients have high numbers of inflammatory cells and inflammatory mediators.[53–55] The inflammatory response comprises a complex cytokine network that activates and deploys cells involved in host defense. Levels of inflammation are controlled by interactions between upregulated proinflammatory cytokines, and anti-inflammatory cytokine and cytokine inhibitors, which are released to dampen the immune response. An imbalance between pro- and anti-inflammatory signaling occurs leading to recruitment of inflammatory cells and ultimately a self-perpetuating cycle of inflammation.[3,56,57]

Neutrophils
Neutrophils are among the first immune cells to be recruited in response to an infection but are also regarded as a key component in the pathophysiology of bronchiectasis. Bronchiectasis is typically considered to be a neutrophilic disease; however, recent studies have suggested that eosinophilic inflammation may be prevalent in up to a third of patients.[58,59]

Neutrophils are recruited to the lung by several mediators, including CXCL-8, IL-1β, IL-17, leukotriene B4, and tumor necrosis factor-α (TNF-α).[54,60,61] Once neutrophils reach the airways, these chemoattractants induce neutrophil activation. The inflamed airways of bronchiectasis patients contain high levels of neutrophil granule products, such as myeloperoxidase, NE, heparin-binding protein, resistin, and matrix metalloproteinases.[62–65] Sputum proteomics has demonstrated that patients with severe disease have an upregulation of neutrophil proteins in the airways, compared with higher levels of antiproteases and epithelial proteins in the sputum of mild patients.[65]

The main stimulant of neutrophil migration into the airway is believed to be bacterial colonization. Bacterial load is associated with markers of airway inflammation such as the key chemoattractants described earlier, and treatment with short- and long-term antibiotic therapies reduce markers of airway and systemic inflammation and therefore reduces neutrophil recruitment to the airway.[66] However, subgroups of patients have been identified for whom inflammation occurred without bacterial infection.[54] This would align with the vortex model of bronchiectasis; neutrophilic inflammation is not induced solely by bacterial infection, meaning antibiotic treatments alone are insufficient to break the cycle of inflammation in all patients.

Once recruited to the site of infection, neutrophils deploy several host defense mechanisms including phagocytosis, degranulation, production of reactive oxygen species, proinflammatory cytokine production, and neutrophil extracellular trap (NET) formation. In blood, bacterial killing by neutrophils occurs primarily through phagocytosis, an efficient intercellular pathway of killing where the cell uses its plasma membrane to engulf particles, resulting in minimal damage to the host. Neutrophil phagocytosis is dependent on the binding of IgG and complement (C3b/Cb4 and iC3b)-coated microbial targets to Fcγ and complement receptors. The cleavage of these receptors by NE, or the cleavage of complement and Ig receptors from the surface of pathogens, is thought to impair phagocytosis in the bronchiectasis airway.[3,67,68] A study by Voglis et al demonstrated that neutrophils isolated from sputum of bronchiectasis patients displayed defective phagocytosis which correlated with high human neutrophil peptide (HNP) concentrations in the lung. The study showed that HNP decreased phagocytic capacity of healthy neutrophils through intracellular calcium and actin cytoskeleton remodeling.[55] Both of these observations suggest a negative feedback loop whereby failure of bacterial phagocytosis leads to extracellular release of NE and HNPs, which further impair phagocytosis.

Several studies have demonstrated that neutrophils in the blood display normal phagocytic function, oxidative burst, and expression of activation receptors.[12,55,69,70] However, a study in 2018 of 24 patients with bronchiectasis (8 mild, 8 moderate, and 8 severe), blood neutrophils had impaired neutrophil phagocytosis and killing of PAO1 increased activation, prolonged viability, and reduced apoptosis compared with healthy controls. Furthermore, bronchiectatic airway neutrophils had significantly reduced bacterial killing and phagocytosis compared with matched autologous blood neutrophils.[71] This study is surprising, as bronchiectasis patients do not exhibit an increased risk in nonpulmonary infections which would be expected with a major systemic defect of neutrophils, and P. aeruginosa infections impact on only a subset of bronchiectasis patients. Further studies are needed to fully understand systemic neutrophil function in patients with bronchiectasis.

