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Cutting Edge: Expression of XCR1 Defines Mouse Lymphoid-Tissue Resident and Migratory Dendritic Cells of the CD8α+ Type

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Subsets of dendritic cells (DCs) have been described according to their functions and anatomical locations. Conventional DC subsets are defined by reciprocal expression of CD11b and CD8α in lymphoid tissues (LT), and of CD11b and CD103 in non-LT (NLT). Spleen CD8α+ and dermal CD103+ DCs share a high efficiency for Ag cross-presentation and a developmental dependency on specific transcription factors. However, it is not known whether all NLT-derived CD103+ DCs and LT-resident CD8α+ DCs are similar despite their different anatomical locations. XCR1 was previously described as exclusively expressed on mouse spleen CD8α+ DCs and human blood BDCA3+ DCs. In this article, we showed that LT-resident CD8α+ DCs and NLT-derived CD103+ DCs specifically express XCR1 and are characterized by a unique transcriptional fingerprint, irrespective of their tissue of origin. Therefore, CD8α+ DCs and CD103+ DCs belong to a common DC subset which is unequivocally identified by XCR1 expression throughout the body. The Journal of Immunology, 2011, 187: 4411–4415.

Dendritic cells (DCs) exert their functions of immune sentinels in different anatomical places. The DCs that reside in the parenchyma of nonlymphoid tissues (NLT) are called interstitial DCs (int-DCs). These DCs shuttle tissue Ags to draining lymph nodes (LNs), where they are called migratory DCs (mig-DCs).

In mouse skin, DCs make up epidermal Langerhans cells (LCs) and three major subsets of dermal DCs: CD11b+CD24− DCs, CD11b−CD24+CD103+ DCs, and CD11b−CD24−CD103− DCs (1), hereafter referred to as CD11b+ DCs, CD103+ DCs, and CD103− DCs (Table I). Although LCs and all dermal DC subsets constitutively migrate from skin to cutaneous LN (CLN), the CD103+ DCs stand out as the most potent subset for presenting keratinocyte-derived Ags to CD8+ T cells in the CLN (1). This capacity is reminiscent of the high efficiency of lymphoid tissue (LT)-resident CD8α+ DCs for cross-presentation (1). CD103+ int-DCs are also found in other anatomical places such as lung and gut. The development of CD103+ int-DCs and LT-resident CD8α+ DCs selectively depends on a common set of transcription factors (2, 3). Hence, these mouse DC populations may belong to a unique category of CD8α+ type DCs (1).

CD8α+ type DCs exist in human and sheep, where their identification was based on their expression of a unique transcriptional fingerprint shared with mouse spleen CD8α+ DCs (4, 5) and on their efficiency for Ag cross-presentation (5–9). We proposed a universal classification of DCs in five major subsets irrespective of tissues and species: monocyte-derived inflammatory DCs, LCs, plasmacytoid DCs, CD11b+ type DCs, and CD8α+ type DCs (1). Yet, a discriminating marker identifying all CD8α+ type DCs across tissues remained to be determined. Moreover, the relationships between LT-resident CD8α+ DCs and CD103+ mig-DCs or CD103− int-DCs need to be confirmed. The chemokine receptor XCR1 is specifically expressed by CD8α+ type DCs in mouse spleen, human blood, and sheep lymph (4–7, 10). The function of XCR1 was first unveiled by the group of R. Kroczek (10), who showed that CD8+ T cell cross-priming Recherche sur le Cancer (to K.C.) and the Ministère de la Recherche (to S.T.), a Marie Curie fellowship (to M.G.), and the Centre National de la Recherche Scientifique and System biology of T cell activation (SYBILLA) project (to H.L.).

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The online version of this article contains supplement material.

Abbreviations used in this article: CLN, cutaneous lymph node; DC, dendritic cell; βGal, β-galactosidase; int-DC, interstitial dendritic cell; LC, Langerhans cell; LN, lymph node; LT, lymphoid tissue; medLN, mediastinal LN; mig-DC, migratory dendritic cell; MLN, mesenteric LN; NLT, nonlymphoid tissue; TN, triple-negative.

