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ORIGINAL ARTICLE

Spatial Transcriptomics Resolve an Emphysema-Specific Lymphoid Follicle B Cell Signature in Chronic Obstructive Pulmonary Disease

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Abstract

Rationale: Within chronic obstructive pulmonary disease (COPD), emphysema is characterized by a significant yet partially understood B cell immune component.

Objectives: To characterize the transcriptomic signatures from lymphoid follicles (LFs) in ever-smokers without COPD and patients with COPD with varying degrees of emphysema.

Methods: Lung sections from 40 patients with COPD and ever-smokers were used for LF proteomic and transcriptomic spatial profiling. Formalin- and O.C.T.-fixed lung samples obtained from biopsies or lung explants were assessed for LF presence. Emphysema measurements were obtained from clinical chest computed tomographic scans. High-confidence transcriptional target intersection analyses were conducted to resolve emphysema-induced transcriptional networks.

Measurements and Main Results: Overall, 115 LFs from ever-smokers and Global Initiative for Chronic Obstructive Lung Disease (GOLD) 1–2 and GOLD 3–4 patients were analyzed. No LFs were found in never-smokers. Differential gene expression analysis revealed significantly increased expression of LF assembly and B cell marker genes in subjects with severe emphysema. High-confidence transcriptional analysis revealed activation of an abnormal B cell activity signature in LFs (q-value = 2.56E-111). LFs from patients with GOLD 1–2 COPD with emphysema showed significantly increased expression of genes associated with antigen presentation, inflammation, and B cell activation and proliferation. LFs from patients with GOLD 1–2 COPD without emphysema showed an antiinflammatory profile. The extent of centrilobular emphysema was significantly associated with genes involved in B cell maturation and antibody production. Protein-RNA network analysis showed that LFs in emphysema have a unique signature skewed toward chronic B cell activation.

Conclusions: An off-targeted B cell activation within LFs is associated with autoimmune-mediated emphysema pathogenesis.

Keywords: lymphoid follicles; autoimmunity; switch-class recombination; centrilobular emphysema; metabolic reprogramming

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At a Glance Commentary

Scientific Knowledge on the Subject: Within chronic obstructive pulmonary disease (COPD), emphysema is characterized by a significant yet poorly understood B cell immune component. An upregulation of the B cell immune compartment in lung tissue is directly linked to the extent of emphysema.

What This Study Adds to the Field: We provide the first comprehensive transcriptomic analysis of lymph follicles from COPD patients with and without emphysema. An off-targeted activation of the B cell compartment with autoimmune features is abundant in the emphysematous lung, either as a consequence or as a concurrent cause of the ongoing emphysematous process. The readouts of activation of the B cell compartment are significantly associated with the extent of emphysema independently of the degree of airflow limitation. A deeper understanding of the B cell responses could define the clinical phenotype (likely emphysema) that could benefit from therapies targeting B cells or B cell products in COPD.

Subjects falling into the same Global Initiative for Chronic Obstructive Lung Disease (GOLD) category of chronic obstructive pulmonary disease (COPD) severity are remarkably heterogeneous, and this diversity is often difficult to handle from a therapeutic standpoint (1). Computed tomography (CT) has been instrumental in identifying COPD subphenotypes, including emphysema (2). The emphysematous lung is characterized by off-targeted immune responses contributing to parenchymal damage (3, 4). Among these immune responses, the B cell compartment plays a key role, but major knowledge gaps still exist regarding their contribution to emphysema pathogenesis (5). We and others have previously shown that patients with severe GOLD COPD stages have abundant lung B cell–rich lymphoid follicles (LFs) (6–8), and LF B cell activation correlates with the extent of CT scan–measured emphysema (9). In COPD, inflammation persists long after smoking cessation, suggesting a self-perpetuating pathogenic process similar to those occurring in autoimmune diseases (7, 10). Consistent with this, increased B cell products (autoantibodies) have been observed in the blood and lungs of patients with emphysema (3, 9).

In this study, we aimed to understand the nature of the B cell immune transcriptional program of LFs within the emphysematous lung. We characterized, for the first time, the transcriptome atlas for LFs in patients with COPD and control subjects, with different degrees of CT scan–defined emphysema. We show that emphysematous changes are associated with LF autoimmune features and that B cell responses are directly correlated with the extent of emphysema even within the same GOLD stage of COPD severity. Part of this body of work was presented as a poster at the 2023 American Thoracic Society meeting (11).

Methods

See the online supplement for further details.

Human Cohort

Following the Declaration of Helsinki's recommendations, all protocols and written consent forms were approved by the institutional review board of the University of Arizona (IRB 1811124026). For this study, we collected formalin-fixed ($n = 21$) or O.C.T.-fixed ($n = 27$) lung sections from 48 subjects undergoing lung volume reduction surgery or transplant for treatment of severe emphysema or lung resection for a solitary peripheral nodule (the lung tissue studied was at least 10 cm away from the nodule) for digital spatial profiling (DSP) of LFs. We included 8 nonsmoker control subjects, 13 ever-smoker control subjects, 17 patients with GOLD 1–2 COPD, and 10 patients with GOLD 3–4 COPD. The nonsmokers were removed from analysis because they did not have LF, as expected (6, 7). In six patients with GOLD 3–4 COPD, two lobes/patient were studied to determine intrasubject variability in the immune responses.

CT Scan Emphysema Measurements

Clinical CT scans were acquired, and thin-section series with smooth reconstruction kernels were manually selected. CT scans with contrast agents and sharp kernels were excluded (*see* Table E1 in the online supplement for technical parameters). The Chest Imaging Platform software (12–14) was used to quantify emphysema as the percentage of low-attenuation areas below the −950 Hounsfield units (HU) threshold (%LAA-$_{950}$) in the specific lobes that were used for the spatial analyses. Emphysema was defined as %LAA-$_{950}$ >5% according to the Fleischner Society criteria (15). Using machine learning methodology (16), we defined %CLE$_{mod}$ as the volume percentage of parenchyma exhibiting moderate centrilobular emphysema (CLE) patterns. For this study, we focused on %CLE$_{mod}$ because this has a better predictive performance in clinical correlations (17). We employed the parenchymal tissue density of moderate CLE (density CLE$_{mod}$ − g/L) as an indicator of tissue integrity within the moderate CLE areas. We focused our analysis on the emphysema metrics specific to the lobe from which the spatial profiling sample was derived. Subjects with %LAA-$_{950}$ <5% were classified as having no emphysema (18); among the subjects with %LAA-$_{950}$ >5%, two emphysema severity groups were established on the basis of %LAA-$_{950}$ median as the cutoff: *1*) moderate emphysema was %LAA-$_{950}$ 5–17.75% and *2*) and severe emphysema was %LAA-$_{950}$ >17.75%. For gene expression comparisons within the GOLD 1–2 COPD group alone and protein-RNA network analysis, subjects were categorized as either with emphysema (%LAA-$_{950}$ >5%) or without emphysema (%LAA-$_{950}$ <5%) (15) because of the smaller sample size used for these analyses.

