Impact of COVID-19 Severity on Lung Tissue-Resident Memory T Cells

Junhun Kwon

Introduction

In 2020, the emergence of SARS-CoV-2 triggered a global pandemic through its respiratory failure, systemic inflammation, and hypoxia, resulting in widespread infection and mortality. Since then, research has increasingly centered on immune cells such as T lymphocytes and macrophages, due to their roles in antiviral defense. Within this context, great attention has been drawn towards lung tissue-resident memory T cells due to their ability to persist in lung tissue after infection and mount rapid responses upon reinfection [14]. Unlike circulating T cells, TRM cells have a distinct niche in the respiratory system, balancing protective immunity with the risk of inflammatory injury during severe disease [3].

This study examined whether SARS-CoV-2 severity alters the abundance and transcriptional programs of lung TRMs isolated from human lung tissue. To address the question, we analyzed publicly available single-cell RNA sequencing data from the Human Cell Atlas. We hypothesized that in patients with severe COVID-19, TRMs would show altered frequencies and increased expression of genes linked to inflammation, exhaustion, and tissue repair compared with patients with mild or no disease.

By focusing on TRMs, the study aims to clarify how severe SARS-CoV-2 infection reshapes local immune memory in the lung, with implications for long-term immunity and tissue pathology. Tissue-resident memory T cells, defined by markers such as CD69 and CD103, remain localized in tissues where they provide rapid pathogen surveillance [4;5;6]. In the lung, they act as a first line of defense against respiratory viruses, but their persistence in inflamed tissue can also intensify disease [7;8]. Altered TRM abundance and transcriptional signatures have been associated with dysfunctional antiviral responses, cytokine-driven inflammation, and impaired resolution of infection in severe COVID-19 [9;10;11]. Because these cells sit at the interface of protection and pathology, examining their transcriptomic profiles offers critical insight into COVID-19 pathophysiology.

Methods

Single-cell RNA sequencing datasets derived from lung tissue samples of COVID-19 patients were retrieved from the Human Cell Atlas using the DCP/Azul API (HCA Project ID: **08fb10df-32e5-456c-9882-e33fcd49077a**; accessible at https://explore.data.humancellatlas.org/projects/08fb10df-32e5-456c-9882-e33fcd49077a; Liao et al., *Nat Med*, 2020). The data, provided in .h5 format, included gene expression matrices for multiple donors alongside metadata specifying clinical severity.

Raw files were imported into Python with the Scanpy function sc.read_10x_h5, and datasets from individual donors were merged into a unified AnnData object (adata_combined). Cells with fewer than 200 detected genes, more than 6000 genes, or over 5% mitochondrial gene content were excluded. Expression values were normalized to 10,000 counts per cell and log-transformed to stabilize variance.

Cell type classification employed CellTypist, a machine-learning tool trained on annotated single-cell datasets. The “Immune_All_Low.pkl” model was applied to the integrated dataset, with predicted cell types stored in the .obs['cell_type'] field. For downstream analysis, the dataset was restricted to tissue-resident memory T cells.

To explore transcriptional heterogeneity, principal component analysis was followed by Uniform Manifold Approximation and Projection for visualization. A k-nearest neighbor graph, based on the top 30 principal components, was constructed, and Leiden clustering identified transcriptionally distinct populations.

According to donor metadata, cells within the TRM subset were stratified by COVID-19 severity (mild, moderate, severe). Differential expression analysis was conducted with sc.tl.rank_genes_groups, using a t-test with multiple testing correction.

Results

Following data preprocessing and comprehensive immune cell annotation using Celltypist, we identified and characterized lung TRM T-cells (**Fig. 1A**). Initially, annotations revealed significant immune populations within the lung, including CD4 and CD8 T-cells, natural killer cells, macrophages, and dendritic cells (**Fig. 1B**). Subsetting of the data to T-cells and Leiden clustering revealed transcriptionally distinct subpopulations (**Fig. 1C**). Canonical TRM markers, specifically CD69, ITGA1 (CD49a), CXCR6, and ZNF683, were strongly expressed within the subpopulations. In particular, clusters 3 and 19 showed high expression of multiple TRM markers, leading to their designation as TRM candidate clusters. The expression of PDCD1 also helped to confirm that these clusters were TRMs.

