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IgG4 expressing plasma cells and altered B cell receptor light chain usage in chronic obstructive pulmonary disease

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Research Article

Keywords: Chronic obstructive pulmonary disease, B cell, IgG4, B-cell receptor repertoire, Cigarette smoke exposure

Posted Date: November 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11262456/v1

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Additional Declarations: No competing interests reported.

Abstract

Background

This study explored B-cell heterogeneity and receptor repertoire features in chronic obstructive pulmonary disease (COPD) and examined their descriptive relationship to immune signatures reported in autoimmune diseases.

Methods

Public single-cell RNA-sequencing datasets from human lung and blood and murine blood and bone marrow were analysed separately by species and tissue to characterise B-cell subsets using differential gene expression, pathway enrichment, protein–protein interaction and cell–cell communication analyses. Serum immunoglobulin G4 (IgG4) was measured by enzyme-linked immunosorbent assay and correlated with clinical parameters in 35 patients with COPD. Peripheral blood B cells were sorted for single-cell B-cell receptor (BCR) sequencing; samples from three patients with COPD and four controls were pooled separately by group. A murine v-Abl/Bcl2 pro-B cell line was exposed to cigarette smoke extract (CSE) to assess developmental gene expression in vitro.

Results

An IGHG4-expressing cluster annotated as plasma cells was detected in the aggregated COPD lung dataset but not in the sampled controls. Serum IgG4 showed exploratory inverse associations with pre- and post-bronchodilator FEV1/FVC (the ratio of forced expiratory volume in 1 s to forced vital capacity). Ligand–receptor analysis predicted candidate interactions involving pulmonary B cells. The small human BCR cohort showed higher pooled λ-chain usage and IGLV1-47 representation. In cigarette smoke exposed mice, pooled blood and bone marrow profiles showed differences in transitional and precursor B-cell composition. CSE exposure reduced the reported λ5 expression readout in the cell line.

Conclusions

These exploratory findings describe B-cell dysregulation in COPD patients and smoke-exposure models. They do not establish autoreactivity, a causal role for IgG4 or a mechanistic link between λ5 suppression and mature λ-chain usage.

Background

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide. It is a chronic inflammatory airways disease characterised by persistent airflow limitation, primarily driven by prolonged exposure to noxious inhaled particles, most notably tobacco smoke and air pollutants,

alongside contributions from genetic susceptibility, developmental factors and social determinants of health [1, 2]. Despite its high prevalence, the precise mechanisms underlying COPD pathogenesis remain incompletely elucidated.

The autoimmune hypothesis of COPD was proposed by Agustí and colleagues in 2003 [3]. Subsequent studies reported autoantibody profiles in smokers with and without COPD [4], associations of BCR signalling with emphysema [5], and B-cell activation signatures in emphysematous lung tissue [6]. These observations motivate investigation of B-cell responses in COPD. However, immune activation and disease-associated expression patterns do not by themselves establish antigen-specific autoreactivity or a pathogenic autoimmune mechanism.

COPD shares several immunological hallmarks with classical autoimmune diseases, including autoantigen exposure, interferon activation and B-cell dysregulation. Certain clinical and immunological features of COPD closely mirror those observed in systemic autoimmune disorders. For example, protein citrullination, a hallmark autoantigenic process in rheumatoid arthritis (RA), is upregulated in COPD lung tissue irrespective of smoking status [7]. Similarly, cigarette smoke exposure in mice induces the release of extracellular double-stranded DNA (dsDNA), triggering inflammatory responses and antinuclear antibody (ANA) production reminiscent of systemic lupus erythematosus (SLE) [8]. A large-scale clinical study has further demonstrated overlapping blood transcriptomic signatures between stable COPD and SLE, suggesting a persistent, interferon-related inflammatory program associated with immune-mediated lung injury [9]. In addition, patients with COPD display an imbalanced ratio of effector B-cells (Beff) to regulatory B-cells (Breg), alongside increased memory B cells and enhanced plasma-cell-related gene expression, abnormalities that parallel those observed in systemic sclerosis (SSc) [10–12].

Given the emerging role of B cells in COPD pathogenesis, B-cell-directed interventions provide additional motivation to investigate these responses, although their therapeutic relevance to COPD remains to be established. Preclinical studies have implicated B-cell activating factor (BAFF) in pulmonary autoantibody production in cigarette smoke-exposed mice [13]. A plasma-cell gene signature has also been associated with disease activity in COPD and with treatment response to inebilizumab in systemic sclerosis [11]. Together, these observations highlight the potential clinical relevance of understanding aberrant B-cell responses in COPD, while findings from other diseases and experimental models require disease-specific evaluation [14].

Despite the growing body of evidence implicating B cells in COPD, the distribution and functional significance of B-cell subsets in this disease remain incompletely characterised. An unresolved question is whether observed B-cell alterations reflect chronic antigen exposure and inflammation, autoreactive responses, or a combination of these processes. Further investigation is therefore warranted to characterise the B-cell subsets, regulatory genes and associated pathways altered in COPD and to determine whether these abnormalities share molecular features with those observed in established autoimmune diseases.

With the rapid advancement of sequencing technologies and bioinformatics, large-scale characterisation of immune-cell heterogeneity has become increasingly feasible. In the present study, we integrated publicly available single-cell transcriptomic datasets from human lung and peripheral blood and from murine blood and bone marrow with serum IgG4 measurements, single-cell BCR sequencing of sorted human peripheral blood B cells, and in vitro cigarette smoke extract (CSE) exposure experiments using a murine v-Abl/Bcl2 pro-B cell line. The human, animal and in vitro components address related but distinct aspects of B-cell biology, while cross-dataset comparisons were used to identify recurrent features of B-cell dysregulation and to generate hypotheses regarding their potential relationship to autoimmune-associated immune responses in COPD.