DSP

To assess lung samples in a spatial manner, DSP technology was used for LF transcriptomics and proteomics according to the manufacturer's instructions (Figures E1A and E1B).

Histological Characterization of the LFs

Lung sections were immunostained for γ-glutamyltransferase-7 as a marker for germinal center (GC) B cells, CD20 as a

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

marker for mature B cells, and CD138 (syndecan-1) as a marker for plasma cells (19).

Statistical Analysis

Demographic and CT emphysema data were analyzed using SigmaPlot. Statistical differences were assessed by Student's *t* test or Mann-Whitney *U* tests. Categorical variables were assessed using the *Z*-test for proportions. Correlations were calculated using Spearman correlation test (ρ) or Pearson's correlation test accordingly. Statistical difference was defined as $P < 0.05$.

All transcriptomic data were analyzed using R script packages (GeoMxTools, NanoStringNCTools, GeoMxWorkflows, lme4, and fgesea). Given the heterogeneous nature of the data, multiple normalizations and corrections were applied (Figures E2A–E2C and Table E2). We chose to use dual thresholding of $P < 0.05$ and $\log_2$ fold change (Log_2FC) >0.5 for differential gene expression analyses.

High-confidence transcriptional (HCT) intersection resolves functional signatures for transcription factors (TFs) within clinical gene sets of interest (20–23). To gather evidence for specific cell populations within the LFs, we applied HCT analysis to compute LF signatures against the Travaglini scRNASeq Lung Atlas (24). To resolve emphysema-specific transcriptional regulatory networks, we applied HCT intersection analysis to genes induced in different emphysema groups. The contrasts were formulated as a hypergeometric distribution or contingency table and solved by Fisher's exact test. All *P* values were adjusted for multiple testing by using the method of Benjamini and Hochberg to control the false discovery rate. Finally, we assessed the activated pathway using PANTHER GO Analysis (25).

Protein–RNA associations were calculated using an unbiased network analysis with a generalized estimating equations model to account for multiple regions of interest per subject (26) (Table E3). Finally, we calculated Pearson's correlations between B cell markers and expression of B cell–related genes. Differential expression was defined as a FC ≥ 1.25 ($\log_2 = 0.32$) and $P < 0.05$.

Results

Study Population

A total of 48 subjects were initially studied, but eight never-smokers were excluded

Table 1. Selected Demographics, Lymphoid Follicles, Lung Function, Emphysema Assessment, Comorbidities, and Medication Use in the Study Population

Selected Demographics	*n*	Ever-Smoker Control Subjects	GOLD 1–2 with Emphysema	GOLD 1–2 without Emphysema	COPD GOLD 3–4	*P* Value*
Total patients, *n*	40	13	5	12	10	
Female sex, %	40	38.5%	0.0%	58.3%	20.0%	NS
Age, yr	40	75.4	68.2	66.3	66.4	0.006
Current smokers, %	40	15.3%	0.0%	41.7%	10.0%	NS
Pack-years	40	35.5	30.5	34	41.2	NS
Lung function						
FEV_1% predicted	40	90.5	71.2	68.9	24	<0.0001
FEV_1/FVC	40	74.5	54.25	64.0	31.6	<0.0001
CT-defined emphysema						
Whole-lung %LAA_{-950}	39†	1.4	15.67	1.21‡	23.0	<0.0001
Lobar %LAA_{-950}	39†	1.4	23.45	1.1‡	20.0	<0.001
%CLE_{mod} (lobar volume)	38§	8.5%	19.9%	5.3%‡	32.0%	<0.0001
Whole-lung volume, g/L	38§	5.19	5.84	5.05	7.3	<0.0001
Comorbidities, %						
Hypertension	40	53.8%	20%	25%	50%	NS
GERD	40	7.69%	—	—	20%	NS
Hyperlipidemia	40	7.69%		8.3%	—	NS
Diabetes mellitus	40	23.07%	20%	0	30%	NS
Squamous cell lung cancer	40	—	0	8.3%	—	NS
Medications, %						
LABA/LAMA/SABA	40	7.69%	60%	50%	80%	NS
Inhaled corticosteroids	40	—	60%	25%	50%	NS
Oral corticosteroids	40	—	20%	8.3%	40%	NS
ACE inhibitors/ARB	40	30.7%	0	8.3%	40%	NS
Calcium antagonists	40	7.69%	0	8.3%	—	NS
β-Blockers	40	23.0%	60%	16.6%	30%	NS
Leukotriene antagonist	40	—	0	8.3%	30%	NS

Definition of abbreviations: %LAA_{-950} = percentage of lung attenuation areas of the lung; emphysema is defined by %LAA_{-950} >5%; ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; CLE = centrilobular emphysema; COPD = chronic obstructive pulmonary disease; CT = computed tomography; DSP = digital space profiling; GERD = gastroesophageal reflux disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease; LABA = long-acting β-adrenoceptor agonist; LAMA = long-acting muscarinic agonist; SABA = short-acting β-adrenoceptor agonist.

**P* values derived from one-way ANOVA between nonsmoker control, ever-smoker control, COPD GOLD 1–2, and GOLD 3–4 groups. *Z*-test was applied for categorical variables such as age or sex.

†One subject from the smoker control subjects did not have a readable CT scan for analysis.

‡$P < 0.01$ between GOLD 1–2 with emphysema versus GOLD 1–2 without emphysema.

§Two subjects did not have conclusive results for the analysis.

because no LFs were found during DSP, as expected (6, 27). Demographic information is shown in Table 1, and the CT-defined lung volumes and emphysema assessments are comprehensively detailed in Table E4. As expected, ever-smokers without COPD did not have emphysema, whereas all patients with GOLD 3–4 COPD had severe emphysema. Within patients with GOLD 1–2 COPD, however, 29% had emphysema (%LAA-$_{950}$ >5%) (15), whereas 71% did not (%LAA-$_{950}$ <5%) (15). We studied a total of 115 LFs, predominantly located in the parenchyma, and fewer in the airways (85.2% vs. 14.8%, respectively; Figure 1A). Interestingly, all airway LFs were found in emphysematous samples (Figure 1B). As expected, 85% of LFs studied were in patients with emphysema versus 15% in patients without emphysema (Figure 1C). We did not segregate LFs by anatomical location for transcriptional analyses.

