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CYSTIC FIBROSIS: MICROBIOLOGY AND HOST RESPONSE

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Abstract

THE EARLIEST DESCRIPTIONS OF LUNG DISEASE IN PEOPLE WITH CYSTIC FIBROSIS (CF) DEMONSTRATED THE INVOLVEMENT OF THREE INTERACTING PATHOPHYSIOLOGICAL ELEMENTS IN CF AIRWAYS: MUCUS OBSTRUCTION, INFLAMMATION, AND INFECTION. OVER THE PAST 7 DECADES, OUR UNDERSTANDING OF CF RESPIRATORY MICROBIOLOGY AND INFLAMMATION HAS EVOLVED WITH THE INTRODUCTION OF NEW TREATMENTS, WITH INCREASED LONGEVITY, AND WITH INCREASINGLY SOPHISTICATED LABORATORY TECHNIQUES. IN THIS CHAPTER, WE WILL REVIEW THE CURRENT STATE OF UNDERSTANDING OF THE ROLES OF INFECTION AND INFLAMMATION AND THEIR ROLES IN DRIVING LUNG DISEASE. WE WILL ALSO DISCUSS HOW THIS CONSTANTLY EVOLVING INFORMATION IS USED TO INFORM CURRENT THERAPEUTIC STRATEGIES, MEASURES AND PREDICTORS OF DISEASE SEVERITY, AND RESEARCH PRIORITIES.

Keywords

CYSTIC FIBROSIS; INFLAMMATION; INFECTION; MICROBIOLOGY; NEUTROPHIL; BIOMARKER

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Introduction

The earliest published descriptions of cystic fibrosis (CF) lung disease described the following triad of pathophysiologic elements that still form the basis of most current models:

- airway obstruction
- infection
- inflammation

In her landmark 1938 publication describing the pathologic and clinical features of children who died from this disorder, Dorothy Andersen identified the common features of “bronchitis, bronchiectasis, pulmonary abscesses arising in the bronchi,” which were plugged with “tenacious, greenish gray mucopurulent material”, and that “*Staphylococcus aureus* was the usual bacteriologic agent”^{1,2}. On the basis of these findings, she and others began treating children with CF with antibiotics targeting *S. aureus*, specifically sulfonamides and penicillin². This approach was followed by several important developments: First, the children generally improved clinically. Second, cultures showed that they were increasingly infected with penicillin-resistant *S. aureus*, as well as pathogens not seen prior to antibiotics, including *Pseudomonas aeruginosa*³.

These early observations are instructive on many levels. The primary defect that causes CF, mutational dysfunction of the CF transmembrane conductance regulator (CFTR), leads to a cycle in the airways of defective mucus clearance, obstruction, infection and inflammation. Improvements in nutrition, mucus clearance, and treatment of inflammation and infection have led to dramatic improvements in CF respiratory morbidity and mortality⁴; however, current treatments have not been able to halt disease progression. As patients live increasingly longer, their respiratory microbiology evolves⁴, perhaps driven by elements of care intended to mitigate their lung disease (such as antibiotics and attending clinics at CF care centers). Put differently, the treatments that have so greatly helped people with CF are often followed by microbiological changes, which in turn may alter their clinical course. The causal relationships between specific pathogens, clinical changes, and our constantly evolving therapies are difficult to sort out.

Andersen and her colleagues made special mention of the apparent link between infection and inflammation². While these are omnipresent features of CF lung disease, the causal relationships between them are controversial. Observations from a variety of studies of patients⁵ and, most recently, animals engineered with CFTR mutations⁶ have led some authors to postulate that infection is required for CF airway inflammation, while others have suggested that inflammation can precede infection⁷. While these disputes continue, great progress has been made in defining the mediators and mechanisms driving the intense inflammation within CF airways, identifying not only new candidate therapeutic targets, but also promising biomarkers of early disease.

In this chapter, we will review the current understanding of the roles of infection and inflammation in CF lung disease pathogenesis. While these two features of the CF airway are mechanistically linked by data from observational studies and, as a result, in our current

pathophysiological models, for simplicity we consider them separately. While we will touch on therapeutic approaches to both infection and inflammation, we are currently experiencing a period of particularly rapid evolution in CF treatment strategies and protocols. Therefore, where appropriate, we will refer the reader to recent, in-depth reviews of CF therapeutic strategies^{8,9} for more information. Similarly, there have been a number of excellent reviews of CF respiratory microbiology and inflammation in general that the reader may find useful^{10–16}.

I. CF MICROBIOLOGY

The microbiology of CF lung disease: The evolving role of methodology

Dorothy Andersen and her contemporaries identified a number of important characteristics of CF lung disease. For example, she and others who later built upon her work^{17–20} noted that the microbes infecting CF lungs were nearly always confined to airway luminal mucus, rather than invading tissue. The role of *S. aureus* was highlighted; however, other bacteria were quickly recognized as important CF pathogens. Blending observations from culture and microscopy, two of the most sophisticated methods available at the time, provided higher sensitivity and yielded a richer depiction of infection pathogenesis than either method alone. As both treatments and laboratory methods have evolved, so has our understanding of the pathogenesis of CF respiratory infections and the spectrum of likely pathogens. Before discussing the most common pathogens, it is instructive to consider how improvements in treatment, innovations in clinical microbiology, and increases in patient longevity combine to create constant evolution in the field of CF microbiology.

CF clinical microbiology generally relies on conventional laboratory cultivation of respiratory samples (e.g. sputum, bronchoalveolar lavage fluid, oropharyngeal swabs, or sinus samples) to identify and study causative organisms. Using these techniques, bacteria and fungi can be identified, enumerated, isolated, and characterized; their growth characteristics and *in vitro* antibiotic susceptibilities are defined. These methods are invaluable for diagnosing infections in individual patients, directing antibiotic treatment, and generating epidemiological information that informs our models of CF infection pathogenesis. Despite the enormous utility and power of these conventional methods, recent work with advanced laboratory techniques has added to the ever-growing list of CF-associated microbes. To show how newer methods might complement culture, we will briefly review how current CF respiratory cultures are performed, and how information is generated and used to direct treatment.

Current methods rely on synthetic laboratory growth media that, together with incubation conditions (generally aerobic and at body-temperature for CF microbiology), have been chosen to select for the microbes customarily associated with lung disease pathogenesis. In addition, current protocols²¹ usually involve selecting a small number of isolates of those “traditional pathogens” from those cultures to test susceptibilities, and to inform treatment.

