Alveolar Vascular Remodeling in Nonspecific Interstitial Pneumonia: Replacement of Normal Lung Capillaries with *COL15A1*-Positive Endothelial Cells

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To the Editor:

Nonspecific interstitial pneumonia (NSIP) is an interstitial lung injury pattern, which can arise idiopathically or secondary to other causes (e.g., connective tissue diseases or drug toxicity). Histologically, fibrotic NSIP (fNSIP) is characterized by uniform widening of the alveolar septa due to homogeneous mild fibrosis with or without inflammation, while the lung architecture remains preserved (1, 2). Recently, we have reported an increased vessel diameter of the capillary network and increased intervascular distances in NSIP compared with control subjects (3), suggesting a vascular contribution to the pathogenesis of fNSIP.

Recent advances in lung vascular biology by single-cell RNA sequencing enabled the discrimination of two capillary cell types in the murine and human lung, termed aerocytes and general capillaries, reflecting previous characterizations of microvasculature zones (4–7). Aerocytes are hyperspecialized cells with a large surface area, which, together with the juxtaposed alveolar type 1 cell, form the air–blood barrier and thus enable gas exchange (4, 5). General capillaries, on the other hand, exhibit more regulatory functions and serve as the progenitor population to aerocytes (4, 5). In lung tissue from patients with idiopathic pulmonary fibrosis, we have observed by single-cell RNA sequencing a dramatic increase in a *COL15A1*$^+$–expressing endothelial population that was transcriptionally indistinguishable from venous endothelial cells around airways and in pleura of healthy control subjects (8). Histologic validation localized *COL15A1*$^+$ vessels to areas of dense fibrosis and to areas surrounding fibroblastic foci (6, 8).

Here, we further investigated the endothelial diversity in fNSIP, because, in contrast to idiopathic pulmonary fibrosis, the lung architecture is preserved, allowing for dissociation of the effect of marked remodeling from the effect of increased fibrotic matrix alone.

Methods

Six diagnostic surgical lung biopsy samples of patients with confirmed fNSIP from Yale University and six human lung explants of patients with end-stage fNSIP obtained during lung transplantation at Hannover Medical School (MHH) were included in this study (for basic patient characteristics, *see* Table 1). The nine nonfibrotic controls were obtained from either surgical size adjustment during lung transplantation or histologically tumor-free specimens from surgical cancer resections. Approval of the relevant ethics committees was obtained (MHH number 2702-2015, Yale 2000031225). Endothelial composition was evaluated by immunofluorescent microscopy using an established staining protocol and established staining panels as described elsewhere (6). Four targets were stained: aerocyte-specific *HPGD*, pancapillary *PRX*, *COL15A1* for fibrotic endothelial cells, and panendothelial *CD31*. Regions of interest were analyzed using the ImageJ color threshold function of ImageJ, with the chosen color corresponding to the targeted protein of interest. We focused our analysis on the lung parenchyma by excluding large airways and large vessels. Randomly selected separate regions of interest were used for analyses within each sample, and nine replicates of intimal diameter and wall thickness measurements were made for each sample, with the mean for the sample reported. Values were compared using the Mann-Whitney *U* test. P values < 0.05 were considered significant.

Results

In two independent cohorts and two clinical settings—diagnostic biopsies and lung explants—we observed a dramatic replacement of normal *PRX*+ lung capillary populations (including both aerocytes and general capillaries) with *COL15A1*$^+$ endothelial cells (reduction of sample area fraction with normal capillaries from 95.6% $\pm$ 0.8% in control subjects vs. 21.6% $\pm$ 3.6% in NSIP, $P < 0.01$, Figures 1A and 1B; with significant increase in *COL15A1*$^+$ staining of the sampled parenchymal tissue from 0.7% $\pm$ 0.3% to 13.9% $\pm$ 2.6%, $P < 0.01$, Figure 1C). The mean *COL15A1*$^+$ area fraction of MHH explant samples was significantly higher than the biopsy samples from Yale

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Table 1. Basic Patient Characteristics