Methods

Ethics approval

All human studies were approved by the Institutional Review Board of Capital Medical University (approval no. Z2021SY025). Written informed consent was obtained from all participants or their legal guardians prior to enrolment. COPD was diagnosed in accordance with the 2023 Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria, defined by a post-bronchodilator forced expiratory volume in one second/forced vital capacity (FEV1/FVC) ratio < 0.70. Diagnosis also required the presence of compatible respiratory symptoms (e.g., dyspnoea and chronic cough) and relevant exposure history, including a smoking history of ≥ 10 pack-years. Patients with a prior diagnosis of asthma or other chronic respiratory diseases were excluded from the study [15].

Demographic and clinical data collection

For serum IgG4 measurement and correlation analyses, clinical data collected for each patient with COPD included age, sex, body mass index (BMI), and smoking-related variables, including cigarettes smoked per day (CPD), smoking duration, smoking cessation status, and years since cessation. Pulmonary function parameters included post-bronchodilator FEV1, FVC and FEV1/FVC ratio. Symptom burden and disease severity were assessed using the modified Medical Research Council (mMRC) dyspnoea scale, the COPD Assessment Test (CAT), GOLD grade (based on predicted FEV1%) and the recorded GOLD clinical grouping. We also recorded the number of acute exacerbations within the previous 12 months. Routine haematological parameters included white blood cell count, neutrophil, lymphocyte, monocyte, eosinophil and basophil counts, neutrophil-to-lymphocyte ratio (NLR), platelet count, haemoglobin level and haematocrit (Table 1).

Table 1
Demographic, clinical and functional characteristics of patients with COPD (n = 35).

Variable	Range	Mean (SD)
Male/Female, n (%)*	—	24/11 (68.57%/31.43%)
Age, years	52–87	67.66 (7.24)
Height, cm	152–176	164.43 (6.88)
Weight, kg	49.0–90.0	66.93 (10.79)
BMI, kg/m²	16.79–32.37	24.72 (3.39)
Smoking (no/yes/quit), n (%)*	—	10/5/20 (28.57%/14.29%/57.14%)
(Lung function)		
FEV1	0.54–2.07	1.22 (0.40)
FEV1REV	0.64–2.29	1.39 (0.43)
FEV1PP	22.40–87.40	49.31 (14.53)
FEV1REV%	25.70–92.40	56.21 (15.40)
FVC	0.93–3.52	2.31 (0.63)
FVCREV	1.17–3.99	2.56 (0.63)
FVCPP	35.50-111.20	73.79 (16.71)
FVCREV%	44.60–131.00	82.12 (17.77)
FEV1/FVC%	33.33–67.96	52.67 (8.84)
Post-bronchodilator FEV1/FVC (%)	33.16–69.30	54.05 (9.13)
Eosinophils%	0.15–9.31	2.01 (1.80)
Leukocytes%	2.67–12.79	6.33 (2.46)
mMRC scale	0–4	1.31 (1.05)
CAT	1–21	11.45 (4.93)

Values are presented as range (minimum–maximum) and mean (standard deviation [SD]), unless otherwise indicated by (*), in which case data are presented as n (%). Abbreviations: N, number of subjects; BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FEV_1REV, post-bronchodilator FEV_1 following reversibility testing; FEV_1PP, percentage predicted FEV_1; FEV_1REV%, percentage change in FEV_1 after reversibility testing; FVC, forced vital capacity; FVCREV, post-bronchodilator FVC following reversibility testing; FVCPP, percentage predicted FVC; FVCREV%, percentage change in FVC after reversibility testing; mMRC, modified Medical Research Council dyspnoea scale; CAT, COPD Assessment Test; GOLD, Global Initiative for Chronic Obstructive Lung Disease; groups, GOLD ABCD classification; AE, acute exacerbation.

Variable	Range	Mean (SD)
GOLD	1–4	2.37 (0.65)
groups	1–4	2.20 (1.08)
AE (last year)	0–3	0.51 (0.66)

Values are presented as range (minimum–maximum) and mean (standard deviation [SD]), unless otherwise indicated by (*), in which case data are presented as n (%). Abbreviations: N, number of subjects; BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FEV_1REV, post-bronchodilator FEV_1 following reversibility testing; FEV_1PP, percentage predicted FEV_1; FEV_1REV%, percentage change in FEV_1 after reversibility testing; FVC, forced vital capacity; FVCREV, post-bronchodilator FVC following reversibility testing; FVCPP, percentage predicted FVC; FVCREV%, percentage change in FVC after reversibility testing; mMRC, modified Medical Research Council dyspnoea scale; CAT, COPD Assessment Test; GOLD, Global Initiative for Chronic Obstructive Lung Disease; groups, GOLD ABCD classification; AE, acute exacerbation.

For single-cell BCR sequencing, demographic and clinical information for patients with COPD and controls included sex, age, height, weight, BMI, tobacco load, smoking duration, FEV1/FVC ratio, mMRC score and CAT score (Table 2).

Table 2
Baseline characteristics of patients with COPD and controls.