However, conventional culture methods can introduce bias or have other shortcomings. For example, it is now known that CF airway mucus has anaerobic niches that alter microbial metabolism¹⁸ and that are not accurately modeled by these laboratory methods. In line with

this finding, both advanced culture-based and culture-independent (sequencing) techniques have identified diverse microbes, including anaerobes and other otherwise undetected bacteria together with traditionally-cultured pathogens, in respiratory samples from many CF patients²². Microscopic and biochemical investigations^{18,23} suggest that microbes infecting CF airways may commonly exist in biofilms, which are gel-encased communities of cells that are resistant to killing compared with the liquid-suspended, dispersed microbial cells usually studied in the laboratory. In-depth studies of traditional CF pathogens (such as *P. aeruginosa* and *S. aureus*)²⁴ have also revealed that they evolve and diversify over time, meaning that one or a few isolates will not accurately reflect the behaviors of an entire, chronically-infecting population of those bacteria. And studies of the microbes in different specimen types (such as swabs, sputum, and lavages) collected concurrently from individual CF patients indicates that each may sample different anatomic sites, raising the question of which sample is best for informing treatment or prognosis.

These new findings highlight the constantly evolving and controversial field of CF microbiology, which has largely been, and continues to be, informed by the results of standard cultures. Accordingly, the rest of this section will focus on what is known from conventional CF microbiology, with a concluding section on how recent developments may change clinical practice.

Traditional CF respiratory pathogens: Epidemiology

The two graphs in Figure 1 demonstrate that CF airway infections exhibit three key features:

- They are diverse.
- They are frequently polymicrobial.
- They are constantly evolving.

Figure 1a shows the US prevalence in 2013 by patient age of the bacteria cultured most commonly from respiratory cultures (as recorded in the Cystic Fibrosis Foundation Patient Registry⁴). This graph shows that *S. aureus* was particularly common among young children, while *P. aeruginosa* was most common in adults. The *S. aureus* subtype methicillin-resistant *S. aureus* (MRSA) had peak CF prevalence in the mid-teens to early 20's. *P. aeruginosa* resistant to multiple antibiotics (MDR-PA) was relatively uncommon but followed the same age distribution as *P. aeruginosa* in general. A brief consideration of these data reveals that, for each age group, many patients must have had more than one of these bacteria during the year.

Figure 1b provides a slightly different perspective of the 2013 data. Consider, for example, how the prevalences of *S. aureus* and *P. aeruginosa* have changed in the US since 1988. *S. aureus* prevalence has dramatically increased, while that of *P. aeruginosa* has decreased somewhat. Therefore, even considering just these 6 commonly-cultured bacterial species, one can appreciate the diverse, polymicrobial, and constantly evolving nature of CF lung infections. Further, some culturable pathogens (such as nontuberculous mycobacteria, NTM) are not depicted on these graphs. In the following sections, we will briefly discuss each of these species, paying particular attention to *S. aureus* and *P. aeruginosa*, given their high prevalence and clinical associations. For brevity, we will provide key points for less common

CF respiratory microbes. We will also discuss clinically-relevant variants of common pathogens, (such as MRSA and MDR-PA), as well as emerging pathogens such as NTM, with attention to their epidemiology, associations with disease, and treatment.

Staphylococcus aureus

Epidemiology and clinical associations: As described above, this gram-positive organism was the bacterium first identified as an important CF respiratory pathogen. The earliest CF antibiotic treatments targeted *S. aureus*, and early proponents of these treatments described the substantial clinical improvements that resulted. However, the role of *S. aureus* in driving CF lung disease, and the appropriate clinical response to its detection, are matters of ongoing debate. A review of published observations regarding the association between *S. aureus* and lung disease illustrates the basis for this controversy.

In addition to the high prevalence of *S. aureus* among children, pediatric CF studies have provided further reason to worry: *S. aureus* has been associated with higher airway inflammation^{25,26}, lower lung function^{27,28}, and even higher subsequent mortality when detected together with *P. aeruginosa*²⁹. Further, these associations were for all *S. aureus*; as described below, specific subtypes of *S. aureus* (such as MRSA and small-colony variants of *S. aureus* (SCVs)) may be associated with even worse outcomes than other *S. aureus*.

However, when considering all *S. aureus* in adults with CF, the picture becomes less clear. Studies of adults found *S. aureus* to be associated with a lower risk of mortality³⁰, better lung function³¹, and a lower risk of exacerbations³². Therefore, *S. aureus* pathogenesis may either be context-dependent (that is, perhaps it is either more pathogenic either in children or in the absence of *P. aeruginosa*, or it adapts over time to become less pathogenic). Alternatively, pathogenesis may be higher for specific subtypes of *S. aureus* (such as MRSA or SCVs), or *S. aureus* may not be universally pathogenic itself but rather serves as a marker of early (as with children) or mild (as with adults) disease.

Answering these questions is particularly pressing because of the recent increase in *S. aureus* prevalence shown in Figure 1b. Recent studies demonstrated similar trends in parts of Europe and Australia³³ by the mid-2000s. However, *S. aureus* prevalence has remained comparatively low in the United Kingdom³³, perhaps related to differences in antibiotic use (discussed below). Importantly, both longevity and lung function have continued to improve in the general CF population⁴, including in the age groups with the highest *S. aureus* prevalences. These combined observations make it difficult to predict the best approach to infection with *S. aureus*.

Subtypes: As hinted above, two subtypes of *S. aureus* are of particular significance for CF lung disease and treatment: MRSA and SCVs.

MRSA (or ORSA for oxacillin-resistant *S. aureus*) are usually identified either by their resistance to these β -lactams or by carriage of the *mecA* gene, which encodes this resistance. MRSA prevalence in CF has increased in the past 2 decades (Figure 1b) in parallel with all *S. aureus* and methicillin-susceptible *S. aureus* (MSSA). MRSA acquisition in CF is strongly associated with exposure to hospitals and to antibiotics^{34–36}, as it is in the general

population, which also experienced a parallel increase in MRSA prevalence in the US. Interestingly, the increase seen in the US CF population has not occurred as dramatically elsewhere³³, suggesting that environmental, treatment, or other factors unique to specific locations are responsible for this epidemiologic change.