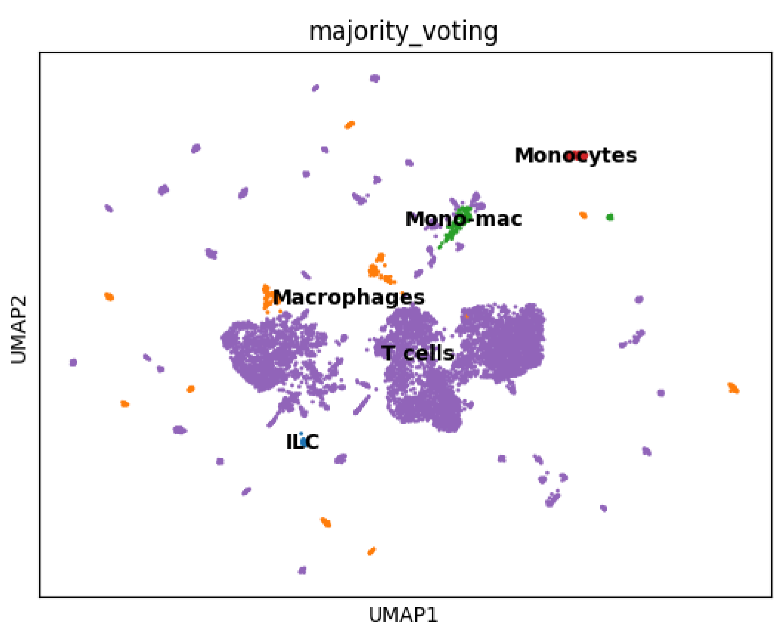

Figure 1A. UMAP of immune cell populations in lung tissue. UMAP projection of all annotated immune cell types in lung tissue, including T cells, macrophages, and Monocytes.

