Constrictive Bronchiolitis in Cystic Fibrosis Adolescents with Refractory Pulmonary Decline

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Abstract

Rationale: Refractory lung function decline in association with recurrent pulmonary exacerbations is a common, yet poorly explained finding in cystic fibrosis (CF). To investigate the histopathologic mechanisms of pulmonary deterioration during adolescence and early adulthood, we reviewed clinically indicated lung biopsy specimens obtained during a period of persistent decline.

Objectives: To determine if peribronchiolar remodeling is prominent in lung biopsy specimens obtained in adolescents with CF refractory to conventional therapy.

Methods: Six adolescents with CF (mean age, 16.2 y; mean FEV₁, 52% predicted at biopsy) with significant pulmonary deterioration over 12–24 months (mean FEV₁ decline of 14% predicted/year) despite aggressive intervention underwent computed tomography imaging and ultimately lung biopsy to aid clinical management. In addition to routine clinical evaluation, histopathologic investigation included staining for transforming growth factor- β (TGF- β , a genetic modifier of CF lung disease), collagen deposition (a marker of

fibrosis), elastin (to evaluate for bronchiectasis), and α -smooth muscle actin (to identify myofibroblasts).

Measurements and Main Results: All computed tomography scans demonstrated a mix of bronchiectasis and hyperinflation that was variable across lung regions and within patients. Lung biopsy revealed significant peribronchiolar remodeling, particularly in patients with more advanced disease, with near complete obliteration of the peribronchiolar lumen (constrictive bronchiolitis). Myofibroblast differentiation (a TGF- β -dependent process) was prominent in specimens with significant airway remodeling.

Conclusions: Constrictive bronchiolitis is widely present in the lung tissue of adolescents with CF with advanced disease and may contribute to impaired lung function that is refractory to conventional therapy (antibiotics, antiinflammatories, and mucolytics). TGF- β -dependent myofibroblast differentiation is prominent in areas of active fibrogenesis and may foster small airway remodeling in CF lung disease.

Keywords: airway remodeling; cystic fibrosis; constrictive bronchiolitis; myofibroblast; transforming growth factor- β

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Pulmonary exacerbations in cystic fibrosis (CF) contribute significantly to longitudinal respiratory decline (1, 2). Approximately one-quarter of patients who experience a pulmonary exacerbation do not recover previous lung function (3–5), with a greater

frequency of exacerbations increasing the likelihood of developing end-stage lung disease (2, 6). Pulmonary decline accelerates during adolescence and early adulthood (7–9), with exacerbations predictive of future respiratory limitation (10).

Recently, we identified an abundance of profibrotic myofibroblasts in areas of collagen deposition in CF lungs (11). Myofibroblasts have increased contractility (manifest by the presence of α -smooth actin contractile fibers) and secrete

extracellular matrix products such as collagen to facilitate wound repair (12). In health, myofibroblasts undergo apoptosis after repair is complete (13, 14). However, persistence of the myofibroblasts in response to ongoing transforming growth factor- β (TGF- β) stimulation and mechanical strain results in a profibrotic phenotype (15–17). Our previous description (11) of extensive small airway remodeling in CF lungs had the limitation of using end-stage CF tissue (transplant or autopsy) in which fibrotic areas were often extensive and paucicellular, with uncertainty as to the timeline of fibrogenesis and the relative contribution to disease. This report of lung histopathology in adolescent subjects with CF undergoing clinically indicated lung biopsy seeks to circumvent this limitation by examining tissue during a period of active, refractory decline.

The Cystic Fibrosis Center at Cincinnati Children's Hospital Medical Center (CCHMC) has incorporated a comprehensive, multidisciplinary treatment algorithm for patients with persistent decline in lung function (18). This algorithm includes a focus on evidence-based care programs, enhanced medication adherence, screening for CF-related diabetes, bronchoalveolar lavage, and lung computed tomography (CT) imaging. When diagnostic evaluation merited further investigation, a few children were referred for clinically-indicated lung biopsy to ascertain more precisely the pathophysiology of respiratory decline.

