In silico immune infiltration profiling reveals the role of naïve B cells in lung tissues of COVID-19 patients

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Abstract

COVID-19 caused by SARS-CoV-2 has rapidly spread to more than 160 countries worldwide since 2020. Despite the tremendous efforts and resources spent around the world trying to explore antiviral drugs, there is still no effective clinical treatment for COVID-19. Approximately 15% of COVID-19 cases progress to pneumonia, patients with severe pneumonia may die from acute respiratory distress syndrome (ARDS). In addition, further pulmonary fibrosis from SARS-CoV-2 infection causes ARDS that often leads to irreversible impairment of lung function. If the mechanisms by which SARS-CoV-2 infection primarily cause immune responses or immune cell infiltration can be identified, it is possible to alleviate or prevent severe lung damage by modulating the infiltration and activation of specific immune cells to mitigate excessive immunity response.

The extent to which subsets of immune cells are significantly altered in the lung tissue of COVID-19 patients remains unclear. This study applied the CIBERSORT method to comprehensively evaluate the immune infiltration landscape in lung tissues of COVID-19 patients, and further compared with the one from lung tissue of patients with idiopathic pulmonary fibrosis (IPF). We found several immune cell subtypes; particularly naïve B cells are highly infiltrated in COVID-19 group. A comparison of functional gene set analysis revealed that non-differentiated naïve B cells may be the main cause of the overactive humoral immune response. We further compared several specific COVID-19 cases receiving therapies targeting B cells and found that appropriate suppression of naïve B cells might be a new strategy to alleviate severe symptoms of COVID-19.

Introduction

COVID–19 (Coronavirus Disease–2019) caused by the SARS-CoV–2 (Severe Acute Respiratory Syndrome coronavirus–2) has rapidly spread to over 160 countries worldwide since the beginning of 2020[1–3]. Nearly 7 million patients with COVID–19 and over 400,000 deaths have occurred up to now. At the same time, the rate of increase in the number of COVID–19 cases is gradually accelerating. Despite the tremendous efforts and resources spent by scientists and clinicians around the world trying to produce vaccines and explore antiviral drugs[4,5], there is still no potent drug or effective clinical treatment for COVID–19 to date[6]. The clinical indications of COVID–19 disease are quite diverse, symptoms including fever, dry cough, loss of smell or taste, fatigue, diarrhea, conjunctivitis, and pneumonia have been reported[7–9]. About 80% of COVID–19 cases are asymptomatic or exhibit mild to moderate symptoms, about 15% of COVID–19 cases progress to pneumonia[10], and patients with severe pneumonia may die from acute respiratory distress syndrome (ARDS) or multi-organ failure[11,12]. Moreover, further pulmonary fibrosis caused by SARS-CoV–2 infection-induced ARDS often result in irreversible impairment of lung function, which may leave permanent or semi-permanent damage even if patients recover from COVID–19[13,14].

The mechanism by which SARS-CoV–2 causes lung injury is only partially understood. It is believed that SARS-CoV–2 infection activates both innate and adaptive immune responses against the virus. However,

excessive inflammatory innate responses and dysregulated adaptive host immune defense may cause harmful tissue damage at sites of SARS-CoV–2 entry, such as the lungs and bronchi, accelerating the process of acute lung injury (ALI) and ARDS. Worse, certain organs, including the lungs, may be irreversibly damaged after infection[15]. Regarding the association of ARDS with lung fibrosis, ARDS is thought to originate from plasma infiltration into the alveolar lumen due to persistent alveolar epithelial damage[16,17]. Activation of the coagulation system in the plasma and production of proinflammatory cytokines and chemotactic factors leads to a massive influx of neutrophils, lymphocytes and monocytes/macrophages into the lungs, resulting in the dysregulated release of potent cytotoxic mediators associated with inflammation, which ultimately leads to damage to the lung endothelium and epithelial cells[18]. When epithelial and endothelial cells are damaged, inflammatory mediators are released to further recruit multiple types of inflammatory immune cell infiltrates, prompting fibroblasts to activate and migrate to the wound center while releasing collagen to remodel the extracellular matrix (ECM). Chronic inflammation and persistent repair can trigger an excessive accumulation of ECM components, which will lead to permanent fibrosis. Therefore, it is generally accepted that persistent inflammation due to infiltration of immune cells is a major contributor to pathological fibrosis in the lungs[19].

While maintaining immune defense against SARS-CoV–2 infection in COVID–19 patients is certainly important, the over-activated inflammatory response is also directly linked to poor prognosis for recovery[20]. If the mechanism by which SARS-CoV–2 infection primarily elicits the immune system response and immune cell infiltration can be found, it would be possible to alleviate the extent of ARDS and lung fibrosis by modulating the infiltration and activation of specific immune cells to attenuate the excessive immune response. We believe that understanding the infiltration status of immune cells in SARS-CoV–2 infected lung tissues is the key to access the originate that raised immune hyperactivation and is also a critical step in developing new therapeutic strategies for COVID–19.

Current clinical studies on COVID–19 have focused on the collection and analysis of peripheral blood or bronchoalveolar lavage fluid from patients[21,22]. However, the extent to which subsets of immune cells are significantly altered in the lung tissues of COVID–19 patients remains unclear. In this study, we applied the CIBERSORT approach developed by Newman et al. to comprehensively evaluate the infiltration status of 22 types of immune cell in the lung tissue of patients who deceased of COVID–19 and further compared it with the one from idiopathic pulmonary fibrosis (IPF) patients[23]. By analyzing immune cell infiltration, we found that several immune cell subtypes, especially naive B cells, are highly infiltrated in the lung tissue of COVID–19 patients. Comparison of functional gene sets revealed that non-differentiated naive B cells may be the main cause of the hyper-activated humoral immune response. We further compared several cases of specific COVID–19 with treatments targeting B cells and showed that suppression of naïve B cells is likely to be a new strategy to alleviate severe symptoms of COVID–19.

Material And Methods

Evaluation of Infiltrating Immune Cells using CIBERSORT

CIBERSORT is a powerful tool for simulating tissue immune infiltration in samples using computational algorithms[23,24]. The application of CIBERSORT accurately quantifies the relative abundance of different immune cell types in a complex gene expression mixture. To characterize and calculate each immune cell subtype, CIBERSORT uses approximately 500 genes with consistent gene expression signatures. Here, we applied the original CIBERSORT gene signature file LM22, which characterizes 22 immune cell subtypes including B cells, T cells, natural killer cells, macrophages, dendritic cells, eosinophils, and neutrophils, and analyzed datasets from COVID–19 patient lung tissues and controls from the same database (NCBI GEO accession number: GSE150317), IPF patient lung tissues and healthy donor lung tissues (NCBI GEO accession number: GSE53845 and GSE124685)[25,26]. After normalization of all sample data, the immune cell profiles were analyzed by CIBERSORT in R. Mean values of individual immunocyte profiles were calculated for each group using GraphPad software.

Gene Set Enrichment Analysis (GSEA) and Enrichment map visualization

GSEA is a computational method used to investigate whether a given whole gene expression profiling with user defined phenotype is significantly enriched in a set of gene sets[27]. The GSE53845, GSE124685 and GSE150317 databases were downloaded from NCBI and normalized before being input into the GSEA program. The BP:GO biological process (7530 gene sets) and the PID subset of CP (196 gene sets) from the C2CP canonical pathways were downloaded from MsigDB and used as functional gene sets[27]. Samples were sorted into “SARS-CoV–2 vs NegControl” and “IPF vs Healthy donor” according to the original database annotations, respectively. *P*-value <0.05 and FDR <0.25 will be considered statistically significant. Enriched gene sets of GSEA identification on “GO:BP” and “PID pathways” were visualized via the Enrichment Map v3.2.1 plugin in Cytoscape 3.8[28]. Enrichment maps were created to represent the degree of enrichment and correlation of functional gene clusters for each disease group compared to control groups.