	Control ($n = 9$)	NSIP Yale ($n = 6$)	NSIP MHH ($n = 6$)
Age, years	55.2 ± 16.4	51.0 ± 11.5	43.7 ± 13.2
Sex, female/male	3/6	5/1	4/2
Race, Black/White	n.a.	2/4	0/6
Diagnosis	n.a.	Myositis ILD ($n = 2$) CTD-ILD ($n = 2$) NSIP ($n = 1$) Unclassifiable ($n = 1$)	Myositis ILD ($n = 1$) SSC-ILD ($n = 2$) CVID ($n = 1$) Unclassifiable ($n = 2$)
Serology	n.a.	Negative ($n = 2$) Jo1 ($n = 1$) Ro ($n = 3$) La ($n = 1$) PL-7 ($n = 1$)	Negative ($n = 1$) Missing ($n = 3$) SS-A/Ro ($n = 1$) Scl70 ($n = 1$)
Immunomodulator therapy, % (n/N)	n.a.	100 (6/6)	67 (4/6)
FEV_1% predicted	n.a.	76.5 ± 13.6	57.6 ± 30.9
FVC% predicted	n.a.	70.0 ± 13.6	42.6 ± 17.0
D_{LCO}% predicted	n.a.	49.7 ± 7.5	24.5 ± 5.3*
Sa_{O_2} room air, %	n.a.	97.0 ± 1.0	87.0 ± 2.5*

Definition of abbreviations: CTD = connective tissue disease; CVID = common variable immunodeficiency; FEV_1 = forced expiratory volume in 1 second; ILD = interstitial lung disease; MHH = Hannover Medical School; n.a. = not available; NSIP = nonspecific interstitial pneumonia; Sa_{O_2} = arterial pulse oximetry measurement; SSC = scleroderma.
*Only two data points were available for this measurement.

(20.3% ± 2.6% compared with 7.6% ± 1.5%; $P = 0.009$). Within NSIP samples, fibrotic parenchymal regions also revealed greater endothelial density (7.2% ± 1.8%) compared with the normal (nonfibrotic) regions (2.4% ± 0.9%) within the same NSIP samples (data not shown; $P < 0.001$).

In NSIP, small circumscribed areas were found with preserved lung capillaries with visually striking thin vascular walls (Figure 1A). Quantitative analysis revealed little difference between the thickness of the alveolar wall in control subjects (2.4 ± 0.1 μm) and in NSIP in areas with preserved *HPGD*$^+$ *COL15A1*$^-$ lung capillaries (2.3 ± 0.1 μm; $P = 0.64$; data not shown), but the vascular walls were significantly thicker in regions with *HPGD*$^-$ *COL15A1*$^+$ vessels in NSIP (3.7 ± 0.1 μm; $P < 0.01$). Furthermore, the diameter of *COL15A1*$^+$ vessels (10.0 ± 0.5 μm; Figure 1D) was much larger than in lung capillaries of control subjects (6.6 ± 0.2 μm; $P < 0.01$) and regions with preserved lung capillaries of NSIP samples (6.4 ± 0.3 μm; $P < 0.01$; Figure 1D), matching our previous observations (3). Neither the intimal diameter nor the vascular wall thickness differed significantly comparing *HPGD*$^-$*COL15A1*$^+$ vessels of MHH samples with Yale samples ($P = 0.10$ and $P = 0.5$, respectively).