The availability of lung biopsy tissue obtained during periods of progressive deterioration allowed the opportunity to investigate the prominence of tissue remodeling in actively progressive disease. We focused specifically on the extent of peribronchiolar remodeling, TGF- β signaling, and myofibroblast differentiation in these lung sections. In this article, we describe the clinical manifestations, radiographic features, and histopathology of six adolescent patients with CF who underwent lung biopsy because of refractory pulmonary disease. These pathologic examinations provide a unique window into the nature of CF lung disease during a period of active decline and emphasize the contribution of myofibroblast differentiation to small airway obstruction in this setting. Some results have been presented previously at the North American Cystic Fibrosis Conference (19).

Methods

Institutional Approval

Examination of human tissue for research purposes and review of the medical record was approved by the CCHMC Institutional Review Board (No. 2014–6774) and the University of Alabama at Birmingham Institutional Review Board (X15073002, X081204008).

Tissue Procurement

The CCHMC database of patients with CF was reviewed for all cases of clinically indicated lung biopsy performed between 2002 and 2015. Lung pathology slides were accessed for review (J.T.B., M.L.B., W.T.H., and J.P.C.) with additional research slides generated from formalin-fixed paraffin-embedded lung tissue. Deidentified patients without CF (patients undergoing clinically indicated lung biopsy) and deidentified patients with CF (with end-stage disease) were used as histopathologic control subjects.

Immunohistochemistry

Lung tissue blocks were sectioned at 4 μ m. Sections of parenchyma were obtained at the pathologists' discretion (J.T.B., M.L.B.), with parallel slides used for serial immunohistochemistry. Typically, the research sample consisted of only one tissue block per subject. Heat-induced epitope retrieval with 0.02 M of citrate buffer (pH 6.0) at 97°C for 20 minutes was performed, and immunohistochemistry was accomplished with an Autostainer 720 semiautomated immunostainer (ThermoScientific, Fremont, CA) and polymer system (UltraVision LP, ThermoScientific). In addition to standard hematoxylin and eosin (H&E), collagen (TriChrome), and elastin (Verhoeff–Van Gieson) stains, research slides for TGF- β 1 (Santa Cruz SC-146, Abcam No. ab170874, Santa Cruz, CA), phosphorylated Smad2 (a marker of TGF- β signaling, Cell Signaling No. 3101 Ser465/467 at 1:100 dilution), p38 MAPK (Cell Signaling No. 9212, Danvers, MA), and α -smooth muscle actin (α -SMA) (a marker of smooth muscle and myofibroblasts, Biocarta AB-1 at 1:100, San Diego, CA) were prepared.

CT Technique and Imaging Review

Radiographic imaging was obtained on the basis of clinical decision making, with

abnormal chest CT findings being the major impetus for lung biopsy. The imaging record was reviewed (A.S.B.), and CT scans were scored using the Brody CT scoring scale, as described previously (20). In brief, the Brody scoring system records the extent and severity of seven features of CF lung disease in each lobe (20–23). For this analysis, the composite score, which includes all seven observations, and subscores for bronchiectasis and air trapping was calculated. For each score or subscore, lobar scores were combined to produce scores reflecting the extent and severity of disease in both lungs.

Over the review period, CT scanning was performed with three different CT scanners (Toshiba Aquilion 1, Toshiba Aquilion 64, Tustin, CA, and General Electric Lightspeed 16, Waukesha, WI). At different times, the interval technique (1 mm at 10 mm inspiratory and 1 mm at 20 mm expiratory) and the volumetric or the contiguous technique were used. All but one subject provided both inspiratory and expiratory images. All studies were performed at 120 or 100 kVp, depending on patient size. Tube current was adjusted for patient size. Scan time was 0.3 seconds for the Aquilion 1, 0.5 seconds for the Aquilion 64, and 1 second for the GE Lightspeed.