The relationship between MRSA and CF lung disease outcomes is complex. While a number of studies have demonstrated an association between MRSA infection and lower lung function in CF patients^{34,35,37}, some studies indicated that patients with MRSA had faster lung function decline prior to MRSA detection, suggesting MRSA may be a marker for severe disease rather than the cause³⁵. Other studies, however, found MRSA to be an independent risk factor for failure to recover lung function during treatment for respiratory exacerbations³⁸ and for increased mortality³⁹.

S. aureus SCVs are slow-growing, antibiotic-resistant variants that are difficult to detect with conventional cultures. Small studies of both European^{40–42} and US CF⁴³ populations have identified SCVs among 8–30% of patients using special laboratory methods. These studies have also found SCVs to be associated with lower lung function, faster lung function decline, and higher rates of preceding treatment with antibiotics known to select for SCVs, including aminoglycosides and sulfonamides, in addition to higher rates of co-infection with *P. aeruginosa*, which may also select for SCVs⁴⁴.

The similarities between MRSA and *S. aureus* SCVs, particularly their relationships with clinical outcomes, antibiotic treatment and resistance, are instructive. Each of these bacterial types is associated with:

- Preceding antibiotic treatment
- Subsequent antibiotic resistance
- Higher lung disease severity

However, these findings are largely from observational studies, and whether MRSA and SCVs cause worse outcomes, whether they reflect patients with worse preexisting disease and resulting higher antibiotic treatment burdens, or both, remains to be seen. Additional observational and interventional studies may resolve these questions of causality. Until then, the best therapeutic approaches to each subtype remains to be defined.

Treatment: Determining the best approach to *S. aureus* infection in CF is a controversial subject. Some countries provide antistaphylococcal prophylaxis during childhood; this practice has been associated in some trials with earlier detection of *P. aeruginosa* which, given its association with worse lung disease, led many countries to not adopt this approach⁴⁵. Some centers provide antistaphylococcal antibiotics aimed at eradicating *S. aureus* on first detection⁴⁶, and strategies and attitudes differ for MRSA versus MSSA; evidence supports the microbiological efficacy of this strategy, but clinical benefit for both MRSA and MSSA remain to be determined^{46,47}. By comparison, for patients with *S. aureus* and no other detected pathogens who have an exacerbation, there is consensus that antistaphylococcal antibiotics should be used. The drugs used most often in this case have been reviewed⁸.

Pseudomonas aeruginosa

Epidemiology and clinical associations: With the advent of effective antistaphylococcal therapy, the gram-negative bacterium *P. aeruginosa* emerged as a common and important pathogen in CF lung disease (reviewed in¹²). *P. aeruginosa* infection has been associated with⁴⁸:

- Worse lung function outcomes
- Higher respiratory tract inflammation
- A greater risk of respiratory exacerbations
- Higher risk of mortality

While chronic *P. aeruginosa* infection usually cannot be eradicated, current evidence indicates that initial *P. aeruginosa* detection presents a “window of opportunity” for eradication with antibiotics. There is also good evidence that suppressing *P. aeruginosa* can improve measures of lung disease. For CF exacerbations among people with *P. aeruginosa*, antipseudomonal antibiotics have been shown to improve outcomes⁴⁹. However, whether early eradication provides similar clinical benefits is still under investigation⁵⁰.

As shown in Figure 1a, *P. aeruginosa* prevalence tends to be higher in adults than in children; its overall prevalence has decreased slightly in the past few decades (Figure 1b), perhaps related to new eradication approaches upon initial detection. Longitudinal analyses indicate that *P. aeruginosa* tends to “exclude” other microbes that were previously detected in the respiratory tract, and that it is frequently the predominant, and often the only, bacterium identified in CF lungs during end-stage disease⁵¹. These aggregate findings have earned *P. aeruginosa* the reputation as the most important CF respiratory pathogen.

P. aeruginosa has a very large genome, encoding numerous potential toxins controlled by a complex array of regulatory elements. During chronic CF infections, *P. aeruginosa* undergoes diverse adaptive changes, including inactivation of many of these “virulence factors” and their regulators⁵². One of the most common phenotypic changes observed for *P. aeruginosa* is the exuberant production of alginate, resulting in a mucoid colony appearance *in vitro*⁵³. Mucoidity has been associated with both persistence and duration of infection; however, it was not associated with failure of early eradication in recent studies. Interestingly, those studies also identified mucoid isolates upon initial detection of *P. aeruginosa*⁵². As mucoidity has traditionally been thought to represent adaptation to the airway, these results may indicate that current detection methods are not perfectly sensitive for earliest infection.

Epidemiologic evidence has identified genetically-related isolates of *P. aeruginosa* (referred to as epidemic strains) among CF populations in several countries (reviewed in reference 54). In some cases, these isolates were associated with worse outcomes than non-epidemic *P. aeruginosa*, including higher risks of death and need for lung transplantation. Similarly, *P. aeruginosa* resistant to multiple antibiotics (MDR) have been found to be associated with worse lung disease by several measures, but as with MRSA and *S. aureus* SCVs, whether

these resistant *P. aeruginosa* identify patients with higher antibiotic exposures, or whether they are more pathogenic than other *P. aeruginosa*, remains to be determined⁵⁵.

Treatment: Prophylaxis for *P. aeruginosa* is not recommended. However, treatment with antibiotics upon initial detection, with the goal of eradication, has proven to be microbiologically effective, and to decrease the risk of subsequent exacerbations. Inhaled antibiotics, such as tobramycin and more recently aztreonam, form the basis of the current guidelines⁵⁶ for eradication, with no benefit found by adding an oral drug of another class (ciprofloxacin). Similarly, inhaled antibiotics are recommended as chronic maintenance medications for CF patients with persistent *P. aeruginosa*. For exacerbation treatment in patients chronically infected with *P. aeruginosa*, limited evidence indicates that treatment with two classes of antipseudomonal antibiotics leads to longer periods of clinical stability than does a single class. Treatments for *P. aeruginosa* in CF have been the subject of recent reviews and guidelines^{9,56}.

Burkholderia cepacia complex (BCC)

Epidemiology and clinical associations: The BCC group of gram-negative bacteria is comprised of at least 18 distinct species (a related species, *B. gladioli*, is often discussed with BCC because it can cause similar infections). Of these, two species are among the most common and most associated with CF lung infections and disease:

- *B. cenocepacia*
- *B. multivorans*

By comparison, other BCC species are less common and their clinical associations less well-defined (as is the case for *B. gladioli*)⁵⁷. As shown in Figure 1, *Burkholderia* species have infected between 3–4% of CF patients in the US for many years^{4,14}, primarily affecting adolescents and adults. *B. multivorans* prevalence was highest, followed by *B. cenocepacia* and *B. gladioli*.