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depends on their ability to secrete the XCR1 ligand XCL1 in experimental models where either the OVA coupled to an anti-CD205 Ab or OVA-expressing allogeneic pre-B cells are administrated in vivo. XCR1 expression on CD8α+ DCs was also found critical for the optimal induction of CD8+ T cell responses upon *Listeria monocytogenes* infection (6). We hypothesized that XCR1 may be the long sought marker defining all CD8α+-type DCs throughout the body. In this study, to investigate whether LT-resident CD8α+ DCs and NLT-derived CD103+ DCs belong to the same DC subset and can be identified by a common specific marker across mouse tissues, we analyzed their expression of XCR1 and compared their gene expression profiles.

### Materials and Methods

#### Mice

Xcr1<sup>tm1Dgen</sup> mice (Xcr1<sup>BGal</sup>) (6, 10) generated by Deltagen were bred in Centre d’Immunologie Marseille-Luminy animal care facilities. C57BL/6J mice were purchased from Charles River Laboratories (L’Arbresle, France). Studies were performed in accordance with institutional regulations governing animal care and use.

#### DC isolation and sorting strategy

DCs were isolated from various organs by a combination of enzymatic digestion, mechanical disruption, and gradient density enrichment (11). Sorting of CLN DCs was performed as described previously (12).

#### Abs and flow cytometry

Most Abs were purchased from eBioscience or BD Biosciences. Identification of mig-DCs was based on their specific pattern of expression of CD11c and MHC class II (Supplemental Fig. 1). CADM1 staining was performed with a chicken anti-SynCAM/TSLC1 Ab (clone 3.E.1) revealed with a goat anti-chicken IgG. XCR1 expression was detected using fluorescein di-β-D-galactopyranoside as a fluorogenic substrate for β-galactosidase (βGal) (6). In CLNs, XCR1 expression was also detected using recombinant mouse XCL1 covalently coupled to the red fluorescent protein mCherry (E. Fossum and B. Bogen, manuscript in preparation).

#### Microarray analyses and real-time PCR

Quality controls and normalization of a public expression data compendium of mouse DC subsets (ImmGen: Gene Expression Omnibus database GSE15907) were performed as described previously (6). The gene set enrichment analysis (GSEA) method is publicly available at http://www.broad.mit.edu/gsea (13). To test whether GeneSets were enriched in pairwise comparisons between CD8α<sup>+</sup> LT-resident DCs or CD103<sup>+</sup> NLT-derived DCs versus their CD11b<sup>+</sup> counterparts, nominal values were calculated as well as false-discovery rate (q value), based on 1000 random permutations between GeneSets. Results were considered significant when p < 0.05 and q < 0.25 (13). Real-time PCR experiments were performed as previously described (6) using the Hprt gene for normalization. Primer sequences are available upon request.

### Results and Discussion

#### High level of XCR1 expression is selective for CD103+ int-DCs in skin and CD103+ mig-DCs in CLNs

To investigate which DC subsets express XCR1 in skin and CLNs, we exploited a reporter mutant mouse model expressing β-galactosidase (βGal) in place of XCR1. In skin, the int-DC subsets encompass the epidermal LCs and the dermal subsets CD103<sup>+</sup> DCs, CD103<sup>-</sup> DCs, CD11b<sup>+</sup> DCs, and CD11b<sup>-</sup>CD24<sup>+</sup> DCs (Fig. 1A, Table I). In skin int-DCs, βGal activity was high in CD103<sup>+</sup> DCs, low in CD103<sup>-</sup> DCs, and undetectable in CD11b<sup>-</sup>CD24<sup>+</sup> DCs, CD11b<sup>+</sup> DCs, and LCs (Fig. 1B, 1C). In CLNs, only LT-resident CD8α<sup>+</sup> DCs and CD103<sup>+</sup> mig-DCs expressed XCR1 (Fig. 1D, 1F). The use of fluorescently labeled recombinant mouse XCL1 to stain CLN cells gave a strong and highly specific...
signal on LT-resident CD8α+ DCs and CD103+ mig-DCs from wild-type mice (Fig. 1E). These data confirmed that the protein XCR1 is specifically expressed on LT-resident CD8α+ DCs and CD103+ mig-DCs and validated the use of βGal activity as a faithful reporter of XCR1 expression. Therefore, in skin and CLNs, a high level of XCR1 expression is selective for CD8α+-type DCs.

