

🔗 Cystic Fibrosis Inflammation: Hyperinflammatory, Hypoinflammatory, or Both?

A major focus of cystic fibrosis (CF) research has been on CF transmembrane conductance regulator (CFTR) ion channel dysfunction in epithelial cells and its impact on dehydration of airway surface liquid, mucus deposition, and impaired mucociliary clearance (1–4). The resultant airway obstruction contributes to chronic infections by opportunistic bacteria such as *Pseudomonas aeruginosa* (5). In patients with CF, airway infection is accompanied by exaggerated inflammation, with evidence of an excess of proinflammatory cytokines, and observed alterations in innate and adaptive immune responses (6–8). The pathophysiology of CF inflammation remains poorly understood. Intrinsic effects of mutant CFTR on the immune system have been described for neutrophils (9), macrophages (10), lymphocytes (11), and dendritic cells (12). For example, alveolar macrophages from CFTR^{-/-} mice exhibit exaggerated inflammatory responses to bacterial LPS (13), and inhibition or mutation of CFTR enhances production of cytokines in both murine and human macrophages (14, 15). However, other studies have indicated that CF airway macrophages in primary culture are hyperinflammatory, a phenotype that is independent of defective CFTR and results from exposure to the CF airway infectious/inflammatory environment (16). Nevertheless, it is currently unclear whether extrinsic signals have a role in the regulation of the CF immune response in peripheral blood. As described in this issue of the *Journal*, Zhang and colleagues (pp. 301–311) developed a novel transcriptomic profiling-based immune cell analysis to test the hypothesis that the response of peripheral immune cells from patients with CF involves an extrinsic regulatory mechanism (17).

To evaluate the effect of extrinsic factors on the pathophysiology of the CF immune response, the authors analyzed gene expression and microRNA (miRNA)–mRNA networks, and performed blood transcriptomics to distinguish immune cell subsets in healthy peripheral blood mononuclear cells (PBMCs) exposed to either autologous plasma or plasma derived from the peripheral blood of patients with CF. Samples from a discovery cohort of 12 patients with CF and 12 healthy control subjects, as well as a validation cohort of 103 patients with CF and 31 healthy control subjects, were analyzed. The authors identified a significant downregulation of immune-related genes in PBMCs in response to CF plasma, including genes involved in immune cell functions such as cell binding and cell adhesion, and transcripts encoding immune receptors, cytokine receptors, and inflammatory mediators. These transcripts were related to key inflammatory signaling pathways, including the TREM1 (triggering receptor expressed on myeloid cells 1), IL-6, and IL-17F pathways, and pathways involved in cellular recognition of bacteria and viruses.

Next, Zhang and colleagues sought to determine whether the transcriptional effects of CF plasma on PBMCs could be explained

by shifts in immune cell subsets in their PMBC samples. For this purpose, they used a gene-based computational approach, which uses a novel marker gene matrix, to systematically infer the composition of 10 immune cell subsets. These included five lymphoid lineage subsets (total T cells, CD8 T cells, CD4 T cells, B cells, and natural killer cells) and five myeloid lineage subsets (monocytes, macrophages, M2 macrophages, general dendritic cells, and activated dendritic cells). This novel application of transcriptomics bioinformatics to distinguish immune cell subsets provides a new approach to evaluate the diversity of immune cell samples when traditional methods, such as flow cytometry, are unworkable due to low overall cell numbers, large sample sets, or a limited repertoire of phenotypic markers. It also provides an opportunity to conduct further analyses and comparisons of historical transcriptome data sets. Using this approach, Zhang and colleagues found that CF plasma exposure resulted in significantly higher percentages of lymphoid cell subsets and significantly lower percentages of myeloid cell subsets. This finding was confirmed by immunomagnetic separation and counting of CD14⁺CD16⁻ monocytes.

Zhang and colleagues then evaluated cell composition scores in PBMCs from a cohort of patients with CF in comparison with PBMCs from their mothers (who had only one copy of the CFTR mutation). The PBMCs from patients with CF exhibited a significantly lower percentage of macrophages than the PBMCs from their mothers. The authors then explored the clinical implications of the CF plasma–induced immune response alterations in PBMCs by comparing the cell composition scores of PBMCs exposed to plasma from *P. aeruginosa*–positive patients with CF and *P. aeruginosa*–negative patients with CF. Using both the gene expression–based computational approach and the traditional cell sorting method, they found that a significant reduction in the monocyte subset correlated with *P. aeruginosa* infection. These findings support previous evidence that *P. aeruginosa* produces virulence factors that inhibit macrophage recruitment (18).

Recent studies have proposed that miRNAs may modulate CF disease progression by affecting the production of either CFTR or various proteins that are dysregulated in the CF airway (19). Therefore, Zhang and colleagues hypothesized that altered miRNA–mRNA associations could explain their findings. The authors identified 44 miRNAs as differentially expressed between PBMCs exposed to autologous plasma or CF plasma. Two miRNA regulators (miR-155 and miR-146a0) connected 11 out of 12 mRNA targets and were increased in response to CF plasma. These miRNAs target a broad range of immune-related genes, including promoting the expression of IL-8 (20), IL-1 β , and TNF- α , and are associated with a proinflammatory status (21). The authors conclude that CF plasma appears to extrinsically regulate these miRNAs in PBMCs.

Zhang and colleagues conclude that the “CF plasma-induced immune response may not contribute to the excessive lung inflammation” and “the secretion of proinflammatory factors in the CF lung is more likely due to the intrinsic CFTR defect.” It will be interesting to evaluate in future studies whether the blunted innate immune response described by Zhang and colleagues also occurs in PBMCs that have been freshly isolated from healthy subjects and exposed to CF plasma, and whether it mimics the immune response of freshly isolated CF PBMCs. This approach would enhance our understanding of the pathophysiology of CF inflammation and the contribution of extrinsic factors present in CF peripheral blood. Another interesting question is whether immune cells from patients with CF being treated with CFTR modulators or correctors such as lumacaftor and ivacaftor behave similarly to healthy immune cells, especially given the potential for these patients to experience chronic lung disease. These types of experiments could further decouple the intrinsic and extrinsic factors that influence inflammation in CF, and provide new opportunities to develop therapies for these patients. ■

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