Burkholderia CF infections are notorious for several characteristics:

- **They are associated with more severe lung disease.** These associations are more pronounced for some *Burkholderia* species than others; some small studies have shown *B. cenocepacia* to be associated with more rapid lung function decline than *B. multivorans*, and a recent Canadian study found the strongest association with subsequent mortality to be with *B. cenocepacia*, followed by *B. multivorans*⁵⁷.
- **They can be transmissible between persons with CF.** Epidemic strains of *B. cenocepacia* have been shown to infect CF patients after interpatient contact at camps and clinics⁵⁸ and have even been shown to replace *B. multivorans* after transmission. This transmissibility was one important driving force behind the institution of stringent infection control measures, which evidence suggests have been highly ineffective for controlling spread of these strains.

- **Clinical outcomes of BCC infections are unpredictable.** Associated outcomes often range from clinical quiescence to rapidly progressive, necrotizing pneumonia and fatal septic disease (“*cepacia* syndrome”).

Treatment: Currently, the practice of antibiotic treatment for eradication on early isolation is controversial, and the utility of this practice is not yet clear^{59,60}. *Burkholderia* species are also notorious for their antibiotic resistance—both intrinsic and acquired—and therapy is usually limited to specific antibiotics as needed. Systematic reviews have recently been published⁶¹.

Considerations for the following microbes in CF infections will be presented briefly:

Stenotrophomonas maltophilia^{13,14}

- Gram-negative
- Increased US CF prevalence in recent years.
- Particularly common among adolescents and young adults.
- Conflicting data regarding clinical associations.
- Both intrinsically and adaptively resistant to many antibiotics
- Inadequate evidence for the clinical impact of therapy; a Cochrane Review of current approaches recently became available.

Haemophilus influenzae¹⁴

- Gram-negative
- Often the first organism detected in CF respiratory cultures; prevalent in children, less common in adults.
- Association with CF clinical outcomes is controversial, but associated with adverse outcomes in other chronic respiratory diseases: non-CF bronchiectasis and chronic obstructive pulmonary disease.
- Difficult to culture, requiring specific conditions for detection.
- Since the introduction in the US of the *H. influenzae* type B (HIB) vaccine, most isolates are “nontypeable” and unencapsulated.
- Can produce β -lactamase; therefore, treatments usually include a β -lactamase inhibitor (e.g., amoxicillin-clavulanate).

Achromobacter xylosoxidans^{10,13}

- Gram-negative bacterium similar to *P. aeruginosa*.
- Increased recent US prevalence, but remains low (<10% of CF patients, Figure 1).
- Associated with worse radiographic and spirometric measures of lung disease.

- Similar to *P. aeruginosa* and BCC, *A. xylosoxidans* can be the dominant, and sometimes only, bacterium isolated from CF patients at end-stage.
- Notorious for resistance to many antibiotics, limiting treatment options.

Nontuberculous Mycobacteria (NTM)^{11,13,14}

- Estimated CF prevalence 6—30%.
- Two groups of Mycobacteria, accounting for 6 species, are currently considered important CF pathogens:
 - *Mycobacterium-avium* complex (MAC)
 - *Mycobacterium abscessus* complex
- NTM treatment approaches usually involve two phases:
 - Multiple intravenous (IV) antibiotics for weeks to months
 - Multiple inhaled and oral antibiotics for months to years
 - Side-effects and toxicities are common and can be troublesome.
- Guidelines for NTM infection diagnosis and treatment from a joint committee based in both the US and Europe expected to be published in 2015-6.

Fungi and viruses^{11,14}

- Numerous fungi are frequently isolated from CF patients, including:
 - Yeasts (particularly *Candida* spp.)
 - Filamentous fungi (including *Aspergillus* spp).
- Whether detection of fungi requires treatment is a matter of current debate.
- **Allergic Bronchopulmonary Aspergillosis (ABPA):** Patients with CF and other chronic airway diseases can develop an IgE-mediated allergic airway disease known as ABPA, the treatment for which primarily involves steroids, although the addition of an antifungal (such as itraconazole) may allow for lower doses of steroids.
- Human respiratory viruses are not thought to chronically infect the CF airway, but they have been shown both to be important and common triggers of CF respiratory exacerbations.

The CF airway microbiome and future directions

The application of DNA-based microbiological techniques to CF respiratory samples has afforded a very different view of their microbiota. To date, dozens of such studies using a variety of methods have been performed. This collective work provides evidence for dynamic, diverse, and highly resilient CF respiratory microbial communities (reviewed in

reference 22). In general, these respiratory microbiota tend to be more diverse, with more microbial species and not dominated by any individual microbe, in CF patients who are younger, have better lung function, and/or have had fewer courses of antibiotics. Conversely, this diversity tends to be lower in patients who are older, who have more severe lung disease, and who have had been treated with more antibiotics. The microbiota in lungs from patients with end-stage CF disease tend to be dominated by one or very few species, most often *P. aeruginosa*, BCC, or *A. xylosoxidans*. However, these associations between age, disease severity, microbial diversity, and antibiotic burden make it difficult to sort out cause and effect; it is not clear yet whether the decrease in diversity “drives” disease severity, or whether patients with worse preexisting disease receive more antibiotics that “prune” the microbiota to select for the most adaptable and resistant microbes. Ongoing and future studies aim to better define the causal relationships between infection dynamics, therapy, and disease progression.

CF microbiology: Summary

There have been remarkable changes and developments since the first descriptions of CF lung infections in the 1930s-40s. Antibiotics have played a central role in improving lung disease outcomes and overall longevity. However, findings over the past 70 years from both culture-based, classical microbiological methods, and those from newer culture-independent techniques, repeatedly demonstrate the resilient and dynamic nature of chronic CF lung infections. These findings warrant ongoing vigilance and innovation, and they highlight the many questions that have arisen in this rapidly-moving field. In the years to come, there are certain to be new revelations regarding the mechanisms of infection and the microbial determinants of both CF lung disease and response to treatment, which will lead to new recommendations for therapy and infection control. However, the many controversies discussed above regarding the relationships between airway microbes and clinical outcomes underscore the importance of host response in driving CF lung disease. The next section will discuss these inflammatory mechanisms.

