Reflux-Induced Collagen Type V Sensitization

Potential Mediator of Bronchiolitis Obliterans Syndrome

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Background: Lung transplantation continues to have poor long-term survival partly because of the high incidence of bronchiolitis obliterans syndrome (BOS). Gastroesophageal reflux disease (GERD) has been implicated in BOS pathogenesis. We investigated the role of collagen type V [col(V)] sensitization in this process.

Methods: Only primary lung transplant recipients were included. Reflux status was assessed with pH monitoring, impedance plethysmography, and esophagogastroduodenoscopy. Sensitivity to col(V) was determined with *trans vivo* delayed-type hypersensitivity reaction (DTH). Kaplan-Meier analyses were performed.

Results: Of the 54 recipients, 26 had proven GERD. There were no significant between-group differences in diagnosis; donor and recipient age; sex; ischemic time; single vs bilateral; human leukocyte antigen A, B, and DR matching cytomegalovirus status; acute rejections; or mean follow-up period. The mean DTH response in the GERD group was 25.7×10^{-4} inches vs 18.3×10^{-4} inches in the non-GERD group ($P = .023$). There was a significant reduction in BOS-free survival in the GERD group for both BOS-I (GERD+, 28.3%; GERD-, 86.6%; $P = .0001$) and BOS-II/III (GERD+, 66.2%; GERD-, 91.7%; $P = .0374$). A second cohort of 53 patients awaiting lung transplantation also was assayed. The mean DTH response in the GERD group was 24.0×10^{-4} inches vs 13.1×10^{-4} inches in the non-GERD group ($P = .003$). There were no differences in age or sex.

Conclusions: GERD is strongly associated with the development of BOS after primary lung transplantation. Col(V) sensitization is associated with reflux and BOS and may play an intermediary role in the pathogenesis of BOS. Trials using col(V) reactivity to assess the impact of antireflux procedures in patients with lung transplantation and idiopathic pulmonary fibrosis are warranted.

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Abbreviations: BOS = bronchiolitis obliterans syndrome; BOS-I = low-grade BOS; BOS-II/III = high-grade BOS; col(II) = collagen type II; col(V) = collagen type V; EBV = Epstein-Barr virus; GERD = gastroesophageal reflux disease; IPF = idiopathic pulmonary fibrosis; TV-DTH = *trans vivo* delayed-type hypersensitivity

Organ transplantation remains the only definitive therapy for most forms of end-stage organ failure. Despite significant advances in surgical technique, organ preservation, immunosuppression, and the post-operative management of recipients, lung transplantation continues to have the poorest long-term survival of all thoracic and abdominal solid organs (registry data available at <http://optn.transplant.hrsa.gov/latestdata/step2.asp>). Although adequate in reversing acute rejection, current immunosuppressive regimens are ineffective in temporizing the progressive fibroproliferative

destruction of chronic rejection.¹ In lung transplantation, this fibroproliferation takes the form of obliterative bronchiolitis, which was first described by the Stanford group in 1984.² To date, multiple risk factors have been associated with the development of obliterative bronchiolitis, yet no clear pathophysiologic etiology has been elucidated.³ These risk factors include early acute rejections,⁴ viral respiratory infections⁵; prolonged ischemic time⁶; human leukocyte antigen mismatch (especially DR loci)⁷; reflux disease⁸⁻¹⁰; and, most recently, collagen type V [col(V)] sensitization.¹¹

Col(V) is classified as a minor fibrillar collagen that serves as a cryptic self-antigen in human lung. It is present with other matrix collagens in a ratio of I:III:IV:V, that is, 86:28:8:1.6.¹² Under normal physiologic conditions, col(V) coassembles into heterotypic fibrils with the major fibrillar collagen type I.^{13,14} The majority of the col(V) monomer is normally partitioned to the interior of col(V)/collagen type I heterotypic fibrils so that disruption of such fibrils with acid, metalloproteases, or low temperature is needed to unmask col(V) epitopes for their immunohistochemical detection.^{13,14} Recent studies have shown a role for col(V) in the pathogenesis of bronchiolitis obliterans syndrome (BOS).^{11,15} Monocyte and T helper 17 cell-mediated fibroproliferative immune responses directed toward the $\alpha 1$ chain of col(V) were tightly linked to the subsequent development of severe BOS and death after transplantation in a cohort of patients with primary lung transplantation.¹¹ The fibroproliferative changes seen in BOS have some histologic similarities, with changes seen in usual interstitial pneumonia and other forms of reflux-associated fibrotic lung disease.¹⁶⁻¹⁸ These similarities caused us to question whether col(V) autoreactivity plays an intermediary role in the observed reflux and BOS association. To answer this question, we evaluated a cohort of patients with primary lung transplantation for their col(V) reactivity as it related to reflux status. A second pretransplantation cohort was analyzed to help to elucidate disease-specific variations in collagen sensitization.