LF Transcriptome Atlas Analysis Reveals an Off-targeted B Cell Response in Emphysema

GCs are sites where high-affinity antibody-producing cells are generated (19). We assessed GCs by triple immunostaining for CD20, GL7, and CD138 (Figure 1D). The presence of GC B cells was predominant in the LFs from patients with emphysema (%GL7^{+} cells, 3 ± 1.9 vs. 1 ± 1.2; $P = 0.021$). Computation of LF-expressed genes against a publicly available lung single-cell RNA-sequencing atlas (24) indicated that the B cell signature was most significantly enriched (Table E5 and Figure E3). To assess transcriptomic signatures from LF-specific immunocytes that might be associated with small airway and parenchymal destruction, we compared the LF DEGs between no, moderate, and severe emphysema groups. All linear mixed effects model comparisons are provided in Table E2.

When comparing moderate versus no emphysema groups, genes associated with memory B cells and switching of the γ-heavy chain subclasses (encoding for IgG2, IgG3, and IgG4), B cell maturation and activation (*CD37*, *IKZF3*, *IKZF1*), LF assembly (*LTB*), regulation of B cell receptor (BCR) downstream signaling (*MARCKS*) (28), and NADPH oxidation (*NCF1*) were significantly enriched in moderate emphysema. These findings suggest that the LFs are undergoing a highly proliferative and differentiating activity (Figure 2A).

When comparing severe versus no emphysema groups, immunoglobulin

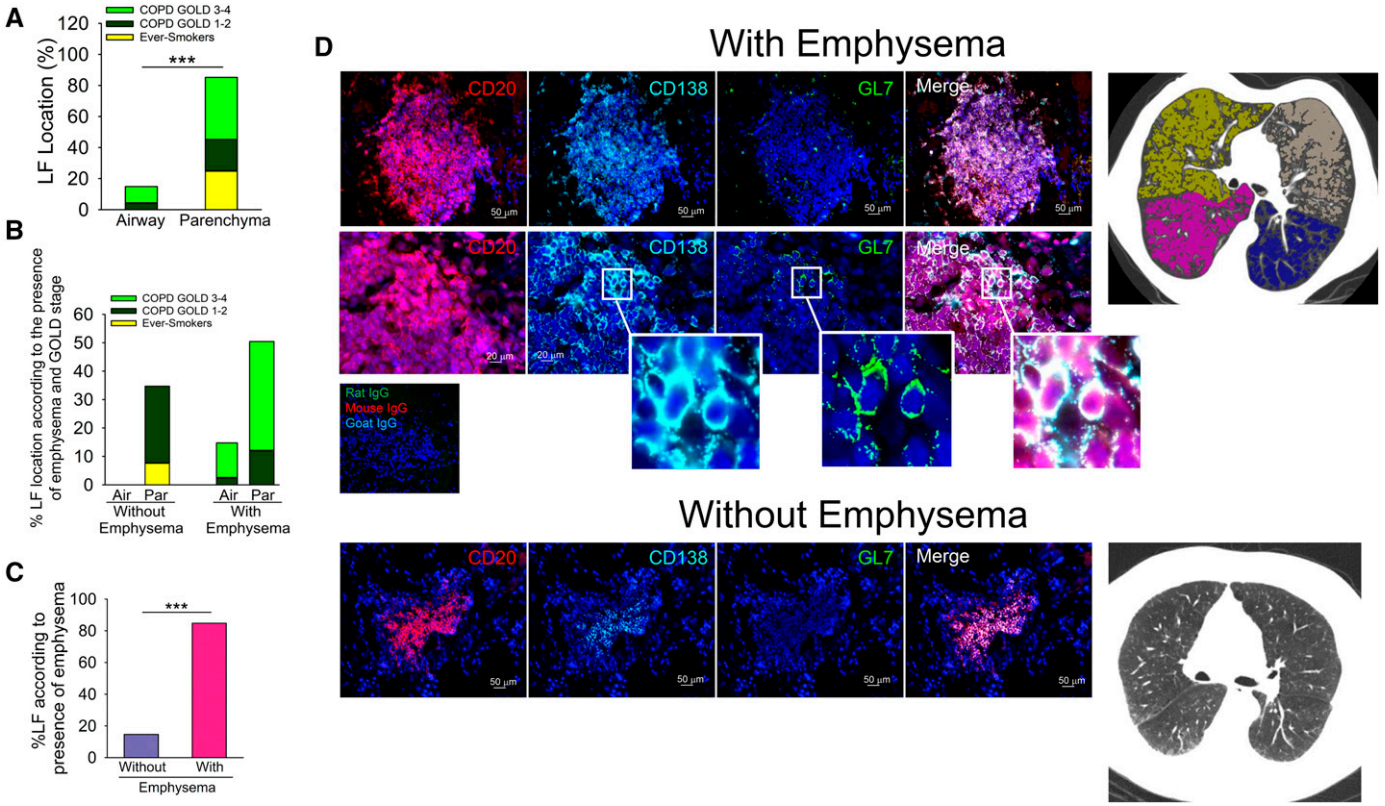

Figure 1. Histological characteristics of the lymphoid follicles (LFs). (*A*) The overall digital spatial profiling–assessed LFs were located predominantly in Par (parenchyma) compared with Air (airway) location (85.2% vs. 14.8%; *Z*-test, 6.171; $P < 0.001$; $n = 115$ LFs). (*B*) The percentage of LFs distributed according to the presence or absence of emphysema within the ever-smokers with and without chronic obstructive pulmonary disease (COPD). (*C*) Eighty-five percent of LFs were located in emphysematous versus 15% of nonemphysematous lobes (*Z*-test, 4.442; $P < 0.001$; $n = 115$ LFs). (*D*) On the left, representative images from conventional immunofluorescence performed in formalin-fixed, paraffin-embedded lung sections from 20 patients with COPD and ever-smoker control subjects with and without emphysema. Formalin-fixed, paraffin-embedded sections were immunostained for GL7 (green), CD20 (red), and CD138 (cyan). The pictures from the first and third rows were taken with 40× magnification (bar, 50 μm), and the second row was taken at 100× magnification (bar, 20 μm). The insets show details of GL7- and CD138-enriched LFs in the emphysematous lung. On the right, axial computed tomography slices are shown from the same subjects represented in *D*. Emphysematous regions are color coded by lobe: RUL (yellow), RLL (pink), LUL (light brown), and LLL (blue), highlighting the disease's distribution. ***$P < 0.001$.