Statistical analysis

For CIBERSORT abs scores, the Student’s t-test was used for comparisons between the two groups with normally distributed data and the Mann-Whitney test was used for comparisons between the two groups with abnormally distributed data. Pearson correlation analysis was used to estimate the consistency among the 22 immune cell infiltration score distributions. Statistical analysis was performed using GraphPad Prism software (GraphPad Software). $P < 0.05$ were regarded as statistically significant differences.

Results

Rationale and design of an in silico simulated immune cell infiltration profiling study

Nearly all of the patients who died from COVID–19 had severe lung tissue damage and pulmonary fibrosis[29]. On the other hand, mortality in IPF is generally the result of progressive fibrotic lung disease. We believe that comparing the gene expression profiling between lung tissues of COVID–19 and IPF will allow us to access the phenotypes that are specific to SARS-CoV–2 infection. Among these, differences in the level of immune cell infiltration are considered to be the most critical factor in the assessment of an over-active immune system.

However, it is difficult to assess experimentally the infiltration of multiple immune cells in clinical. The first step is to obtain ethical approval and valuable COVID–19 lung samples, followed by tissue sampling and analysis in laboratories with adequate biosafety levels, and then staining and analysis of various immune cell populations with specific biomarkers. Moreover, the biomarkers for the analysis were limited, and the proportion of multiple immune cells could not be analyzed simultaneously. In recent years, algorithms to precisely simulate the proportion of multiple immune cells infiltrating tissue samples using whole gene mapping have emerged and have been used in many studies[30,31]. The simulation results were confirmed to correlate significantly with the proportion of actual immune cells in several studies[24,32]. In this study, CIBERSORT was utilized for tissue immune cell infiltration scoring, which was based on 22 types of immune cell subsets profiling, and abs mode was performed to enable cross-database comparison[23]. For sample collection, we used the valuable COVID–19 patient organ RNA-sequencing whole gene expression results uploaded to the NCBI GEO database by Ting et al. as the target for analysis (SARS-CoV–2 infected lung tissue sample N = 16, Negative Control lung tissue sample N = 5). GSEA was then used to evaluate the enrichment score of functional gene sets associated with disease groups. The functional gene set significance filter was set at *P*-value <0.05, FDR <0.25. Further, Cytoscape Enrichment map was applied to visualize the GSEA results and perform related gene set clustering, the whole process is shown in Figure 1A.

In the lung tissue of COVID–19 patients and negative controls, 22 immune cell infiltration scores were calculated by CIBERSORT and visualized in Morpheus developed by Broad institute[33]. Comparison of the differences by T test showed that T cell $CD8^+$, B cell plasma, monocyte, and Macrophage M1 were significantly increased in the lung tissues of COVID–19 patients (Figure 1B). Similarity matrix analysis of the distribution of 22 immune cells showed that B cell naïve, monocyte, T cell $CD8^+$, B cell plasma, mast cell activated, and T cell $CD4^+$ mem clustered together, indicating that the distribution of these immune cells was similar, and the percentage of infiltration was increased in the lung tissue of COVID–19 patients (Figure 1C).

Comparison of T-cell lineage infiltration in lung tissue between COVID–19 and IPF patients.

In order to understand the detailed differences between the immune cell infiltration landscape in COVID–19 and IPF, CIBERSORT was carried out on two IPF databases (GSE124685 and GSE53845), and the results were divided into T cell lineage, B cell lineage, Myeloblast lineage, and other cells respectively.

T cell lineage analysis showed significant increases in $CD8^+$, $CD4^+$ naïve, and $CD4^+$ memory activated in both SARS-CoV–2 infection and fibrotic lung tissues (Figure 2). In contrast, there was no consistent comparisons among $CD4^+$ memory resting, regulatory T cell and γδ T cell subgroups.

Related studies showed that $CD8^+$ T cells significantly increased bronchoalveolar lavage fluid in SARS-CoV–2 infected patients[22], and $CD4^+$ memory T cells have been reported to respond to viral spike protein after SARS infection[34]. In addition, both $CD4^+$ naïve T cells and $CD8^+$ T were reported to be significantly increased by mass cytometry (CyTOF) analysis[35].

Comparison of B-cell lineage infiltration in lung tissue between COVID–19 and IPF patients.

The results of the B-cell lineage analysis showed that the CIBERSORT abs score of Plasma B cells was significantly increased in both COVID–19 and IPF patients (Figure 3). In contrast, there were significantly elevated naïve B cells in the lung tissue of SARS-CoV–2-infected patients and significantly elevated memory B cells in the lung tissue of IPF patients respectively, suggesting that SARS-CoV–2 infection is associated with the infiltration of naïve B cells rather than memory B cells in the lungs, a phenomenon exactly the opposite of the observations in the IPF. In addition, the infiltration of follicular T cells was significantly increased in one of the IPF databases and was not significantly different in COVID–19 group.

Correlative studies have shown significantly increased expression of memory B cells, plasmablast and BAFF (B cell-activating factor of the TNF family) in lung tissue and peripheral blood of IPF patients[36,37], where BAFF is considered important for the survival of plasma cells[38]. In addition, the proportion of T follicular helper cells in the peripheral blood of IPF patients increased significantly[39,40]. In COVID–19 patients, recent studies have shown a significant increase in plasma cells and a significant decrease in naïve B cells in peripheral blood[21]. In an analysis of B cell compartment of SARS-CoV–2 infected patients, Nielsen et al. found that most of the B cells recruited to respiratory tracts in the early stage of infection lacked significant somatic mutation, suggesting that the recruited B cells were similar to naïve B cells[41].

Comparison of myeloblast lineage and other immune cell infiltration in lung tissue between COVID–19 and IPF patients.

The analysis of myeloblast lineage showed an increase in monocyte in the lung tissue of SARS-CoV–2 infected patients, and a decrease in the lung tissue of IPF patients (Figure 4). In common, the CIBERSORT abs score of M1 macrophage was significantly increased in both disease groups. The results of M0 and M2 macrophage infiltration do not vary significantly or are not consistent between the two IPF databases.

Monocytes and lung macrophages are thought to be involved in the pathogenesis of pulmonary fibrosis[42]. Previous studies have shown that higher monocyte counts observed in the peripheral blood of patients with IPF are significantly associated with poor prognosis[43]. Recent studies have shown that

alveolar macrophages (AM), which are primarily involved in the pathogenesis of pulmonary fibrosis, arise more from in situ proliferation than from bone marrow supplementation[44,45]. These studies suggest that during pulmonary fibrosis, cytokine released from immune cells may locally induce AM polarization to M1 or M2 subtypes and thus influence the progression of fibrosis[46]. The role of monocyte in the lungs of IPF patients remains to be defined.

On the other hand, a high degree of monocyte infiltration has been observed in the lung tissue of COVID–19 patients[22,35], macrophage infiltration has also been reported recently[47]. The infiltration of monocytes in the lung may be activated by the immune response to form macrophages, which may ultimately promote acute inflammation and cause lung damage through increased M1-polarized macrophages[48]. Other types of immune cells were not significantly different in the lungs of COVID–19 patients, as shown in Supplementary Figure 1.

GO:BP analysis based on GSEA reveals specific activation of B-cell-mediated humoral responses in the lungs of COVID–19 patients

To understand the similarities and differences in gene functional enrichment of lung tissues from COVID–19 and IPF patients compared to controls, the GO:BP (Gene Ontology Biological Processing) gene set was used to evaluate a variety of biological responses including immune responses. Based on the GO:BP gene set, the top 20 most enriched functional gene sets for COVID–19 and IPF are listed respectively in Figure 5A.