MATERIALS AND METHODS

Trans Vivo Delayed-Type Hypersensitivity Assay

The *trans vivo* delayed-type hypersensitivity (TV-DTH) assay was used to detect antigen-specific memory responses as previ-

ously described.¹⁹⁻²¹ Briefly, 7×10^6 to 9×10^6 mononuclear cells derived from peripheral blood were injected along with antigen into the footpads of CB.17 severe combined immunodeficiency mice. Animals were housed and treated in accordance with Association for Assessment and Accreditation of Laboratory Animal Care International and National Institutes of Health guidelines. Swelling was measured after 24 hours using a dial thickness gauge, and postinjection measurements were compared with preinjection measurements to determine specific swelling response. TV-DTH reactivity was evidenced by the change in footpad thickness, using units of 10^{-4} inches relative to background swelling due to injection of mononuclear cells and phosphate-buffered saline alone. This value was subtracted from the swelling responses measured for each individual test antigen to obtain a net swelling value. Typical recall TV-DTH responses to Epstein-Barr virus (EBV) or tetanus toxoid in seropositive individuals are between 25×10^{-4} inches and 60×10^{-4} inches net swelling. Net swelling responses $\geq 25 \times 10^{-4}$ inches, therefore, were considered positive. Responses $< 25 \times 10^{-4}$ inches were considered negative.

Antigens and Immunizations

Inactivated EBV (Viral Antigens, Inc; Memphis, TN) was used as a positive control recall antigen. Human col(V) [$\alpha 1(1)/\alpha 2(2)$] was purified from placenta as previously described²² and used for TV-DTH monitoring. Native, nondenatured bovine collagen type II was purchased from Sigma-Aldrich Corporation (St Louis, MO). Collagens were tested in TV-DTH at 5 μ g per injection.

Human Subjects Design

The Human Subjects Institutional Review Board approved all aspects of this study protocol prior to any patient enrollment, data collection, or data analysis. From May 1998 to May 2005, 200 lung transplantations were performed at our institution. During this period, 54 patients underwent posttransplantation analysis for TV-DTH reactivity to col(V). All were primary lung transplantation recipients, and all were eligible for study. Inclusion was determined by patient interest and personnel availability. Peripheral blood mononuclear cell samples were collected and analyzed during routine follow-up visits after transplantation, and BOS onset was prospectively monitored for by concordant pulmonary function analysis. Reflux status was retrospectively analyzed. Inclusion into the group with gastroesophageal reflux disease (GERD) required prospective confirmation by positive endoscopic evidence, impedance plethysmography, and manometry and pH monitoring in patients with persistent clinical history of reflux disease. Patients demonstrating clinical signs and symptoms of GERD underwent routine upper endoscopy. If the findings were consistent with reflux, the patients were included in the GERD positive (GERD+) cohort. In patients without positive endoscopic signs, confirmatory pH or impedance plethysmography was completed. Patients who developed progressive BOS were interrogated regardless of clinical symptoms. Only those patients having objective clinical evidence for reflux disease were included in the GERD+ analyses. Overall survival and BOS-free survival were monitored during the course of this study.

A second cohort of 53 patients on the lung transplantation wait-list also were analyzed for reflux status and col(V) sensitization from May 2005 to May 2007. These patients were approached for enrollment at the time of transplantation listing and evaluation. Those who chose to participate underwent TV-DTH analysis at that time. Due to the unpredictable timing of transplantation, no standardized pretransplantation interval could be established. For patients who spent extended periods on the wait-list, repeat analysis was conducted as patient status allowed. Disease-specific variance in col(V) sensitization and reflux status were analyzed in these patients.

