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| **COVID-19-associated lung injury evolution characterised by significant immune cytometric flux.** |
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| **Introduction/Aim:** Diffuse Alveolar Damage (DAD) is the dominant histological manifestation of severe COVID-19 in the lungs. SARS-CoV-2 can transform tissue from a preserved state to an early exudative phase of DAD (EDAD) and later a proliferative and organising phase (ODAD). Few analyses directly compare immune cell infiltration and interactions between these discrete phases. We aimed to determine the degree of flux in immune cell subset representation and interactions as DAD evolved.  **Methods:** Lung tissue was obtained from patients who died from COVID-19. Pathologist-selected 500µm2 regions of interest (ROIs) were classified as exudative DAD (EDAD) or organising (ODAD) by light microscopy. ROIs free of DAD from SARS-CoV-2-negative donors and positive patients served as controls. ROIs were stained with 40 immune, functional and stromal markers and ablated using imaging mass cytometry. Segmented cells were classified by FlowSOM clustering. Cell populations and spatial relationships were compared between ROI classes to look for characterising cellular signatures.  **Results:** Forty patients (80% male, age 22-98), 345 ROIs and >900k single cells were analysed. DAD progression was marked by progressive and significant increases in mononuclear phagocytes, T and B lymphocytes. With respect to immune cell interactions, the most marked differences were observed between SARS-CoV-2-negative and positive preserved tissue areas. Notably there were increased interactions between neutrophils with alveolar type 2 cells which are the primary lung target of SARS-CoV-2, neutrophils with inflammatory mononuclear phagocytes and CD8+ T lymphocytes with a repair subset of macrophages (CD206HI).  **Conclusion:** The immunopathogenesis of severe DAD in COVID-19 lung disease is characterised by sustained increases in MnPs and lymphocytes. Key innate-stromal and innate-adaptive interactions occur early, prior to overt tissue damage, likely establishing a program of aberrant inflammation and repair which severe COVID19 is known for.  **Grant Support**: UK Research and Innovation/Medical Research Council through the UK Coronavirus Immunology Consortium, Barbour Foundation, General Sir John Monash Foundation, Newcastle University, JGW Patterson Foundation, Wellcome Trust. |