CT images of 10 age-matched control subjects with CF were compared with the 6 CF biopsy cases. In addition to the general scores, the lobe that was biopsied was described specifically and was scored for bronchiectasis and hyperinflation. Comparison of Brody score used *t* test analysis with significance assigned at $P \leq 0.05$.

Spirometric Analysis

Spirometry was performed according to American Thoracic Society/European Respiratory Society guidelines (24), with comparisons to normative values using National Health and Nutritional Examination Survey III reference equations (25). FEV₁ progression over time was fitted using a linear mixed-effects model with random intercepts and slopes. Each subject's predicted slope was used to estimate the individual rate of decline. Data used to fit the model included maximum quarterly data taken over the 2 years before biopsy, and each patient included in the calculation had to have ≥ 1.5 years of data and at least five

measurements as described previously (9). Rate of decline estimates are expressed as FEV₁% predicted/year.

Results

Demographics

Lung biopsy was performed in six (three male, three female) patients during the period of chart review. Mean age was 16.2 years, with a range of 11 to 19 years. FEV₁ at the time of biopsy averaged 52% predicted, with a mean FEV₁ decline in the preceding 24 months of 14% predicted/year. This rate of decline was >2 SD beyond the center mean. Overall patient characteristics are described in Table 1.

Brief Clinical Case Summaries

Patient A was a 19-year-old male who had normal lung function (measured as FEV₁% predicted) until 1 year before the time of lung biopsy. Lung function diminished from 110% predicted to a minimum of 65% predicted during the year before biopsy, until plateauing at 90% predicted at the time of biopsy. The patient had two CT scans that revealed ground-glass parenchymal opacities, which prompted the lung biopsy. The biopsied right middle lobe was mildly hyperlucent overall, with small areas of consolidation within the hyperlucent area. Lung function improved to 100% predicted 1 year out from biopsy.

Table 1. Demographics and clinical characteristics

Age at biopsy, yr	16.2 (11–19)
Female, %	50
BMI at biopsy, kg/m ²	18.2 (7.0–24.4)
Microbiology at time of biopsy, %	
<i>Pseudomonas aeruginosa</i>	50
<i>Staphylococcus aureus</i>	33
Gram-negative bacteria	83
Genotype, %F508del/F508del	80
Exacerbations in last 12 mo	4.0 (1–7)
FEV ₁ % predicted at biopsy	52 (33–90)
FEV ₁ rate of decline, % predicted/yr	14

Definition of abbreviation: BMI = body mass index.

Data are presented as mean (range) unless indicated otherwise.

Patient B was a 19-year-old female with lung function of 52% predicted at the time of biopsy and an annual rate of decline of 13%. She had two CT scans before lung biopsy, with the second CT scan notable for worsening areas of ground-glass and tree-in-bud opacities on a background of stable bronchiectasis. Her lung function continued to deteriorate after biopsy for the following year before plateauing at 20% predicted 1 year after biopsy.

Patient C was an 11-year-old female with a lung function of 51% predicted at the time of lung biopsy, who had an annual rate of decline of 11.5% predicted/year for the 3 years before lung biopsy. She had grown mycobacteria, *Pseudomonas*, *Burkholderia gladioli*, and *Candida lusitanae*. She had been receiving antimycobacterial therapy for a year, without improvement. She had two CT scans before lung biopsy that showed widespread bronchiectasis, centrilobular nodules, and mosaic attenuation (volume loss and air trapping). Imaging of her biopsied lingula indicated hyperlucency with areas of consolidation. After lung biopsy, she had transient improvement in her lung function to 70% predicted before resuming her lung function decline. One year after biopsy, her lung function again had declined to 50% predicted.

Patient D was a 17-year-old male with a lung function of 44% predicted at the time of biopsy and a rate of decline of 12.5% predicted/year, with multiple pneumothoraces. At the time of pleurodesis, a lung biopsy was performed to help guide his therapeutic management. His CT scan showed moderate bronchiectasis, numerous diffuse nodular densities, and mild scattered air trapping. After lung biopsy, his lung function continued to deteriorate, requiring lung transplant 6 months after biopsy.

