



Isolation and analysis of tissue-derived CD11c⁺ mouse dendritic cells

Background

Dendritic cells (DCs) are the sentinels of the immune system¹ and are naturally located in all tissues, especially in those located at the interface between the body and the external environment, such as skin², lung³, intestine^{4,5}, and in the lymphoid organs^{6,7}, where they activate T cells and thus start immune responses. The role of tissue DCs in the initiation of diseases such as autoimmunity, allergy, and food intolerance, as well as their key function in vaccinations brought DCs more and more into the focus of basic and translational research in the last decade. However, the study of tissue DCs is hampered by several limitations, including the lack of ability to isolate viable cells in numbers that would allow for detailed investigation. In order to study tissue DCs it is important to be equipped with optimal tools for the dissociation of tissues as well as the isolation of DCs, yielding cells with high viability and epitope integrity. Both criteria are essential for downstream applications such as flow analysis and cell sorting, as well as transcriptional studies.

DCs constitutively express the surface hematopoietic markers CD45, MHC class II, Flt3, and CD11c and lack T cell, natural killer (NK) cell, B cell, granulocyte, and erythrocyte lineage markers.¹ In mice CD11c is a well-established, yet not exclusive DC marker that can be conveniently used for the isolation of pan DCs and also CD11c⁺ macrophages.¹ The protocol below describes the procedure to dissociate mouse spleen, lung, and intestine using the gentleMACS™ Dissociator and specific tissue dissociation kits in order to obtain viable subsets of DCs that can be further isolated using MACS® Cell Separation Technology or flow sorting. Miltenyi Biotec's tissue dissociation kits ensure a high viability of CD11c⁺ cells and preservation of epitopes for cell isolation and staining. CD11c MicroBeads UltraPure have been optimized for the rapid and simple isolation of mouse DCs from single-cell suspensions generated from lymphoid and non-lymphoid tissues. These MicroBeads greatly improve recovery and purity of the sorted population by specifically enriching viable cells.

Materials and methods

Tissue dissociation

Spleens, lungs, and small intestines were dissected from BALB/c mice and dissociated into single-cell suspensions according to the respective dissociation protocols:

- Spleens and lungs were dissociated using the Spleen Dissociation Kit and Lung Dissociation Kit, respectively, in combination with the gentleMACS™ Octo Dissociator with Heaters.
- Small intestines were cleared from feces and Peyer's patches were removed. The tissues were dissociated using the Lamina Propria Dissociation Kit in combination with the gentleMACS Octo Dissociator with Heaters: The intraepithelial lymphocytes (IELs) were first removed and the tissues were further digested to obtain the lamina propria lymphocytes by following the protocol included in the kit.

The resulting single cells were suspended in PEB buffer and the cell number was determined. The cell suspensions were then ready for subsequent cell isolation.

CD11c⁺ cell enrichment and flow analysis

1. Cells were kept cold and pre-cooled solutions were used to prevent capping of antibodies on the cell surface and non-specific cell labeling.
2. PEB buffer, i.e., a solution containing PBS (pH 7.2), 0.5% bovine serum albumin (BSA), and 2 mM EDTA was prepared by diluting MACS BSA Stock Solution at a ratio of 1:20 with autoMACS® Rinsing Solution. The buffer was kept cold (2–8 °C) and degassed before use, as air bubbles might block the column.
3. Cell suspensions were labeled with CD11c MicroBeads UltraPure, mouse according to the corresponding data sheet.

- Cell separation was done by following the protocol provided in the data sheet. The negative flow-through fraction was discarded, and the positive fraction containing the CD11c⁺ cells was analyzed by flow cytometry.
- Isolated CD11c⁺ cells were stained in PEB buffer with the respective antibodies for 10 minutes at 4 °C.

Cell surface antigen	Clone	Fluorochrome
CD11c	N418	VioBlue®/APC/PE
MHC class II	M5/114.15.2	APC/FITC
CD11b	REA592	FITC/APC
XCR-1	REA707	PE
PDCA-1	JF05-1C2.4.1	PE

- Flow cytometry was performed on the MACSQuant® Analyzer 10 and data were analyzed with Flowlogic™ V7.

Results

Single-cell suspensions of spleen, lungs, and small intestine obtained with the gentleMACS™ Octo Dissociator with Heaters and Spleen Dissociation Kit, Lung Dissociation Kit, or Lamina propria Dissociation Kit, respectively, showed DC subsets with high viability. Cell surface epitopes were well preserved for labeling with antibodies against relevant phenotyping markers, as shown by flow cytometry analysis (fig. 1A). CD11c MicroBeads Ultrapure, mouse, resulted in an effective enrichment of viable pan DCs, including specific myeloid (CD11b⁺ and XCR-1⁺) and plasmacytoid (PDCA-1⁺) DC subsets (fig. 1B, C).

