# Exploring mononuclear phagocyte diversity in the human gut with single-cell sequencing and flow cytometry

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Introduction. DC and macrophages make crucial but distinct contributions to the maintenance of homeostasis in the gut, and to the pathogenesis of inflammatory bowel disease. Although DC are specialised to activate the adaptive immune system, and macrophages to clear debris and remodel tissue, the two cell types share a number of functions and surface markers. Because of the phenotypic overlap, it has been historically challenging to separate some MNP subsets from one another.
Methods. We used single-cell sequencing to characterise the HLA-DR+ populations present in the human gut, in both the lamina propria and in isolated lymphoid tissues. Guided by single-cell sequencing data, we developed a panel of antibodies allowing analysis of MNP subsets by flow cytometry.
Results. We identified pDC, cDC1, cDC2, monocytes, macrophages, and other putative monocyte-derived cells, including a monocyte-derived subset with a cDC2-like phenotype, using single-cell sequencing. pDC were found almost exclusively in lymphoid tissue and not in the lamina propria, while macrophages were highly enriched in the lamina propria. Using flow cytometry, we identified subsets of putative monocyte-derived cells which have not been described before in the human gut. Several monocyte-derived subsets were found to be enriched in the colon compared to the small intestine, and some were enriched in the lamina propria of Crohn's disease patients compared to non-inflamed controls.
Conclusion. Characterisation of human gut HLA-DR+ cells by single-cell sequencing and flow cytometry identified a number of distinct MNP subsets, and showed they have unique distributions in health and in disease.

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Keywords : MNP, DC, macrophages, gut, intestine
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