In IPF group, normalized enrichment score (NES) of COLLAGEN FIBRIL ORGANIZATION and EXTRACELLULAR STRUCTURE ORGANIZATION associated with pulmonary fibrosis was the highest. B-cell immune responses including REGULATION OF B CELL PROLIFERATION, REGULATION OF B CELL ACTIVATION, REGULATION OF B CELL DIFFERENTIATION, PROCESSING AND PRESENTATION OF ENDOGENOUS ANTIGEN, B CELL HOMEOSTASIS were also highly enriched in IPF group. On the other hand, in SARS-CoV–2 infected lung tissues, the NES for a series of gene function sets associated with the humoral immune response was the highest, such as HUMORAL IMMUNE RESPONSE MEDIATED BY CIRCULATING IMMUNOGLOBULIN, PHAGOCYTOSIS RECOGNITION, COMPLEMENT ACTIVATION, and FC RECEPTOR MEDIATED STIMULATORY SIGNALING PATHWAY. The next is a set of gene functions related to B-cell immune response, such as B CELL MEDIATED IMMUNITY, B CELL RECEPTOR SIGNALING PATHWAY, IMMUNOGLOBULIN PRODUCTION, and ADAPTIVE IMMUNE RESPONSE.

In addition, the results of gene-set enrichment were visualized using Enrichment map app in Cytoscape (Figure 5B). Clusters of immune response were enriched in both COVID–19 and IPF groups compared to controls. Clusters of metabolic processes, tissue development and molecular functions were mostly enriched only in the IPF groups. Based on the enriched gene set, the cluster of immune response was further classified as follows: B cell mediated immunity, Humoral immune response, Fc receptor signaling pathway, Phagocytosis, and T cell activation. Edges between gene sets showed that B-cell-mediated immunity, Humoral immune response, Fc receptor signaling pathway, and phagocytosis were highly

correlated in COVID–19 except T cell activation (edges with dark blue). Interestingly, in the cluster of B cell mediated immunity, gene sets such as B cell proliferation, homeostasis and differentiation were enriched only in the IPF groups, indicating that the processes of B cell proliferation and differentiation did not take place in COVID–19 group. In addition, the enriched IFNγ signaling pathway in COVID–19 may be involved in differentiation inhibition of activated naïve B cells[49]. These results are consistent with the data from the CIBERSORT analysis of B cell lineage (Figure 3). That is, in the lungs of COVID–19 patients, undifferentiated naïve B cells may be the main immune cells that elicit humoral immune response, which has been observed in clinical practice[50,51].

PID pathway analysis reveals correlation between immune cell signaling and migration responses and shared signaling pathways in damaged lung.

The function of immune cells is thought to be associated with multiple signaling pathways. Through a comprehensive analysis of the enrichment of signaling pathways, not only the status of specific immune cells can be assessed, but also potential therapeutic targets can be identified. The PID (Pathway Interaction Database) pathway gene set is known for its accuracy in reflecting specific signal pathways, which helps us to precisely define the specific variation in COVID–19 group[52]. Figure 6 shows the visualized results of the GSEA analysis using PID pathway. In terms of the relevance of immune cell-associated signaling pathways, TCR signaling in Naïve $CD4^+$/$CD8^+$ T cells was significantly activated in both COVID–19 and IPF groups. In addition, CD40/CD40L signaling were negatively correlated only in SARS-CoV–2 infected lung tissues, which was suggested to be associated with the differentiation and proliferation of activated B cells in germinal center[53]. In the cluster of cytokine signaling, IL–1 and IL–6 signaling pathways were negatively correlated, while IL–4 and IL–12 signaling pathways were positively correlated, shows that IL–4 and IL–12 have more significant effects on COVID–19 lung tissue than other cytokines.

Among the integrin associated interactions, β1, 3, and 5–7 integrin cell surface interaction were all negatively correlated in COVID–19 group. In contrast, β2 integrin (LFA–1) and α4β1 integrin were positively correlated, which are thought to be essential for B-cell activation and adhesion[54,55]. Furthermore, among the many signaling pathways, the Insulin pathway showed a significant negative correlation in both COVID–19 and IPF groups, the physiological significance of which needs to be further verified. Most of the other signaling pathways are unknown or require further study in relation to the immune response, all of which are listed in Supplementary Figure 2.

Discussion

Based on in silico simulation of immune infiltration, this study reveals the impact of SARS-CoV–2 infection on the immune infiltration landscape of lung tissue. Significantly increased infiltration of $CD4^+$/$CD8^+$ T cells (Figure 2), Plasma cells (Figure 3) and M1 macrophage (Figure 4) was a common observation in patients with SARS-CoV–2 infection or IPF. It is believed that the increased infiltration of these immune cells is a major contributor to lung damage or fibrosis. However, the elevated naïve B cell

infiltration (Figure 3), uncorrelated gene-sets of B cell proliferation and differentiation in the cluster of B cell mediated immune response (Figure 5), and the negative correlation of CD40/CD40L signaling are all specific to the lung tissue of COVID–19 patients (Figure 6). These results imply that the onset of the immune response in the lung tissue of COVID–19 patients may be correlated with elevated infiltration of naive B cells.

The following hypothesis was developed based on the results of functional gene-set analysis combined with in silico immune infiltration profiling, which is similar to the context of influenza-specific B cell response[56]: SARS-CoV–2 infection may activate and promote the adhesion and accumulation of naïve B cells to the mediastinal lymph node through the activation of β2 integrin (LFA–1) and α4β1 integrin[55,57], resulting in a decreased proportion of naïve B cells in the peripheral blood[58,59]. The increased infiltration of naïve B cells activated by spike proteins of SARS-CoV–2 secretes large amounts of IgM to promote humoral immune response[56,60]. Monocytes are recruited and differentiate to macrophage in response to the amplified humoral immune response[61]. Secreted IgM simultaneously activates the complement system and Fc receptor in dendritic cells and macrophage to increase antigen presentation and phagocytosis to facilitate innate and adaptive immunity[62,63]. In addition, abundant spike protein from SARS-CoV–2 presented by antigen-presenting cell (APC) may leads to rapid induction of extrafollicular (EF) response through stimulating IL–12-dependent plasma cell differentiation in naïve B cell to produce more IgM[56,64], instead of promoting B cell proliferation and differentiation by CD40/CD40L signaling mediated germinal center formation[65].

Based on this hypothesis, we believe that by suppressing the growth or migration of naïve B cells, it may help to reduce the excessive immune response caused by naïve B cells associated humoral immune response to reduce the risk of lung damage after SARS-CoV–2 infection, which have also been reported recently[66]. Remarkably, Quinti et al. reported several COVID–19 patients with primary antibody deficiencies (PAD) who clinically exhibited strikingly different extents of symptoms[67]. Five of the patients with Common Variable Iummune Deficiency (CVID) had severe COVID–19 symptoms. B cells in patients with CVID fail to differentiate into memory B cells, which maintain their properties similar to naïve B cells and continue to release IgM and IgG[68]. In contrast, 2 COVID–19 patients with agammaglobulinemia had mild symptoms and favorable outcome. These patients were congenitally deficient in B cells and plasma cells due to mutations in the gene encoding bruton kinase, which is essential for B cell survival[69]. This report increases the likelihood of our hypothesis that naïve B cells act as the trigger of severe respiratory and pulmonary symptoms of COVID–19.

In the clinical treatment of abnormal increases in serum IgM or neoplastic B cells, ibrutinib, which inhibits the activity of Bruton Kinase in the B cell receptor signaling pathway[70], is now commonly used to reduce the abnormal proliferation of B cells[71,72]. Treon et al. reported that the use of ibrutinib may be useful to reduce lung damage from SARS-CoV–2 infection by suppressing the number of B cells[73]. Fingolimod, as an agonist of the S1P1/3 receptor, is thought to inhibit B cell egress out of lymph node through overstimulation of the B cell S1P1/3 receptor[74,75]. Foerch et al. reported that multiple sclerosis patient

with severe COVID–19 infection was being treated with Fingolimod. The patient improved rapidly right after appropriate therapy[76]. Integrin complexes have multiple implications in immune cell migration and viral infection. Immune cells use LFA–1 and α4β1 integrin for migration and adhesion[77,78], which are also important for B cell[54,55]. In addition, SARS-CoV–2 viral protein is known to bind to ACE2 or integrin heterodimers to facilitate virus entry and infection[79]. Borriello et al. reported on a COVID–19 patient who was using the α4β1 integrin targeted monoclonal antibody natalizumab[80], which functions to reduces B-cell migration by blocking α4β1 integrin and have significant effect in increasing circulating B cells[81,82]. The patient improved significantly with appropriate treatment and no new symptoms developed or worsened. In terms of targeting interleukin, increased expression of IL–4 is thought to be associated with IPF, and dupilumab was also effective in asthma exacerbations, implying that inhibition of IL–4 may alleviate lung damage caused by SARS-CoV–2 by attenuating inflammation[83,84]. Conversely, IL–12 is associated with the suppression of pulmonary fibrosis, and the clinical application in COVID–19 remains need to be further clarified[85,86].