II. HOST- RESPONSE IN CF LUNG DISEASE

As emphasized above, chronic airway infections are a major driver of progressive airways destruction in CF. Much of the damage seen with chronic infection is mediated by a vigorous and abnormal host-response to airway infections, resulting in a vicious cycle that leads to irreversible bronchiectasis and lung function decline^{8,62} (Figure 2a). Over the past several decades, numerous studies have documented a heightened, primarily neutrophilic inflammatory response in CF airways. Much of the inflammatory response is driven by the presence of bacterial pathogens, but the reaction is exaggerated even when accounting for the presence of infection^{63,64}. Debate continues over how much of the inflammatory response is related to underlying CFTR dysfunction and whether airway inflammation precedes the onset of infection.

In this section, we will consider evidence for the role of CFTR dysfunction in altered host-defense; how imbalances in the airway environment contribute to disease pathophysiology and may lead to new therapies; and how specific measures of inflammation, particularly

neutrophil elastase (NE), may be useful biomarkers of disease. Finally, we will consider current and future approaches to anti-inflammatory therapies and highlight challenges in moving these therapies from the bench to bedside.

CFTR, impairments in host-defense and inflammation

The role of CFTR in altered host-defense has been examined extensively using *in vitro* cell culture systems, *in vivo* animal models and human subject studies. Two recent review articles explore this issue in depth^{16,15}. Briefly, several lines of evidence now link CFTR dysfunction to altered host-defense through primary and secondary mechanisms (Figure 2b). Airway surface liquid dehydration and acidification results from lack of CFTR function in the epithelial cells lining the airways. Loss of the airway surface liquid layer results in abnormal mucociliary clearance, a first layer of defense against microbes. Acidification, due to lack of bicarbonate secretion by CFTR, likely alters periciliary mucins, resulting in a dense and overly adherent mucus layer lining the cilia^{65,66}. Neutrophils and macrophages also express CFTR, and lack of functional CFTR channels likely reduce bacterial killing and clearance by these cells¹⁵.

In many ways, CF airways disease can be best understood as imbalances initiated by CFTR dysfunction and perpetuated by chronic infection, a necessary but overly exuberant inflammatory response, and airway structural injury that progressively worsens mucociliary clearance. Bacterial burden is typically high in CF airways; however, the neutrophilic inflammatory response is elevated for a given bacterial load in CF compared to non-CF patients^{63,64}. The neutrophilic response is worsened by defects in neutrophil apoptosis and impaired clearance of debris. Defects in mucociliary clearance and macrophage dysfunction lead to accumulation of neutrophils and their products^{15,67}. Proteases, primarily NE, are released by neutrophils and overwhelm the normally counteracting anti-proteases (e.g. alpha-1-antitrypsin, a serine protease inhibitor, and secretory leukocyte protease inhibitor), leading to airways damage^{68,69}. Antioxidants in the CF airways are also overwhelmed by oxidative stress from persistent infection and inflammatory responses. CFTR dysfunction may also lead to worsening oxidative imbalance through impairment of Nrf2, a transcription factor crucial in maintaining oxidative reducing capacity. Finally, lipid mediators that regulate antiinflammatory responses to insults are likely abnormal in CF, further limiting the ability of the host to mitigate an overly vigorous inflammatory reaction. Therefore, therapies aimed at restoring airway equilibrium may help reduce airway injury.

Host response and inflammation as biomarkers of disease

In addition to contributing to the pathophysiology of CF airway disease, mediators of host-response may serve as important biomarkers of disease progression. NE has been the most well-documented inflammatory biomarker to date. NE is more abundant in induced sputum in CF compared to control children⁷⁰; in school-aged children with CF, elevated NE in induced sputum has been associated with subsequent lung function decline⁷¹. Elevated NE in BALF is associated with bronchiectasis in infants and preschool aged children⁷² and with lung function measurements in infants with CF²⁷. Sputum measures of airway inflammation have also been shown to decrease after treatment of a pulmonary exacerbation with IV

antibiotics⁷³. In a study of IV antibiotics, higher sputum NE on day 14 of therapy for a pulmonary exacerbation was associated with increased risk of subsequent exacerbation⁷⁴.

Circulating inflammatory proteins have also been examined as potential biomarkers of disease in CF. In a multicenter study measuring a panel of plasma proteins at onset of a pulmonary exacerbation and following treatment with IV antibiotics, significant reductions in 10 of 15 proteins were observed⁷⁵. Baseline measurements of IL-8, NE-protease complexes and alpha-1-antitrypsin, when combined with changes in protein markers and lung function, predicted a better treatment response, suggesting that a panel of circulating markers may be useful clinically and for future therapeutic trials. A clinical trial in CF demonstrated reduction in systemic inflammation following six months of treatment with chronic azithromycin⁷⁶, although treatment with several agents, including ibuprofen and ivacaftor in patients with the G551D mutation, that were expected to decrease inflammation did not demonstrate this anticipated effect^{77,78}.

Host-response to *Pseudomonas aeruginosa*

A number of studies have examined the host response to *P. aeruginosa* in an effort to understand the clinical associations with this pathogen^{79–81}. The interaction between *P. aeruginosa* and the CF host is complex and beyond the scope of this review. However, it is likely that CFTR dysfunction predisposes the host to infection with *P. aeruginosa*; subsequent adaptation by the bacteria then allows for chronic infection and reduced opportunity for eradication. It is also likely that *P. aeruginosa* interacts with other bacterial pathogens including *Staphylococcus aureus* and *Burkholderia cepacia* complex in a way to further alter the inflammatory response^{25,82}.

Anti-inflammatory therapies in CF

As described above, an overly exuberant inflammatory response in CF leads to airways damage and worsening bronchiectasis. However, the CF immune response also plays a critical role in containing microbes within the airways and preventing invasive disease. While therapies that blunt the inflammatory response theoretically may limit airway damage, they could potentially lead to worsening infections. Several anti-inflammatory therapies have been studied with variable results, likely due to difficulties in maintaining this balance.