Variable	HC group (n = 4)	COPD group (n = 3)	*P*
Male/Female, n (%)*	3/1 (75%/25%)	3/0 (100%/0%)	0.052
Age, years	57.75 ± 14.22	56.00 ± 12.77	0.545
Height, m	1.70 ± 0.07	1.74 ± 0.02	0.155
Weight, kg	73.63 ± 13.39	83.67 ± 18.04	0.648
BMI, kg/m²	25.44 ± 3.38	27.63 ± 5.96	0.345
Tobacco load (pack-years)	197.75 ± 152.03	324.33 ± 70.44	0.382
Smoking (years)	27.50 ± 22.17	38.67 ± 22.59	0.049
FEV1/FVC (%)	76.50 ± 3.42	58.31 ± 16.83	0.083
mMRC scale	0 ± 0.00	0.33 ± 0.58	0.005
CAT	0 ± 0.00	15.33 ± 8.50	0.008

Values are presented as mean ± standard deviation (SD), unless otherwise indicated by (*), in which case data are presented as n (%). Abbreviations: BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; mMRC, modified Medical Research Council dyspnoea scale; CAT, COPD Assessment Test.

Human PBMC collection and cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated using HISTOPAQUE-1077 (Sigma-Aldrich) according to the manufacturer's instructions. Briefly, 2 mL of fresh peripheral blood collected in EDTA anticoagulant tubes were layered onto HISTOPAQUE-1077 and centrifuged. The mononuclear cell layer at the plasma-HISTOPAQUE interface was collected, transferred to a new tube and washed twice with phosphate-buffered saline (PBS; Gibco). Cells were subsequently treated with red blood cell lysis buffer (Miltenyi Biotec) on ice for 1–2 min, washed twice, and resuspended in fluorescence-activated cell sorting (FACS) buffer consisting of PBS supplemented with 2% foetal bovine serum (FBS) at a final concentration of approximately 1 × 10^6 cells/mL.

FACS for B-cell sorting

For live B-cell sorting, PBMCs were stained with anti-CD45-FITC (BioLegend, clone HI30), anti-CD19-PerCP-Cy5.5 (BioLegend, clone HIB19) and fixable viability stain 506 (FVS506; BD Horizon™) for 30 min on ice in the dark. Following staining, cells were washed twice and resuspended in MACSQuant Tyto running buffer (Miltenyi Biotec) at a concentration of 2–4 × 10^6 cells/mL. Cell sorting was performed using a MACSQuant Tyto cell sorter (Miltenyi Biotec) equipped with 405 nm, 488 nm, and 561 nm lasers and a closed cartridge-based system. Sorted cells with a purity ≥ 90% were collected into the positive chamber, transferred into 1.5 mL centrifuge tubes using gel-loading tips and maintained on ice prior to single-cell BCR sequencing. All procedures were performed under sterile conditions at 4°C.

Single-cell 5' BCR sequencing

Immediately after sorting, live $CD45^+CD19^+$ B cells were centrifuged (300 × g, 5 min, 4°C) and resuspended in PBS containing 0.04% bovine serum albumin (BSA) at a concentration of 700–1200 cells/μL. Single-cell libraries were generated using the Chromium Next GEM Single Cell 5' Reagent Kit v2 (10x Genomics, PN-1000265) according to the manufacturer's protocol. For BCR libraries, nested PCR amplification of immunoglobulin heavy-chain (IgH) and light-chain (Igκ and Igλ) transcripts was performed from 5' barcoded cDNA following the 10x Genomics V(D)J workflow. Final libraries were quantified using a Qubit 4.0 fluorometer (Thermo Fisher Scientific), and fragment size distributions were evaluated using a Bioanalyzer 2100 (Agilent Technologies) with a High Sensitivity DNA chip. Paired-end sequencing was conducted on an Illumina NovaSeq X Plus platform using the Xp workflow, targeting a minimum sequencing depth of 5000 read pairs per cell for BCR libraries. FASTQ files were processed using Cell Ranger v7.1 (10x Genomics) with the Cell Ranger V(D)J pipeline aligned to the GRCh38 reference genome (refdata-gex-GRCh38-2020-A). Productive and non-productive V(D)J rearrangements were identified for each cell.

Single-cell RNA-seq data collection from GEO

Public datasets comprised human emphysema and normal lung (GSE196638), cigarette smoke- and air-exposed mouse blood and bone marrow (GSE205078), human systemic sclerosis and normal lung within

a mixed-species lung series (GSE132771), human SLE and healthy blood B cells (GSE193867), and murine collagen-induced arthritis (CIA) bone marrow (GSE221704) [16–20]. GSE196638 contains three emphysema and three normal lung samples. The relevant human portion of GSE132771 contains two systemic sclerosis and three normal donors, each with distinct sorted fractions. GSE193867 contains three SLE and three healthy donors with paired transcriptome and BCR libraries; paired libraries are not additional donors. GSE205078 contains one pooled library per exposure group per compartment. GSE221704 contains one listed library each for CIA, treated CIA and DBA control.

Quality control of scRNA-seq data

Quality control was performed using Seurat (v4.4.0) to remove doublets, damaged cells and dead or dying cells prior to downstream analysis [21]. The Harmony package (v0.1.0) was used for dataset integration and reduction of batch effects where appropriate.

For publicly available scRNA-seq datasets, cells expressing fewer than 150 genes or more than 5000 genes, as well as cells with > 10% mitochondrial gene expression, were excluded. For the single-cell 5' BCR-associated datasets, cells expressing fewer than 200 genes or more than 2500 genes, together with those with > 10% mitochondrial gene expression, were excluded.

Data normalisation was performed using the NormalizeData function following the standard Seurat workflow. Gene expression values were log-transformed and subsequently scaled at the gene level using the ScaleData function.

Cluster annotation and quantification

Principal component analysis (PCA) was performed using the top 2000 variable features. Depending on the dataset, the top 5–17 principal components were selected for downstream analyses. Cell clustering was performed in Seurat using a resolution range of 0.2–0.5. Clusters were visualised using Uniform Manifold Approximation and Projection (UMAP).

Marker genes used for cluster annotation were identified using the FindAllMarkers function. Clusters were initially annotated according to highly expressed marker genes and subsequently manually verified using CellMarker 2.0 and previously published marker-gene references [22–25].