In summary, in silico simulated immune infiltration combined with gene function enrichment analysis provide us novel perspective on the immune system impact of SARS-CoV–2 infection. It is hoped that these findings will lead to new opportunities for the clinical treatment of COVID–19. All hypothetical mechanisms and locations of B cell targeted treatment acting are shown in Figure 7.

Declarations

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Limitations of the Study

We understand that computer modelling of immuno-infiltration has its limits and problems of over-interpretation, and further experimental validation is often required. However, in the midst of the COVID–19 pandemic, we are eager to provide any significant data that can be used for interpretation or to increase confidence in COVID–19 treatment. Given the support of grant funding, the difficulty of

collecting samples from COVID–19 patients, the limitations of the research environment and resources, and the fact that time is of the essence, we are strong-minded to announce the data as soon as possible, in the hope that we can do our part to contribute to the treatment of COVID–19.

Author Contributions

All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript; YYW organized clinical information about B-cell targeted treatment, HFL and CHW performed the data pre-treatment and analysis, SHW conducted the organization and introduction of IPF, LCY conducted the GSEA analysis and the Enrichment map visualization. CLH supplied critical clinical consultation. The manuscript was written by YLC and YYW. YLC conceived the whole study design.

Competing interests

The author(s) declare no competing interests.

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Figures

Figure 7

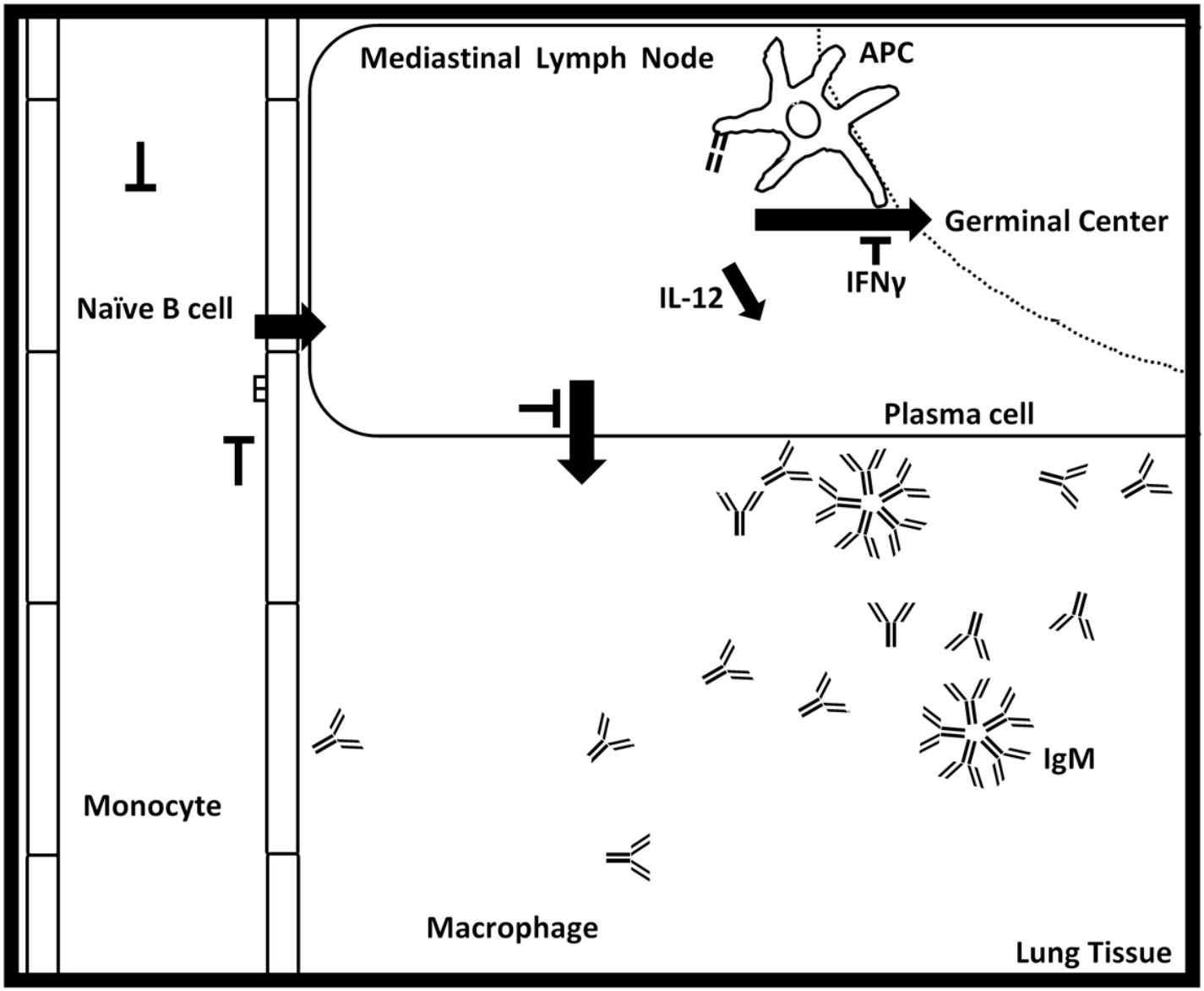


Figure 1

Diagrammatic representation illustrates the postulated role of naïve B cell in triggering humoral immune response in lung tissues of COVID-19 patients and the locations where B cell-targeted therapeutic strategies function. SARS-CoV-2 infection may activate and promote the adhesion and accumulation of naïve B cells to the mediastinal lymph node with β2 integrin (LFA-1) and α4β1 integrin. The increased infiltration of naïve B cells activated by spike proteins from SARS-CoV-2 secretes large amounts of IgM to promote extrafollicular response and humoral immune response. Monocytes are recruited and differentiate to macrophage in response to the robust humoral immune response. Secreted IgM simultaneously activates the complement system and Fc receptor in dendritic cells and macrophages to increase antigen presentation and phagocytosis to facilitate innate and adaptive immunity. On the other hand, high affinity or abundant SARS-CoV-2 presented by antigen-presenting cell (APC) such as dendritic

cell may stimulate IL-12-dependent plasma cell differentiation in naïve B cells to produce more IgM, instead of promoting B cell proliferation and differentiation by CD40/CD40L signaling mediated germinal center formation. Ibrutinib inhibits B-cell growth by specifically inhibiting Bruton kinase, which is thought to be critical for the BCR signaling pathway; Natalizumab reduces B-cell migration by blocking α4β1 integrin. Fingolimod reduces B-cell egress out of the lymph node by stimulating S1PR1/3 to internalize the receptors.