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RESULTS

During a 9-year period from May 1998 to May 2007, 107 patients donated blood for measurement of TV-DTH responses to col(V), 54 of whom had already undergone lung transplantation. A second cohort of 53 patients was assayed while on the lung transplant wait-list as a confirmatory cohort. All 54 patients with primary lung transplants were monitored prospectively from the time of transplantation to the onset of BOS. Among these 54 patients, 26 had an objectively documented history of GERD by impedance plethysmography, manometry and pH monitoring, or endoscopy. Ten patients had clinically documented and confirmed GERD prior to lung transplantation, whereas 16 were found to have GERD after lung transplant. These 26 patients were compared with 28 lung transplantation recipients who had no objective evidence for GERD (GERD−). There were no significant differences in sex of recipients, diagnosis leading to transplantation, transplant type (single vs bilateral), or age at the time of transplantation (Table 1). Kaplan-Meier analysis was performed for BOS-free survival for both low-grade (BOS-I) and high-grade (BOS-II/III) BOS. There was a statistically significant difference in BOS-free survival between patients with and those without documented reflux (Fig 1). Patients with documented GERD had a significantly higher incidence of both low BOS-I ($P = .0001$) and BOS-II/III ($P = .037$).

Table 1—Patient Demographics

Category	GERD+ (n = 26)	GERD− (n = 28)	P Value
Recipient sex ^a			$P = .1$
Male	10 (38.5)	18 (64.3)	
Female	16 (61.5)	10 (35.7)	
Diagnosis ^b			$P = .14$
α 1-antitrypsin deficiency	2 (7.7)	8 (28.6)	
Cystic fibrosis	6 (23.0)	6 (21.4)	
COPD	12 (46.2)	7 (25.0)	
Idiopathic pulmonary fibrosis	4 (15.4)	3 (10.7)	
Other ^c	2 (7.7)	4 (14.3)	
Transplantation type ^a			$P = .59$
Single	10 (38.5)	13 (46.4)	
Bilateral	16 (61.5)	15 (53.6)	
Mean $\pm$ SD recipient age, y ^d	48 $\pm$ 0.5	47 $\pm$ 0.4	$P = .76$

Table shows demographic data for 54 primary lung transplantation recipients. Data are presented as No. (%), unless otherwise indicated. GERD = gastroesophageal reflux disease.

^aFisher exact test.

^bAnalysis of variance.

^cThe other diagnostic category includes IgG deficiency (n = 1), atrial septal defect (n = 1), lymphangiomyomatosis (n = 1), silicosis (n = 1), polymyocitis (n = 1), and primary pulmonary hypertension (n = 1).

^dStudent *t* test, two-tailed, equal variance.

FIGURE 1. BOS-free survival based on reflux status in 54 patients after primary lung transplantation. Solid lines denote the positive gastroesophageal reflux disease (GERD) group (n = 26); dotted lines represent the group with no objective evidence of GERD (n = 28). Patients with objectively proven reflux had significantly worse BOS-free survival for both low-grade BOS (A) and high-grade BOS (B) over the 7-year study period. BOS = bronchiolitis obliterans syndrome; BOS-I = low-grade BOS; BOS-II/III = high-grade BOS.

Multiple risk factors for BOS have been identified to date. In an attempt to explain the discrepancy in BOS rates between the GERD+ and GERD− groups, we examined these risk factors in our patient cohort (Table 2). There were no significant differences in donor age or sex. Additionally, there was no significant difference in total ischemic time, human leukocyte antigen matching at the A, B, and directly repeated sequence loci, cytomegalovirus exposure, and number of early acute rejections, despite equivalent long-term follow-up (GERD+, 4.54 years; GERD−, 3.93 years; $P = .27$). There was, however, a significant difference in col(V) sensitization among the GERD+ group. The GERD+ patients reacted significantly stronger to col(V) ($25.7 \pm 0.6 \times 10^{-4}$ inches) than the GERD− group ($18.3 \pm 0.3 \times 10^{-4}$ inches) ($P = .02$). This disparity in reactivity was not seen in