B-cell and plasma-cell populations were distinguished using established lineage-associated marker genes [26], with plasma cells characterised by expression of plasma-cell-associated genes such as JCHAIN, MZB1 and immunoglobulin transcripts, together with reduced expression of canonical B-cell markers relative to other B-cell populations.

Cell counts and proportions were calculated for each annotated cluster. Cell-type proportions were calculated at the level of individual biological replicates or pooled samples, depending on the sampling

design of each dataset, as detailed in Supplementary Table 1. Individual cells were not treated as independent biological replicates in comparisons between experimental groups.

Differential gene expression analysis

The cell-level workflow used Seurat marker comparisons to characterise clusters and Wilcoxon rank-sum comparisons to explore expression differences between conditions. Reported settings included a minimum expression fraction of 0.1, a $\log_2$ fold-change threshold > 0.25 and nominal $P < 0.05$ for condition comparisons. For the IgG4-expressing cluster, FindAllMarkers was used, with enrichment relative to other cell populations defined by $\log_2$ fold change > 0.25 and adjusted $P < 0.05$.

Pathway analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis was performed using the R packages clusterProfiler and enrichplot [27]. For disease-versus-control analyses, pathway enrichment was performed using differentially expressed genes identified from the corresponding donor-level comparisons. Adjusted $P < 0.05$ was used as the significance threshold for pathway enrichment analyses.

Protein-protein interaction (PPI) analysis

DEGs were mapped to the STRING database to construct PPI networks using a confidence score threshold ≥ 0.400. For hub gene visualisation, interaction data containing $\log_2$ fold change information were imported into Cytoscape (v3.10.0) and analysed using the cytoHubba plugin [28].

Cell-cell communication analysis

Potential cell-cell communication between B-cell subclusters and other cell populations was computationally assessed using the iTALK package, which evaluates cytokine, growth factor, immune checkpoint and other receptor-ligand interactions [29]. Candidate receptor-ligand pairs were identified using the STRING database and selected through the FindLR function.

BCR analysis

Cells containing at least one productive heavy-chain rearrangement and one productive light-chain rearrangement (κ or λ) were retained for analysis. Using the R package scRepertoire (v1.6), we evaluated clonal diversity (Shannon entropy and inverse Simpson index), clonal expansion (defined as the proportion of cells belonging to expanded clones containing > 1 cell), V(D)J gene usage, somatic hypermutation frequency and immunoglobulin isotype distribution, including IgM, IgD, IgG1-4, IgA1-2 and IgE.

ELISA detection of serum IgG4

Peripheral blood samples were collected, and serum was isolated and stored at − 80°C until analysis. Prior to ELISA, serum samples were thawed on ice and centrifuged at 10 000 × g for 5 min at 4°C to

remove cryoprecipitates and cellular debris. Serum IgG4 concentrations were measured using a commercially available human IgG4 ELISA kit (Thermo Fisher Scientific; cat. no. BMS2095TWO) according to the manufacturer's instructions.

Correlation analysis

Associations between serum IgG4 concentration and clinical characteristics in the 35-patient COPD cohort were explored using Spearman rank correlations in GraphPad Prism (version 10.1).

Culture and stimulation

The murine v-Abl/Bcl2 pro-B cell line was maintained in complete RPMI 1640 medium (Thermo Fisher Scientific) supplemented with 10% FBS, 50 U/mL penicillin, 50 µg/mL streptomycin, 2 mM L-glutamine, 1× MEM non-essential amino acids, 1 mM sodium pyruvate, 50 µM 2-mercaptoethanol (Sigma-Aldrich), and 20 mM HEPES (pH 7.4). Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 and maintained at approximately 1 × 10^6 cells/mL by subculturing every 2–3 days [30].

To synchronise cells in the G1 phase and induce V(D)J recombination, exponentially growing v-Abl/Bcl2 pro-B cells were treated with the Abl kinase inhibitor imatinib (STI-571; 3 µM) for 48 h as a positive control. Experimental groups were treated with CSE at concentrations of 1.25%, 2.5%, 5%, and 10% for 48 h. Following treatment, the expression of BCR-related genes, including λ5, λ5-promoter, Igκ, VpreB-promoter and IL-7R, was quantified. Primer sequences are listed in Supplementary Table 2.

Data were analysed and visualized using GraphPad Prism (version 10.1). The relative expression of target genes (normalized to β-actin) was calculated using the $2^{-\Delta\Delta Ct}$ method. Results are presented as the mean ± standard error of the mean (SEM) from four independent experiments (n = 4 per group). One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test was performed to compare each treatment group with the untreated control group. A P value < 0.05 was considered statistically significant.

Statistical analysis

Unless otherwise specified, statistical analyses were performed using R and GraphPad Prism (version 10.1). Biological donors or independent experiments were treated as the experimental units for group-level statistical comparisons.

For two-group comparisons of independent samples, normally distributed data were analysed using a two-tailed Student's t-test or Welch's t-test as appropriate, whereas non-normally distributed data were analysed using the Wilcoxon rank-sum test. For comparisons involving more than two groups, one-way ANOVA followed by the appropriate multiple-comparisons procedure was applied where assumptions of parametric testing were satisfied.

Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Where multiple hypotheses were tested simultaneously, P values were adjusted for

multiple comparisons using the Benjamini-Hochberg false discovery rate procedure unless otherwise specified. Data are presented as the mean ± SD or mean ± SEM, as indicated. All statistical tests were two-sided, and adjusted $P < 0.05$ was considered statistically significant where multiple-testing correction was applied.