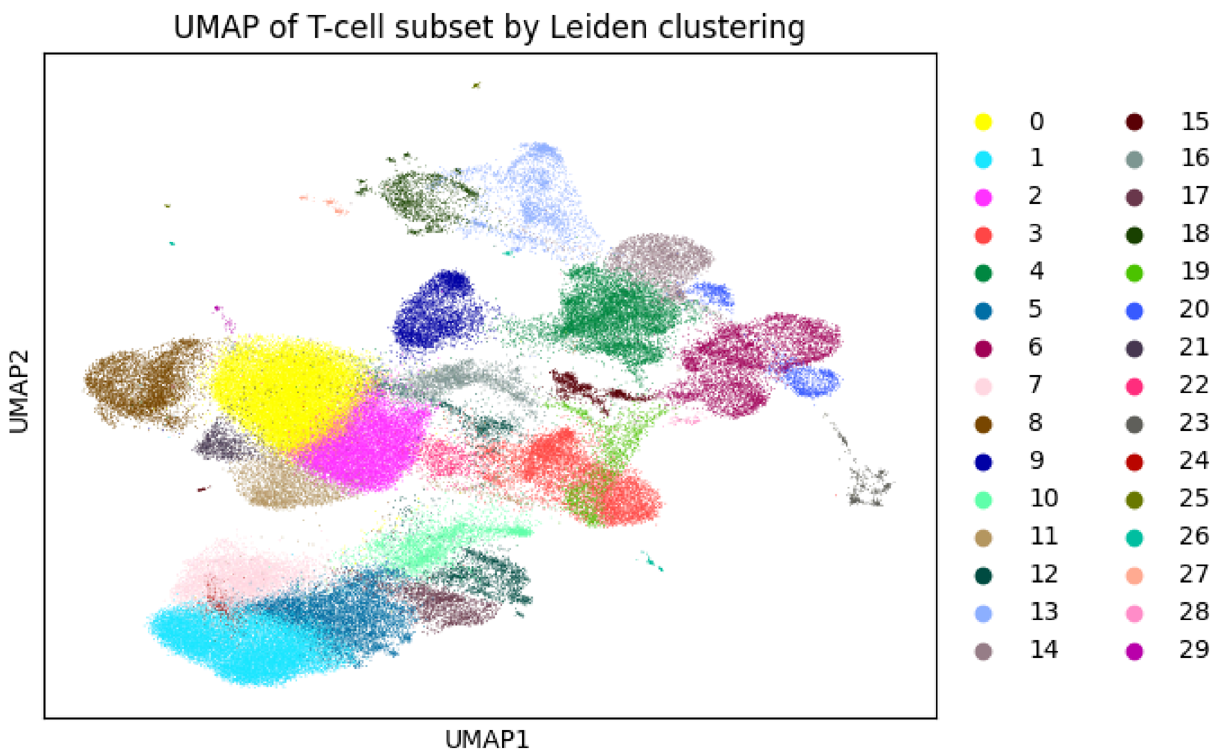

Figure 1B. UMAP of T-cell subset by Leiden clustering. UMAP of T-cell subset showing transcriptionally distinct subclusters following Leiden clustering.

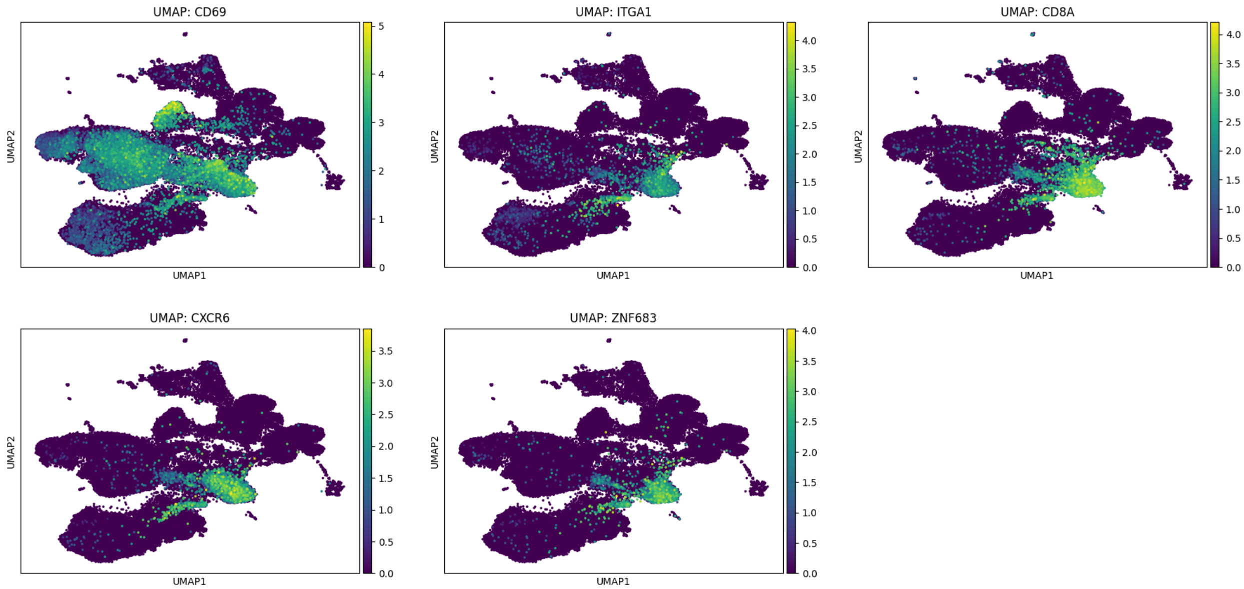

Figure 1C. Expressions of canonical TRM markers across T-cell clusters. Expression of canonical TRM markers (CD69, ITGA1, CD8A, CXR6, ZNF683)

Next, we compared TRM cell abundance across non-, moderate-, and severe-COVID lung samples (**Fig. 2A**). Through this comparison, we found that non-COVID lungs had the lowest percentage of TRMs, followed by moderate, with the largest belonging to the severe lungs. Using Welch's t-test and Mann-Whitney U tests, this difference was tested to be significant ($p < 0.05$; **Fig. 2B**).