Table 2—Bronchiolitis Obliterans Syndrome Risk Factor Analysis

Category	GERD+ (n = 26)	GERD– (n = 28)	P Value
Donor age, y ^a	31 ± 0.5	32 ± 0.5	P = .74
Donor sex ^b			P = .16
Male	13 (50.0)	20 (71.4)	
Female	13 (50.0)	8 (28.6)	
Ischemic time, min ^a	382 ± 5.0	442 ± 6.1	P = .28
HLA matches ^c			
HLA-A	0.58 ± 0.03	0.50 ± 0.02	P = .76
HLA-B	0.27 ± 0.02	0.32 ± 0.02	P = .87
HLA-DR	0.42 ± 0.02	0.43 ± 0.02	P = .92
New cytomegalovirus exposures ^b	5 (19.2)	8 (28.6)	P = .48
Median acute rejections, No. (range) ^c	1 (0-4)	1 (0-4)	P = .25
Col(V) TV-DTH response ^a	25.7 ± 0.6	18.3 ± 0.3	P = .02
Mean follow-up, y ^a	4.54	3.93	P = .27

Data are presented as mean ± SEM or No. (%), unless otherwise indicated. Collagen type V sensitization appeared to be the only significant discriminating risk factor. col(V) = collagen type V; HLA = human leukocyte antigen; TV-DTH = *trans vivo* delayed-type hypersensitivity. See Table 1 legend for expansion of other abbreviation.

^aStudent *t* test, two-tailed, equal variance.

^bFisher exact test.

^cMann-Whitney *U* test, two-tailed.

response to recall antigen (EBV) or control antigen [col(II)]. Of the GERD+ group, 10 of 26 patients developed progressive BOS-II/III. Demographic data, reflux status, and time to BOS/col(V) DTH are presented in Table 3.

Additionally, patients were stratified by col(V) reactivity status (TV-DTH+ or TV-DTH–), and Kaplan-Meier analyses for severe BOS and mortality were conducted. As previously described,¹¹ col(V) autoreactivity appeared to be significantly associated with an increased risk of BOS-II/III (*P* = .01) (Fig 2A). Col(V) autoreactivity appeared to carry an independent increased risk for death after lung transplantation.

There were two deaths in the TV-DTH– cohort (*n* = 34) during the 7-year follow-up period as opposed to six in the TV-DTH+ group (*n* = 20) (*P* = .04). Five-year survival in the col(V) TV-DTH+ vs col(V) TV-DTH– groups was 65.0% and 93.7%, respectively (*P* = .026).

To determine whether there was a disease-specific predilection to col(V) reactivity and reflux disease, a second cohort of 53 pretransplantation patients was analyzed. Patients with idiopathic pulmonary fibrosis (IPF) were found to have the highest proportion of reflux disease (67% overall). When compared with patients with cystic fibrosis, α1-antitrypsin deficiency, COPD, and other conditions (primary ciliary dyskinesia, sarcoidosis, graft vs host disease, and Wegner granulomatosis), IPF had the highest incidence of reflux (*P* = .0124) (Fig 3A). When we analyzed col(V) reactivity by reflux status in this second cohort, a similar pattern of reactivity was found (Fig 3B). Patients in the GERD+ group (*n* = 17) had a similarly significant and strong anti-col(V) response compared with the patients in the GERD– group (*n* = 34) ($24.0 \pm 3.0 \times 10^{-4}$ inches vs $13.1 \pm 1.7 \times 10^{-4}$ inches, respectively; *P* = .001). This association between reflux status and col(V) sensitization was significant in the complete cohort of 107 patients. The odds ratio for col(V) sensitization given reflux disease was 2.81 (*P* = .02). When examining this association in each subgroup, the odds ratio was 3.58 (*P* = .05) for pretransplantation and 2.11 (*P* = .22) for posttransplantation.

DISCUSSION

Long-term survival in lung transplantation recipients continues to be limited despite a substantial increase in the 1-year survival rate.²³ BOS, which is characterized by fibrous obliteration of the small airways, is the most common cause of death 6 months after transplantation.² The syndrome affects 50% to

Table 3—Patients With Progressive Bronchiolitis Obliterans Syndrome

Patient No.	Primary Disease	Age at Lung Tx	Reflux Diagnosed	Time to BOS-I, y	Time to BOS-II/III, y	Col(V) TV-DTH Testing Time Points, y
1	AAD	60	Post-Tx	3.82	3.82	1.55, 2.07
2	COPD	59	Post-Tx	3.01	7.56	1.55, 2.41, 2.60, 5.01, 6.05
3	PPH	37	Post-Tx	0.79	0.90	0.54, 0.97, 1.20
4	CF	31	Pre-Tx	3.41	3.41	0.53, 1.03, 2.71
5	IPF	58	Pre-Tx	2.10	4.46	0.86, 3.06
6	COPF	63	Post-Tx	2.30	6.15	0.78, 2.30, 5.16
7	CF	20	Post-Tx	3.37	3.37	0.52, 1.01, 2.03, 3.53
8	COPD	56	Pre-Tx	2.86	5.13	0.88, 1.35, 1.78, 2.86
9	CF	18	Post-Tx	4.69	5.00	0.69, 1.60, 2.04
10	COPD	56	Post-Tx	0.78	2.81	0.50, 1.77, 2.52