**XCR1 expression defines CD8α+-type DCs in visceral organs and their draining LNs**

We next analyzed XCR1 expression on DCs residing in different tissues. As in skin and CLNs, three main populations were defined in the liver, lungs, and the small intestine: CD11b+ DCs, CD103+ DCs, and CD103+ DCs (Fig. 1A, Table I). In these organs, XCR1 expression was high in CD103+ int-DCs, intermediate in CD103+ int-DCs, and not detected in CD11b+ int-DCs (Fig. 1H), as observed in the skin. In the mig-DCs from mesenteric LNs (MLNs) and mediastinal LNs (MedLNs) draining, respectively, the intestine and the lung, XCR1 expression remained highest in the CD103+ subset (Fig. 1J). Despite the use of an inhibitor of endogenous Gal activity, the CD103+ int-DCs in the intestine and the CD103+ mig-DCs in MedLN showed substantial levels of βGal activity in wild-type mice. However, a clear increase over that background signal could be detected in the corresponding subsets isolated from XCR1−βGal mice. Within LT-resident DCs, XCR1 expression remained confined to the CD8α+ subset (Fig. 1J).

In most of the NLT we have examined, CD103− int-DCs expressed intermediate levels of XCR1. CD103−XCR1low DCs may be precursors of CD103+XCR1high DCs. In bone marrow FLT3 ligand cultures, CD11b−CD24−CD103− DCs represent an immature stage of CD11b−CD24−CD103− DCs not yet endowed with full cross-presentation potential. In these cultures, the acquisition of cross-presenting competence correlates with the expression of CD103 upon TLR or cytokine stimulation (14). The acquisition of XCR1 in vivo might be exploited to follow the development of CD8α+-type DCs into full-fledged cross-presenting DCs in tissues.

**LT-resident CD8α+ DCs and NLT-derived CD103+ DCs share a common gene signature**

To investigate the potential similarities between LT-resident CD8α+ DCs and skin-derived CD103+ mig-DCs, which
both specifically expressed XCR1, we analyzed the expression of several transcripts in DC subsets sorted from CLNs. We focused on Xcr1, Clec9a, Cadm1, Clink, and Rab7b (5430435G22Rik), because these genes are expressed selectively in CD8α+ type DCs from mouse spleen, human blood, and sheep lymph (4, 5). CLEC9A, XCR1, and CADM1 contribute to confer their specific functions on spleen CD8α+ DCs by promoting the cross-presentation of dead cell-associated Ags, the priming of CD8+ T cells, and the induction of IL-22 in CD8+ T cells (6, 10, 15, 16). CLNK belongs to the SLP-76 family of adaptors critical for signal transduction by ITAM-coupled receptors. CLEC9A contains a hemi-ITAM motif and may signal through CLNK downstream of the Syk tyrosine kinase (15). The small GTPase RAB7B expressed in endosomes may promote assembly of the machinery required for crosspresentation as suggested by the functions of its paralogs (17). In CLNs, Xcr1, Clink, Rab7b, Clec9a, and Cadm1 were expressed the highest in LT-resident CD8α+ DCs and CD103+ mig-DCs (Fig. 2A). Tlr3 was expressed identically in all subsets of mig-DCs. As reported for spleen DC subsets (3), Batf3

![Graphs showing gene expression levels](image)

