

By CyTOF Heterogeneity of Human Monocytes

P'ng Loke, Timothy B. Niewold

Human monocyte subsets have traditionally been defined based on expression of CD14 and CD16 into classical, nonclassical, and intermediate monocytes. Using CyTOF, Thomas et al¹ identified CCR2, CD36, HLA-DR, and CD11c as additional cell surface markers that provide better resolution of intermediate and nonclassical monocyte subsets. These markers can be used in traditional flow cytometry to identify alterations in subset frequencies during clinical studies.

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Monocytes are a heterogeneous population of blood cells derived from the bone marrow that can be recruited to inflamed tissues and differentiate into macrophages and dendritic cells.² In mice, monocytes have been separated into vessel-patrolling monocytes that are Ly6C^{low} CCR2^{low} CX₃CR1^{hi} and tissue-infiltrating monocytes that are Ly6C^{hi} CCR2^{hi} CX3CR1^{low}. Although the Ly6C^{hi} CCR2^{hi} CX3CR1^{low} monocytes are often regarded as inflammatory because they are the primary source of inflammatory M1 macrophages,² they are also the predominant source of reparative alternatively activated M2 macrophages,³ and hence their differentiation and activation properties are driven primarily by the tissue environment they encounter. In humans, there is an analogous separation of monocytes into classical (CD14^{hi}CD16⁻), nonclassical (CD14^{low}CD16^{hi}), and intermediate (CD14^{hi}CD16^{hi}) phenotypes,⁴ although the function of each subset is not as well defined as in mice. Transcriptional profiling studies defined using the schema above document significant differences between the subsets in healthy human blood, which support the idea that these monocyte subsets are functionally different.⁵ In human disease states, there are alterations in relative frequencies of monocyte subsets, which correlate with inflammatory and clinical features. For example, in rheumatoid arthritis, there is an increased number of intermediate

monocytes compared with controls.⁶ The increased level of intermediate monocytes in rheumatoid arthritis patients has been correlated with decreased responsiveness to therapy⁶ and increased coronary artery calcification.⁷ Thus, monitoring the frequency and phenotype of human monocyte subsets may be useful biomarkers for clinical outcomes in inflammatory diseases or immunotherapy and also provides insights into the contribution of the different monocyte subsets to disease processes.

In the study by Thomas et al,¹ they selected 36 cell surface markers to phenotype monocytes using CyTOF, or mass cytometry, to provide a comprehensive profile of surface markers to better define monocyte subsets. By clustering cell populations based on cell surface markers, they found that many intermediate monocytes clustered with classical and nonclassical monocytes. Using many bioinformatics approaches to identify the surface markers that would best discriminate between the different monocyte subsets, they selected CD14, CD16, CD11c, HLA-DR, CD36, and CCR2 as the best markers for separating monocyte subsets clearly into classical, nonclassical, and intermediate. Classical monocytes most highly expressed CD14, CD36, and CCR2, intermediate monocytes expressed the highest level of HLA-DR and high levels of CD14, CD16, CD11c, and CD36, whereas nonclassical monocytes expressed CD16 and CD11c with less HLA-DR. Although the goal of this study was to identify better markers for separating predefined monocyte subsets, the unsupervised clustering of intermediate monocytes into the other populations is also an indication that there is considerable heterogeneity within this population of monocytes.

In another recent publication, Villani et al⁸ used a different strategy by FACS sorting single cells for RNA-seq to examine HLA-DR⁺ cells from the peripheral blood, also from healthy individuals. They collected quality sequencing data from 339 monocytes FACS sorted based on CD14 and CD16 expression. These cells fell into 4 transcriptional clusters, with the 2 largest clusters constituting the classical and nonclassical monocytes, but some of the intermediate monocytes clustered with both classical and nonclassical monocytes, similar to the CyTOF study. The 2 smaller clusters also contained intermediate monocytes but shared some transcripts with classical monocytes, indicating considerable transcriptional heterogeneity for intermediate monocytes, some of which may have cytotoxic functions. Hence, transcriptionally, there may be 4 distinct monocyte subsets.

Depending on whether the goal is to more cleanly define established monocyte subsets (eg, in the study by Thomas et al¹) or more accurately identify new and distinct subsets (eg, by Villani et al⁸), the appropriate computation strategies can be used (eg, taking supervised versus unsupervised approaches) toward addressing that question. In the study by Thomas et

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al,¹ they used their new gating strategy with conventional flow cytometry antibodies to both validate that they can get clearly more distinct monocyte subsets and could apply this conventional FACS approach on peripheral blood samples collected from patients with cardiovascular disease.

The rapid technological improvements in single-cell analysis⁹ are providing us with unprecedented views of the heterogeneity of immune cells, such as blood monocytes. Although we are still mostly in the observation phase (some would deride as descriptive) for many of these studies, we should not underestimate the power of observation and reflection of interesting biological patterns, as best exemplified by Charles Darwin and his perceptions on evolution as the basis of biology as we know it. For those insistent on establishing causal mechanisms, several CRISPR-based perturbation approaches have now been developed for interrogating molecular circuits at the single-cell level,^{10–12} although they have yet to be applied directly to diseased patient samples, which will be a new frontier.

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