Discussion

Replacement of normal lung capillaries with *COL15A1*$^+$ endothelial cells reflects alveolar vascular remodeling in NSIP, in which the parenchymal architecture of the lung is preserved but fibrotic thickening of the alveolar wall is occurring. When we compared early-stage disease diagnostic samples of the Yale cohort with the end-stage disease samples of the MHH cohort, we observed an association between the extent of the alveolar vascular remodeling and disease severity, in which a significant increase of *COL15A1*$^+$ vessels associated with decreases in FVC and D_{LCO}. Whether *COL15A1*$^+$ cells are the result of a change in phenotype of native lung capillary cells, metaplastic or aberrant differentiation from local endothelial precursors, encroachment from venules or arterioles, or new cells migrating from the bronchial vasculature is not known. However, the consequences of the shift from differentiated lung capillaries to *COL15A1*$^+$ blood vessels in NSIP are manifold. First, the obvious consequence of the loss of specialized capillaries is an impaired gas exchange in patients with NSIP. Second, lung capillaries are continuous, nonfenestrated vessels, whereas *COL15A1*$^+$ vessels are fenestrated, as illustrated by the expression of the marker gene *PLVAP* (6), which may explain the increased lung permeability in fibrotic lung diseases (9). Third, aerocytes are the major source of *HPGD* (hydroxyprostaglandin dehydrogenase). *HPGD* is responsible for the first-pass degradation of most prostaglandins by the lung (6, 10), suggestive of disruption of prostaglandin homeostasis in patients with NSIP.

In conclusion, NSIP is characterized by the replacement of alveolar capillaries with *COL15A1*$^+$ blood vessels. The role of *COL15A1* in lung fibrosis is unclear, highlighting the need for further research regarding the endothelial contribution to the pathogenesis of NSIP and to fibrotic lung diseases in general. ■

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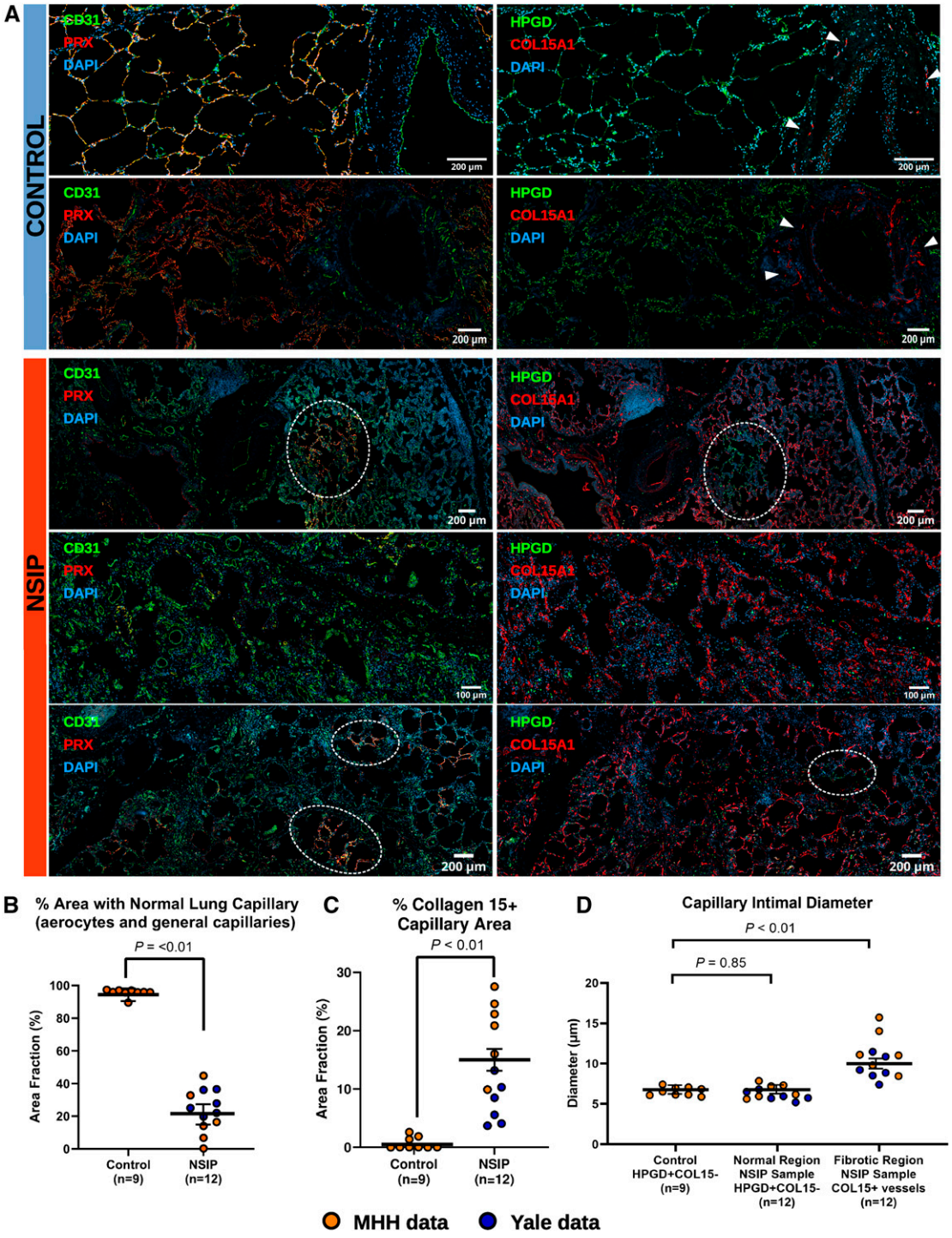