Figure 6

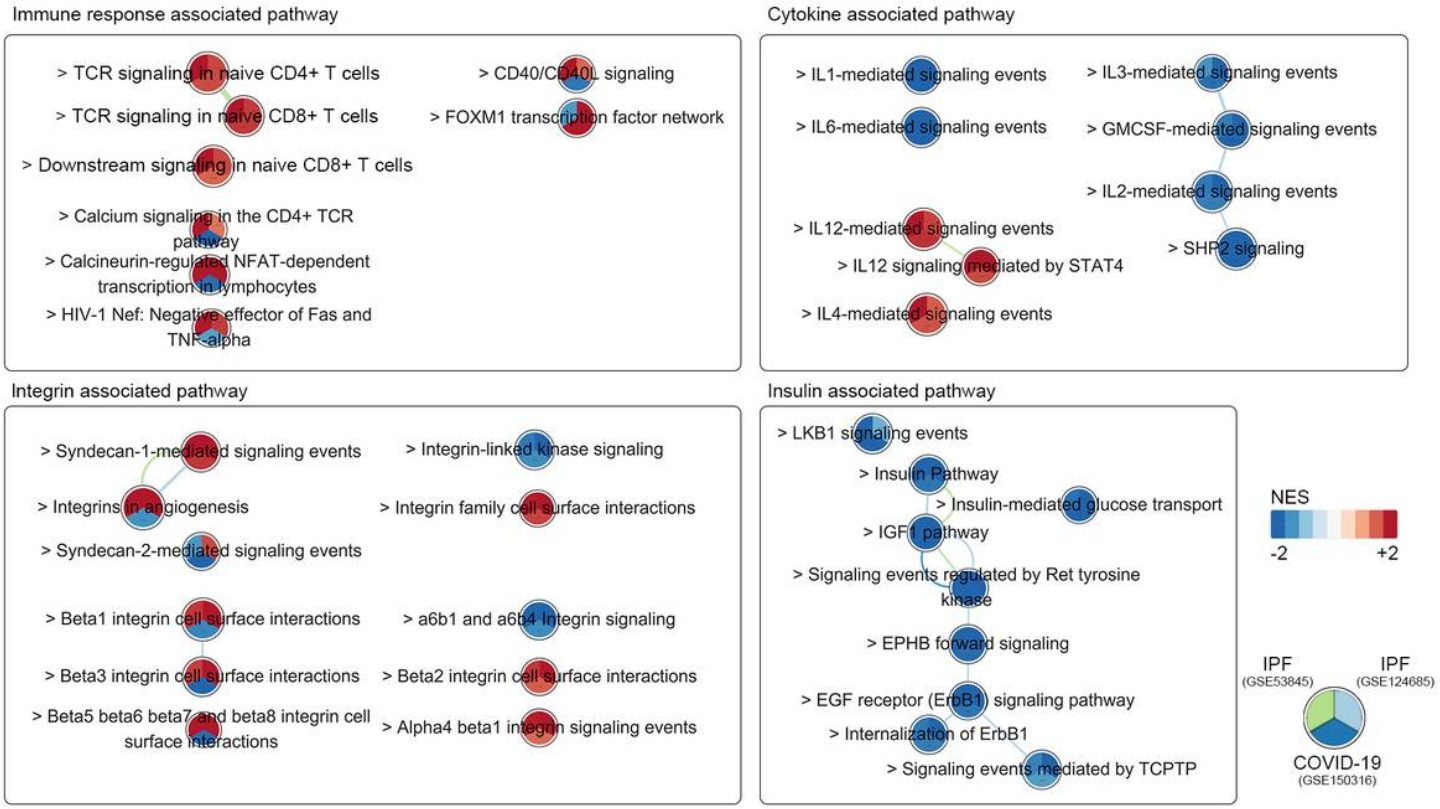
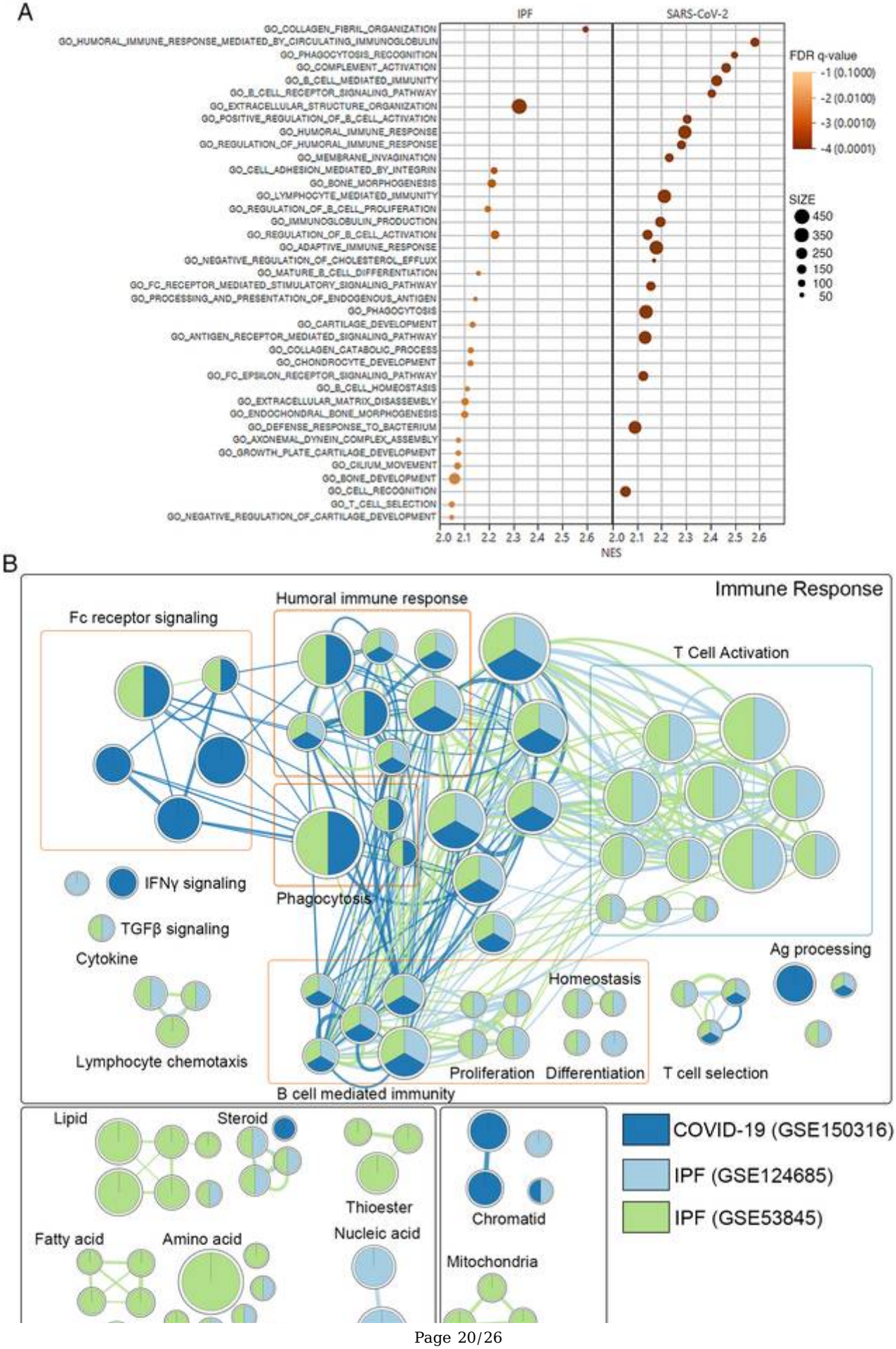


Figure 2

PID pathway functional enrichment map summary of GSEA in the lung tissues of COVID-19 and IPF patients. Commonality of positive or negative correlations in the PID pathway gene sets after GSEA assessment using enrichment map visualization in COVID-19 and IPF. Color represents the NES of the gene set in the database as assessed by GSEA. Gene sets that are associated with immune responses or that have a high degree of consistency and overlap in enrichment are listed here.