It is likely that the reduced functional properties of bacterial killing and phagocytosis are perpetuating the vicious vortex. Additionally, Watt et al demonstrated that bronchiectasis neutrophils have delayed apoptosis and prolonged viability.[56] A study in CF patients found that neutrophils had a longer life span, which resulted in increased NET formation.[72]

We recently observed in a series of independent international bronchiectasis cohorts that NET formation was a key component of bronchiectasis pathophysiology.

NETs are highly ordered, web-like structures released by neutrophils in response to multiple stimuli including bacterial infection. The webs contain antimicrobial neutrophil granule proteins including NE and histones which are also toxin to microbes. It is likely that NET formation represents the dominant mechanism of NE release into the bronchiectasis airway. It remains controversial whether NETs are truly antimicrobial or are only able to trap bacteria and prevent infection from being disseminated. Our study found that NETs were present in sputum of patients with bronchiectasis and correlated with increasing severity, mortality, severe exacerbation, and a reduction in time to next exacerbation. Additionally, NET levels can be reduced through antibiotic treatment with patients who had the largest reduction in NET concentrations showing the greatest clinical benefit.[65] This study indicates that NETs may actively contribute to the pathophysiology of bronchiectasis. A central role for neutrophil serine proteases and NETs in the pathophysiology of bronchiectasis is suggested by the recent demonstration of prolonged time to next exacerbation and reduced frequency of exacerbations in patients treated with two doses of a novel dipeptidyl peptidase-1 (DPP1/cathepsin-C) inhibitor compared with placebo in 256 patients with bronchiectasis. The WILLOW trial showed marked reductions in NE with treatment that correlated with clinical response. DPP1 directly promotes NETosis and indirectly promotes NETosis through the action of NE. Neutrophils from individuals with Papillon–Lefevre syndrome, a congenital syndrome arising through genetic absence of DPP1, cannot make NETs but have preserved bacterial killing. The results of the WILLOW study demonstrate the potential importance of neutrophil serine proteases in bronchiectasis exacerbations as well as the potential for translating basic research findings into novel therapies.[73]

Macrophages

Macrophages play a critical role in immune response and are involved in the detection, phagocytosis, and eradication of pathogens as well as the initiation of the inflammatory response through cytokine release.[74] In bronchiectasis, macrophages in the lung are increased compared with healthy controls.[32,75] Macrophages are responsible for regulating neutrophil numbers in the airway through efferocytosis, the clearance of apoptotic neutrophils, and the release of neutrophil chemoattractants. Phagocytosis of apoptotic neutrophils by macrophages, before they undergo secondary necrosis, prevents the release of inflammatory cytokine, proteases, and oxygen radicals which exacerbate tissue injury and inflammation.[76,77] Several studies have shown indirect evidence that apoptosis and apoptotic cell clearance are reduced in bronchiectasis.[72,78,79] Watt et al investigated the effects of antibiotic treatment for exacerbations on

neutrophil apoptosis and necrosis in 15 patients with idiopathic bronchiectasis.[56] Neutrophil apoptosis and necrosis were analyzed using morphology and flow cytometry, on sputum samples taken on day 1 of an exacerbation and again after 2 weeks of antibiotic treatment. The study found that bronchiectasis patients had a significantly lower percentage of macrophages present in their sputum and a significantly higher percentage of secondary necrotic cells compared with healthy controls on day 14, suggesting impaired efferocytosis possibly due to low numbers of macrophages.[56] Impaired efferocytosis may also be caused by cleavage of the phosphatidylserine receptor by NE, a potential mechanism of the reduced phagocytosis of apoptotic cells.[79] In the study of Watt et al, no relationship was seen between NE and the percentage of secondary necrotic cells, suggesting that the mechanisms of efferocytosis are not fully understood. The impairment of efferocytosis in bronchiectasis is likely to contribute to pathophysiology through the increase in the release of molecules which damage lung tissue and aggravate inflammation.

