

Anatomical distribution analysis reveals lack of Langerin⁺ dermal dendritic cells in footpads and tail of C57BL/6 mice

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Abstract: Epidermal Langerhans cells (LCs) and dermal dendritic cells (dDCs) capture cutaneous antigens and present them to T-cells in lymph nodes (LNs). The function of LCs and Langerin⁺ dDCs was extensively studied in the mouse, but their anatomical repartition is unknown. Here, we found LCs in back skin, footpads and tail skin of C57BL/6, BALB/c, 129/Sv and CBA/J mice. Langerin⁺ dDCs were readily observed in back skin of all strains, but only in footpads and tail of BALB/c and CBA/J mice. Similarly, while LCs were equally present in all LNs and strains, Langerin⁺ dDCs were found in popliteal LNs (draining footpads)

only in BALB/c and CBA/J mice. The sciatic LNs, which we identified as the major tail-draining lymphoid organ, were devoid of Langerin⁺ dDCs in all strains. Thus, functionally different DCs reside in different skin areas, with variations among mouse strains, implying a potential impact on the cutaneous immune reaction.

Key words: dermal dendritic cells – footpad skin – Langerhans cells – skin-draining lymph nodes – tail skin

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Background

Although they represent only a small proportion of total skin DCs, epidermal Langerhans cells (LCs) and Langerin⁺ dermal dendritic cells (dDCs) have been the subject of many functional studies in the mouse, discrepancies still exist as to their functional specialisation (1–6). LCs often appear tolerogenic, while Langerin⁺ dDCs have a critical role in cross-presentation of skin-derived antigens and type 1 T helper responses (3,5,7–9).

Questions addressed

The anatomical repartition (10,11) and strain-associated variations of Langerin⁺ DC subsets have not been thoroughly investigated, although such differences could have an important impact on the interpretation of results and better understanding of skin-specific immune responses. We analysed Langerin⁺ DC subsets in back skin, footpads and tail and in corresponding skin-draining LNs of C57BL/6, BALB/c, 129/Sv and CBA/J mouse strains.

Experimental design

Animals

C57BL/6, CBA/J, 129/Sv and BALB/c mice (Charles River Laboratories France) were housed in specific pathogen-free conditions, and all experiments were carried out in conformity to the animal bioethics legislation.

Preparation of skin and LN cell suspensions

Back skin, footpad and tail skin as well as inguinal, popliteal, sciatic and mesenteric LNs were collected. Small pieces of tissues were incubated at 37°C in DMEM medium supplemented with 2% foetal calf serum (FCS; PAN-Biotech, Aidenbach, Germany), 1 mg/ml collagenase D, 1 mg/ml dispase II, 50 µg/ml DNase I (all enzymes from Roche Diagnostics, Meylan, France) for 1 h (LNs) or 2 h (skin) on a shaker. 5 mM

EDTA was added to stop enzymatic digestion, and cells were filtered through a 40 µm cell strainer (BD Biosciences).

Flow cytometry

All reactions were performed at 4°C for 20 min in PBS complemented with 2% FCS and 2.5 mM EDTA. Fc-receptors were blocked with 5 µg/ml CD16/CD32 antibody (2.4G2, BD, San Diego, CA, USA). Dead cells were excluded by labelling with Fixable Viability Dye (eBioscience, Frankfurt, Germany) in PBS for 30 min. Surface stainings were performed with 1 µg/ml of the following reagents: streptavidin-APC, anti-CD103-PE or –PerCP-Cy5.5 (M290), anti-CD11c-PerCP-Cy5.5 (N418) or –PE-Cy7 (HL3), anti-CD3-PE (145-2C11), anti-CD45-APC (30-F11; all from BD) and anti-CCR7-biotinylated (4B12, eBioscience) or their isotype controls. Cells were fixed, permeabilised (Cytotfix/Cytoperm, BD) and labelled with 1 µg/ml anti-Langerin-FITC (929F3.01, Dendritics, Lyon, France). Flow cytometry was performed on a Gallios (Beckman-Coulter, Fullerton, CA, USA) and analysed with FlowJo (Treestar, Ashland, OR, USA).