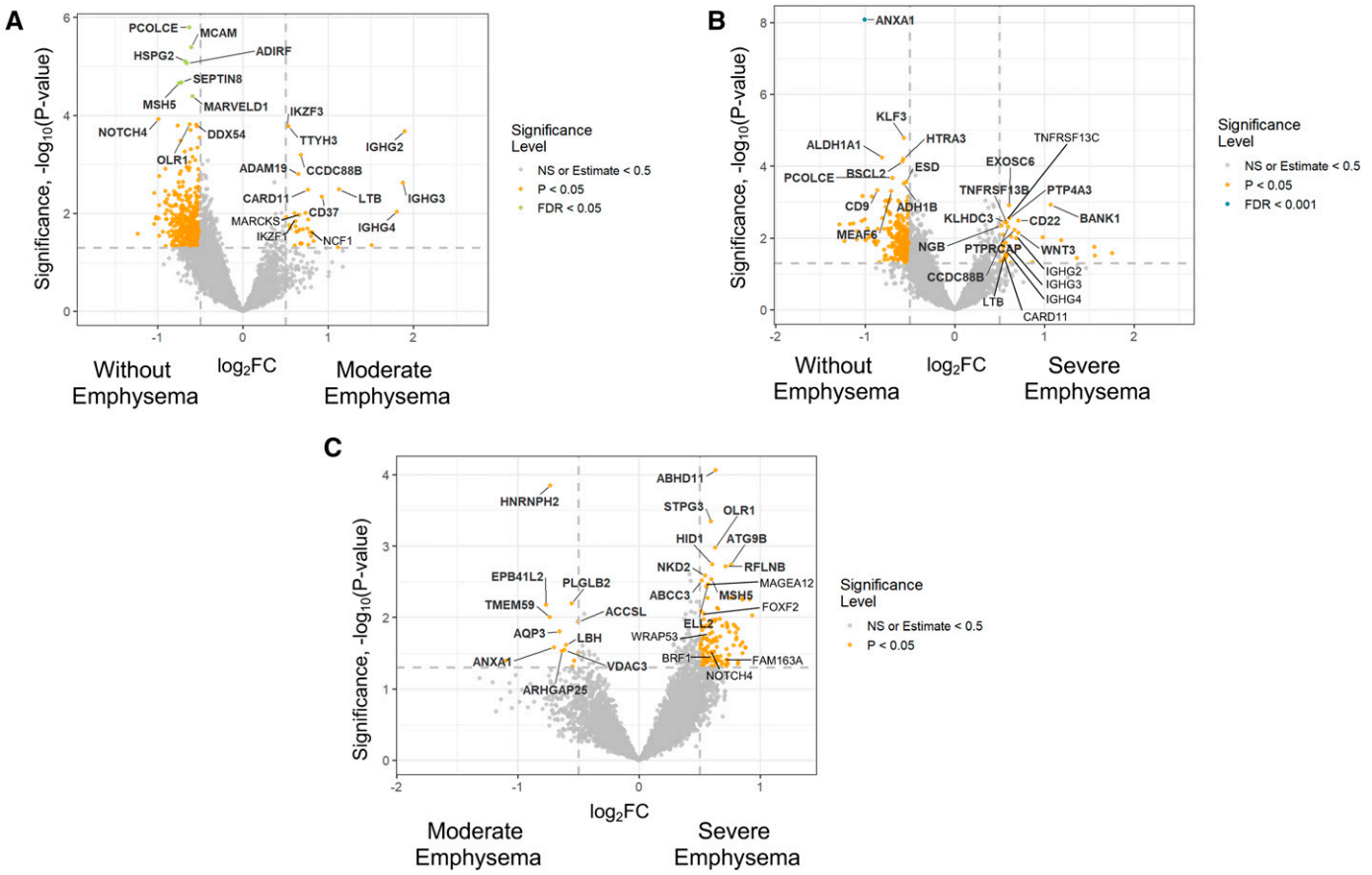

Figure 2. Lymphoid follicle (LF) distinctive differentially expressed genes according to emphysema severity. (*A*) Volcano plot shows moderate emphysema versus no emphysema contrasts. (*B*) Volcano plot shows severe emphysema versus no emphysema contrasts. (*C*) Volcano plot shows severe versus moderate emphysema contrasts. Differentially expressed genes were assessed by using dual thresholding of $P < 0.05$ and $\log_2$ fold change >0.5. The subjects were classified according to the percentage of low-attenuation areas below the −950 Hounsfield units (%LAA-$_{950}$) metric in (*A*) no emphysema (%LAA-$_{950}$ <5%), (*B*) moderate emphysema (%LAA-$_{950}$ between 5% and 17.75%), and (*C*) severe emphysema (%LAA-$_{950}$ >17.75%). FDR = false discovery rate.

production factors such as *BANK1*, *CD22*, *TNFRSF13C* (B-cell activating factor receptor [BAFFR]), and *TNFRSF13B* (transmembrane activator and CAML interactor) were significantly enriched in severe emphysema (Figure 2B). It is noteworthy that the expression of the *IGHG2*, *IGHG3*, *IGHG4*, *CCDC88B*, *LTB*, and *CARD11* genes was significantly higher in both moderate and severe emphysema groups than in the no emphysema group (Figures 2A and 2B). Pathway analysis reported enrichment in lymphocyte activation pathways ($P = 1.43E-05$) in LFs from patients with emphysema versus no emphysema.

When comparing severe versus moderate emphysema groups, cell proliferation (*NOTCH4*, *FAM163A*, *NKD2*, *FOXF2*), glucose metabolism (*ABHD11*), and DNA repair (*WRAP53*, *BRF1*) genes were significantly increased in severe emphysema. These results indicate an active B cell metabolism associated with antigen processing (Figure 2C).

When comparing the two lobes from the six patients with GOLD 3–4 COPD, we did not find significant differences in gene expression, despite differing extents of emphysema in each lobe. This finding suggests that within the same subject, an overall B cell immune signature may exist throughout the lung, regardless of varying levels of parenchymal destruction.

LF Emphysema-associated Gene Sets Contain Regulatory Footprints for Critical B Cell TFs

To identify TFs associated with emphysema in LFs, we applied HCT intersection analysis to LF genes induced in severe versus no emphysema and moderate versus no emphysema (*see* Figure 3 and Tables E6 and E7). We hypothesized that if B cell infiltration was characteristic of emphysema, then genes induced in emphysema would contain footprints for B cell regulatory TFs. To test this hypothesis, we used the Mouse Genome Informatics resource to retrieve a set of TFs whose ablation in mice results in abnormal B cell function, and we referred to this set of TFs as "critical B cell TFs." Consistent with our hypothesis, we observed enrichment of human orthologs of critical B cell TFs among TFs with significant regulatory footprints in both severe and moderate emphysema versus no emphysema (Figures 3A and 3B, respectively). Interestingly, many of the critical B cell nodes with footprints in emphysema-induced gene sets have previously been linked to autoimmune disorders, including TCF3 and TCF4 (29, 30), PAX5 (31), HIF1A (32), RUNX1 (33), IRF4 (34), and MYC (35). Overall, our results indicate that the induction of signatures regulated by TFs