Figure 5


A
IPF
SARS-CoV-2
GO_COLLAGEN_FIBRIL_ORGANIZATION
GO_HUMORAL_IMMUNE_RESPONSE_MEDIATED_BY_CIRCULATING_IMMUNOGLOBULIN
GO_PHAGOCYTOSIS_RECOGNITION
GO_COMPLEMENT_ACTIVATION
GO_B_CELL_MEDIATED_IMMUNITY
GO_B_CELL_RECEPTOR_SIGNALING_PATHWAY
GO_EXTRACELLULAR_STRUCTURE_ORGANIZATION
GO_POSITIVE_REGULATION_OF_B_CELL_ACTIVATION
GO_HUMORAL_IMMUNE_RESPONSE
GO_REGULATION_OF_HUMORAL_IMMUNE_RESPONSE
GO_MEMBRANE_INVAGINATION
GO_CELL_ADHESION_MEDIATED_BY_INTEGRIN
GO_BONE_MORPHOGENESIS
GO_LYMPHOCYTE_MEDIATED_IMMUNITY
GO_REGULATION_OF_B_CELL_PROLIFERATION
GO_IMMUNOGLOBULIN_PRODUCTION
GO_REGULATION_OF_B_CELL_ACTIVATION
GO_ADAPTIVE_IMMUNE_RESPONSE
GO_NEGATIVE_REGULATION_OF_CHOLESTEROL_EFFLUX
GO_MATURE_B_CELL_DIFFERENTIATION
GO_FC_RECEPTOR_MEDIATED_STIMULATORY_SIGNALING_PATHWAY
GO_PROCESSING_AND_PRESENTATION_OF_ENDOGENOUS_ANTIGEN
GO_PHAGOCYTOSIS
GO_CARTILAGE_DEVELOPMENT
GO_ANTIGEN_RECEPTOR_MEDIATED_SIGNALING_PATHWAY
GO_COLLAGEN_CATABOLIC_PROCESS
GO_CHONDROCYTE_DEVELOPMENT
GO_FC_EPSILON_RECEPTOR_SIGNALING_PATHWAY
GO_B_CELL_HOMEOSTASIS
GO_EXTRACELLULAR_MATRIX_DISASSEMBLY
GO_ENDOCHONDRAL_BONE_MORPHOGENESIS
GO_DEFENSE_RESPONSE_TO_BACTERIUM
GO_AXONEMAL_DYNEIN_COMPLEX_ASSEMBLY
GO_GROWTH_PLATE_CARTILAGE_DEVELOPMENT
GO_CILIUM_MOVEMENT
GO_BONE_DEVELOPMENT
GO_CELL_RECOGNITION
GO_T_CELL_SELECTION
GO_NEGATIVE_REGULATION_OF_CARTILAGE_DEVELOPMENT
2.0 2.1 2.2 2.3 2.4 2.5 2.6
NES
FDR q-value
-1 (0.1000)
-2 (0.0100)
-3 (0.0010)
-4 (0.0001)
SIZE
450
350
250
150
100
50
B
Immune Response
Humoral immune response
Fc receptor signaling
T Cell Activation
IFNγ signaling
TGFβ signaling
Cytokine
Phagocytosis
Ag processing
Homeostasis
Lymphocyte chemotaxis
Proliferation
Differentiation
T cell selection
B cell mediated immunity
Lipid
Steroid
Thioester
Chromatid
Fatty acid
Amino acid
Nucleic acid
Mitochondria
COVID-19 (GSE150316)
IPF (GSE124685)
IPF (GSE53845)

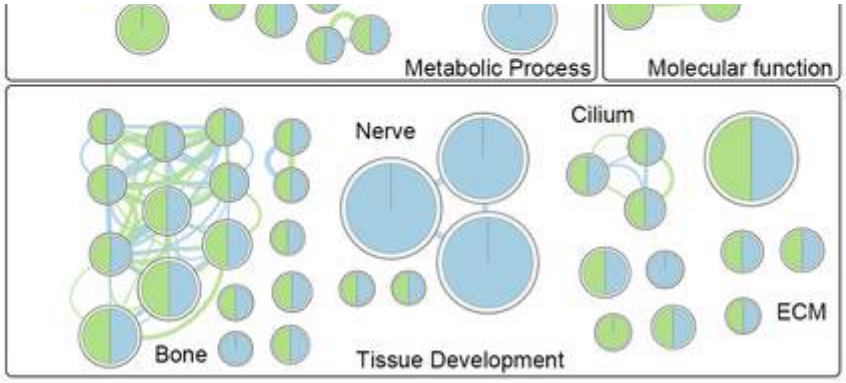


Figure 3

GO:BP functional enrichment map summary of GSEA in the lung tissues of COVID-19 and IPF patients. (A) The top 20 positively enriched results of biological processing by gene ontology (GO) functional enrichment analysis in IPF (GSE124685) or COVID-19 (ranked by FDR). The normalized enrichment score (NES) was presented in the abscissa, and the names of enriched functional gene sets were shown in the ordinate. The color gradation on the right side indicates false discovery rate (FDR). Size represents the enriched gene set size. (B) Commonality of positive correlations in the GO:BP gene sets after GSEA assessment using enrichment map visualization in COVID-19 (GSE150316, dark blue) and IPF (GSE124685, light blue; GSE53845, light green). Single color: the node is positively correlated in only one database; halved: the node is positively correlated in two databases; equally divided: the node is correlated in all databases. Lines connecting nodes represent the degree of overlapping between two gene sets. Criteria for significance screening: P-value <0.05, FDR <0.25.

Figure 4

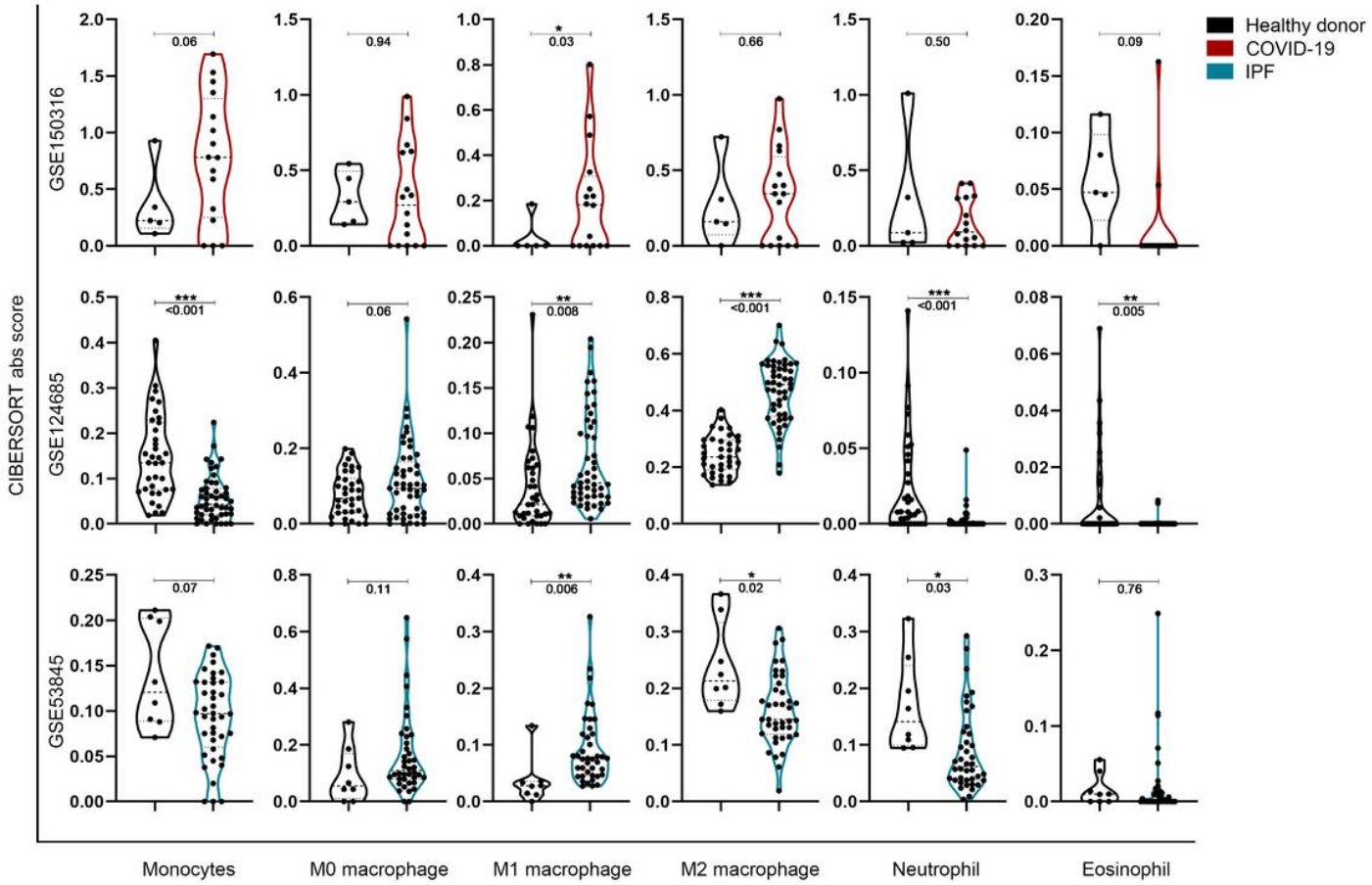


Figure 4

In silico simulated myeloblast lineage infiltration in lung tissue between COVID-19 and IPF patients. *P<0.05, **P<0.01 and ***P<0.001, with comparisons indicated by brackets

