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Isolation of murine skin resident and migratory dendritic cells via enzymatic digestion

Sakeen W. Kashem[1] and **Daniel H. Kaplan**[2,*]

[1]Center for Immunology, Department of Dermatology, University of Minnesota, Minneapolis, MN

[2]Department of Dermatology, Department of Immunology, University of Pittsburgh, Pittsburgh, PA

Abstract

Dendritic cells (DCs) are a highly specialized subset of professional antigen-presenting cells (APCs) that reside in peripheral and lymphoid tissues. DCs capture antigen in the periphery and migrate to the lymph node where they prime naïve T cells. In addition, DCs have been recently appreciated to have function in innate immunity within tissues. In the skin, heterogeneous populations of DCs reside within the epidermis and the dermis. Analysis of the cutaneous DC subsets is complicated by requirements of distinct enzymatic digestion protocols for isolation of APCs from distinct anatomical compartments of the skin. Here, we detail specific approaches for isolation of DCs from the epidermis, dermis and the skin draining lymph nodes of mice.

Keywords

Dendritic cell; Langerhans cell; Antigen-presenting cell; Skin

INTRODUCTION

This unit presents methods for preparing dendritic cells (DCs), a highly potent type of antigen-presenting cell (APC), from the murine skin. The first method involves isolation of epidermal Langerhans cells (LCs) from the epidermis using trypsin digestion. Trypsin digestion offers efficient and quick separation of the epidermis for the isolation of LCs, the only APC of the epidermis, at the cost of loss of certain cell surface markers after enzymatic cleavage. Alternatively, dispase may be utilized for digestion instead of trypsin to better preserve expression of cell surface markers. The second method includes collagenase and hyaluronidase digestion for isolation of DCs and migrating LCs from the dermis. This method can be performed on the whole skin or on the residual dermis after trypsin separation of the epidermis. Finally, the third method offers collagenase digestion of secondary lymphoid organ suspension to increase yield of migratory and resident DCs from the skin draining lymph nodes. We also detail the appropriate cell surface markers and antibodies for the potential characterization of murine epidermal, dermal and lymph node DC subsets by flow cytometry or cell sorting.

*Corresponding author.

Note: All solutions and equipment coming in contact with cells must use proper sterile technique accordingly.

Note: All incubations are performed in a humidified, 37°C, 5% CO_2 incubator unless noted otherwise.

BASIC PROTOCOL 1

Preparation of epidermal single cell suspension for analysis and isolation of murine Langerhans cells

Langerhans cells (LCs) are the only major histocompatibility class II (MHC-II) positive cells of the epidermis. In the mouse, they can be characterized by the expression of markers CD45, CD11c, MHC-II, CD207, CD11b and the absence of marker CD103 and XCR1. In addition to LCs, the epidermis also contains a rich population of keratinocytes, and dermal epidermal T cells. Single cell isolation of the epidermis can be prepared from the mouse ear, ventral or dorsal skin. Trypsin digestion facilitates easy separation of the epidermis from the dermis but leads to cleavage of cell surface markers such as CD11c from DCs and CD3e from dermal epidermal T cells. As an alternative, dispase digestion may be used to improve preservation of these markers partially, but not completely. Skin explant models may also be used for isolation of mature LCs, and is discussed elsewhere(Stoitzner et al., 2010).

Materials

1. Trypsin GNK solution (see reagents below)
2. DNAse solution (see reagents below)
3. Cell media (see reagents below)
4. Dispase solution (if necessary, see reagents below)
5. FACs staining media (SM; see reagents below)
6. Razor blades (Personna 74-0001)
7. Stainless steel forceps and curved bottom scissors
8. 10 cm petri dishes
9. Primary antibodies for staining (Table 1)

Protocol steps—*Step annotations*

1. Euthanize a 7- to 10- weeks old mouse, preferably female.
    a. Aggressive male mice are more prone to fighting. This may lead to increased wounding and inflammation of the dorsal skin, with increased variation amongst the cellular composition of the skin of male mice. Thus, we prefer to use female mice for greater consistency amongst samples. In addition, mice undergo synchronized hair growth from 5 to 7 weeks of age and undergo hair follicle pigmentation during this time. Harvesting the skin during outside of the range of 7- to 10- weeks of

age may lead to contamination with incontinent melanin, which can further lead to variation of analysis and potential instrument clogging during flow cytometry.