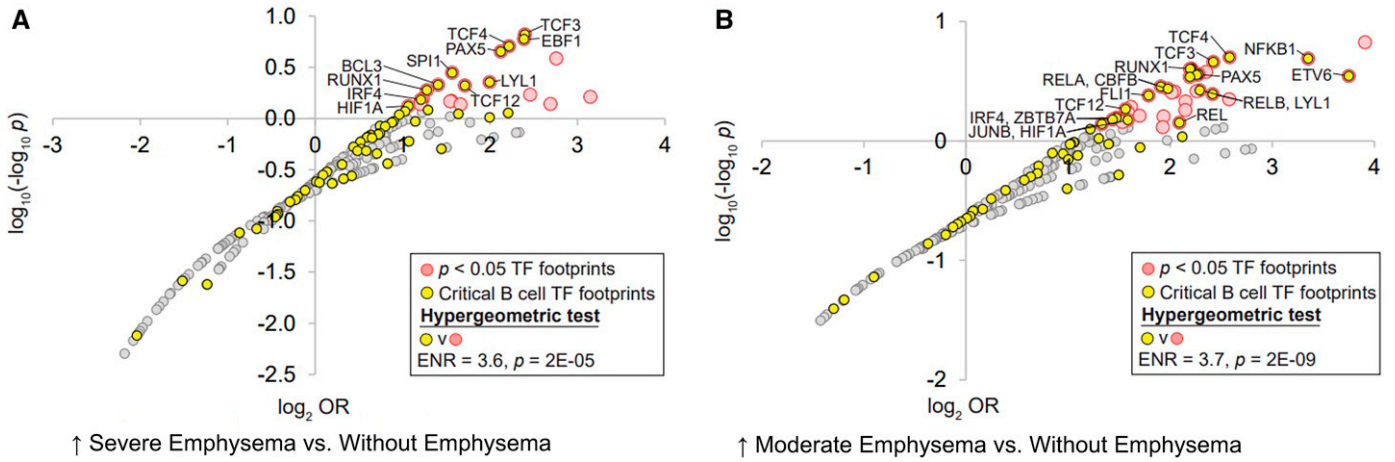

Figure 3. High-confidence transcriptional target (HCT) intersection analysis of transcription factors (TFs) within lymphoid follicle signatures from moderate and severe emphysema groups. (*A* and *B*) TFs with the strongest and most significant HCT footprints among the indicated gene sets are distributed in the top right quadrant of the plot (P = 2E-05 and P = 2E-09, respectively). *See* Table E5 for full numerical data. (*A*) Regulatory network plot of TF HCT intersections with severe emphysema-induced genes ($\log_2$ fold change, >0.32; $P < 0.05$). Hypergeometric test demonstrates enrichment of critical B cell TFs among $P < 0.05$ TFs. (*B*) Regulatory network plot of TF HCT intersections with moderate emphysema-induced genes ($\log_2$ fold change, >0.32; $P < 0.05$). Hypergeometric test demonstrates enrichment of critical B cell TFs among $P < 0.05$ TFs. The subjects were classified according to the percentage of low-attenuation areas below the 2950 Hounsfield units (%LAA-$_{950}$) metric in (*A*) no emphysema (%LAA-$_{950}$ <5%), and in (*B*) moderate emphysema (%LAA-$_{950}$ between 5% and 17.75%). ENR = enrichment number; FDR = false discovery rate; v = versus.

with critical roles in B cell biology is associated with emphysema pathogenesis and that these TFs represent potential novel points of therapeutic intervention in this condition.

Subjects with Emphysema Have Upregulated and Off-targeted B Cell Signatures Independent of the GOLD Stage

When comparing patients with GOLD 1–2 COPD with emphysema versus those without emphysema, DEG analysis showed significantly higher levels of *ADAM15*, factors related to major histocompatibility complex processing (*BTBD6*, *FBXW12*), monocarbon metabolism enzymes (*MTHFD1*, *METTL17*, *FTCD*), and proinflammatory mediators such as serum amyloid A2 (*SAA2*) and *IL2RA* (Figure 4A). On the contrary, LFs from patients with GOLD 1–2 COPD with no emphysema had significant expression of *DUSP8*, *LRP1*, *TIMP3*, and *GRN*. These genes are associated with the dampening of tumor necrosis factor–mediated inflammation pathways and inhibition of extracellular matrix destruction. HCT intersection analysis indicated enrichment of critical B cell TF footprints within emphysema-associated genes (P = 5E-05), including those of PAX5, TCF4, MYC, NFATC2, and IRF2 (Figure 4B).

To assess whether there was a direct correlation between B cell–related genes and

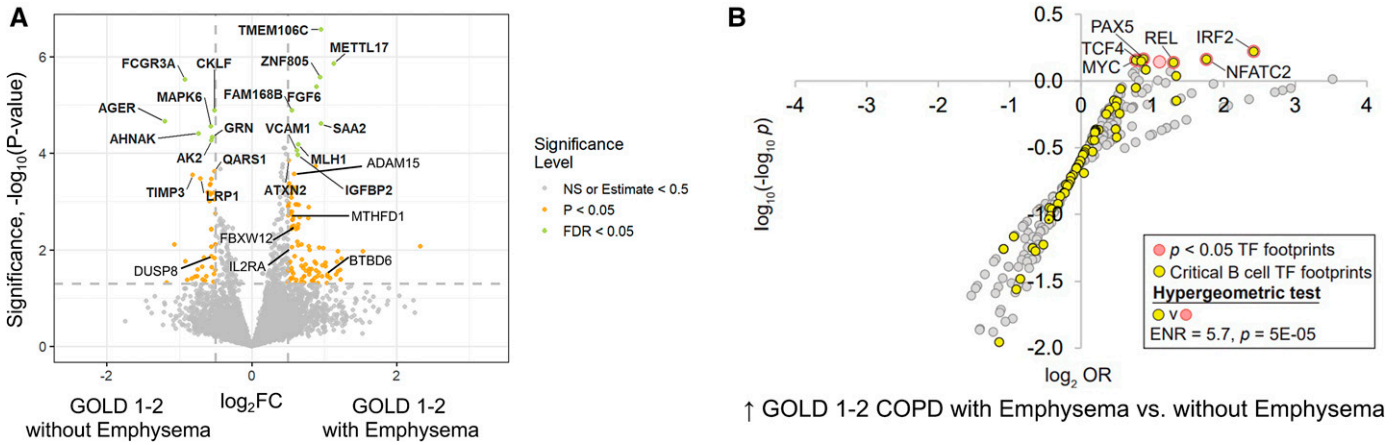

Figure 4. Lymphoid follicle transcriptomic characterization between patients with Global Initiative for Chronic Obstructive Lung Disease (GOLD) 1–2 COPD with and without emphysema. (*A*) Volcano plot showing differentially expressed genes between GOLD 1–2 COPD with emphysema and GOLD 1–2 COPD without emphysema. Differentially expressed genes were assessed by using dual thresholding of $P < 0.05$ and $\log_2$ fold change >0.5. (*B*) Regulatory network plot of transcription factor (TF) high-confidence transcriptional target (HCT) intersections with GOLD 1–2 emphysema-induced genes ($\log_2$ fold change, >0.32; $P < 0.05$). Hypergeometric test demonstrates enrichment of critical B cell TFs among $P < 0.05$ TFs. The subjects were classified according to the percentage of low-attenuation areas below the −950 Hounsfield units (%LAA-$_{950}$) metric in (*A*) no emphysema (%LAA-$_{950}$ <5%) and (*B*) with emphysema (%LAA-$_{950}$ >5%). ENR = enrichment number; v = versus.

the extent of emphysema and parenchymal destruction, we performed correlation analyses between differential gene expression, %LAA$_{-950}$, and %CLE$_{mod}$, which has been shown to have the strongest associations with COPD pathogenesis (36, 37). Genes associated with switching of the γ-heavy chain subclasses and immunoglobulin production (*IGHG1*, *IGHG3*, *IGHG4*), BAFFR (*TNFRSF13C*), and B cell differentiation (*YAP1*) (38, 39) were found to be directly correlated with lobe-specific %LAA$_{-950}$ and/or %CLE$_{mod}$ (Figures 5A–5H).