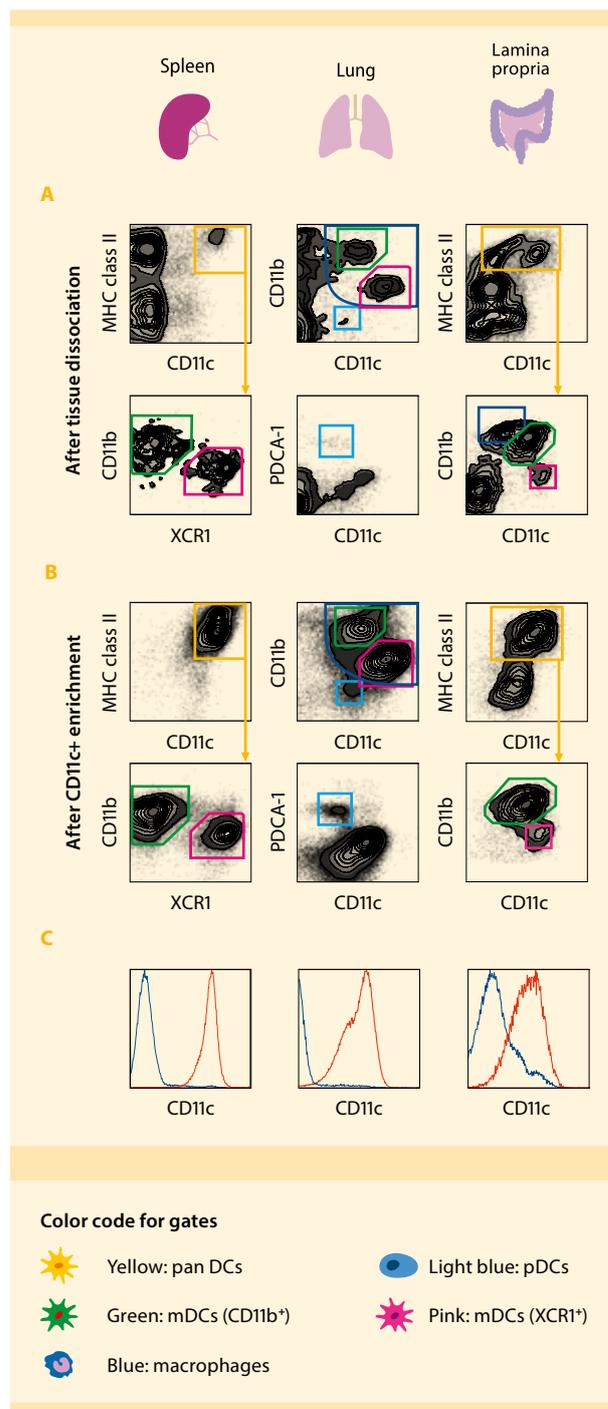


Figure 1: (A) DC and macrophage phenotypes after dissociation of mouse spleens, lungs, and small intestines with gentleMACS Octo Dissociator with Heaters and the respective MACS Tissue Dissociation Kit. Dissociated cells were stained with the specified antibodies and analyzed by flow cytometry using Flowlogic software. Cells were gated on the PI⁻ population. (B) Enrichment of pan DCs and CD11c⁺ macrophage populations with CD11c MicroBeads Ultrapure. Enriched cells were stained with the specified antibodies and analyzed by flow cytometry. Cells were gated on the PI⁻ population. (C) Comparison of CD11c⁺ populations before (blue) and after (red) enrichment with CD11c MicroBeads UltraPure, mouse. Cells were gated on the PI⁻ population.

Conclusion

- DCs with high viability and preserved epitopes can be obtained from mouse spleen, lung, and lamina propria using the gentleMACS™ Octo Dissociator with Heaters and the appropriate tissue dissociation kit from Miltenyi Biotec.
- Pan DCs can be enriched from dissociated tissues with CD11c MicroBeads UltraPure.
- Isolated tissue-derived pan DCs show specific surface markers enabling the detailed analysis of distinct DC subsets.

References

1. Merad, M. *et al.* (2013) The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. *Annu. Rev. Immunol.* 31: 563–604.
2. Malissen, B. *et al.* (2014) The origins and functions of dendritic cells and macrophages in the skin. *Nat. Rev. Immunol.* 14: 417–428.
3. Plantinga, M. *et al.* (2010) Origin and functional specializations of DC subsets in the lung. *Eur. J. Immunol.* 40: 2112–2118.
4. Denning, T. L. *et al.* (2007) Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat. Immunol.* 8: 1086–1094.
5. Fujimoto, K. *et al.* (2011) A new subset of CD103⁺CD8α⁺ dendritic cells in the small intestine expresses TLR3, TLR7, and TLR9 and induces Th1 response and CTL activity. *J. Immunol.* 186: 6287–6295.
6. Steinman, R. M. and Cohn, Z. A. (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* 137: 1142–1162.
7. Steinman, R. M. and Cohn, Z. A. (1974) Identification of a novel cell type in peripheral lymphoid organs of mice. II. Functional properties *in vitro*. *J. Exp. Med.* 139: 380–397.

MACS Product	Order no.
gentleMACS Dissociator	130-093-235
gentleMACS Octo Dissociator	130-095-937
gentleMACS Octo Dissociator with Heaters	130-096-427
gentleMACS C Tubes (25 or 100 tubes)	130-093-237 130-096-334
Spleen Dissociation Kit, mouse	130-095-926
Lung Dissociation Kit, mouse	130-095-927
Lamina Propria Dissociation Kit, mouse	130-097-410
MACS SmartStrainers (70 µm)	130-098-462
Pre-Separation Filters (70 µm)	130-095-823
MACS BSA Stock Solution	130-091-376
autoMACS Rinsing Solution	130-091-222
CD11c MicroBeads UltraPure, mouse	130-108-338
Red Blood Cell Lysis Solution (10x)	130-094-183
LS Columns	130-042-401
MACSQuant Analyzer 10	130-096-343
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