Patient E was a 17-year-old female with a lung function of 42% at the time of lung biopsy, with an annual rate of decline of 20%. Increasing exacerbations to seven in 1 year and declining pulmonary functions prompted the biopsy. Biopsy results revealed occasional foci of bronchiolitis obliterans of various temporal stages, together with bronchopneumonia and bronchiolitis. Two months after biopsy, she died of respiratory complications of influenza infection.

Patient F was a 13-year-old male with a lung function of 33% predicted at the time of lung biopsy, with annual rate of

decline of 9% predicted/year. His chest CT scan was concerning for bronchiolitis obliterans and fungal infection, prompting his lung biopsy. His biopsied lingula had hyperlucent areas abutting a ground-glass opacity. His lung function continues to deteriorate, currently less than 30% predicted, as he awaits lung transplant.

The graphs of FEV₁ decline over time and representative CT scans for the subjects are shown in Figure 1.

Radiographic Imaging

All CT scans demonstrated bronchiectasis and hyperinflation that were variable across lung regions and within patients. Mean total Brody score was 47.5 (range, 17–78), with a bronchiectasis subscore of 19.1 (range, 5–40) and a total hyperinflation subscore of 17.0 (range, 10–24). The mean hyperinflation score of the biopsied lobe was 3.0 (range, 2–4.5). The sum of all six lobes (lingula scored as a separate lobe) is the total hyperinflation score. Total Brody scores were similar between the CF biopsy cases and 10 age-matched control subjects (CF biopsy (±SD), 47.5 ± 23.9; CF control subjects: 37.9 ± 24.6; *P* = 0.49).

Histopathology

Examination of H&E slides revealed increasing airway remodeling and parenchymal distortion in association with increasing FEV₁ impairment. The patient with mild disease (Patient A: FEV₁, 90%) had increased cellularity consistent with inflammation and mucus obstruction. The extent of airway remodeling in this patient was limited. Patients with more advanced disease (Patients C, D, E, and F) had less overt intraluminal inflammation and increased impingement on the small airways (constrictive bronchiolitis). In Patient B (FEV₁, 52%), intraluminal obstruction was present, with early-stage airway remodeling. Comparison of H&E slides with chest CT and spirometry results are shown in Figure 1.

Examination of TriChrome (collagen deposition) and Verhoeff–Van Gieson (elastin degradation) stains further emphasized the development of airway remodeling in these patients (Figure 2). With advancing lung disease, overt intraluminal inflammation waned, and the extent of airway remodeling progressed. In the patient with mild

Figure 1. Comparison of spirometry (FEV₁ %), radiology (computed tomography [CT] scan), and histopathologic findings (hematoxylin and eosin [H&E]) at the time of lung biopsy. With advancing and refractory disease, the extent of constrictive bronchiolitis (arrows) and lung tissue distortion became more prominent (series C, D, E, and F vs. series A). Patients are arranged in order of lung function decline with prominent constrictive bronchiolitis developing in patients with advancing lung disease. CT findings are representative images. Images are at original magnification $\times 100$. PFT = pulmonary function test; Pt = patient.

disease that normalized after biopsy (Patient A), the lumen was plugged with mucoid debris, but the airway structure itself was essentially normal, with no significant fibrosis and preservation of

the elastin lamina. In the patient with moderate but advancing lung disease (Patient B: FEV₁, 52% predicted), peribronchovascular inflammation was accompanied by increased collagen

deposition (airway remodeling) with disruption of the elastic lamina (bronchiectasis). In the patient with advanced disease awaiting transplant (Patient F: FEV₁, 33% predicted),