Figure 3

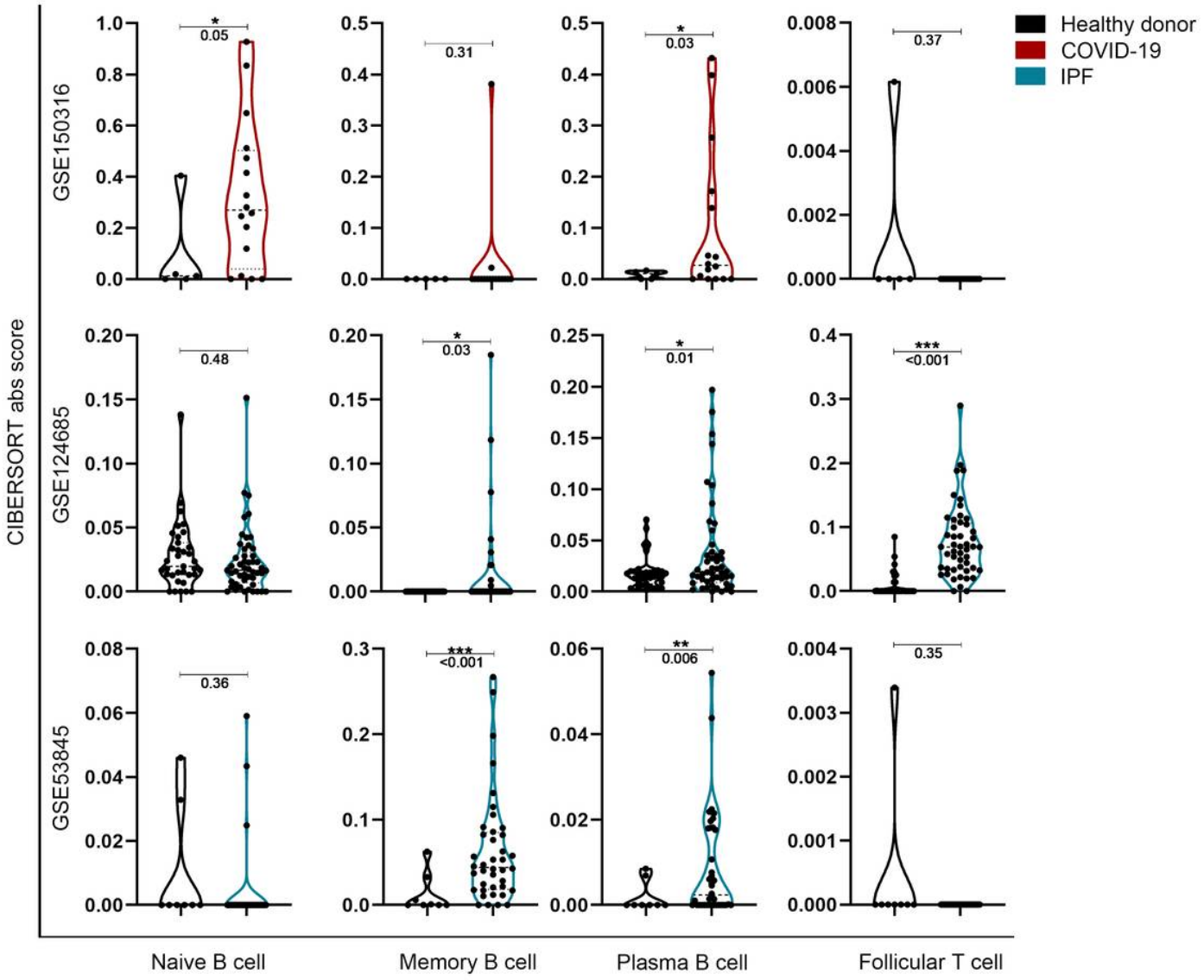


Figure 5

In silico simulated B-cell lineage infiltration in lung tissue between COVID-19 and IPF patients. *P<0.05, **P<0.01 and ***P<0.001, with comparisons indicated by brackets