Ibuprofen—In cell and animal models, ibuprofen has been shown to inhibit neutrophil migration and aggregation, including in CF animal models⁸³. The effect of chronic high-dose ibuprofen was evaluated in a two-center, randomized, double-blind, placebo controlled trial⁸⁴. Patients in the treatment group had a slower rate of lung function decline overall, although in a subgroup analysis by age, the difference was significant only in patients younger than 13 years. There was no reduction in hospitalization rate seen with treatment. Several follow-up studies confirmed these findings, and showed reasonable safety data, although gastrointestinal bleeding was more common in those on chronic therapy⁸⁵. A recent Cochrane review concluded that high-dose ibuprofen could slow the progression of lung disease in CF, particularly in children with mild disease⁸⁶. Despite these findings, clinical use of ibuprofen is relatively uncommon particularly compared to other CF

therapies⁸⁷. Reasons are likely related to persistent clinician concerns about adverse effects (e.g. kidney impairment, gastric bleeding), and need for careful pharmacokinetic monitoring to achieve and maintain therapeutic dosing. Based on pulmonary clinical guidelines published in 2013, the CF Foundation recommends chronic use of ibuprofen in children ages 6–17 years with FEV₁ > 60% (Grade B, moderate net benefit), but does not recommend treatment in adults with CF⁸⁸.

Chronic azithromycin—Azithromycin is a macrolide antibiotic with immunomodulatory effects, which are likely the basis for its effectiveness in CF and other chronic inflammatory conditions⁸⁹. Chronic azithromycin has been studied in two CF populations: patients with and those without chronic *P. aeruginosa* infection^{90,91}. Patients with chronic *P. aeruginosa* infection treated with thrice weekly azithromycin for 6 months had improved FEV₁, decreased risk of pulmonary exacerbation and improved weight gain compared to placebo⁹⁰. In a second study, patients without *P. aeruginosa* treated with azithromycin had a 50% reduction in pulmonary exacerbations and a modest improvement in weight⁹¹. However, lung function did not improve compared to placebo. CF clinical guidelines recommend azithromycin for people with CF ages 6 years and older with chronic *P. aeruginosa* (Grade B, moderate net benefit), and consideration of treatment for those without *P. aeruginosa* (Grade C, moderate net benefit)⁸⁸. The durability of effect with azithromycin remains unclear however, and there are concerns about emergence of bacterial resistance^{92,93}; thus, treatment should be reassessed every 6–12 months. In addition, patients with NTM should not receive azithromycin unless it is prescribed in combination with other anti-mycobacterial medications as part of NTM therapy.

Corticosteroids and leukotriene receptor antagonists—Systemic and inhaled corticosteroids have been studied in patients with CF. Although systemic corticosteroids have been shown to improve lung function, the adverse effects, including poor somatic growth, outweigh any benefit⁹⁴. Inhaled corticosteroids have also not been shown to be effective in CF⁹⁵. Thus, the CF Foundation recommends against the use of chronic systemic or inhaled corticosteroids⁸⁸. Montelukast, a leukotriene receptor antagonist, showed modest effects on lung function and inflammatory markers in a small pediatric study (n=28), however more robust data are lacking⁹⁶. A phase 2 clinical trial of a leukotriene B₄ receptor antagonist, BIIL 284 BS, was terminated after an interim analysis revealed increased pulmonary-related, serious adverse events and incidence of pulmonary exacerbations in those on treatment, highlighting the potential risks of suppressing the immune response in patients with chronic infections⁹⁷.

Clinical trials: Several compounds are currently in the CF drug development pipeline. Alpha-one-proteinase inhibitor (Alpha-1 HC) is an aerosolized protease inhibitor with anti-inflammatory properties. A phase 2 randomized, double-blind, placebo-controlled study of Alpha-1 HC given for three weeks to adults with CF showed safety and tolerability at two dose levels⁹⁸. CTX-4430 is a LTB₄ receptor antagonist that is currently in phase 2 testing; risk of pulmonary adverse events and exacerbations will be closely monitored particularly given the previous above results with BIIL 284 BS (Trial number NCT02443688, clinicaltrials.gov). Ajulemic acid (JBT-101) is an oral synthetic cannabinoid agonist that

binds to the CB2 receptor expressed on activated immune cells and fibroblasts, and thought to help resolve inflammation and limit fibrosis⁹⁹. A 12-week, phase 2 study in adults with CF is currently recruiting (Trial number NCT02465450, clinicaltrials.gov).

III. CONCLUSIONS

Airway obstruction, infection and inflammation lead to much of the morbidity and mortality associated with CF. *P. aeruginosa*, *S. aureus* and BCC are the bacteria most frequently associated with CF lung disease; improved and aggressive antimicrobial treatment of these pathogens has contributed greatly to improvements in life expectancy. However, airway microbial communities are complex and likely influenced by environment, host characteristics, disease progression, and antibiotic treatment. Host-response to microbes in the CF airways is robust, likely due in part of underlying CFTR protein dysfunction. The overly exuberant host-inflammatory response and decreased host ability to clear microbes and inflammatory debris from the airways leads to further damage. Therapies targeting airway infections and host-inflammatory response are effective in reducing disease activity, but do not fully stop progression. Fortunately, a number of anti-inflammatory and antimicrobial therapies are in the therapeutic development pipeline. In addition, CFTR modulator therapy, newly available for ~50% of people with CF, and with the hope of expanded treatments for all with CF, will undoubtedly influence both airway microbiology and inflammatory responses.

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Key points

- CYSTIC FIBROSIS LUNG DISEASE INVOLVES A CYCLE OF MUCUS OBSTRUCTION, INFLAMMATION, AND INFECTION IN THE AIRWAYS, AND EACH OF THESE ELEMENTS IMPACTS THE OTHERS AS DISEASE PROGRESSES.
- OBSERVATIONS FROM THE EARLIEST STUDIES OF CF DISEASE IN THE 1930S-40S DEMONSTRATED THESE ELEMENTS, BUT OUR UNDERSTANDING AND TREATMENT OF EACH HAS EVOLVED OVER TIME.
- CF RESPIRATORY MICROBIOLOGY HAS ALSO EVOLVED AS NEW TREATMENTS HAVE BEEN INTRODUCED, AS PEOPLE HAVE LIVED LONGER WITH THIS DISEASE, AND AS DETECTION METHODS HAVE BECOME MORE SOPHISTICATED.
- THE CF AIRWAY ALSO DEMONSTRATES ALTERED HOST-DEFENSE, AND IMBALANCES IN THE AIRWAY ENVIRONMENT ARE IMPORTANT FOR PATHOPHYSIOLOGY AND MAY INDICATE BOTH NEW TREATMENT DIRECTIONS AND USEFUL DISEASE BIOMARKERS.
- BECAUSE MOST INFORMATION REGARDING THE RELATIONSHIPS BETWEEN INFECTION, INFLAMMATION, AND DISEASE SEVERITY COME FROM OBSERVATIONAL STUDIES, THEIR CAUSAL RELATIONSHIPS ARE NOT ALWAYS CLEAR.