Results

CD45⁺ CD11c⁺ skin DCs include CD103[–] EpCAM^{high} Langerin⁺ LCs and CD103⁺ EpCAM^{low} Langerin⁺ dDCs (1,2,7,9,12,13). We found LCs in all skin areas and mice studied (Fig. 1a), but their proportion relative to CD11c⁺ DCs varied with their location (Fig. 1b). LCs were abundant in back skin and footpads, but rare in tail. The low proportion of LCs in tail was confirmed by immunofluorescence on epidermal sheets (Figure S2a,b; 10). Compared to LCs, the percentage of Langerin⁺ dDCs was lower (Fig. 1a), with a manifest variability across skin locations and mouse strains (Fig. 1c). Indeed, while this DC subset was consistently found in back skin of all strains tested, it was only detectable in footpads of BALB/c and to a

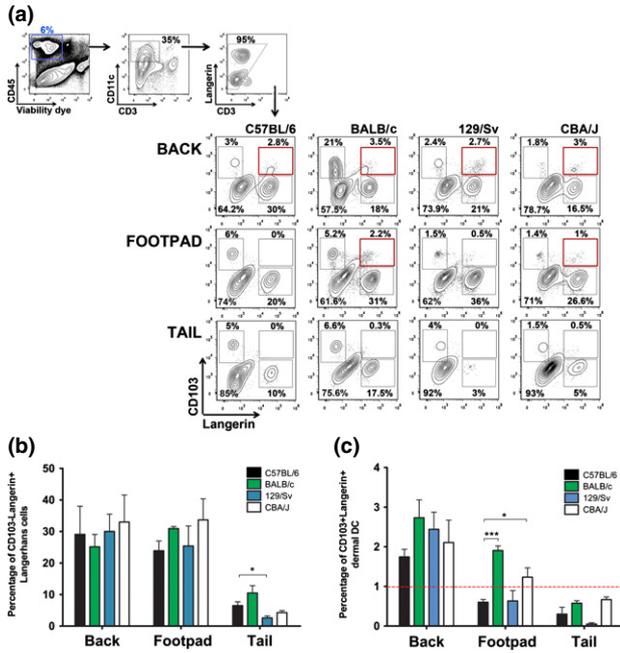


Figure 1. Skin dendritic cell (DC) subset composition varies according to the localisation and mouse strains. (a) Single-cell suspensions were prepared from back, footpad and tail skin of C57BL/6, BALB/c, 129/Sv and CBA/J mice and analysed by flow cytometry. CD11c+ CD3- DCs were selected among viable CD45+ cells. Additional gating of Langerin-/- CD3- allowed to further eliminate T-cells with low levels of CD3. The corresponding isotype controls are shown in Figure S1a. Red gates highlight a percentage of Langerin+ CD103+ cells above 1%. Panels (b) and (c) present the compiled percentage data of both skin DC subsets in different skin areas and across the mouse strains, respectively (mean of two experiments, $n \geq 4$ mice in each condition; significant values: * <0.05 ; *** <0.0001). Dashed red line indicates the 1% threshold below which staining was considered as background. Percentages of Langerin- CD103+ dDCs are available in Figure S4a.

lesser extent of CBA/J mice. Langerin+ dDCs were missing from tail skin in all strains. Immunofluorescence staining confirmed the scarcity of Langerin+ dDCs in footpads of C57BL/6 but their presence in BALB/c mice (Figure S2c).

Variations in thickness and hairiness might impede uniform enzymatic digestion of the different skin areas. Because they are migratory, skin DCs can be isolated from skin-draining lymph nodes (LNs). However, the LNs that drain the tail remain unclearly defined (14). We identified the sciatic LN as a major draining site for a coloured dye injected into the tail (Figure S3a). Mice with skin-draining LN hypertrophy (15,16) also had enlarged sciatic LNs (Figure S3b). Tg(Grm1)EPv mice that spontaneously develop melanomas on the tail (17) present with melanin-loaded sciatic LNs (Figure S3c). Finally, tail skin inflammation triggered by the TLR7 agonist imiquimod (18) or the irritant chemical dibutylphthalate (19) induced emigration of LCs into sciatic LNs (Figure S3d,e).

We therefore studied inguinal (draining back skin), popliteal (footpads) and sciatic (tail) LNs, using mesenteric (intestine) LNs as a control. Skin-derived DCs were distinguished from LN-resident DCs by the expression of the chemokine receptor CCR7 that guides them upon emigration (Fig. 2a; 20). LCs were found in all skin-draining LNs (Fig. 2b), but the percentage of Langerin+ dDCs varied greatly and was in line with our analysis at skin level (Fig. 2c). They were clearly present in inguinal LNs of all strains, in popliteal LNs of BALB/c and CBA/J mice only, and were totally absent from sciatic LNs in all strains.