Eosinophils

Although bronchiectasis is classically thought of as a neutrophilic disease, emerging evidence suggests the presence of an eosinophilic subtype of bronchiectasis. Up to 30% of bronchiectasis patients show airway eosinophilia in sputum using established cut-offs such as 3% eosinophils.[11,58,59] Gaga et al also found increased infiltration of eosinophils into bronchial biopsies from bronchiectasis patients compared with controls. Inhaled corticosteroid (ICS) use is not recommended in the treatment of bronchiectasis, out with ABPA or severe asthma which are both T-helper (Th) type 2 cell/eosinophil-driven conditions.[14] ICS use has been demonstrated to be associated with an increased risk of hospitalized respiratory infections[80] in the general bronchiectasis population; however, the identification of eosinophilic subtypes in bronchiectasis may be useful to guide personalized treatment. In chronic obstructive pulmonary disease (COPD), eosinophilia is a recognized endotype which can be used to predict response to ICS.[81,82] A recent post hoc analysis of two randomized controlled trials of ICS use, which stratified patients according to blood eosinophil count, found that patients with peripheral eosinophilia of ≥4% had a significant reduction in number of exacerbations during follow-up compared with patients with noneosinophilic inflammation on ICS treatment. As a post hoc analysis, this study did have limitations, including differences in ICS treatment type and doses, but does, however, highlight the potential benefits of personalized treatment approaches surrounding ICS use in bronchiectasis.[83]

Immunodeficiency

Immunodeficiency has been identified as part of the underlying etiology of bronchiectasis in several conditions.

Lymphocytes

Less is known about B- and T-cell function in bronchiectasis, but the previously mentioned study examining bronchial biopsies by Gaga et al found increased infiltration of CD4+ T lymphocytes in the airways of bronchiectasis patients.[32] It is clear that patients with abnormal B- and T-cell function are at increased risk of bronchiectasis. Chronic lymphocytic leukemia and transporter antigen presentation deficiency syndrome are both disorders with abnormal T-cell functions which are associated with bronchiectasis.[84,85] HIV has also been implicated in the development of bronchiectasis.[86] Patients with common variable immunodeficiency (CVID) have a lack of general and local protective humoral immunity due to reduced levels of Ig. The progression of bronchiectasis development can be slowed down by Ig replacement therapies in CVID patients.[87–89] The advancement in genomic and direct approaches is likely to improve our understanding and identify new mechanisms of immune dysfunction in bronchiectasis pathogenesis.

In patients with established bronchiectasis, there are limited published data. One study found lower T-cell responses, measured using interferon-gamma release, to bacterial pathogens in bronchiectasis patients compared with healthy volunteers. Interestingly, a negative correlation was observed between the bronchiectasis severity index and T-cell responses to *Haemophilus influenzae*, the most common bronchiectasis pathogen in northern Europe, indicating that patients with more severe disease had impaired to reduced T-cell immunity to *H. influenzae*.[90]

Quigley et al studied T-cell responses to OprF, an outer membrane protein of *P. aeruginosa* as the other critical pathogen in this disease. They found that patients with chronic *P. aeruginosa* surprisingly had reduced T-cell immunity to OprF determined by interferon-gamma release but had enhanced release of multiple cytokines and chemokines involved in neutrophil, monocyte, and NK cell recruitment, with additionally an increase in release of interleukin-4. These data suggest T-cell responses may be important in the impaired immune response permitting chronic *P. aeruginosa* infection while additionally promoting the chronic neutrophilic inflammation that accompanies it.[91]