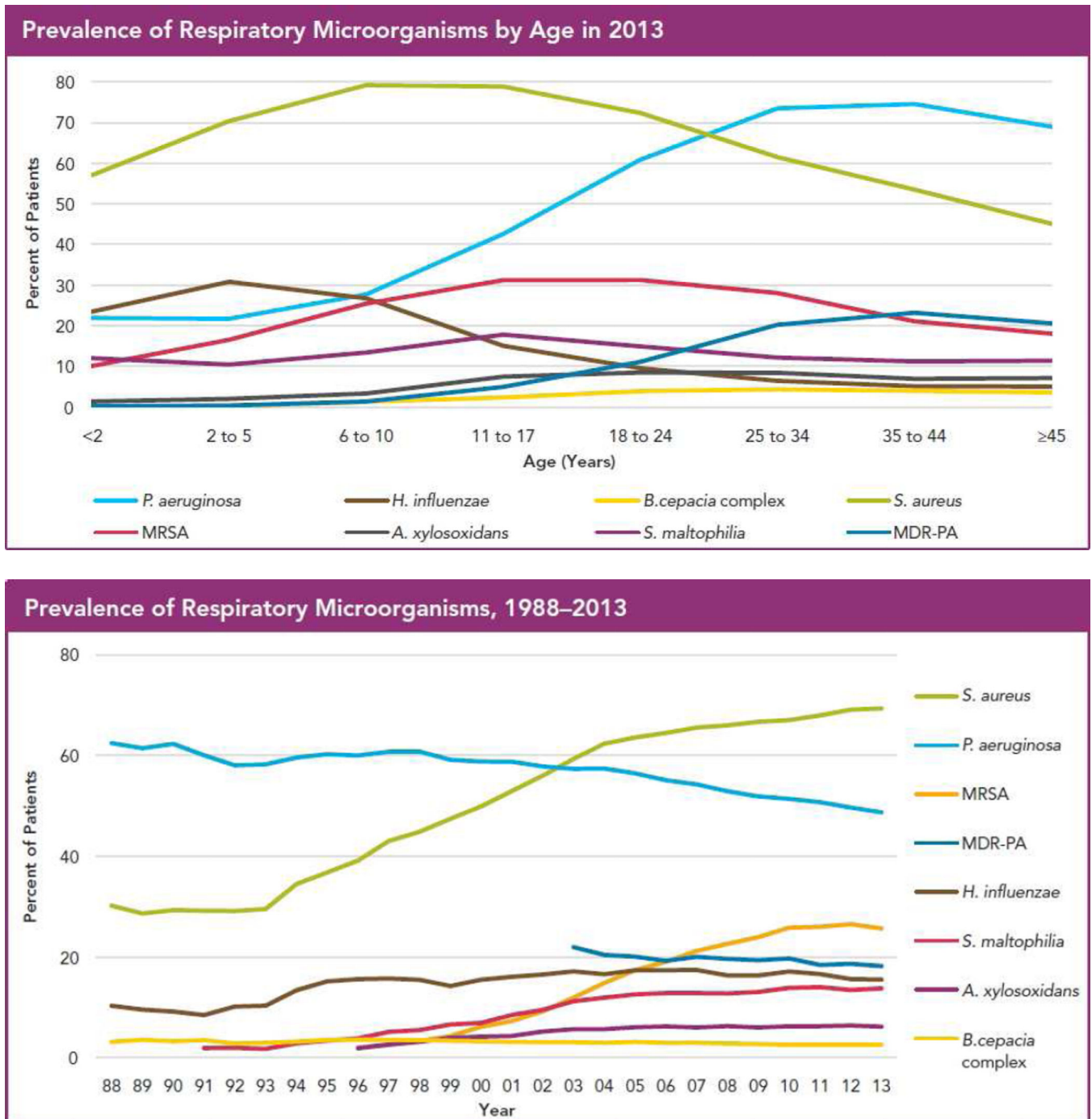
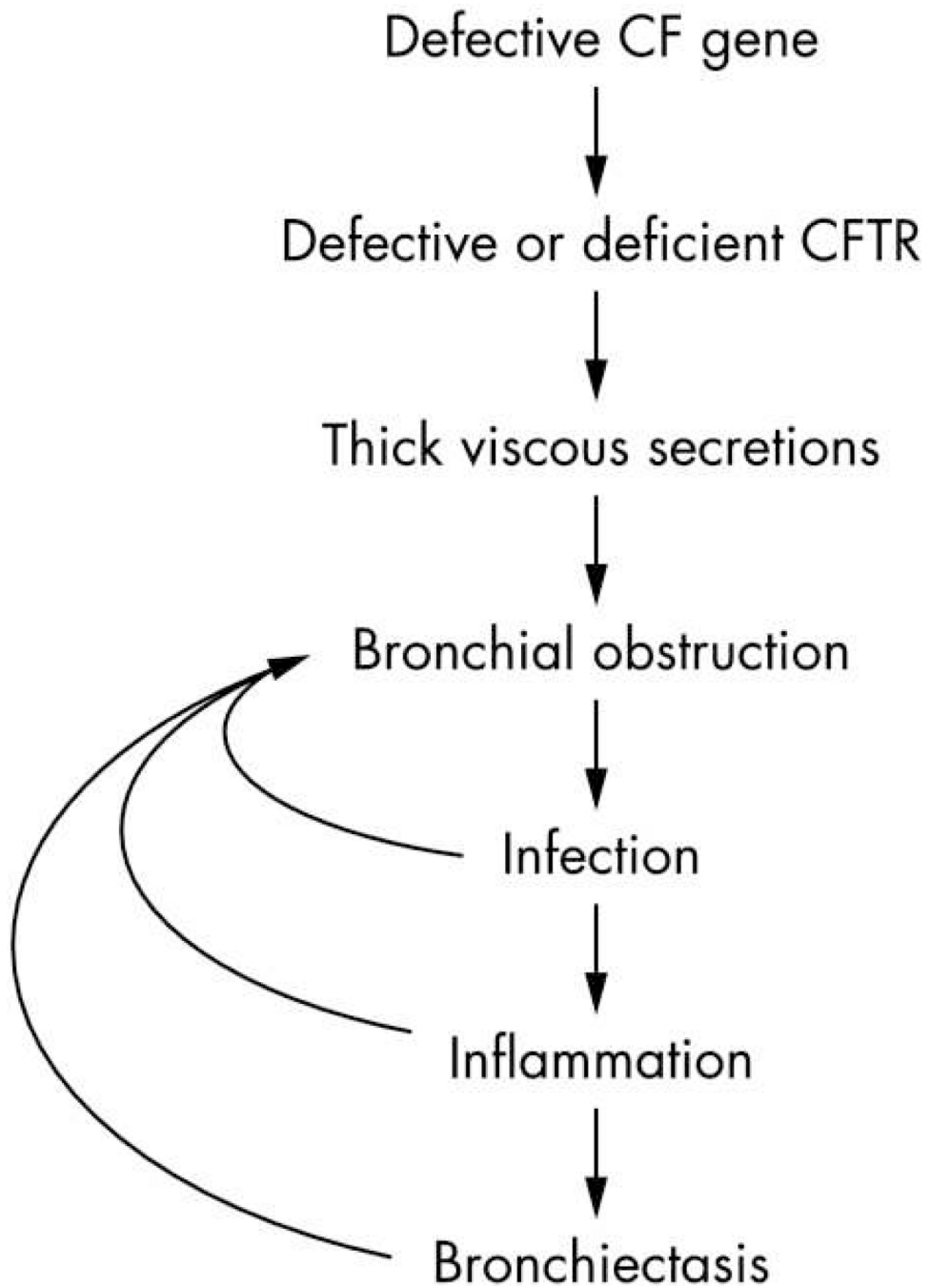