Figure 2. Extent of airway remodeling in mild, moderate, and severe cystic fibrosis lung disease detailed by histochemistry (collagen [blue] and elastin [black]). Arrows point to the elastic lamina. (A) In mild disease, no significant remodeling was seen, with minimal fibrosis and an intact elastic lamina. (B) In moderate disease, peribronchiolar fibrosis was developing, and the elastic lamina was interrupted. (C) In severe disease, extensive fibrosis had developed, with complete obstruction of the bronchiolar lumen (constrictive bronchiolitis). The remnant of the bronchiolar elastic lamina highlights the edge of the obliterated bronchiole. Images are at original magnification $\times 100$. Pt = patient; VVG = Verhoeff-Van Gieson (elastin).

inflammation had abated, and the small airway lumen had been eliminated completely by the surrounding, impinging fibrosis (constrictive bronchiolitis). The elastic lamina has been interrupted similarly, now serving to delineate what was previously an airway lumen. The irreversible lung function impairment observed by serial spirometry (Patient F) was associated with fibrotic obliteration of small airway lumens.

Figure 3 shows representative images of the extent of myofibroblast proliferation in areas of CF remodeling. In Figure 3A, parallel sections are stained for α -SMA, Trichrome, and H&E. Staining for α -SMA was increased in the areas of extensive tissue remodeling that obstructed the small airway lumen (constrictive bronchiolitis) in a patient with CF with extensive disease (Patient E: FEV₁, 42% predicted). Figure 3B shows multiple areas of myofibroblast proliferation in a patient with CF with moderate disease (Patient C: FEV₁, 51% predicted). Myofibroblast proliferation was clearly extensive around bronchioles and prominent in another area of constrictive bronchiolitis. These data suggest that TGF- β -dependent myofibroblast proliferation is active in CF lung disease progression, particularly in areas of airway remodeling.

We also evaluated TGF- β signaling (pSmad2), myofibroblast proliferation

(α -SMA), and collagen deposition (Trichrome) in parallel sections from all six biopsied patients with CF (Figure 4). Both total TGF- β and its downstream signal pSmad2 are nearly ubiquitous in CF lung tissue, becoming more prominent in areas of increased cellularity. This is similar to that which we have reported

in end-stage CF samples (11). Staining for α -SMA highlighted the presence of a TGF- β -dependent myofibroblast phenotype that is known to be involved in fibrogenesis (12, 26, 27). In sections with mature fibrosis, specimens become less cellular, and collagen staining becomes more prominent.

These data in Figure 4 further underscore the heterogeneity of CF pulmonary disease. TGF- β signaling and myofibroblast differentiation are prominent in CF lungs. The arrangement of histopathology by lung function emphasizes progressive loss of small airway patency in patients with advancing pulmonary impairment. Patient A (FEV₁, 90%) represents a patient with relatively less small airway involvement. Histology shows intraluminal inflammation, but only modest myofibroblast involvement between the epithelial and smooth muscle layers. Patient C (FEV₁, 51%) has extensive peribronchiolar myofibroblasts that completely envelop the airway, yet collagen deposition is modest. The three patients with the lowest FEV₁ (Patients D, E, and F) have loss of small airway patency with more extensive collagen deposition.

Comparison of adolescent CF biopsies with those of control subjects without CF and with end-stage CF tissue further

Figure 3. Myofibroblast proliferation in cystic fibrosis (CF). Myofibroblasts are transforming growth factor- β -activated fibroblasts that stain positive for α -smooth muscle actin (α -SMA). Persistence of the myofibroblast phenotype leads to fibrosis and tissue contracture. (A) Myofibroblast proliferation (α -SMA plus fibroblasts [brown]) in regions of obliterative fibrosis and associated loss of bronchiolar lumen (constrictive bronchiolitis) in a patient with CF with advanced disease (FEV₁, 42%). (B) Myofibroblast proliferation in multiple areas of airway remodeling in a patient with CF with moderate disease (FEV₁, 51%). Images are at original magnification $\times 200$. H&E, hematoxylin and eosin; Pt = patient.