Epithelial Inflammation

The role of epithelial cells in the immune response is not limited to mucus clearance; they also release several inflammatory factors which induce, amplify, and modulate ongoing inflammation. Bronchial epithelial cells synthesize and release proinflammatory mediators, including CXCL-8 and TNF-α, which trigger neutrophil migration to the site of inflammation.[92,93] ET-1 production by epithelial cells, which promotes neutrophil adhesion to endothelial cells and migration to areas of inflammation, is increased in the serum of bronchiectasis patients with *P. aeruginosa* infection.[94–96] Both epithelial cell communication and interactions with bacteria cause bronchial epithelial cells to express ICAM-1.[97] ICAM-1 plays multiple roles in the modulation of inflammation, including upregulating airway response to pathogens and inducing neutrophil adhesion to airway epithelial cells through neutrophil surface receptors CD11/CD18.[97–100] The release of antimicrobial peptides from epithelial cells, such

as LL-37, has been shown to be higher in patients with more severe disease, although LL-37 can also be neutrophil derived.[101,102] A study by Sibila et al identified that patients with severe bronchiectasis had raised levels of the proinflammatory mediators lactoferrin and cathelicidin LL-37 and reduced levels of anti-inflammatory secretory leucocyte protease inhibitor (SLPI). Raised LL-37 coupled with lower SLPI levels was associated with a reduction in forced expiratory volume in 1 second (FEV1), *P. aeruginosa* infection, and reduced time to next exacerbation. This study highlights a subgroup of bronchiectasis patients who have dysregulated antimicrobial peptide levels which are associated with disease severity.[102] Bronchial epithelial release of secretory leukocyte protease inhibitor may be an important mechanism of defense against chronic neutrophilic inflammation. Our proteomic analysis found that higher levels of SLPI in sputum were among the strongest predictors of a benign disease course, and an independent study by Sibila et al found that among antimicrobial peptides, SLPI was most strongly associated with outcomes. In particular, lower SLPI was associated with a higher risk of exacerbation. SLPI is the dominant inhibitor of NE in secretions (in contrast to α-1 antitrypsin which is thought to be the dominant inhibitor in tissues and blood). The study by Sibila et al also showed that elastase exposure blocked release of SLPI from epithelial cells through a mechanism that was independent of its protease activity. This is supported by our recent findings using proteomics which saw that response to intravenous antibiotic treatment was associated with reductions in NE and increases in SLPI and other epithelial antiproteases. This supports a model in which severe disease is associated with an imbalance of proteases and proinflammatory mediators which suppress epithelial anti-inflammatory, antiprotease, and antimicrobial responses, while successful treatment is associated with a restoration of normal epithelial defense.[102]

Airway Infection

Bacterial Infection

Airways infection is believed to be key to the pathophysiology of bronchiectasis, through both direct structural damage and contributing to the chronic cycle of inflammation. Bacterial colonization is one of the main drivers of neutrophil migration into the airway and is thought to be a major driver of disease progression.[103–105] Bacterial load has been directly correlated with markers of airway inflammation, which can be reduced through short- and long-term antibiotic treatment.[66] Although bacterial pathogens are most commonly discussed clinically, virus, fungi, and mycobacteria have also been identified in the airways of bronchiectasis patients and likely further disease progression.[106–110]

The most common organisms that chronically colonize the airways of bronchiectasis patients are the gram-negative pathogens from the Proteobacteria phylum such as *P. aeruginosa*, *H. influenzae*, *Moraxella catarrhalis*, and Enterobacteriaceae, or Firmicutes pathogens such as *Staphylococcus*

aureus or *Streptococcus pneumoniae*. Proteobacteria dysbiosis of the microbiome is associated with more severe disease and worse clinical outcomes.[6,65,111–113]

