Single-cell landscape of bronchoalveolar immune cells in COVID-19 patients

Journal Article, Retrospective study

May12, 2020

Liao M, Liu Y, Yuan J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19.Nature Medicine, 2020 May 12. doi: 10.1038/s41591-020-0901-9.

Summary

Methods:

- 13 patients (3 moderate, 2 severe, 8 critical) with COVID-19,at Shenzhen Third People's Hospital, January to February 2020.
- Disease severity was defined as moderate, severe and critical, according to the 'Diagnosis and Treatment Protocol of COVID-19 (the 7th Tentative Version) by the National Health Commission of China issued on 3 March 2020.
- Enrolled 3 patients with moderate infection (fever, respiratory symptoms and pneumonia evidenced by computed tomography [CT] imaging).
- Diagnosis of severe infection was made if one of the followingcriteria were present: (i) respiratory distress with respiratory rate ≥30 times per minute; (ii)fingertip oxygen saturation ≤93% at rest; (iii) ratio of partial pressureof arterial oxygen to fraction of inspired oxygen (PaO2/FiO2) ≤300 mmHg(1 mmHg=0.133 kPa); and (iv) obvious progression of lesions in 24–48 hours shownby pulmonary imaging >50% increase in size of lesions (via CT scans).
- Diagnosis of critical infection was based on one of the following criteria: (i) respiratory failure and an artificial airway required for invasive mechanical ventilation; (ii) shock; and (iii) combined failure of other organs that required intensive care unit monitoring. All8 patients with critical infection received invasive mechanical ventilation.
- The median age of the patients was 57 years, and the participants included both male and female patients.
- Robust and exhaustive single-cell RNA sequencing (scRNA-seq) andcytokine analysis was performed on bronchoalveolar lavage fluid (BALF) cells isolated from 3 patients with moderate, 6 with severe/critical infection, 3healthy controls and a publicly available BALFsample.
- Differentially expressed gene (DEG) analysis, gene ontology (GO) analysis and gene set enrichment analysis (GSEA) was further performed between various cell groups.

Results:

- Clustering analysis showed 31 distinct clusters composed of macrophages (CD68), neutrophils (FCGR3B), myeloid dendritic cells (mDCs) (CD1C, CLEC9A), plasmacytoid dendritic cells (pDCs) (LILRA4), natural killer (NK) cells (KLRD1), T cells (CD3D), B cells (MS4A1), plasma cells (IGHG4) and epithelial cells (TPPP3, KRT18), identified by their respective signature genes.
- Major cell types, including mDCs, pDCs, mast cells, NK cells, T cells and B cells, were observedin most samples, whereas macrophages showed specific enrichment in different groups.
- BALFs of patients with severe/critical COVID-19 infection contained higher proportions of macrophages and neutrophils and lower proportions of mDCs, pDCs and T cells than those with moderate infection.
- A highly proinflammatory macrophage microenvironment was observed in the lungs of patients with severe COVID-19, which is consistent with previous knowledge of macrophage populations during steady-state, inflammation and recovery.
- Six major clusters of T and NK lymphocytes were observed based on the expression of canonical genes.
- Lower CD8+ T cell and higher proliferating T cell proportions were observed in patients with severe/critical infection than in patients with moderate infection.
- CD8+ T cells in BALFs from patients with severe/critical infection were less expanded, more proliferative and more phenotypically heterogeneous, whereas a larger proportion of CD8+ T cell effectors with tissue-resident and highly expanded features were present in BALFs from patients with moderate infection.
- Compared to patients with moderate COVID-19 infection, patients with severe/critical infection had much

higher levels of inflammatory cytokines, particularly interleukin (IL)-8, IL-6 and IL-1β, in their BALFs.

Conclusion:

- The study characterized BALF immune cells from patients with varying severity of COVID-19 and from healthy people by using single-cell RNA sequencing.
- Proinflammatory monocyte-derived macrophages were abundant in the BALF from patients with severe COVID-9. Moderate cases were characterized by the presence of highly clonally expanded CD8+ T cells.
- These data suggest that lung macrophages in patients with severe COVID-19 infection may contribute to local inflammation by recruiting inflammatory monocytic cells and neutrophils though CCR1 and CXCR2, whereas macrophages in patients with moderate COVID-19 infection produce more T cell-attracting chemokines through engaging CXCR3 and CXCR6, thereby re-establishingthe fact that cytokine storm is associated with disease severity in COVID-19.
- This atlas of the bronchoalveolar immune microenvironment suggests potential mechanisms underlying pathogenesis and recovery in COVID-19.

Appraisal

- Only 13 subjects included: will impact significance and validity
- The study has several shortcomings including limited and heterogeneous patients along with a lack of longitudinal sample collected before and after infection. In addition, the B cell response could not be analyzed due to low cell numbers. The effects of age, pre-existing conditions and immunoregulatory therapies could also not be fully assessed.

Opinion

The study explores for the first time the landscape of bronchoalveolar immunecells revealing aberrant macrophage and T cell responses underlyingimmunopathogenesis in COVID-19 and thus could be a landmark both in the pathogenesis and management of COVID-19.

Appraisers

Rupesh Srivastava, Bhupendra Verma, Sumit Rathore; Department of Biotechnology, AIIMS New Delhi