Figure 1. (*A*) Representative immunofluorescent image staining for pan-endothelial *CD31* and pancapillary *PRX* (first column), as well as aerocyte-specific *HPGD* and bronchial and fibrosis-specific *COL15A1* (second column) in control and nonspecific interstitial pneumonia (NSIP) lungs. In control subjects, *PRX* stains *bona fide* lung capillaries, whereas *COL15A1* stains bronchial vessels (white arrows) but not the alveolar microvasculature. In NSIP, PRX^{+} and $HPGD^{+}$ microvasculature is mainly lost; inversely $COL15A1^{+}$ vessels are dramatically expanded. However, small, circumscribed areas were found in NSIP with preserved *bona fide* lung capillaries (white dashed circles). (*B*) Comparison of sample area fraction containing PRX^{+} lung capillaries (i.e., aerocytes and general capillaries) in control and NSIP samples reveals a significant decrease in the area of PRX^{+} lung capillaries in the parenchyma of NSIP samples. (*C*) *COL15A1* staining is significantly higher in NSIP samples. (*D*) Average microvascular diameter of $COL15A1^{+}$ NSIP vessels was significantly greater than normal regions ($HPGD^{+}$ $COL15A1^{-}$) of NSIP and controls. MHH = Hannover Medical School.

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Can Pulmonary Arterial Compliance Be a Prognostic Marker for Pulmonary Hypertension?

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To the Editor:

Pulmonary hypertension (PH) is a group of fatal diseases characterized by elevated mean pulmonary arterial pressure (mPAP) due to increased pulmonary vascular resistance (PVR). The diagnosis of PH is established through right heart catheterization, which provides an evaluation of hemodynamic parameters, including mPAP, pulmonary arterial wedge pressure, and PVR (1). These parameters are critical for the diagnosis, classification, and prognostication of PH (2). Unfortunately, there are very few reports of patient survival with PH. The early identification of patients at risk for poor prognosis is crucial for improving the quality of life of patients with PH.

Pulmonary artery compliance (PAC) refers to the degree of resistance of pulmonary vessels to blood inflow, which can also be understood as the elasticity and ability of blood vessels to expand. PAC is determined by the ratio of stroke volume to pulmonary artery pulse pressure, and it plays a role in regulating perfusion and oxygenation in the lung (3). PAC may be a potentially useful prognostic marker for PH (4). In a recent issue of the *Journal*, Wang and colleagues (5) used a network medicine framework to identify 21 unique subgroups with variable clinical and outcome characteristics from 79 clinical variables. The authors analyzed the discovery cohort, which comprised 37,744 patients from the VA-CART (Veterans Affairs Clinical Assessment, Reporting, and Tracking) program with mPAP $\geqslant$ 19 mm Hg. They found a protective association between PAC and all-cause mortality in patients with PH when PAC was between 3 and 7 ml/mm Hg. The authors further verified their findings using 1,514 patients from Vanderbilt University Medical Center as a validation cohort, and the results were consistent with those of the discovery cohort. They also discovered that higher PAC had a consistent protective effect across different values of pulmonary arterial wedge pressure and

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