**FIGURE 2.** Gene expression profiling of DC subsets from nonlymphoid and lymphoid organs. A, Relative levels of expression of selected transcripts in DC subsets sorted from spleen and CLNs as assessed by real-time PCR. Graphs represent pooled results from three independent experiments. B, Gene expression ratio (Log2) of CD8α+-like DC/CD11b+-like DC gene expression ratio (Log2) to confer their specific functions on spleen CD8α+-type DCs from mouse spleen, human blood, and sheep lymph. C, Gene set enrichment analysis for comparison of putative CD8α+-type versus CD11b+-type DCs in a number of mouse tissues. CD8α+ DC-specific genes ("CD8α+ DC" GeneSet encompassing 92 genes) and CD11b+ DC-specific genes ("CD11b+ DC" GeneSet encompassing 75 genes) were examined for global enrichment of their expression in the ImmGen public microarray data when comparing CD8α+-type versus CD11b+-type DCs. The color of circles corresponds to the font color of the DC subset where the GeneSet is enriched. The circle surface area is proportional to the normalized enrichment score, which varies from 1 (no enrichment) to 5 (all genes of the GeneSet are expressed to higher levels in the same DC subset). The color intensity of circles is indicative of the false-discovery rate (FDR) statistical q value, which estimates the likelihood that the enrichment of the GeneSet represents a false-positive finding. ND, not detectable; TN, triple-negative CD4−CD8α−CD11b− DCs.
expression was comparable in all CLN DC subsets (Fig. 2A). As expected, Igga (CD103) was expressed selectively in CD103+ mig-DCs and in CD8α+ LT-resident DCs. Therefore, the common expression of a specific gene signature, including Xcr1, Clec9a, Clnk, Rab7b, and Cadm1, by LT-resident CD8α+ DCs and skin-derived CD103+ mig-DCs confirms their belonging to the CD8α+-type DC subset. To determine whether XCR1 expression defined CD8α+-type DCs across tissues, we exploited public data from the Immunological Genome project, which spans DC subsets from many organs and their draining LNs (18). Xcr1 transcript was expressed to high levels in all LT-resident CD8α+ DCs in lung and liver CD103+ int-DCs as well as in MLN CD4+CD8+CD11b+ triple-negative (TN) mig-DCs, as compared with CD11b+ DCs (Fig. 2B). Xcr1 transcripts were below the level of detection in CD103+ mig-DCs from CLNs, likely because of limitations in the sensitivity of the microarray technology. Consistently, Xcr1 expression levels assessed by real-time PCR were ~100-fold lower in CLN CD103+ mig-DCs as compared with CLN-resident CD8α+ DCs (Fig. 2A). A similar reduction was observed for Clec9a, Cadm1, Clnk, Igga, and Rab7b (Fig. 2A, 2B). However, Xcr1 and CADM1 were both still highly expressed in these cells at the protein level (Fig. 1D, 1E, 1G). Therefore, the transcription of some genes may decrease upon maturation of CD103+ int-DCs into mig-DCs, whereas expression of the corresponding proteins is maintained as a result of a sufficiently long half-life.

To further verify the degree of similarity that exists between DC subsets expressing XCR1, we examined in the conventional subsets of DCs isolated from the same tissues the enrichment of gene expression signatures (GeneSets) specific of splenic CD8α+ DCs or of splenic CD11b+ DCs (Fig. 2Q). In all pairwise comparisons, we observed that the CD8α+ DC-specific GeneSet was significantly enriched in LT-resident CD8α+ DCs, CD103+ int-DCs, CD103+ mig-DCs, or TN mig-DCs (Fig. 2Q). Reciprocally, the CD11b+ DC-specific GeneSet was significantly enriched in CD11b+ DCs from all tissues. Nineteen genes from the CD8α+ DC GeneSet were consistently expressed to higher levels in all CD8α+type DCs as compared with their CD11b+type DC counterparts (Supplemental Fig. 2). Some genes from the CD8α+ DC GeneSet were not expressed on all CD8α+type DCs. This observation pinpoints the necessity to assess the relationships between two cell subsets, based on their comparative expression of many markers. Although highly expressed by all CD8α+type DCs, Clec9a and Cadm1 are also expressed by cell subsets other than CD8α+type DCs in mice and humans, contrary to Xcr1 (6). This genome-wide expression profiling analysis confirmed the existence of CD8α+-type DCs characterized by XCR1 expression across all examined tissues in mice.

In conclusion, we identified for the first time to our knowledge a robust and specific marker defining mouse CD8α+ DCs in all the LT and NLT examined. This marker, the chemokine receptor XCR1, is also specifically expressed by human blood BDCA3+ DCs and sheep lymph CD26+ DCs and conserved during evolution (5, 6). Thus, the community of the researchers working on DCs may consider using the term “XCR1+ DCs” to designate CD8α+-type DCs in a consistent manner across all mammalian species and tissues to further implement the definition of a universal nomenclature for mouse and human DC subsets (19).

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Disclosures

The authors have no financial conflicts of interest.

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