LF Gene-Protein Network Shows Blunted Control of B Cell Proliferation in Emphysema

Proteins CD20, HLA-DR (major histocompatibility complex [HLA]-II cell surface receptor for antigen presentation and antibody responses), CD27 (memory antibody-producing B cell marker), and β_2-microglobulin (B2M; part of the HLA-I complex on B cells) were the most significantly enriched proteins within the LFs (*see* Table E2). The overall protein-RNA network for all subjects is shown in Figure E4. In patients without emphysema (Figure 6A), CD20 was associated with the enrichment of regulatory genes (*ALDH3A1*) (40), genes associated with control of cell proliferation (*CIRBP*, *PTPRF*), and a reduction of activation markers *CD14* and *AIF1* (41). Interestingly, genes associated with the control of immune responses, such as *ALDH3A1*, *TNK2* (42), *CORO7*, and *UBQLN1*, connect CD20 and CD27, HLA-DR, and B2M.

In patients with emphysema (Figure 6B), CD20 was associated with enrichment of autoimmune-related gene markers *IL33* and *FCAR* (43–45) and lung injury marker *TTF1* (46). *NUTM2A*, a gene associated with uncontrolled B cell proliferation (47), was positively correlated with CD20 and CD27 protein expression, and *PAIP2B*, a gene that controls chromatin accessibility and cell proliferation (48), was negatively correlated instead. HLA-DR protein was strongly associated with immune activation genes such as *CD79B*, *MT2A*, *CXXC5*, and *HDAC10*. Finally, using Pearson's correlation, we found that HLA-DR, CD20, B2M, and CD27 are significantly correlated with significant B cell genes, including *BANK1*, *CD37*, *IGHG1*, *IGHG2*, *IGHG3*, *IGHG4*, *IKZF1*, *MARKCS*, *NOTCH4*, transmembrane activator and CAML interactor, and BAFF receptors (Table E8).

Discussion

In this study, we present the first comprehensive LF transcriptome atlas in patients with COPD obtained using high-throughput DSP. We show a specific exaggerated B cell lung transcriptomic signature in patients with COPD with emphysema with increased antibody production, which is absent in patients with COPD with no emphysema, regardless of the degree of airflow limitation indicated by the GOLD stage (proposed model in Figure 7). In particular, we found that LFs from patients with moderate emphysema have increased expression of B cell proliferation markers, whereas LFs from patients with severe emphysema exhibit chronic B cell activation and enrichment in autoimmune-associated genes. These findings suggest that LFs are more a feature of emphysema than airflow obstruction and that LFs are not passive bystanders of the small airway and alveolar destruction but likely develop as the emphysematous damage progresses. Nonetheless, recent data suggest that B cell infiltrate strongly correlated with the loss of small airways occurring in the very early stages of COPD (49).

In patients with moderate versus no emphysema, B cell activation markers were significantly increased. Particularly true of this is *MARCK*, a plasma membrane surface protein that regulates BCR downstream signaling (28). *LTB*, *CD37*, *IKZF1*, and *IKZF3* are fundamental for B cell survival, differentiation, and LF formation (50, 51) and serve as B cell "metabolic gatekeepers" (52). *NCF1*, a part of the NADPH oxidase enzyme (53), is associated with oxidative

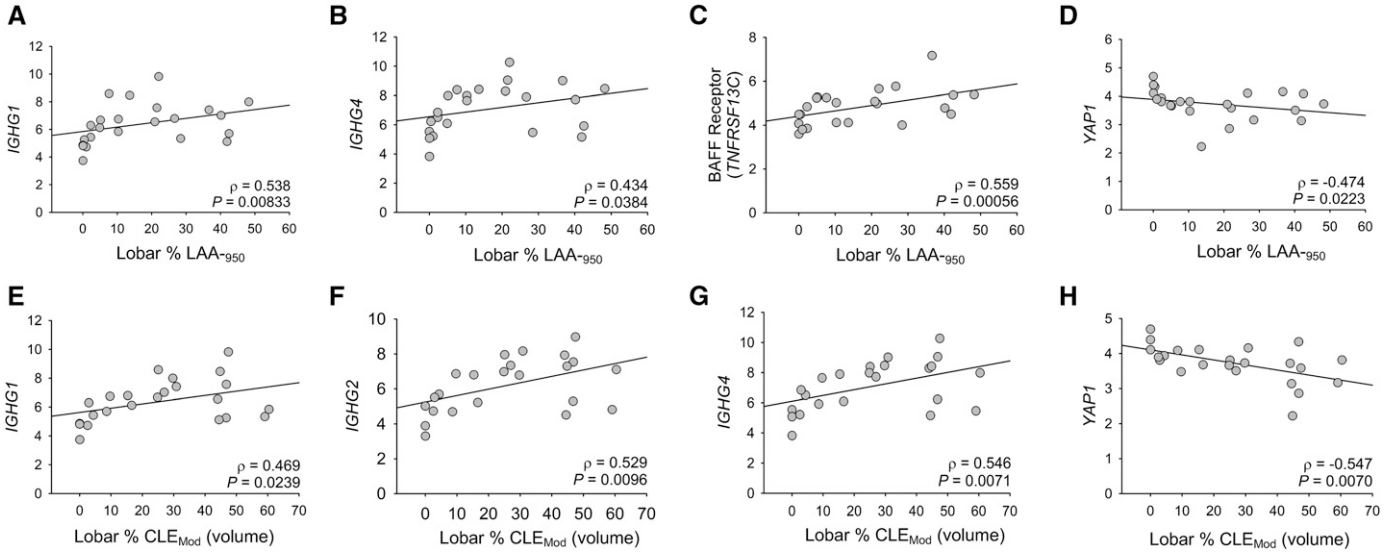

Figure 5. Correlation between emphysema, tissue destruction measurements, and B cell–related genes within the lymphoid follicles. Differentially expressed genes were correlated with digital spatial profiling percentage of low-attenuation areas below the −950 Hounsfield units (%LAA-$_{950}$) scores (*A–D*) or %CLE$_{Mod}$ (*E–H*). Data were analyzed with Spearman correlation (ρ); $n = 23$. Significant when $P < 0.05$. Emphysema is defined by %LAA-$_{950}$ >5%. CLE = centrilobular emphysema.