Ten of 26 patients in the GERD group experienced progressive BOS. Diagnosis, age, reflux status, and time-specific data are presented. Time is reported as years following lung transplantation. AAD = α1-antitrypsin deficiency; BOS = bronchiolitis obliterans syndrome; BOS-I = low-grade BOS; BOS-II/III = high-grade BOS; CF = cystic fibrosis; IPF = idiopathic pulmonary fibrosis; PPH = primary pulmonary hypertension; Tx = transplantation. See Tables 1 and 2 for expansion of other abbreviations.

FIGURE 2. BOS-free survival (A) and overall survival (B) after primary lung transplantation based on *trans vivo* delayed-type hypersensitivity (TV-DTH) reactivity to collagen type V [col(V)]. Solid lines denote TV-DTH+ (n = 20); dotted lines represent TV-DTH- (n = 34). Patients sensitized to col(V) were significantly more likely to develop severe BOS after transplantation. Additionally, col(V) reactivity was associated with a higher mortality after lung transplantation. See Figure 1 legend for expansion of other abbreviation.

60% of patients by 5 years posttransplantation. Survival at 5 years posttransplantation is 20% to 40% lower than that in patients who do not develop BOS.²⁴ Once BOS ensues, it continues to progress without resolution, with 5-year survival only 30% to 40%.^{24,25} Treatment options for BOS are limited. Combination therapy with azithromycin and fluticasone and salmeterol holds some promise for stabilization of function but does not necessarily reverse damage that has already occurred.^{25,26}

GERD has been linked to the pathogenesis of BOS.^{8,9} D'Ovidio et al²⁷ showed that the prevalence of GERD is high in patients with advanced lung disease secondary to esophageal dysmotility and poor lower esophageal sphincter tone. After lung transplantation, pulmonary defense mechanisms, such as cough reflex and mucociliary clearance, are impaired.²⁸

FIGURE 3. Fifty-three patients before lung transplantation were analyzed while on the transplantation wait list. Reflux status was objectively confirmed as previously described in the "Materials and Methods" section. Patients with idiopathic pulmonary fibrosis (n = 15) had a significantly higher proportion of GERD (solid bars) than all other conditions (n = 38) (A). Additionally, patients with proven reflux (solid bars, n = 17) had a significant and specific response to col(V) not seen in the group with no objective evidence of GERD (dotted bars, n = 36) (B). This response was near the magnitude of the positive recall antigen Epstein-Barr virus. There was no response to collagen type II. AAD = α 1-antitrypsin deficiency; CF = cystic fibrosis; col(II) = collagen type II; col(V) = collagen type V; EBV = Epstein-Barr virus; IPF = idiopathic pulmonary fibrosis. See Figure 1 legend for expansion of other abbreviations.

In addition, standard immunosuppression regimens prolong gastric emptying, causing increased reflux and potential aspiration. Animal models suggest increased allrecognition in response to chronic reflux after lung transplantation.²⁹ The present study data add to the broadening consensus that reflux is associated with lung transplantation outcomes in a statistically significant way. Both survival and BOS-free survival were reduced in our patient population with documented reflux. The incidence of GERD in patients with end-stage lung disease and lung transplantation is staggering and may be underestimated in our study population. However, reflux alone does not seem sufficient for the initiation of BOS. This finding suggests a link between reflux and an intermediate agent of immunoreactivity that has variable expression and is host dependent.

There is increasing evidence that col(V) plays a role in both acute and chronic rejection in lung transplantation as a cryptic self-antigen.³⁰ To our knowledge, we present the first evidence that col(V) TV-DTH reactivity is associated with GERD. We propose that the injury created by aspiration of laryngopharyngeal reflux is the initiating event causing col(V) exposure. Patients who then have the appropriately primed T-cell and monocyte immunoreactive milieu progress to BOS at significantly increased rates. Our data strongly correlate reflux disease with col(V) sensitization in both pretransplantation and posttransplantation populations as well as in patients with IPF. Although this relationship was statistically significant, it may be even stronger given the potential for silent reflux and aspiration. We relied on clinical symptoms to prompt objective documentation of GERD in this study. We acknowledge that a proportion of patients will have reflux without outward clinical symptoms; thus, we advocate for routine objective screening for GERD in patients awaiting lung transplantation.³¹ The association between GERD and col(V) TV-DTH reactivity was statistically significant ($P = .02$); however, additional study is needed to confirm this connection. We hope to further elucidate this connection and the effect of fundoplication on col(V) sensitization in an ongoing study.