P. aeruginosa is the most commonly identified pathogen in bronchiectasis patients worldwide and has been associated with increased exacerbation frequency, reduced quality of life, and increased mortality.[112,114,115] The frequency of *P. aeruginosa* colonization is likely due to its ability to evade killing by inflammatory cells and antimicrobial peptides. Hilliam and colleagues used whole-genome sequencing to characterize *P. aeruginosa* adaptations in bronchiectasis.[116] In total, 191 isolates from the sputum of 91 bronchiectasis patients were sequenced and revealed that during infection, *P. aeruginosa* populations adapt by accumulating loss-of-function mutations which lead to changes in biofilm formation and nutrient acquisition, suggesting adaption of the organism to survive in the hostile lung environment. A separate study of *P. aeruginosa* isolated from 40 patients with bronchiectasis found that pathogen virulence reduced over time, an adaption that has also been identified in CF that enables the organism to hide from the immune system and establish a chronic infection.[117,118] Both the studies conducted by Hilliam et al and Woo et al found that patients can experience multiple infections with *P. aeruginosa* where they acquire different lineages over time.[116,117] *P. aeruginosa* may also evade killing by inflammatory cells through the stimulation of NET formation.[65] Numerous studies have found that *P. aeruginosa* can mediate NETosis through multiple stimulants including LPS, flagellum, and release of virulence factors.[119–122] The induction of NET formation gives *P. aeruginosa* a survival advantage, as NET formation inhibits and kills competitor microorganisms, while *P. aeruginosa* persists due to an ability to degrade NETs and a resistance to killing.[123–125] The induction and evasion of NETs is not limited to *P. aeruginosa*. Pathogens *H. influenzae* and *S. aureus* are also able to induce and evade NETosis. *H. influenzae*, *S. aureus*, *Streptococcus suis* and group A *Streptococcus* all release nucleases as part of NET resistance, which may account in part for their colonization of the airways.[126,127]

H. influenzae is a common but less well studied pathogen in bronchiectasis. Infection with *H. influenzae* results in a complex interactions between multiple microbial adhesins, host responses to microbial antigens, and both mucosal and systemic immune responses which takes place intra- and extracellularly.[128–131] Nontypeable *H. influenzae* (NTHi) is typically found in the upper respiratory tracts of healthy individuals, but can cause a strong adaptive immune response if infection occurs in the lower airway of patients with bronchiectasis.[132] *H. influenzae* have been associated with a loss of microbial diversity and the formation of NETs[65,133] as well as an increase in serum CRP, sputum IL-1β, and CXCL-8.[54,134,135] In addition to a failure of neutrophil killing, it is likely that pathogenesis *H. influenzae* is also dependent on ciliary dysfunction. Several studies have found that NTHi can reduce ciliary beat frequency and cause damage to epithelial cells in the respiratory tract, and it is

among the most common pathogens isolated from patients with PCD.[132,136–138]

NTM can be a cause or a consequence of bronchiectasis and is thought to occur in approximately 9% of patients.[139] *Mycobacterium avium* complex (MAC) is the most frequently isolated NTM species in bronchiectasis.[109,140] Patients with NTM may have unique body and immune phenotypes including pectus excavatum, scoliosis, and mitral valve prolapse and are often tall, slender females.[141,142] The genetic components involved in NTM infection are complex; patients with NTM have low-frequency genetic variants in immune, cilia, connective tissue, and CFTR genes compared with healthy control individuals.[35] Patients with NTM often have coinfection with *P. aeruginosa* and *Aspergillus*-related lung disease, which suggests there may be a shared susceptibility across different infections.[140,143] Treatment of bronchiectasis patients with NTM is complicated by concerns that long-term macrolide therapy can increase the risk of macrolide-resistant MAC,[144] which is problematic as several randomized controlled studies have shown benefits of long-term macrolide treatment.[14,145] A preliminary study of 410 patients from the United States Bronchiectasis Research Registry found low rates of NTM positivity in patients receiving long-term macrolide therapy, suggesting that long-term therapy could be protective against NTM infection.[108]

The advent of next-generation sequencing technologies has allowed researchers to look at the microbiome in bronchiectasis in more detail than was previously available through culture. 16S rRNA sequencing is the most commonly used technique and several studies have identified dominant organisms which are concordant with those found using culture-based approaches, such as *Pseudomonas* and *Haemophilus*.[146–148] The bronchiectasis microbiome is heterogenous and highly complex, with multiple bacterial genera.[146,149] Woo et al conducted a longitudinal study of 29 bronchiectasis patients over 16 years and demonstrated a strong stability of the microbiome over time.[148] Loss of microbial diversity has been associated with a reduction in FEV1 and lung function decline, while dominance of Proteobacteria *Pseudomonas* and *Haemophilus* has been associated with increased exacerbations and neutrophilic inflammation including NET formation.[65,134,147,150] Real-time quantitative polymerase chain reaction can be used to determine bacterial load as 16S rRNA sequencing is not quantitative. A study by Cox et al found that bacterial remained relatively consistent between baseline and during an exacerbation in 76 patients with bronchiectasis. Additionally, the microbiome composition remained relatively stable between baseline and exacerbation.[146] This suggests current clinical concepts that exacerbations are caused by bacterial infections or changes in bacterial load are likely overly simplistic. There are still important limitations to sequencing that are worth considering, including a limited resolution in terms of defining bacterial species and a loss of low abundance taxa which may be identified by culture.[146]