Figure 4. Comparison of transforming growth factor- β (TGF- β) signaling (pSmad2 [brown]), myofibroblast differentiation (α -smooth muscle actin [α -SMA; brown]), and collagen deposition (collagen [blue]). TGF- β and peribronchiolar myofibroblast differentiation are prominent in patients with cystic fibrosis. With advancing pulmonary decline, the presence of myofibroblasts and tissue fibrosis becomes more conspicuous, with loss of small airway lumens. The dotted line separates the subject with less small airway remodeling (Pt A: FEV₁, 90%) from the five other biopsied patients with more extensive myofibroblast proliferation and collagen deposition. Pt = patient.

underscores the progression of disease (Figure 5). In normal tissue (Figure 5A), staining for α -SMA is limited to the smooth muscle lining vessels and larger airways. Similarly, collagen staining is present in supportive structures with absence of tissue fibrosis. In contrast, end-stage CF lung tissue is notable for extensive collagen deposition that significantly disrupts the pulmonary architecture (Figure 5C). Fibrosis extends from airway to lung parenchyma, with obliteration of both bronchioles and alveolar structures. Lung biopsies from adolescents with

CF have a more preserved structure that allows for identification of focal areas of constrictive bronchiolitis with less mature collagen deposition (Figure 5C).

In addition to airway remodeling, impressive vascular remodeling was also observed in some patients with CF (Patient B, FEV₁ 52% predicted; Patient D, FEV₁ 42% predicted). This was best demonstrated by the elastin stain that demarcated the internal laminae (Figure 6). In these patients, prominent intimal hyperplasia occluded the vascular lumen. Pulmonary hypertension

complicates a majority of cases of CF and has a multifactorial cause (28, 29). Pulmonary arterial remodeling in CF is generally considered hypoxia-related and most typically presents histologically as nonspecific, concentric smooth muscle hypertrophy and hyperplasia in the tunica media with or without reduction of arterial luminal diameter. Arterial remodeling progresses with the establishment of an internal elastic lamina, as seen in systemic arteries, and distal “extension” of smooth muscle into (normally) nonmuscularized precapillary vessels. Smooth muscle cell migration through the internal elastic lamina, with proliferation in the tunica intima (Figure 6), is seen less commonly in cases of sustained pulmonary hypertension in general and may contribute to the rapid deterioration of lung function in a subset of patients with CF. Smooth muscle migration into the tunica intima is often a response to injury and inflammation with the release of growth factors and extracellular matrix proteins that parallels the mechanisms involved in constrictive bronchiolitis and bronchiolar remodeling.

Discussion

In this report, we describe the clinical, radiographic, and pathologic features of six adolescent patients with CF with refractory progressive lung disease poorly responsive to standard interventions. These patients represent a spectrum of disease, with FEV₁ ranging from 33 to 90% of predictive values. The lung pathology and subsequent clinical course separated into two groups. One patient with milder disease had lung disease that was limited to airway inflammation and mucus obstruction that eventually was responsive to aggressive antibiotic, mucolytic, and antiinflammatory therapy. The other five patients had evidence of airway remodeling on lung biopsy, followed by a subsequent deterioration of lung function that was not responsive to conventional therapy. Examination of the lung biopsy specimens of the refractory group emphasized the extent to which small airway remodeling may contribute to unfettered lung deterioration in CF.

Although CF pulmonary decline is attributed frequently to progressive mucus plugging and bronchiectasis (30–35), peribronchiolar stenosis has been reported

Figure 5. Comparison of adolescent cystic fibrosis (CF) biopsies with those of normal control subjects and end-stage CF samples. (A) In control subjects without CF, collagen deposition is generally limited to supportive structures, and α -smooth muscle actin (α -SMA) is present only in smooth muscle. No significant tissue fibrosis or myofibroblast activation is present. (B) In adolescent CF biopsy specimens, focal areas of constrictive bronchiolitis are identified easily with associated myofibroblast differentiation. Collagen deposition is present but is not predominant in these sections. (C) In end-stage CF specimens, extensive areas of fibrotic tissue destruction are present, with obliteration of lung parenchyma, including loss of small airway lumens. Myofibroblasts are present in fibrotic areas. For each condition, parallel sections from three separate subjects are shown with images at original magnification $\times 100$. H&E, hematoxylin and eosin.

