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DENDRITIC CELLS

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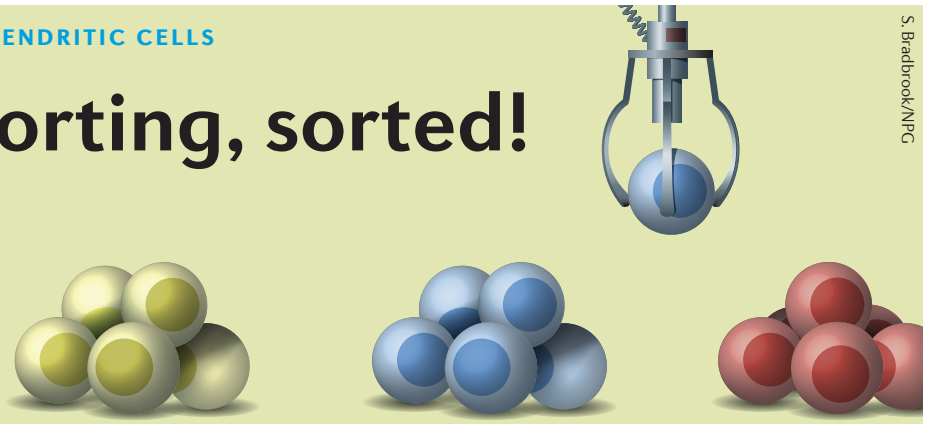

S. Bradbrook/NPG

A major challenge in the dendritic cell (DC) field has been to accurately and consistently define DC subsets in distinct tissues and across species. Different groups have used distinct strategies to identify the same DC population, and populations of 'DCs' have often been contaminated with macrophages. A collaborative study from the Lambrecht, Malissen and Ginhoux groups now suggests that automated gating strategies can overcome these issues and robustly identify distinct DC subsets from different tissues and species.

It was recently proposed (see Further reading) that DCs be classified into three main lineages according to their ontogeny: conventional type 1 DCs (cDC1s; which depends on the transcription factors IFR8, BATF3 and ID2), cDC2s (which depend on IRF4 and ZEB2) and plasmacytoid DCs (pDCs; which develop from distinct E2-2-dependent progenitors). However, there has been a lack of consensus on how to reliably identify these subsets based on phenotypical markers. In this study, Guilliams *et al.* describe a more accurate flow cytometry method for identifying mouse cDC1s and cDC2s in isolates of $CD45^+$ leukocytes. Briefly, their protocol involves excluding macrophages as $CD64^+F4/80^+$ cells, excluding cells that express lymphocyte lineage markers (CD3, B220, CD19 and NK1.1) and selecting for MHC class II^+ cells; cDCs are then identified as $CD11c^+CD26^+$ cells and can be subdivided based on expression of XCR1 and CD172a into cDC1s ($XCR1^{hi}CD172a^{low}$) and cDC2s ($XCR1^{low}CD172a^{hi}$). pDCs express B220 and are identified among the lymphocyte lineage as $120G8^+B220^+CD11c^+LY6C^+CD11b^-$ cells. The authors validated their approach by staining for subset-specific transcription factors and, importantly, found that this protocol could accurately identify these DC subsets in all mouse tissues. Furthermore, the inclusion of a CD24 gate allowed Langerhans cells ($CD26^{low}CD24^{hi}$) to be distinguished from dermal cDC2s ($CD26^{mid}CD24^{low}$) in both the skin and draining lymph nodes, something that had previously only been achieved using bone marrow chimaera systems.

> "automated approaches... should enable a more robust analysis of high-dimensional data"

Having determined a more accurate manual gating strategy, the same phenotypical markers were used to develop automated methods for identifying DC lineages. The authors used FlowSOM and t-SNE (which allow high-dimensional datasets to be visualized in low-dimensional data trees) to generate nodes corresponding to cDC1s and cDC2s; this automated gating strategy accurately identified these DC subsets in all mouse tissues. Furthermore, the automated method was more reliable than manual gating for identifying DC subsets in mutant mice that show altered expression of lineage-associated markers. By slightly adapting their protocol, the authors were able to identify cDC1s, cDC2s and pDCs in mice, humans and macaques using a similar gating strategy across species. In these experiments, macrophages in humans and macaques were excluded as $CD14^+CD16^+$ cells, CD1c was included to more reliably identify cDC2s, and CADM1 was substituted for XCR1 (as there is not yet a suitable antibody for detecting XCR1 in humans or macaques). These modifications allowed cDC1s and cDC2s to be identified in all tissues of each of the species as $CADM1^{hi}CD172a^{low}CD11c^{mid/hi}CD26^{hi}$ cells and $CADM1^{low}CD172a^{hi}CD1c^{hi}$ $CD11c^{hi}$ cells, respectively.

Finally, the authors used mass cytometry (CyTOF) and a One-SENSE computational approach to compare heterogeneity in mouse and human cDC1s, cDC2s and pDCs from different tissues, based on their expression of other phenotypical markers. These analyses indicated that DC heterogeneity is mainly determined by tissue residence. CyTOF and One-SENSE was also used to progressively monitor the phenotypical changes that occur in cDC1s, cDC2s and monocyte-derived cells during tissue inflammation.

Such automated approaches for flow and mass cytometry should enable a more robust analysis of high-dimensional data and improved reproducibility. However, the authors caution that we still need better methods for identifying other cell lineages, such as tissue macrophages, across species.

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ORIGINAL ARTICLE Guilliams, M. *et al.* Unsupervised high-dimensional analysis aligns dendritic cells across tissues and species. *Immunity* http://dx.doi.org/10.1016/j.immuni.2016.08.015 (2016)

FURTHER READING Guilliams, M. *et al.* Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat. Rev. Immunol.* **14**, 571–587 (2014) | Saeys, Y., Gassen, S.V. & Lambrecht, B.N. Computational flow cytometry: helping to make sense of high-dimensional immunology data. *Nat. Rev. Immunol.* **16**, 449–462 (2016)