Results

Identification of an IgG4-expressing plasma-cell population in COPD lung samples

To characterise B-cell abnormalities in COPD lung tissue, we extracted B cells from the integrated human lung single-cell dataset GSE196638. Dimensionality reduction and clustering identified four major B-cell subclusters (Fig. 1A). In the pooled dataset, B cells accounted for a higher proportion of recovered cells in COPD samples than in controls (6.72% vs. 3.52%) (Supplementary Fig. 1A, C, E). We annotated the subclusters using cluster-enriched genes (Fig. 1B) and established lineage markers [31, 32] (Fig. 1C). Pooled COPD samples had a higher proportion of memory B cells and a lower proportion of $IgG1^+$ plasma cells than controls (Fig. 1D). A distinct IgG4-expressing plasma-cell population accounted for approximately 5% of recovered B cells in the COPD group but was not detected in the sampled controls (Fig. 1A, D).

Exploratory cell-level comparisons identified transcriptional differences between COPD and control samples in memory B cells and $IgA1^+$ and $IgG1^+$ plasma cells (Fig. 1E). PPI network analysis identified MT2A among the genes with higher expression across these subsets (Fig. 1F-H). KEGG enrichment analysis identified immune-related pathways, including type 1 diabetes mellitus (T1DM), inflammatory bowel disease (IBD) and Th17 differentiation (Fig. 1I-K).

We further characterised the IgG4-expressing plasma-cell population by examining genes enriched in this cluster relative to other cell populations. These included the immunoglobulin variable-region genes IGLV1-47, IGHV1-24 and IGHV3-23, as well as BCL2, FOS, HSPA6, HSPB1, HSP90AA1 and IRF1 (Fig. 1L). KEGG analysis of these genes identified TNF, NF-κB, MAPK and p53 signalling terms (Fig. 1M).

After identifying this pulmonary population, we measured serum IgG4 concentrations in a separate cohort of 35 patients with COPD and examined their relationships with clinical parameters (Table 1). Exploratory correlation analyses indicated an association with age and inverse trends with pre- and post-bronchodilator FEV1/FVC ratios (Fig. 2A, O, T).

Predicted ligand-receptor networks involving pulmonary B-cell subsets

We used transcript-based cell-cell communication analyses to examine potential interactions between B-cell subsets and the pulmonary microenvironment. The analyses included immune populations, such as

monocytes, macrophages, NK cells and T cells, and non-immune populations, including fibroblasts, endothelial cells, basal cells, alveolar epithelial cells and stromal cells.

For IgA1$^+$ plasma cells, the predicted networks included HLA-A/HLA-B interactions with KIR2DL3 in immune populations and THBS1/TGFB1-associated integrin interactions involving fibroblasts and endothelial cells. The analysis also identified CCL13-associated connections. ICOSLG, IL1R1, CD44 and TRAF3 appeared among the accompanying expression-associated network features (Fig. 3A-B; Supplementary Table 3).

IgG1$^+$ plasma-cell networks included IL-1β/IL-6-related interactions, collagen-integrin interactions and HLA-B-associated connections involving monocytes and macrophages. Chemokine-related connections included interactions of CCL2/CCL3/CCL4 with CCR5. CD44, MYLK, IL6R and TRAF3 were also identified among the expression-associated features of these networks (Fig. 3C-D; Supplementary Table 3).

Memory B cells had predicted connections with surrounding immune and stromal populations involving IL-1β, FN1 and CCL21. Accompanying expression-associated features included CAV1, CCR7 and CD44 (Fig. 3E-F; Supplementary Table 3).

These analyses identified candidate ligand-receptor connections involving inflammatory mediators, chemokines and extracellular matrix-associated molecules.

Transitional B-cell profiles in peripheral blood from cigarette smoke-exposed mice

We analysed mouse peripheral blood B cells from GSE205078 to examine responses to cigarette smoke exposure and identified seven major subclusters [27, 33] (Fig. 4A-D; Supplementary Fig. 2). Pooled smoke-exposed samples had a higher proportion of naïve B cells and a lower proportion of transitional B cells than air-exposed controls (Fig. 4B).

Exploratory cell-level comparisons identified transcriptional differences in immature and memory B-cell populations (Fig. 4E). KEGG analysis of immature B-cell gene lists identified pathways associated with immune responses and inflammation (Fig. 4F). Gene lists from cell-cycling B cells were enriched for pathways annotated as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), autoimmune thyroid disease (AITD), IBD, Th1/Th2/Th17 differentiation and the intestinal immune network for IgA production (Fig. 4G). Other B-cell subsets had distinct enrichment patterns (Supplementary Fig. 3A-C).

We examined transitional B cells in more detail because they occupy a developmental stage between immature bone marrow B cells and mature peripheral B cells and are frequently altered in autoimmune diseases [34]. PPI analysis identified Uba52, Rpl29 and Gna13 among genes with lower expression in smoke-exposed samples (Fig. 5A). KEGG pathway annotations included RA, T1DM, SLE, IBD, AITD and Th17 differentiation (Fig. 5B).

We divided transitional B cells into T1 and T2 subsets using the expression patterns of Ighm, Ighd, Cd24a and Fcer2a (Cd23) [31, 33] (Fig. 5C-E). T1 cells accounted for a larger proportion of the transitional compartment in smoke-exposed samples (Fig. 5C, E). We also identified Wnt10a-expressing T1 and Sell-expressing T2 populations and compared them with the corresponding transitional populations in controls (Fig. 5D, F-G).

KEGG analyses of both T1 and T2 gene lists identified pathways annotated as RA, T1DM and IBD (Fig. 5H-I). Additional enrichment patterns in Sell-expressing T2 and Wnt10a-expressing T1 populations involved stress responses, mitochondrial processes, DNA damage responses and inflammation. FoxO signalling appeared among the terms associated with genes showing lower expression in Wnt10a-expressing T1 cells (Fig. 5J-K).