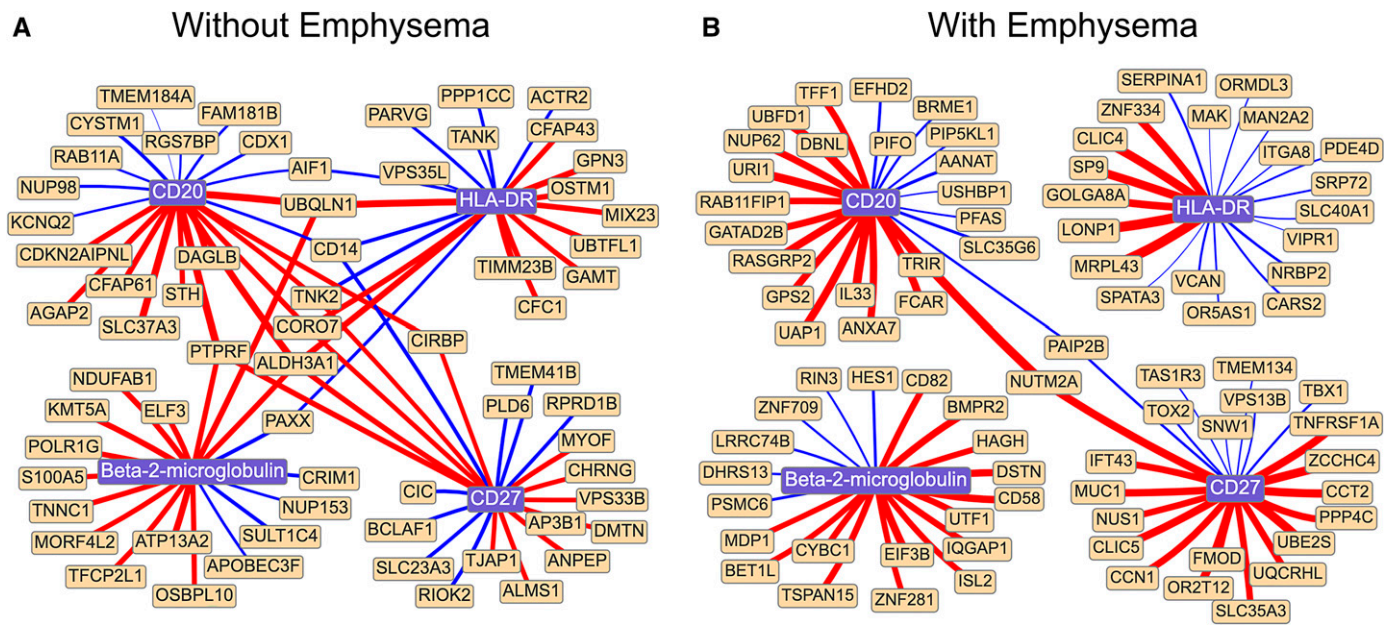

Figure 6. Network of associations between proteins CD20, HLA-DR, CD27, β_2-microglobulin (B2M), and related genes within the lymphoid follicles. The figure shows the protein-RNA network in subjects without emphysema (*A*) and with emphysema (*B*). The network plot shows the top 15 associated RNA (ranked by *P* value) of each protein (CD20, HLA-DR, CD27, B2M). The purple node represents protein, and the yellow node represents RNA. The red edge indicates a positive association between RNA and protein. The blue edge indicates a negative association between RNA and protein. The edge width is proportional to the association effect size (a thicker edge means a stronger association).

stress and mitochondrial dysfunction in autoimmune diseases.

Notably, LFs from both emphysematous groups were characterized by the switching of the γ-heavy chain subclasses. IgG2 is mostly restricted to bacterial protection, IgG3 is the effector/proinflammatory antibody, and IgG4 is expressed after long-term exposure to antigens, becoming the dominant Ig subclass overtime (54). Specifically, IgG4 is often upregulated in autoimmune diseases because it arises during

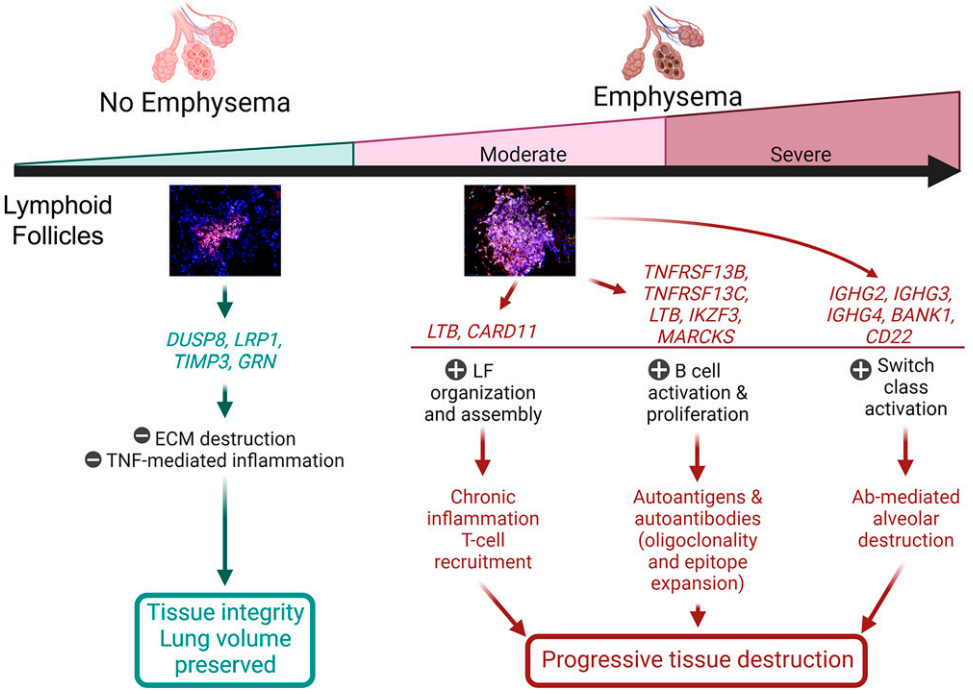

Figure 7. Hypothetical model of our findings. Patients with chronic obstructive pulmonary disease can dampen chronic inflammation and extracellular matrix turnover, which results in preserved tissue integrity. The assembly of lymphoid follicles, the activation and expansion of B cells, and the presence of switch-class recombination are key factors in the progression and acceleration of emphysematous changes. Parenchymal destruction is associated with antibody-mediated mechanisms, aided by oligoclonality and epitope expansion processes.

chronic (self-)antigen exposure (55–57). The known pathogenic role of IgGs in autoimmune diseases suggests that their increase in moderate and severe emphysema might be associated with autoimmune features contributing to lung tissue destruction in this subset of patients.