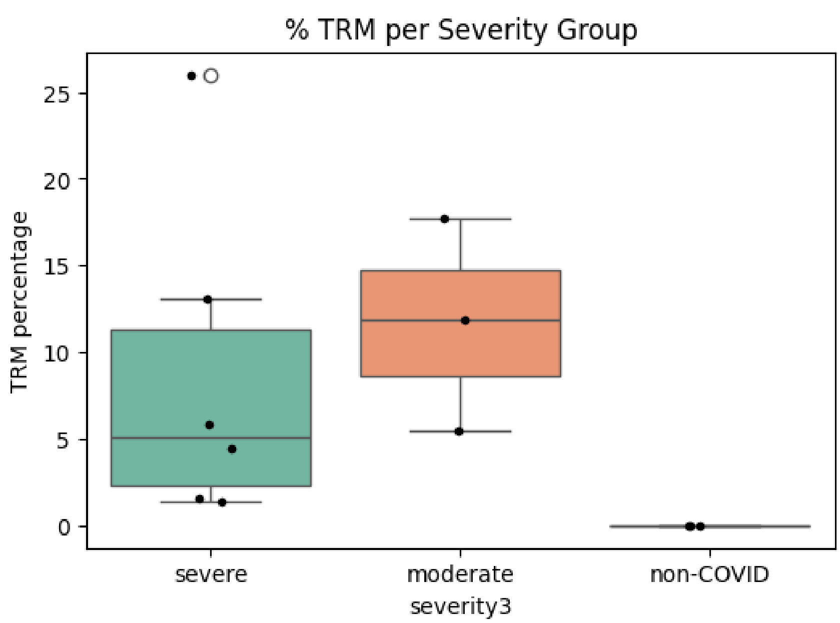

Figure 2A. Proportion of TRM cells by COVID-19 severity. Proportion of TRM cells among total T cells in non, moderate, and severe-COVID lung samples.

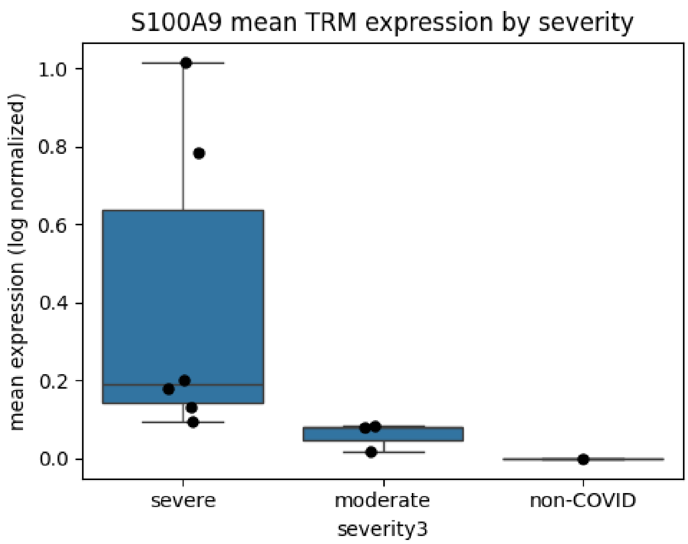

Figure 2B. S100A9 means TRM expression by severity. Mean expression of S100A9 in TRM cells by COVID-19 severity