2. Gently spray the mouse body with 70% ethanol. This will facilitate smoother shaving and removal of mouse hair from harvested organs.

3. Shave the mouse with the razor blade in a linear, longitudinal manner in the cephalocaudal direction.

4. Harvest ventral skin, dorsal flank skin or ear of desired size. Generally, a transverse cut is made near the base of the tail of the back and cut upwards towards the neck. We place the skin, dermis side down, in petri dishes and ensure that the skin samples are dry.

    a. For harvesting the ears, the murine ears are cut off at the base to include the cartilage. At the base of the ear, you will notice a separating pocket of cartilage. Mouse ears are split into dorsal and ventral halves (containing the cartilage) with two strong forceps and placed dermal side down. To ensure uniform separation of the dorsum and ventral halves, separate at the base and go side to side in separating the two sides slowly until reaching the opposing tip of the ear.

5. Cut the murine body skin into ~1 cm wide and 2 cm long strips. Remove any adipose and subcutaneous tissues and float the skin with its dermal side down in the Petri dish containing pre-warmed 37°C Trypsin GNK solution. Approximately 10 mL of the enzymatic solution can be utilized in a 10 cm petri dish and can contain multiple strips (3–4). For smaller samples like the ears, less of the buffer in a smaller petri dish may be used.

    a. Trypsinization will lead to cleavage of certain cell surface markers such as CD11c and CD3. T cells may be characterized as CD45 positive, MHC-II negative cells that are CD103 positive. Alternatively, skin samples can be immersed on dispase solution for 1 to 2 hours to avoid excessive cleavage of cell surface markers although there may still be some loss of these markers. It is also critical to remove any adipose tissue from the digestion process as digestion of adipose tissue as cell death of any contaminating fat may lead to toxicity and decreased viability of the desired epidermal cells.

6. Incubate the samples in CO_2 incubator at 37°C. Generally, ears take 25–45 minutes for digestion while the flank and ventral skin takes 60–90 minutes. Check for completion with attempted separation after 30 minutes. The epidermis should generally peel off in one motion.

    a. Skin should float on the enzymatic solution to achieve thorough digestion of the dermal side. Floating, rather than submersion, prevents over digestion of epidermis and allows the epidermis to be peeled off in one piece.

7. To separate the epidermis, put the skin on the top of a petri dish. Collect the epidermis by scraping off the epidermis with forceps. Epidermis should come off as a thin white layer. This process will leave behind a thicker dermal layer, which can be saved for further dermal digestion, discussed below.

8. Transfer the epidermis into a 50ml conical tube containing 1 mL of DNAse solution and 9 mL of pre-warmed cell media, pulse vortex the tube and incubate in 37°C water bath/shaker for 20–30 min. Brief Vortex every 10 minutes.

    a. This step is critical for inactivation of trypsin as to stop over digestion and cell death. Furthermore, extracellular DNA may lead to cellular aggregation and trapping which reduces yield of single cell suspensions and decreased staining. Treatment with DNAse is critical for addressing these concerns and obtaining optimal single cell preparation.

9. Filter through 40 μm strainer into a new 50ml tube. Wash with 10 ml staining media, and centrifuge at 1200rpm 8min, swinging bucket at 4° C.

10. Decant supernatant and re-suspend pellet in 10 ml SM, and centrifuge as step 7.

11. Count cells via trypan blue exclusion and stain them for FACS (see Table 2 for epidermal markers).

    a. LCs should make for 1–3% of the cell suspension. If isolation of pure LC suspension is desired, cell sorting can be performed on pooled samples from multiple mice. To facilitate faster sorting, density gradient centrifugation or with MHC-II or CD11c microbead positive selection may be performed.

BASIC PROTOCOL 2

Preparation of dermal single cell suspension for analysis and isolation of dermal dendritic cells

The dendritic cell network of the dermis consists of four major subsets of migratory antigen presenting cells. All dendritic cells can be distinguished by the markers CD45, MHC-II and CD11c. Conventional DC2 subsets, marked by CD11b+CD103−CD207−, make up for majority of the dermal dendritic cells, while cDC1 dermal DC (CD103+CD207+CD11b−), migratory Langerhans cells (CD11b+CD207+CD103−) and the DN dermal DC (CD103−CD207−CD11b−) subsets make up for rest of the network in the dermis. Isolation of these dermal DCs can be achieved via collagenase digestion of the whole skin or after the digestion of the epidermis as described above on basic protocol 1. Digestion of the whole skin will lead to contamination of some, but not all, of the epidermal cells. On the other hand, digestion of the dermis post trypsin digestion of the epidermis can lead to enhanced isolation of epidermal cells, decreased contamination of the epidermis during the digestion of the dermis. Once isolated, DCs may be analyzed via flow cytometry or sorted for functional or sequencing assays.