HCT intersection analysis of LFs in severe emphysema resolved footprints for critical B cell TFs, including TCF3 and TCF4 (29, 30), PAX5 (31), HIF1A (32), RUNX1 (33), IRF4 (34), and MYC (35). These TFs are key determinants of B cell lineage, differentiation into plasma cells, and class-switch recombination (58). In particular, PAX5 (31) and TCF3/4 (29, 30, 59) are key regulators of VDJ rearrangement. In emphysema, the enrichment we observed in genes associated with B cell maturation and antibody production is likely associated with the enrichment in IgGs we observed in the same group. We confirmed that LFs in moderate and severe emphysematous tissues harbor activated class-switch–specific B cells (19), which are not present in the absence of emphysema. Last, we found enrichment for BAFFR, which we have previously shown to be crucial in LF formation in COPD and in particular in the emphysema subtype (6, 9, 60).

A clonal process has previously been observed in LFs in COPD, and the presence of ongoing somatic mutations was observed in 75% of the LFs, indicating oligoclonal, antigen-specific proliferation (27). The DEG and network analysis performed here showed enrichment for B cell metabolic reprogramming signatures in moderate and severe emphysema. Metabolic reprogramming is essential for B cell maturation and class switching (52, 61) because these processes have a high energy demand. Thus, our data suggest an ongoing antibody production characterizing the emphysematous lung.

Within patients with GOLD 1–2 COPD, those with emphysema exhibit a specific LF signature involving DNA methylation, monocarbon metabolism, and cell replication regulation (*MTHFD1*, *METTL17*, *FTCD*) (62) that was absent in patients with GOLD 1–2 COPD without emphysema. Antigen processing and presentation is a costly process, requiring several rounds of ubiquitin-dependent protein degradation (63). These findings suggest that in emphysema, LF cells begin to replicate and expand, adding to the cellular burden inside the LFs and likely contributing to exaggerated and off-targeted antibody formation.

Interestingly, all patients with GOLD 1–2 COPD were male. It is common knowledge that women have a higher risk than men for autoimmune diseases (64, 65). At the same time, COPD is more common in males than in females (66). However, this risk is more correlated with systemic than with organ-specific autoimmunity and with cell-mediated autoimmune disease than with antibody-mediated diseases (65). Further clinical studies are needed to evaluate the role of sex in the autoimmune-like signature we found in emphysema.

Patients without emphysema have a very different protein-RNA network from those with emphysema. LF CD20 protein was associated with oxidative stress resistance (*ALDH3A1*) (40), cell proliferation, mitochondrial dysfunction and inflammation (*CIRBP*, *PTPRF*), and reduction of B cell activation (*CD14*, *AIF1)* (41) in the top-ranked genes, whereas we found significant enrichment for BAFF-mediated antibody production activator and autoimmunity inducer *IL33* (67) and *FCAR* (43–45) in patients with emphysema. These features speak of active LFs, intensely proinflammatory and primed for autoimmunity, possibly driven by the IL33 axis (67, 68).

These findings might indicate the presence of a "preemphysematous prodrome-like" state in which LFs are present and are actively scanning BCR-ligated antigens, which are generated by chronic injury and subclinical basal membrane damage. This "prodromic destructive state" is a window of opportunity for either preventing antigen generation or loss of tolerance.

Previous network analyses of lung transcriptomics in COPD have found a B cell signature present only in emphysematous tissue (5) and not in bronchiolitis, indicating that the B cell compartment is indeed involved in emphysema onset and/or progression. We and others have previously reported that the COPD lung incurs in aberrant B cell responses (6, 7, 9), including antielastin autoantibodies (3), with increased IgG^+ B cells and plasma B cells, correlating strongly with the number of LFs (9). These off-targeted B cell responses were associated with the presence of emphysema more than the GOLD stage of COPD severity (9).

It is important to note that within the same lungs, we did not find any difference in B cell signature between lobes with more and less emphysema, suggesting that other factors contribute to emphysema progression, such as a dysregulated immune response to inhaled particles and gases resulting in protease/antiprotease imbalance and aberrant extracellular matrix repair and remodeling (69, 70).

Limitations

This study has a few limitations. First, the overall number of subjects with LFs was small because not all lung sections from ever-smokers and patients with COPD had identifiable LFs. Second, our HCT intersection analysis was based on a set of TFs derived from mouse studies, albeit this data analysis has been published and validated with human *in vitro* assays (20, 21). Third, it is impossible to establish whether the presence of a B cell signature associated with emphysema is the consequence or a concurrent cause of the ongoing emphysematous process. Importantly, we found genes of B cell activation and antibody production strongly and directly correlated with the extent of parenchymal destruction. Nonetheless, we acknowledge that the association between B cells and emphysema in our cross-sectional study does not provide proof of a causal association (cause–effect) and could be due to chance, bias, confounding, and/or reverse causation (effect–cause), the effects of which need to be explored in future studies analyzing longitudinal cohorts of subjects and *in vitro* assays. Fourth, lung sampling did not comply with stereological requirements proposed by the American Thoracic Society (71). Because our specimens are not autoptic but were obtained during lung surgery, the tissue obtained was limited by what was available at the time of the surgery. In some cases, such as the COPD explants, we were able to obtain several specimens from the same lungs. In these patients, we have indeed compared the nature of the LFs obtained from different lung regions. For most of the patients, only one lung sample/subject was obtained. However, this is a common limitation in most of the studies of the human lung. We recognize that the sampling and assessment of the LFs may have been biased by our approach. To partially overcome this sampling bias, lobe morphological assessment of emphysema was performed using CT scans to account for overall tissue pathology. Last, we do not have genotyping information to diagnose alpha-1

antitrypsin deficiency disease; nevertheless, our population sample exhibited CLE, which differs from the panlobular emphysema typically seen in subjects with alpha-1 antitrypsin deficiency (15).

Conclusions

Our results show that LFs in subjects with emphysema have an exaggerated and off-targeted B cell signature, which potentially contributes to emphysema pathogenesis (Figure 7). Currently, there is a lack of disease-modifying therapies for COPD, mainly because available therapies target patients with COPD as a whole and cluster them simply according to their airflow limitation. Our data suggest that patients with COPD, even within the same GOLD stage, are pathobiologically diverse and that the presence of emphysema is more strongly associated with B cell responses than airflow limitation. The complexity of B cell maturation presents opportunities for therapeutic interventions for COPD with an emphysema phenotype (72, 73). A deeper understanding of the B cell population will pave the way to novel immunomodulatory and antiinflammatory therapies in emphysema-predominant COPD, which will target specific B lineage cells (monoclonal antibodies) and/or their products, such as (auto)antibodies (74–76), and/or reset the threshold for B cell activation. ■

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