The mechanisms leading to pathogenic changes in the microbiome are complex and are likely to be derived from several factors including pathogen virulence, dysregulated immune response, local nutrient availability, and ciliary function. There is also evidence to suggest that long-term antibiotic therapy may change the microbiome. A *post hoc* analysis of the BLESS study, a randomized controlled trial of erythromycin, found that in patients without *P. aeruginosa* infection, treatment reduced *H. influenzae* resulting in an increase in macrolide-tolerant pathogens including *P. aeruginosa*.[149] The authors suggest caution in long-term erythromycin treatment in patients without *P. aeruginosa* infection. There are still many unanswered questions around the effect of antibiotic treatment on the microbiome in bronchiectasis and more longitudinal studies are needed to answer these questions.

16S sequencing is limited to the study of bacteria which ignores potential contributions to the microbiome from virus, fungi, mycobacteria, and potential other microorganisms. Studies of other kingdoms are limited by the availability of sequencing technologies and reference databases. There are relatively few studies of viruses in bronchiectasis, but Gao et al found viruses in 49% of bronchiectasis exacerbations compared with 18.9% of patients in stable state. This suggests a contribution of respiratory viruses to bronchiectasis exacerbations.[151] The first study of the lung microbiome in bronchiectasis was the CAMEB (Cohort of Asian and Marched European Bronchiectasis), published in 2018. The study analyzed sputum from 238 Asian and matched European bronchiectasis patients. *Candida*, *Saccharomyces*, and *Penicillium* were the most frequently detected genera in bronchiectasis and healthy controls, while differentially abundant, bronchiectasis genera included *Aspergillus*, *Cryptococcus*, and *Clavispora*. High frequencies of *Aspergillus*-associated disease including sensitization and allergic bronchopulmonary *Aspergillus* were detected, with *Aspergillus terreus*–dominant profiles associating with an increase in exacerbations.[107] The major clinical manifestations of fungal disease in bronchiectasis is ABPA which affects up to 10% of patients with bronchiectasis. ABPA is unusual, in the normally neutrophil-dominant inflammatory profile of bronchiectasis syndromes, being dominated by a Th2-driven, hypersensitivity response with elevated levels of total and specific IgE and eosinophilic inflammation. Patients often have frequent exacerbations and thick tenacious mucus.[152,153] Testing for ABPA is mandated by international guidelines because it requires specific treatment with corticosteroids with or without antifungal drugs.[14,154] The earlier-mentioned CAMEB study suggested that ABPA may be underdiagnosed.

Implications of Pathophysiology for Treatment

The concept of identifying individual pathophysiological mechanisms in each individual with bronchiectasis and then targeting treatment to the appropriate part of the cycle is known as the "treatable traits" approach and is summarized in ►**Fig. 2**. New treatable traits are being identified regularly, but the figure below summarizes pathophysiological mechanisms that are linked to exacerbation and may be targetable by treatment.
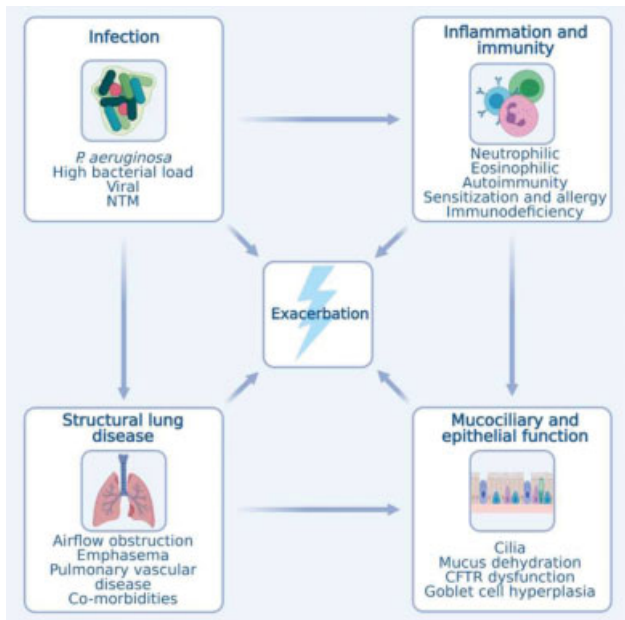
Fig. 2 Summary of the underlying pathophysiological mechanisms of bronchiectasis that could be targeted in a "treatable traits" approach to treatment in bronchiectasis. CFTR, CF transmembrane conductance regulator; NTM, nontuberculosis mycobacterium. (Created with BioRender.com.)