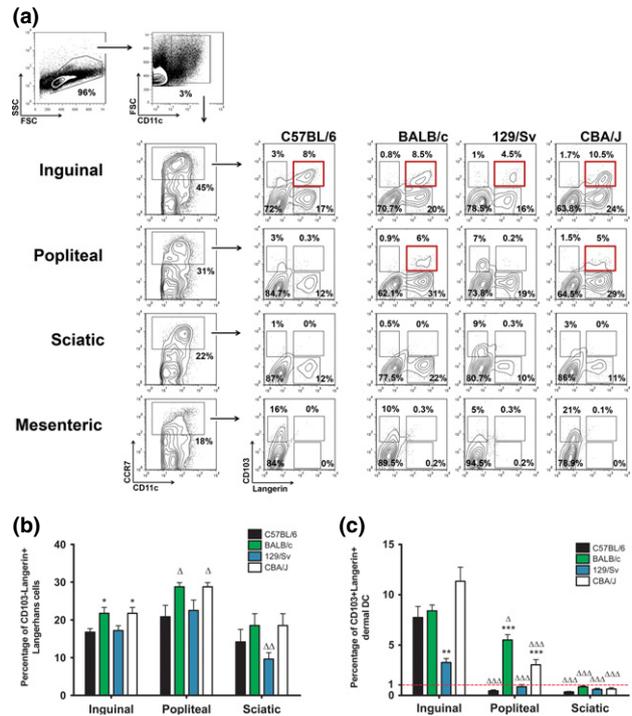


Figure 2. Skin-draining LN DC composition depends on localisation and mouse strain. (a) Inguinal, popliteal, sciatic and mesenteric LNs from C57BL/6, BALB/c, 129/Sv and CBA/J mice were prepared to obtain a single-cell suspension to identify viable CD11c+CCR7+ DCs by flow cytometry. The corresponding isotype controls are shown in Figure S1b. Red gates highlight a percentage of Langerin+ dermal dendritic cells (dDCs) above 1%. Panels (b) and (c) represent the compiled percentage data of Langerin+ cells and Langerin+ dDCs, respectively, in different LNs and mouse strains (mean of two experiments, $n \geq 5$ mice in each condition). Asterisks (*) indicate a significant difference between a given strain and C57BL/6 mice. Triangles (Δ) indicate a significant difference between a given LN and inguinal LNs (* or Δ : $P < 0.05$; ** or $\Delta\Delta$: $P < 0.005$; *** or $\Delta\Delta\Delta$: $P < 0.0001$). Percentages of Langerin- CD103+ dDCs are available in Figure S4b.

Conclusions

While LCs are present in all skin types of all mouse strains, the distribution of Langerin+ dDCs appears site- and strain-specific. There was an absence of Langerin+ dDCs from footpads and popliteal LNs of C57BL/6 mice and 129/Sv and from tail and tail-draining LNs of all strains. The major tail-draining LNs were identified as the sciatic LNs based on a number of criteria including mobilisation by skin inflammation. Even upon skin DC mobilisation, no Langerin+ dDCs were observed in sciatic LNs. Inefficient hapten sensitisation on tail skin has been associated with the low density of LCs in this area (10), but the lack of Langerin+ dDCs might contribute to this hyporesponsiveness (3). A recent report suggested a complete absence of dDCs from footpads (11), but we found Langerin- dDCs in all sites investigated (Figure S4).

Such differences among skin DC subsets are reminiscent of mouse-strain-specific expression of the endocytic receptor Langerin by LN-resident CD8+ DCs and gut DCs (21,22), and by the variations of plasmacytoid DC numbers linked to genetic polymorphisms (23,24). Footpads and the tail have a particularly protective stratum corneum, which might explain why immunosurveillance does not necessarily require the highly immunocompetent Langerin+ dDCs. Alternatively, a lack of Langerin+ dDCs could limit immune responses in these areas that are constantly subject to physical stress.

Our findings strongly encourage side-by-side analyses of human DC subsets from different skin locations, that is areas exposed to UV or mechanical stress (25,26), and across ethnic groups. This may shed light on the frequencies of site-specific dermatitis or tumor formation in diverse genetic backgrounds. Finally, our results suggest new means to target specific DC subsets to manipulate the outcome of immunisation (27,28).

Acknowledgements

C.G.M. and V.F. designed the research study. B.V. and D.G.M. performed experiments. P.S. and S.C. contributed the tg(Grm1)EPv mouse strain. B.V. and V.F. analysed data. B.V., C.G.M. and V.F. wrote the paper.

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Conflict of interest

The authors have declared no conflicting interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Isotype control stainings for flow cytometry analyses.

Figure S2. Identification of Langerhans cells (LCs) and Langerin+ dermal DCs *in situ*.

Figure S3. The sciatic LNs drain tail skin.

Figure S4. Quantification of Langerin– CD103+ and Langerin– CD103– dermal DCs in the skin and in skin-draining LNs.