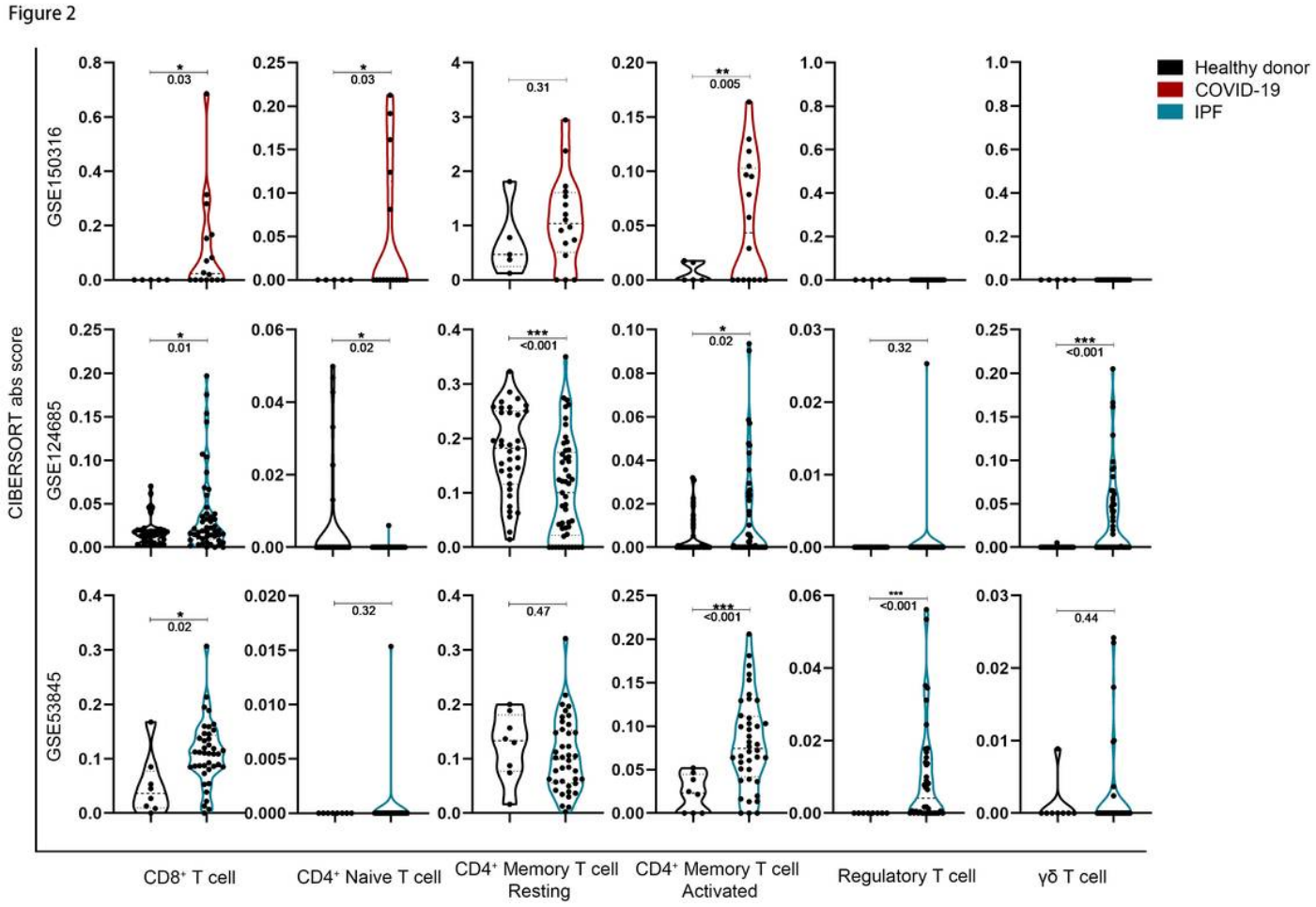
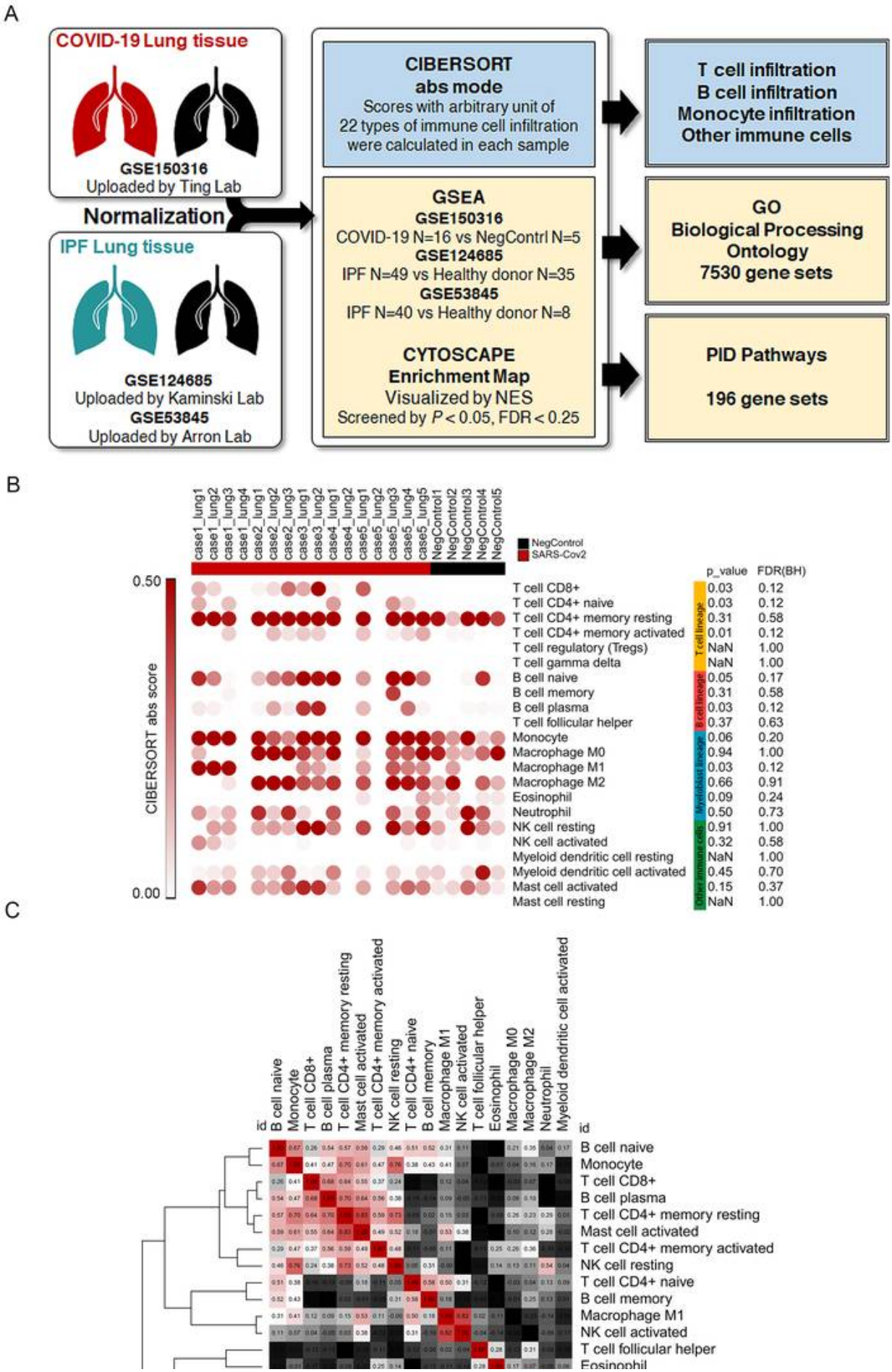


Figure 6

In silico simulated T-cell lineage infiltration in lung tissue between COVID-19 and IPF patients. *P<0.05, **P<0.01 and ***P<0.001, with comparisons indicated by brackets.

Figure 1


A
COVID-19 Lung tissue
GSE150316
Uploaded by Ting Lab
Normalization
IPF Lung tissue
GSE124685
Uploaded by Kaminski Lab
GSE53845
Uploaded by Arron Lab
CIBERSORT
abs mode
Scores with arbitrary unit of
22 types of immune cell infiltration
were calculated in each sample
GSEA
GSE150316
COVID-19 N=16 vs NegContrl N=5
GSE124685
IPF N=49 vs Healthy donor N=35
GSE53845
IPF N=40 vs Healthy donor N=8
CYTOSCAPE
Enrichment Map
Visualized by NES
Screened by P < 0.05, FDR < 0.25
T cell infiltration
B cell infiltration
Monocyte infiltration
Other immune cells
GO
Biological Processing
Ontology
7530 gene sets
PID Pathways
196 gene sets
B
NegControl
SARS-Cov2
CIBERSORT abs score
0.50
0.00
p_value
FDR(BH)
T cell lineage
B cell lineage
Myeloid/stem lineage
Other immune cells
T cell CD8+ 0.03 0.12
T cell CD4+ naive 0.03 0.12
T cell CD4+ memory resting 0.31 0.58
T cell CD4+ memory activated 0.01 0.12
T cell regulatory (Tregs) NaN 1.00
T cell gamma delta NaN 1.00
B cell naive 0.05 0.17
B cell memory 0.31 0.58
B cell plasma 0.03 0.12
T cell follicular helper 0.37 0.63
Monocyte 0.06 0.20
Macrophage M0 0.94 1.00
Macrophage M1 0.03 0.12
Macrophage M2 0.66 0.91
Eosinophil 0.09 0.24
Neutrophil 0.50 0.73
NK cell resting 0.91 1.00
NK cell activated 0.32 0.58
Myeloid dendritic cell resting NaN 1.00
Myeloid dendritic cell activated 0.45 0.70
Mast cell activated 0.15 0.37
Mast cell resting NaN 1.00
C
id
B cell naive
Monocyte
T cell CD8+
B cell plasma
T cell CD4+ memory resting
Mast cell activated
T cell CD4+ memory activated
NK cell resting
T cell CD4+ naive
B cell memory
Macrophage M1
NK cell activated
T cell follicular helper
Eosinophil
Macrophage M0
Macrophage M2
Neutrophil
Myeloid dendritic cell activated

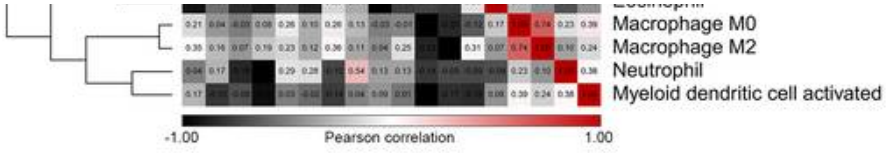


Figure 7

Overall procedure of the study and infiltration of 22 types of immune cells in the lungs of COVID-19 patients. (A) Diagram showing the comprehensive procedure of the study; (B) CIBERSORT abs score of 22 immune cells, colors represent the classification of the following: T cell lineage, B cell lineage, Myeloblast lineage, and other immune cells; (C) Similarity matrix representing the correlation among 22 immune cells in GSE150316.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure.pdf