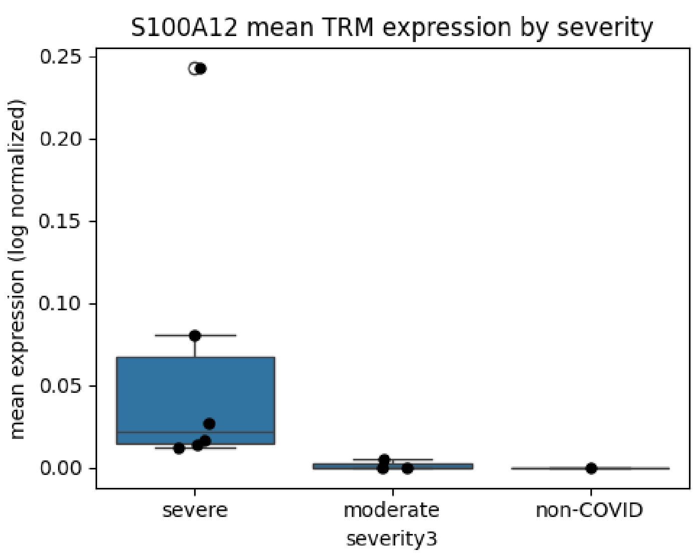

Figure 2C. S100A12 means TRM expression by severity. Mean expression of S100A12 in TRM cells by COVID-19 severity

To further understand the molecular impact of COVID-19 severity on TRM cells, differential gene expression analysis was performed within the identified TRM populations (Fig. 3A). As the non-COVID group was too small for reliable comparisons, we focused on the severe vs moderate. Through the comparisons, we found that severe TRMs upregulated inflammatory genes, such as CCL2, CCL8, CCL3L1, S100A8, S100A9, and S100A12. The upregulation of S100A9 and S100A12 in severe COVID-19 patients suggests an inflammatory TRM phenotype (**Fig. 2B and 2C**). Severe TRMs also had higher PDCD1, suggesting they were activated and exhausted.

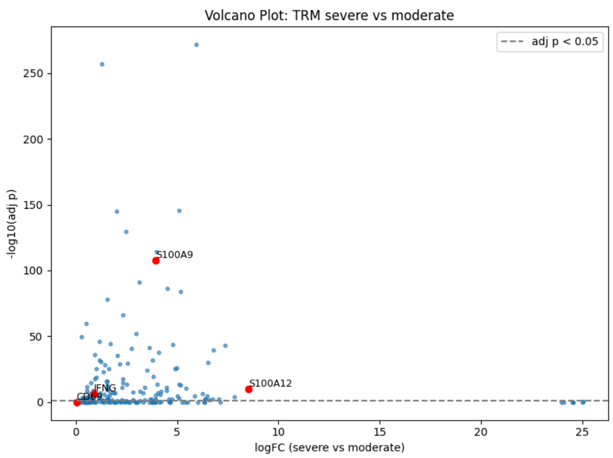

Figure 3A. Differentially expressed genes between moderate and severe TRMS. Volcano plot showing genes differentially expressed between moderate and severe TRM cells

Pathway enrichment analysis was performed on the marker genes for the identified TRM clusters (specifically clusters 3 and 19) to infer their associated biological functions. The Enrichr analysis of cluster 3 markers revealed enrichment in pathways related to T cell activation, cytokine signaling, and response to viruses. For cluster 19, enriched terms included pathways associated with mitochondrial function, oxidative phosphorylation, and metabolic processes. These findings suggest distinct functional roles for these TRM subpopulations in the context of COVID-19.

In summary, the results show that TRM cells represent a distinct portion of the T cell subpopulation in the lung. This study shows that COVID-19 severity is associated with altered abundance and gene expression profiles of lung tissue-resident memory T cells. A significantly higher percentage of TRM cells was observed in severe and moderate COVID-19 cases compared to non-COVID individuals. Furthermore, TRM cells from severe cases exhibit distinct gene expression patterns compared to moderate cases, including the upregulation of genes involved in inflammation and immune modulation. These findings collectively suggest that lung TRM cells may play a dynamic and severity-dependent role in the immune response to COVID-19.