Infection

As described earlier, sputum culture is a critical component in the assessment of patients. It enables the identification of organisms such as *P. aeruginosa*, which has a worse prognosis, and organisms such as NTM which require specific treatment (or avoidance of specific treatment such as macrolide monotherapy). Culture is insensitive and future application of molecular techniques may improve detection of infection and therefore treatment. As an example of a treatable trait, patients with *P. aeruginosa* infection respond better to macrolide therapy than patients without *P. aeruginosa* infection according to an individual participant data meta-analysis of three randomized trials in bronchiectasis patients.[145] In vivo, we found that macrolides reduce NETs in patients with *P. aeruginosa* infection, suggesting an anti-inflammatory effect may be responsible for this beneficial effect.[65] Higher bacterial loads of *P. aeruginosa* and other pathogens are associated response with inhaled antibiotics and in future bacterial load may be a useful biomarker of response. Other potential contributors to exacerbation under the heading of infection are dealt with in the earlier section. Future directions in this area may include vaccination against specific pathogens (e.g., *H. influenzae*).

Inflammation

As noted, eosinophilia in the airway or blood identifies patients more likely to respond to inhaled corticosteroid treatment in COPD and asthma and there are emerging data that show this is also true in bronchiectasis.[155] In the future, this may allow treatment of bronchiectasis patients with Th2-mediated inflammation with specific drugs such as anti-IL-5 or anti-IL-5 receptor blockers or emerging options such as anti-TSLP (thymic stromal lymphopoietin). Targeting neutrophilic inflammation has been more challenging because most approaches historically have attempted to block neutrophil recruitment to the lung. Drugs such as CXCR2 antagonists have effectively done this, but have been associated with increased infections or exacerbations, as illustrated by AZD5069 which was tested in a 28-day study in bronchiectasis,[156] and by a recent larger study in COPD with the CXCR2 antagonist danirixin.[157] Similar results were obtained with leukotriene B4 antagonism in CF where it was tested in adults and children and while associated with reduced neutrophilic inflammation this produced an increase in exacerbations and a worsening of lung function.[158] Subsequent studies in mice found increased translocation of *P. aeruginosa* into the blood and increased airway *P. aeruginosa* loads indicating the risks associated with reducing neutrophils in the airways.[159] NE inhibition has been tested in two randomized studies in bronchiectasis. One study by Stockley et al missed its primary endpoint but had encouraging trends including a 100-mL improvement in FEV1 and an improvement in St Georges Respiratory Questionnaire.[160] The second study by Watz et al enrolled 94 patients and, despite showing reductions in elastase activity in blood, found no clinical benefits.[161]

These data suggest that antineutrophil treatment is challenging. The ideal antineutrophil strategy would reprogram dysfunctional bronchiectasis neutrophils before they reach the lung and would not impair neutrophil recruitment or neutrophil function once they arrive in lung, while still reducing harmful neutrophilic inflammation. Encouraging results with DPP1 inhibition as noted earlier suggest the potential for this type of immune modulation, but results of a phase 3 trial are awaited.[73]

There are therefore significant challenges in implementing anti-inflammatory treatment in bronchiectasis, as it is not immediately obvious how to identify patients with eosinophilic disease in clinical practice. Blood eosinophils counts are validated in COPD and asthma,[162–165] but more work is needed to validate them in bronchiectasis. Sputum counts are not practical to implement in widespread clinical care. Nevertheless, work is underway to establish the optimal treatment for eosinophilic bronchiectasis. Our increasing understanding of the inflammatory pathways in different subtypes of bronchiectasis is likely to lead to the emergence of multiple new anti-inflammatory therapies.