In the separate human peripheral blood cohort, which comprised three COPD donors and four controls, KEGG analyses of T1-cell gene lists also identified RA, IBD, AITD, T1DM and graft-versus-host disease annotations. Other terms included Th1/Th2 differentiation, Th17 differentiation and the intestinal immune network for IgA production (Table 2; Supplementary Fig. 4).

These findings support further study of transitional B cells in smoke-associated and COPD-associated B-cell changes. The human and mouse observations, however, came from separate comparisons.

Bone marrow B-cell precursor profiles in cigarette smoke-exposed mice

To examine earlier stages of B-cell development, we classified bone marrow B cells from cigarette smoke-exposed and control mice in GSE205078 into seven subsets (Supplementary Fig. 5A, C, E; Fig. 6A-D). Pooled smoke-exposed samples had a lower proportion of small pre-B III cells and higher proportions of pre-B I, immature and mature B cells than controls (Fig. 6B).

Figure 6E shows the exploratory expression differences within each subset. KEGG analysis of pre-B I cell gene lists identified pathways annotated as T1DM, AITD, graft-versus-host disease and the intestinal immune network for IgA production (Fig. 6F). In immature and large pre-B II cells, genes with lower expression mapped to several immune-disease pathways, whereas genes with higher expression mapped to BCR-signalling pathways (Fig. 6H; Supplementary Fig. 3D). KEGG analysis of small pre-B III cell gene lists identified FoxO and JAK-STAT signalling pathways (Fig. 6G).

These analyses revealed differences in the composition of recovered early B-cell populations and their transcriptional profiles following cigarette smoke exposure. The associated developmental pathways provide candidates for further study.

Peripheral blood BCR repertoire and light-chain distributions in COPD

We performed single-cell profiling with V(D)J sequencing on peripheral blood B cells from three patients with COPD and four controls to examine BCR features associated with COPD (Table 2). Nine major B-cell subsets were identified (Fig. 7A-C). The analysed dataset contained 5,474 B cells from COPD donors and 9,942 from controls.

Pooled COPD samples had lower proportions of resting naïve (rNAV) and resting memory (rM) B cells than controls, together with higher proportions of transitional (Tran), early activated naïve (eaNAV) and activated class-switched memory (SA-MEM) B cells (Fig. 7B-C).

Pooled BCR repertoire analysis showed a higher proportion of rare clonotypes and a lower proportion of medium-sized clonotypes in COPD samples. Resting class-switched memory (SR-MEM) B cells were predominantly represented by small clonotypes (Fig. 7D-E). Isotype analysis showed higher IgM and lower IgD representation in COPD samples (Fig. 7F-G).

Light-chain analysis showed greater λ-chain representation across several B-cell subsets in pooled COPD samples, with no apparent dominant monoclonal expansion in the displayed repertoire. λ-chain usage was relatively prominent in transitional B cells, whereas κ chains were more highly represented in naïve and memory B-cell populations (Fig. 7H-I). These findings describe differences in light-chain distribution across the recovered populations.

Within the λ-chain-associated repertoire, COPD samples had higher IgG and lower IgA representation across multiple B-cell subsets (Fig. 7J-K). Thus, the relationship between isotype distribution and light-chain usage differed with B-cell subset composition.

Analysis of the displayed IGLV genes showed greater representation of several λ-chain variable-region genes, including IGLV1-47, in COPD samples (Fig. 7L). Pooled diversity analysis also showed higher BCR diversity in COPD B-cell populations. Memory B cells had higher Shannon entropy and inverse Simpson indices, as well as higher Chao1 and ACE richness estimates (Supplementary Fig. 6).

In a separate experiment, we exposed murine v-Abl/Bcl2 pro-B cells to CSE in vitro to examine its effect on a component of the early B-cell developmental programme. CSE treatment reduced λ5 expression, with lower expression at increasing concentrations (Fig. 8B). This finding suggests a CSE-associated response in developmental gene expression in these cells.

Exploratory comparisons with autoimmune-disease datasets

We placed the observed B-cell profiles in context through exploratory comparisons with human systemic sclerosis (SSc) lung tissue from GSE132771, human SLE peripheral blood from GSE193867 and mouse collagen-induced arthritis (CIA) bone marrow from GSE221704 (Supplementary Figs. 1, 2, 5 and 7–11). We compared cell composition and reported transcriptional features within each dataset, accounting for differences in species, tissue, sample processing and study design when interpreting the results.

In the SSc lung dataset, pooled samples had lower proportions of $IgA1^+$ plasma cells and memory B cells and a higher proportion of $IgG1^+$ plasma cells than their controls. This differed from the pattern in COPD lungs (Fig. 1D; Fig. 9A). Reported transcriptional similarities included antigen-presentation-associated genes such as HLA-B. IGHG4 expression was higher within the annotated memory B-cell population in both datasets (Fig. 9D-F; Supplementary Table 4). KEGG analyses identified overlapping immune-related pathways, including T1DM and graft-versus-host disease (Fig. 1I; Supplementary Fig. 7H). Predicted communication networks also differed in their HLA-associated, inflammatory mediator-associated and extracellular matrix-associated features (Fig. 3; Supplementary Tables 3 and 5; Supplementary Fig. 8).

Peripheral blood profiles from cigarette smoke-exposed mice and patients with SLE showed lower pooled proportions of transitional B cells and higher proportions of memory B cells than their respective controls (Fig. 4B; Fig. 9B; Supplementary Fig. 9B). Transitional B-cell gene lists shared pathway annotations related to immune diseases, antigen presentation and Th1/Th2 differentiation (Fig. 5H-I; Supplementary Figs. 9–10). Figure 9G-I and Supplementary Table 4 show the reported gene-list comparisons.