Discussion

The study's results show important information for the understanding of the association of the severity of COVID-19 and lung tissue-resident memory T cells. Our analysis identified two key findings: severity-dependent TRM cell abundance change and disease severity-correlated TRM cell gene expression profiles with characteristic signatures. We initially noticed an increased frequency of TRM cells in the lung tissues of moderate to severe COVID-19 patients compared to non-COVID controls. This increase in the TRM fraction following infection with SARS-CoV-2 shows that the lung recruits TRMS during infection, especially in severe phases. We also noted there was variability in TRM proportions among patients, particularly between severe COVID-19 and non-COVID cases. This variance suggests that TRMs have a role centered on antiviral defense. The increase in TRMS may help protect the lungs, as these cells can both destroy viruses and monitor local tissues for infection, as previous findings have shown that TRM expansion occurs during influenza and other lung infections [14;13;7]. On the other hand, excessive TRM activation could cause immunopathology, which can indicate severe COVID-19. We advise caution in interpreting these observations due to the small sample size of non-COVID patients, and larger cohort studies in the future may justify these in terms of cell number.

Additionally, our gene expression analysis profiles of the TRM cells showed significant differences between the moderate and severe COVID-19 patients. Recognition of differentially expressed genes, such as upregulated CCL2, S100A8, S100A9, CCL8, and CCL3L1 in severe TRM cells, is consistent with an activated and potentially inflammatory state. S100A8 and S100A9 are established alarmins that encode calprotectin—an alarmin complex associated with host defense and inflammation—and are frequently upregulated in life-threatening inflammatory states, such as COVID-19 [16;17]. The presence and upregulation of these markers in TRMs suggest that the cells may be contributing to or indicative of heightened inflammation in severely infected COVID-19 lungs.

Likewise, upregulated expression of chemokines such as CCL2, CCL8, and CCL3L1 suggests TRM may be recruiting other immune cells into the inflamed lung tissue during inflammation [14;15]. Recognizing the upregulated PDCD1 expression in certain TRM subsets, we think that the host's T cell compartment is in a state of activation and potential exhaustion, and that this could inhibit their effector functions in the setting of severe disease [18;19].

Further analysis of the pathway enrichment regarding the identified tissue-resident memory (TRM) clusters 3 and 19 offers additional context regarding these molecular modifications. This identified enrichment in cytokine-cytokine receptor pathway-associated interactions, chemokine signaling, and neutrophil degranulation further reinforces the hypothesis that TRM cells in patients with severe COVID-19 exhibit a markedly activated and pro-inflammatory phenotype. In contrast, pathways relevant to tissue repair and the assembly of cytotoxic granules were found to be differentially regulated, indicating a deviation from traditional tissue-protective and cytotoxic functions towards the facilitation of inflammatory processes.

All together, these observations imply that lung TRM cells function as active contributors to the immune response, and that disease severity during the course of COVID-19 influences their functional status.

Although this work provides significant insight into key correlations between the severity of COVID-19 and TRM attributes, we also note certain limitations. First, the sample size is relatively small, specifically for the non-COVID group, and thus limited broad-scope, three-group comparison statistics, notably for gene expression studies. Also, due to the cross-sectional nature of these data, it is not possible to make inferences toward causality or the temporal nature of these changes. Longitudinal follow-ups, in larger groups, coupled with functional rather than PCR assay analysis, would, in the future, serve to confirm these observations, explore the particular effector functions of these TRM populations, and establish their exact role in protection versus pathology in COVID-19.