frequently in “older” patients with CF (36–38). Lesions in our biopsied adolescent patients reflect similarities to findings in end-stage disease, suggesting the important contribution of distal airway limitation to respiratory decline. The careful quantification of lung morphometry by Sobonya and Taussig (37) addressed the dynamic pathology of the small airway across the disease state, emphasizing the multifaceted contributions to CF disease. More recent work by Boon and colleagues (39) overlaying multidetector and micro-CT with thin-section histopathology revealed focal areas of bronchiolar obliteration with distal reopening that complements well our findings of constrictive bronchiolitis in these adolescents with CF.

Recurrent respiratory exacerbations herald a progressive pathophysiology against which current therapy has limited effect. Recent evidence suggests that the benefit of intravenous antibiotics plateaus, on average, at 10 days (8, 40), and epidemiologic data suggest that frequent intravenous antibiotic courses predict an increased rate of lung function decline (1). A study of adult patients with CF by De Boer and colleagues (6) found that subjects having more than two exacerbations/year carried a fourfold increased risk of death or transplant during 3-year follow-up, compared with subjects without an exacerbation. Sanders and colleagues (10) proposed two possibilities to explain the connection between frequent pulmonary exacerbations and persistent lung function decline: (1) unresolved but clearly delineated triggers of disease that require sustained treatment (e.g., increased infection or inflammation causes respiratory decline) or (2) poorly understood mechanisms of pulmonary decline that are unresponsive to conventional therapy. The lung biopsy histopathology described in this adolescent cohort suggests the latter and emphasizes the potential role of peribronchiolar remodeling in refractory pulmonary disease.

Lung biopsy is not performed commonly in CF (nor is it typically recommended), but the clinical scenarios represented by these patients are familiar to the practicing CF clinician. These patients had a concerning clinical course that prompted a comprehensive review of the cause of lung function decline with heterogeneous CT imaging. The members

Figure 6. Vascular remodeling in cystic fibrosis. Vascular remodeling was present in a patient with cystic fibrosis with moderate lung disease (FEV₁, 52%). Prominent intimal hyperplasia (*thin arrows*) significantly impinged on the cross-sectional luminal diameter. Images are at original magnification $\times 200$ using hematoxylin and eosin (H&E) and elastin (Verhoeff-Van Gieson) stains.

of the Cincinnati CF care team were not satisfied that the lung function decline in these patients had exclusively conventional causes (infection, inflammation, and mucus obstruction), and the histopathologic findings were supportive of their insight.

Beyond mucus obstruction, extensive airway remodeling with obliteration of the peripheral bronchioles is a common histopathologic finding in these CF samples with advancing disease. This pathologic finding has been called constrictive bronchiolitis (or obliterative bronchiolitis); in this condition, inflammation engenders a fibrotic response external to the airway, which compresses the airway lumen (41, 42). We use the term constrictive bronchiolitis to describe a chronic, irreversible, airway subepithelial fibrotic process that, by definition, does not involve intraluminal proliferation. Although constrictive bronchiolitis is often referred to (not incorrectly) as obliterative bronchiolitis or “pure” bronchiolitis obliterans, we have abstained from the use of these terms to avoid confusion with the nonspecific histologic pattern of organizing pneumonia with intraluminal polyps, formerly referred to as bronchiolitis obliterans organizing pneumonia. This latter, potentially reversible process is a relatively acute or subacute response to a single insult or illness of short duration, consisting of airway and alveolar luminal plugs of organizing granulation tissue, and, by definition, it is not an interstitial process (43).