In bone marrow, pooled compositional differences in pre-B I, pre-B III and immature B-cell populations appeared less pronounced in CIA samples than in the smoke-exposure dataset (Fig. 6B; Fig. 9C; Supplementary Fig. 11B). Immune-disease pathway annotations occurred mainly in pre-B III and immature B-cell gene lists in CIA samples, but predominantly in pre-B I cell gene lists in smoke-exposed samples (Fig. 9J-L; Supplementary Fig. 11F-G).

Reported genes with higher expression in both datasets included Ifi27l2a, Pcna, Atp5k and Cenpf in pre-B I cells; Il7r, Mnd1 and Gm10076 in small pre-B III cells; and Hsp90b1 in immature B cells (Fig. 9J-L; Supplementary Table 4).

These comparisons identified recurring immune-response annotations alongside differences in B-cell composition and transcriptional profiles across datasets. The findings provide hypotheses for further study of B-cell dysregulation in COPD.

Discussion

The autoimmune hypothesis has prompted studies of B-cell responses in COPD [3], but whether these responses reflect chronic immune activation or pathogenic autoreactivity remains unresolved. In this study, we examined human lung and peripheral blood samples, profiles from cigarette smoke-exposed mice, and responses in a transformed murine pro-B cell line. These analyses identified several features of possible B-cell dysregulation: an IgG4-expressing pulmonary cluster, exploratory differences in peripheral light-chain usage, and altered precursor-cell profiles in pooled mouse samples. The findings support further study of a possible autoimmune component in COPD. They do not, however, establish causal links between these compartments or demonstrate low-grade autoimmunity.

An IgG4-expressing cluster was detected in the sampled COPD lungs, with no clearly corresponding population in controls. This finding suggests a possible local IgG4-associated plasma-cell response. IgG4-related disease and IgG4-mediated disorders offer biological context [35, 36], although the relationship between this population and established autoimmune pathology is uncertain. The cluster was annotated using transcript expression, and the pooled data cannot show whether it was consistently present across individual COPD donors. The independent serum cohort allowed assessment of correlations within COPD but lacked matched non-COPD measurements. The evidence therefore supports the identification of a candidate IgG4-expressing pulmonary plasma-cell population; it does not establish elevated circulating IgG4 or a pathogenic role for these antibodies. Further work should assess cell abundance in individual donors, validate the population at the protein level in lung tissue, and determine the antigen specificity of its IgG4 antibodies.

We also observed differences in light-chain usage. IGLV1-47 appeared both in the gene list associated with the pulmonary cluster and among the V genes with greater representation in the small peripheral blood cohort. These observations came from different samples and provide no evidence of shared clones or recognition of a common antigen. They do raise the question of whether differences in light-chain usage are reproducible across COPD donors and associated with defined antibody specificities. BCL2 expression and TNF-, MAPK- and NF-κB-related annotations suggest additional cellular programmes for study, but their effects on cell survival and inflammatory activity remain unknown. Replication in individual donors and functional characterisation are needed to assess these findings.

The MT2A findings raise a related question about how B cells respond to oxidative stress and inflammation. Exploratory comparisons showed increased MT2A expression in several pulmonary B-cell populations. Our previous study identified metallothionein-high T-cell populations in COPD [37], although differences in cell populations and study design prevent direct functional comparison. These observations suggest that metallothionein-related programmes may occur in distinct immune-cell populations exposed to the inflammatory and oxidative environment of COPD. The established roles of metallothioneins in stress responses and inflammation provide a biological basis for exploring this possibility [38, 39]. The co-occurrence of MT2A expression and chemokine-related features does not, by itself, establish a role in monocyte recruitment or lung remodelling. Perturbation studies in relevant B-cell populations are needed to distinguish protective, pathogenic and compensatory functions.

Changes in transitional B-cell representation and early developmental profiles suggest possible alterations in B-cell maturation. These include transitional-cell profiles in cigarette smoke-exposed mice and the small human COPD cohort, as well as differences in precursor-cell representation and the expression of Il7r, Vpreb and Rag-family genes in mouse bone marrow. Altered λ5 expression after CSE exposure in a transformed murine pro-B cell line provides a complementary observation at an early developmental stage. Whether developmental changes in bone marrow are related to B-cell alterations in peripheral blood and lung tissue remains an open question. The datasets cover distinct biological settings and do not establish a developmental continuum, defective receptor editing or impaired negative selection. λ5 expression and mature λ-chain usage are also distinct biological readouts, and

autoreactive clones were not traced across compartments. Addressing these questions will require paired sampling and direct assessment of developmental checkpoints and clonal relationships.

The peripheral blood BCR analysis revealed exploratory differences in repertoire composition. Differences in pooled λ-chain and IGLV1-47 usage, together with greater observed diversity, suggest possible repertoire remodelling in the sampled COPD cohort. This analysis included only three COPD donors and four controls, however. Variation between individuals, smoking exposure, subset composition, clone size and sequencing depth could account for the pooled differences. The findings remain candidate associations that require replication before they can be considered disease markers. Larger cohorts, donor-level effect estimates and antigen-specific analyses are needed to establish their reproducibility and biological significance. Experimental studies of B1 cells in elastase-induced emphysema have directly examined B-cell function in a separate setting [40]. Those experiments and our descriptive analyses address complementary aspects of B-cell biology, but differences in cell populations and models limit the mechanistic conclusions that can be drawn across studies.