Mucociliary Clearance

Airway clearance exercises are the mainstays of treatment and should be practiced by all patients. Many patients are unable to control their disease with just airway clearance exercises, but the role of mucoactive drugs or adjuncts is not established. Trials that have attempted to address mucociliary clearance in bronchiectasis have had mixed results with some suggesting benefit and others suggesting no benefit or potential harm in the case of recombinant DNAse.[166] This is likely due to the extremely heterogeneity of the patient population. The recent study by Ramsey et al

which examined mucus properties in patients with bronchiectasis gives some clues as to why drug approaches to mucociliary clearance have been so challenging.[36] Hypertonic saline treatment hydrates mucus and is widely used, but evidence of its benefit is lacking. Ramsey et al showed profound diversity in mucus properties across a bronchiectasis patient population with some having severely dehydrated mucus and others having mucus properties within the normal range.[36] These data are consistent with our own clinical experience that patients have very different mucus properties and therefore responses to mucoactive drugs. Similarly, DNA concentrations in sputum varied in the study of Ramsey et al by more than 1,000-fold in the bronchiectasis population indicating remarkable diversity. It is highly likely that patients at the extremes of this distribution would have very different responses to DNAse therapy. Patients with CF derive benefit, and it is reasonable to assume some patients with a "CF-like phenotype" with bronchiectasis may also respond. The reasons why DNAse did not work in bronchiectasis have been the subject of much debate, but likely it is explained by the different mucus properties between CF and non-CF bronchiectasis, and greater heterogeneity in mucus properties. Release of proteases entrapped within DNA has been shown to be enhanced by DNAse treatment,[167] suggesting a potential mechanism for harm in those with neutrophilic inflammation but without highly viscous mucus as is commonly the case in bronchiectasis.

All of this argues for a personalized medicine or "treatable traits" approach to mucoactive therapy. Biomarkers of mucus properties are currently not easily available in clinical practice, but there is early evidence supporting this approach. In a post hoc analysis of the mannitol trials, Gao et al showed that the presence of increased symptoms, a surrogate of more difficult mucus in bronchiectasis, was associated with response.[168] In keeping with the aforementioned hypothesis, there was a trend to more exacerbations in patients who had few baseline symptoms. Further research into this area is needed, as mucociliary clearance is the least researched area of bronchiectasis pathophysiology. Exciting future directions in this area include the potential for CFTR modulation in bronchiectasis which will shortly be tested in a phase 2 trial (NCT04396366).

Structural Lung Disease

Currently, there are no therapies shown to reverse bronchiectasis once established, although there are early-phase studies of regenerative medicine approaches reported on international clinical trial registries.[169] Most approaches therefore aim to deal with the consequences of structural lung damage, such as treating airflow obstruction with bronchodilators or in extreme cases removing damaging areas of the lung through surgery.[170] In the future, it would be nice to think that there may be approaches to reverse established lung damage. In the medium, the most likely approach to reduce this would be to introduce preventative measures that reduce the likelihood of development of bronchiectasis. Pre-bronchiectasis syndromes are increasingly recognized, particularly the concept of persistent bacterial bronchitis in children which has also been reported in adults.[171,172] It is highly likely that inflammation and infection precede structural lung damage in a majority of cases, as has been observed in CF.[173] Further studies are needed to understand the mechanisms preceding the development of structural bronchiectasis, but it is tempting to speculate that anti-inflammatory or immunomodulatory approaches early in the disease could prevent the development of chronic disease. Studies of early and pre-bronchiectasis are urgently needed.

Conflict of Interest
None declared.

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