Figure 1. Epidemiology of traditional CF pathogens in 2013, from the US CF Patient Registry. (a) Percent of patients reported to be culture-positive for the indicated bacteria in 2013, by age group. (b) Overall prevalence in CF patients per year of these bacteria. Cystic fibrosis patients under care at CF Foundation-accredited care centers in the United States, who consented to have their data entered. Courtesy of CF Foundation, Broomall, PA.



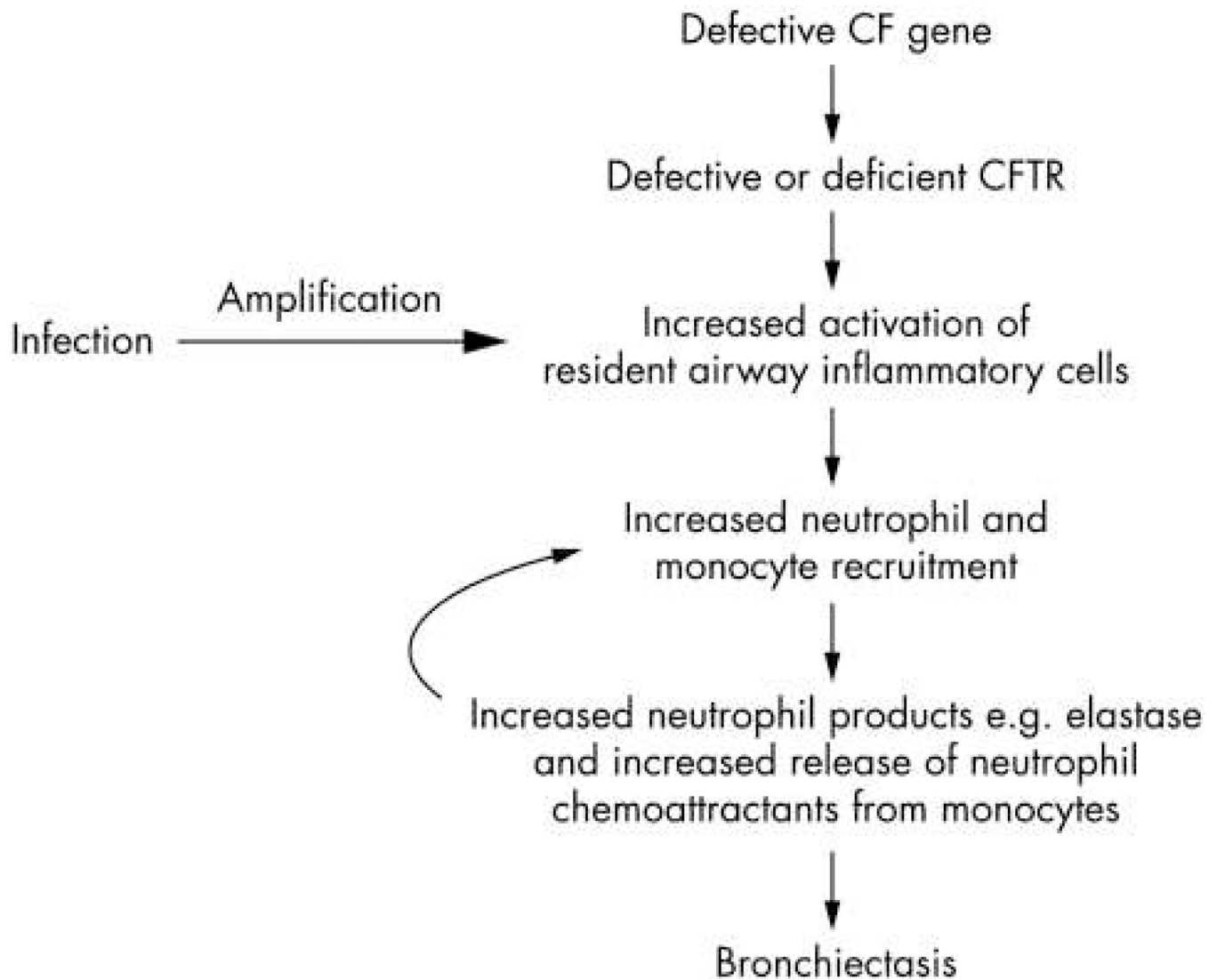


Figure 2.

Pathophysiology of lung disease in CF. (a) Traditional view of pathology of lung disease in CF. Inflammation is triggered by chronic bacterial infection. (b) Potential alternative mechanism for airway inflammation in CF. Underlying CFTR dysfunction contributes to altered host-response.

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