The effect of laryngotracheal reflux in patients with asthma and the subsequent improvement in symptoms after fundoplication has been well documented.³² GERD also has been associated with the development of BOS-associated pneumonia or diffuse bronchiolitis in the non-lung transplantation population.³³ In addition, there has been an observational association between IPF and GERD, although a mechanistic causal relationship has not yet been demonstrated.^{31,34,35} Our study provides a potential linking intermediate in the development of col(V) immunoreactivity.

IPF is characterized by inflammation and fibrosis of the alveolar septae and terminal bronchioles and is believed to be immune mediated, with activation of inflammatory cytokines. The refluxate may trigger an immune response targeting the alveoli, resulting in fibroblast proliferation and scarring. Control of GERD with surgical antireflux procedures has been associated with a slowing of the progression of IPF as measured by oxygen requirement.³⁶ Additionally, Cantu and colleagues³⁷ demonstrated that early fundoplication leads to significant improvements in freedom from the development of BOS. In our own lung transplantation recipients, we recommend surgical fundoplication if reflux is documented. As yet, we do not have sufficient numbers to determine the significance of the intervention on BOS development and subsequent long-term survival outcomes. In ongoing

studies, we are prospectively evaluating potential lung transplantation recipients for GERD with impedance plethysmography and determining col(V) TV-DTH sensitivity pre- and postfundoplication in those with documented reflux. These additional data will address the complicating factor of silent reflux and help us to determine whether col(V) TV-DTH status is affected by interrupting the recurrent injury from reflux.

This study has several limitations. First, during the study period, 200 patients received lung transplantation of whom 54 underwent col(V) testing. This series of patients was not consecutive; however, there were no specific exclusion criteria from the study. The number of patients was limited by timing of transplantation and follow-up visits in coordination with research team availability. Thus TV-DTH testing was performed at variable time points posttransplantation. The outcomes of the 54 patients involved in the study did not differ significantly from the total population. Second, GERD status was not part of our prospective database collection but was confirmed retrospectively. Objective data, such as esophagogastroduodenoscopy, pH and impedance, or esophagram, were required to categorize patients as having GERD. Third, we may be underestimating the prevalence of reflux in our study population and overall transplantation cohort. Pretransplantation testing was not routinely performed during the study period. Asymptomatic patients were not studied prior to 2007. Symptomatic patients underwent pH or impedance testing unless GERD was documented on EGD. Patients with progressive BOS often were interrogated regardless of symptoms. We currently obtain impedance testing on all candidates as part of the lung transplantation evaluation. We have not altered selection or care of these patients based on this study. We believe that the literature supports the impact of GERD on BOS and lung transplantation recipient survival. Work previously published by our lung study group demonstrates the role of col(V) on primary graft dysfunction, BOS, and patient and graft survival. We are trying to identify patients at risk for poor outcomes and, in the follow-up study, to determine whether we can decrease relative risk by pre- or post-transplantation fundoplication.

In conclusion, long-term survival after lung transplantation continues to be poor partly due to the high incidence on BOS. We have confirmed that the development of BOS after lung transplantation is associated with reflux disease. Additionally, we have shown that this association is linked to col(V) sensitization, resulting in a higher incidence of severe BOS and death after transplantation. Col(V) immunoreactivity likely plays an intermediary role in the development of BOS as a result of chronic reflux-induced lung disease. In our pretransplantation population, we confirmed

the existence of col(V) sensitization in patients with proven reflux disease prior to transplantation and identified that patients with IPF were at most risk, potentially providing a mechanistic explanation for the poor outcomes after lung transplantation in these patients. Further evaluation of long-term lung function, BOS development, and outcomes after fundoplication is needed. We would advocate that prospective determination of col(V) immunoreactivity is warranted in gauging the success of these antireflux procedures. These efforts currently are ongoing at our institution.

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