Cross-disease comparisons also need to account for the genes underlying pathway annotations. KEGG pathways labelled as SLE, RA, type 1 diabetes, autoimmune thyroid disease or coronavirus disease include genes involved in shared inflammatory, interferon-response and antigen-presentation processes. As with the autoimmune-labelled pathways, recurrent enrichment of the coronavirus disease pathway may reflect shared immune-response programmes; it does not establish SARS-CoV-2 infection or a disease-specific mechanism. Interpretation depends on the contributing genes and the background used for enrichment analysis. Differences in species, tissue processing, cohort composition and assay design further prevent quantitative comparisons of the extent of autoimmunity in COPD, SSc, SLE and CIA. These analyses can generate hypotheses about shared transcriptional features, but cannot determine the relative extent of autoimmunity across these conditions.

Several limitations affect the interpretation of our findings. The IgG4-expressing pulmonary cluster requires donor-level assessment of abundance and independent protein-level validation in tissue. Serum correlations in 35 patients were obtained without a non-COPD comparator, correction for multiple testing or covariate-adjusted estimates. The BCR cohort was small, and the reported differential expression analyses lacked donor-level pseudobulk confirmation. In GSE205078, pooling produced one library per condition and compartment, preventing independent animal-level inference. Incomplete information on medication use, disease severity, exacerbation history, comorbidities and autoimmune status may also confound the human observations. The ligand-receptor networks are computational predictions, and comparisons across studies are limited by differences in species, sampling and orthology. The transformed murine cell-line experiment lacked validation at the protein level, viability assessment, confirmation in primary cells and rescue experiments. Neither autoreactivity nor antibody pathogenicity was assessed directly. These constraints keep the findings exploratory and identify the validation needed for stronger biological conclusions.

Conclusions

This study identifies exploratory differences in B-cell composition, gene expression and repertoire features in human COPD samples and cigarette smoke-exposure models. The IgG4-expressing pulmonary cluster and pooled differences in light-chain usage deserve priority in replication studies, but neither establishes that IgG4 or λ-chain remodelling drives autoimmunity. Determining their relevance to COPD pathogenesis will require donor-level validation, appropriately controlled clinical analyses, and direct antigen-specific and functional studies.

Abbreviations

ACE
abundance-based coverage estimator
AE
acute exacerbation
AITD
autoimmune thyroid disease
ANA
antinuclear antibody
ANOVA
analysis of variance
BAFF
B-cell activating factor
BCR
B-cell receptor
Beff
effector B cell
BMI
body mass index
Breg
regulatory B cell
BSA
bovine serum albumin
CAT
COPD Assessment Test
CIA
collagen-induced arthritis
COPD
chronic obstructive pulmonary disease
CPD
cigarettes per day
CSE

cigarette smoke extract
DEG
differentially expressed gene
dsDNA
double-stranded DNA
ELISA
enzyme-linked immunosorbent assay
FACS
fluorescence-activated cell sorting
FBS
foetal bovine serum
FEV1
forced expiratory volume in 1 second
FVC
forced vital capacity
GEO
Gene Expression Omnibus
GOLD
Global Initiative for Chronic Obstructive Lung Disease
GSA
Genome Sequence Archive
IBD
inflammatory bowel disease
Ig
immunoglobulin
KEGG
Kyoto Encyclopedia of Genes and Genomes
mMRC
modified Medical Research Council
NK
natural killer
NLR
neutrophil-to-lymphocyte ratio
PBMC
peripheral blood mononuclear cell
PBS
phosphate-buffered saline
PCA
principal component analysis
PCR

polymerase chain reaction
PPI
protein–protein interaction
RA
rheumatoid arthritis
scRNA-seq
single-cell RNA sequencing
SD
standard deviation
SEM
standard error of the mean
SLE
systemic lupus erythematosus
SSc
systemic sclerosis
T1DM
type 1 diabetes mellitus
UMAP
Uniform Manifold Approximation and Projection
V(D)J
variable,diversity and joining
WBC
white blood cell.

Declarations

Ethics approval and consent to participate

Human specimen collection was approved by the Institutional Review Board of Capital Medical University (approval no. Z2021SY025). Written informed consent was obtained from all participants prior to enrolment.

Consent for publication

Not applicable. All data presented in this study are anonymized and do not contain any identifying personal information.

Availability of data and materials

Publicly available scRNA-seq datasets analyzed in this study were obtained from the GEO database under accession numbers GSE196638 [16], GSE205078 [17], GSE132771 [18], GSE193867 [19], and GSE221704 [20]. The single-cell BCR sequencing data generated in this study have been deposited in the

Genome Sequence Archive (GSA) of the National Genomics Data Center under accession number HRA018181 (Project ID: PRJCA063022) [41]. These data will be released upon publication. All other data supporting the findings of this study are available within the article and its supplementary information files, or from the corresponding authors upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Beijing Natural Science Foundation (grant no. 7242002) and the National Natural Science Foundation of China (grant no. 82090013) and sponsored by Beijing Nova Program (No. 20240484639). The funders had no role in the study design, data collection, data analysis, data interpretation, manuscript preparation or the decision to submit the work for publication.

Authors' contributions

H.H.Y. conceived the research project following initial discussions with W.W., Y.C. and Y.S. L.D. led the single-cell RNA-seq analyses. H.Z. and Y.Q.X. contributed to early single-cell data analysis and interpretation. Z.Q.L. and M.M. collected human samples. L.D. and H.H.Y. performed the in vitro experiments. H.H.Y. drafted the initial version of the manuscript with assistance from H.Z. and Y.Q.X. Y.S. and W.W. reviewed the data and critically revised the manuscript. C.J.C. offered valuable comments on the manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements

We thank Professor Jiazhi Hu from the School of Life Sciences, Peking University, for generously providing the v-Abl/Bcl2 pro-B cell line used in this study.

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