The pathophysiology of constrictive bronchiolitis is generated typically by

ongoing bronchiolar inflammation (often in association with infectious bronchiolitis) that then progresses to fibrotic disease (44). The data presented here suggest that TGF- β -dependent myofibroblast proliferation is an important link between chronic inflammation and obstructive fibrosis in CF. Previously, we have demonstrated elevated protein levels of TGF- β , a genetic modifier of disease severity (45–47), in CF bronchoalveolar lavage fluid (48) and circulating plasma (49) during a CF exacerbation and increased TGF- β signaling in association with prominent myofibroblast differentiation in end-stage CF lung tissue (11). Recent studies by our group and others have also described the direct effect of TGF- β to reduce CFTR transcription, protein expression, and channel function that limits CFTR correction (50, 51).

Myofibroblast differentiation is also potentiated further by mechanical strain, which is likely elevated in CF by mucus plugging (52) and heterogeneous air trapping (53). The extensive increase in myofibroblast proliferation and collagen deposition (Figure 5) in end-stage CF specimens suggest increased mechanical strain in the terminal CF lung, perhaps exacerbated by tension generated between the fibrotic and emphysematous regions. From these data, we hypothesize that heterogeneous mechanostimulation may accelerate CF pulmonary decline. Studies of matrix rigidity (54–56) and regional peaks in matrix stiffness (57), such as those performed in idiopathic pulmonary fibrosis, may offer additional insight into mechanisms of disease progression.

Although we are not the first group to describe constrictive bronchiolitis in CF (58) or myofibroblast differentiation in chronic fibrotic disease (59), to the best of our knowledge, this article offers the first opportunity to identify the importance of myofibroblast-driven constrictive bronchiolitis in the setting of rapid and refractory CF pulmonary decline.

The radiographic findings and clinical course that led to the lung biopsy are also not unfamiliar. Manifestations of constrictive bronchiolitis on CT scan include peribronchiolar thickening, centrilobular nodules, tree-in-bud opacities, air trapping, and mosaic attenuation. Typically, bronchiectasis is expected on chest CT, but a pattern of mosaic attenuation is also common in adolescents and young adults with CF (23); it is observed in almost two-thirds of patients (60). Using the established Brody CT scoring system (20), we confirmed the presence of bronchiectasis, centrilobular nodularity, and mosaic attenuation in these patients, consistent with the pathologic findings. The overlap in Brody score between these biopsy subjects and the CF control subjects suggests that many of these histologic processes and radiographic findings may be applicable to a wider spectrum of patients with moderate to severe pulmonary dysfunction.

The conclusions of this study are constrained by the limitations of histopathologic investigation. Although we have identified a pattern of airway remodeling in patients with CF experiencing a decline in lung function, causality cannot be confirmed. Similarly, the relative contribution of constrictive bronchiolitis to disease progression cannot be defined. By emphasizing the extent of constrictive bronchiolitis in these specimens, we do not intend to minimize the contribution of infection, inflammation, and mucus plugging to CF lung disease pathology. Rather, our results emphasize that some of the irreversibility of CF lung function deterioration may be secondary to small airway remodeling that is not responsive to conventional therapy. Similarly, we cannot prove that myofibroblast proliferation is secondary to increased TGF- β in CF lung disease. Rather, we are translating established principles of fibrogenesis to the CF context. Finally, we recognize that selection bias of these adolescents for lung

biopsy may limit the generalizability of these pathologic findings to all patients with CF. However, in patients with abnormal CT scans and refractory lung function decline, similar disease pathology may be applicable.

Conclusions

We have taken the unique opportunity to review a group of clinically driven lung biopsy samples in patients with CF experiencing persistent pulmonary deterioration that was poorly responsive

to conventional therapy. This article emphasizes the significance of airway remodeling during refractory disease with prominent constrictive bronchiolitis in these samples. Positive staining for TGF- β -dependent myofibroblasts in areas of active remodeling suggests an important mechanism through which TGF- β may modify CF progression. Our findings augment the current paradigm of CF lung pathology and suggest the need for interventional strategies to limit fibrogenic pathways in patients with CF who are

experiencing refractory pulmonary decline. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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