Conclusion

This study demonstrates that COVID-19 severity significantly influences both the abundance and the gene expression profiles of lung tissue-resident memory T cells. There was a greater percentage of TRM cells in the lungs of individuals with moderate to severe COVID-19 than in non-COVID individuals, which suggests an increase in these cells during infection. Additionally, TRM cells from severe cases showed distinct gene expression patterns compared to moderate cases. These were characterized by the upregulation of genes associated with inflammation and immune cell recruitment, such as CCL2, S100A8, S100A9, CCL8, and CCL3L1. These molecular alterations, alongside enriched functional pathways, indicate a dynamic shift in the functional state of lung TRM cells in response to increasing disease severity. These findings hilight the critical and severity-dependent role of lung TRM cells in shaping the local immune landscape during COVID-19. Further research is warranted to fully elucidate the functional consequences of these changes and their implications for host protection and disease pathogenesis.

Works Cited

[1] Szabo, P. A., et al. (2019). *Tissue-resident memory T cells in human health and disease.* Nature Reviews Immunology, 19, 682–697.

[2] Zheng, M. Z. M., et al. (2022). *Tissue resident memory T cells in the respiratory tract.* Mucosal Immunology, 15, 94–104.

[3] Parga-Vidal, L., et al. (2021). *Hobit identifies tissue-resident memory T cell precursors.* Science Immunology, 6(63), eabg5667.

[4] Kumar, B. V., Connors, T. J., & Farber, D. L. (2017). *Human tissue-resident memory T cells are defined by core transcriptional and phenotypic features.* Cell Reports, 20(12), 2921–2934.

[5] Kumar, B. V., Ma, W., Miron, M., et al. (2017). *Functional heterogeneity of human tissue-resident memory T cells.* JCI Insight, 2(1), e93401.

[6] Reilly, E. C., et al. (2021). *CD49a identifies polyfunctional memory CD8 T cells in the human airway.* PNAS, 118(1), e2025458118.

[7] Grant, R. A., et al. (2021). *Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia.* Nature Immunology, 22, 1229–1240.

[8] Tse, S. W., et al. (2019). *CXCR6 regulates the localization of tissue-resident memory CD8 T cells to airway compartments.* Journal of Experimental Medicine, 216(12), 2748–2762.

[9] Liao, M., et al. (2020). *Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19.* Nature Medicine, 26, 842–844.

[10] Wang, Z., et al. (2019). *PD-1hi CD8+ resident memory T cells balance immunity and fibrosis in the human lung.* Science Immunology, 4(36), eaaw4892.

[11] Grant, R. A., et al. (2021). *Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia.* Nature Immunology, 22, 1229–1240.

[12] Snyder, M. E., et al. (2019). *Generation and persistence of human tissue-resident memory T cells in lung transplantation.* Immunity, 51(4), 617–629.e5.

[13] Szabo, P. A., et al. (2019). *Tissue-resident memory T cells in human health and disease.* Nature Reviews Immunology, 19, 682–697.

[14] Deshmane, S. L., et al. (2009). *Monocyte chemoattractant protein-1 (MCP-1): An overview.* Journal of Interferon & Cytokine Research, 29(6), 313–326.

[15] Melief, S. M., et al. (2022). *CXCL chemokines shape immunity and immunotherapy in cancer.* Nature Immunology, 23, 1324–1336.

[16] Silvin, A., et al. (2020). *Elevated calprotectin and abnormal myeloid cell subsets discriminate severe from mild COVID-19.* Cell, 182(6), 1401–1418.e18.

[17] Guo, Q., et al. (2021). *Induction of alarmin S100A8/A9 mediates activation of aberrant neutrophils in the pathogenesis of COVID-19.* Signal Transduction and Targeted Therapy, 6, 342.

[18] Wherry, E. J., & Kurachi, M. (2015). *Molecular and cellular insights into T cell exhaustion.* Nature Reviews Immunology, 15, 486–499.

[19] De Biasi, S., et al. (2020). *Marked T cell activation, senescence, exhaustion, and skewing towards TH17 in patients with COVID-19 pneumonia.* Nature Communications, 11, 3434.