Materials

- Collagenase XI/hyaluronidase solution (see reagents below)
- DNAse solution (see reagents below)
- Cell media (see reagents below)
- Staining media (see reagents below)
- Curved edge stainless steel scissors
- 10cm Petri dishes

Protocol steps—*Step annotations*

1. Euthanize a 7- to 10- week old female mouse. Harvest ear, ventral or dorsal skin as described above to desired size.
    a. Alternatively, this protocol may begin after the separation of the epidermis after protocol 1 described above.
2. Finely mince the entire skin or the dermis using curved edge scissors. Place dermis into a 10cm Petri dish. Add collagenase/hyaluronidase digestion buffer, 10ml/mouse for the entire dorsal skin. Pipet the mixture multiple times.
    a. The dermis will digest faster with finer mincing of dermis. Pipetting with a 5 or 10 mL syringe may aid dissociation through increased friction.
3. Incubate dermis at 37°C degrees 45–90 minutes in CO_2 incubator. Check every 30 minutes, by pipetting rigorously for single cell suspension.
    a. Alternatively, one can also use 50 ml conical and 37°C shaking incubator. Vortex periodically
4. Filter through 40 μm strainer into a new 50ml conical tube. Wash with 10 ml staining media and centrifuge at 1200 rpm for 8min in a swinging bucket centrifuge at 4° C.
5. Decant supernatant and re-suspend cells in 10 ml SM, and centrifuge as step 4.
6. Count cells and stain them for FACS accordingly as listed in Table 3.
    a. If DC purification is desired, MHC-II or CD11c microbead positive selection may be used on pool samples from multiple mice.

BASIC PROTOCOL 3

Preparation of draining lymph node suspension for analysis of skin migratory dendritic cells

Skin draining lymph nodes harbor subsets of dendritic cells that have migrated from the skin as well as lymph node resident DCs. Collagenase digestion of secondary lymphoid organs significantly increase the yield of dendritic cells over disruption by non-enzymatic means.

As with other organ systems, the dendritic cell suspension can by analyzed by flow cytometry or isolated for sequencing or functional assays.

Materials

- Collagenase D solution (see reagents below)
- Cell media (see reagents below)
- Staining media (see reagents below)
- 70% ethanol
- 3.5 cm petri dish
- 3 mL syringe

Protocol steps—*Step annotations*

1. Euthanize a mouse accordingly and gently spray with 70% ethanol.
2. Harvest the desired axillary, brachial and cervical lymph nodes. Place lymph nodes in 3.5 cm petri dish.
    a. Note that in an ear tagged mouse, the cervical lymph nodes from the draining tagged ear may be inflamed and enlarged. In this case, consideration can be given to not harvest the cervical lymph nodes.
3. Using a stainless steel mesh or via scratching the bottom of the petri dish vertically and horizontally with 25-gauge needle, disrupt the lymph nodes with the back side of a syringe plunger.
4. Add 2 mL of RPMI + 10% FBS media and 200 μL of collagenase D solution aliquots. Pipet disrupted tissue homogenously into the solution.
5. Incubate in 37°C CO_2 incubator for 1 hour.
6. Pipette tissues for disruption and transfer the suspension through a 40um filter into a 50 mL conical tube. Further disrupt tissue through the rubber plunger of a 3 mL syringe through the filter.
7. Wash with 8 mL staining media through the filter and centrifuge the 50 mL tubes at 1200 rpm for 8 minutes.
8. Decant supernatant and wash with 5 mL staining media and centrifuge as in step 7.
9. Decant supernatant and re-suspend in 1 mL staining media.
10. Count cells via trypan blue exclusion and re-suspend at desired concentration for further analysis. See Table 4 for markers of resident conventional DC subsets. Migratory DCs may be stained similarly as in Table 3.

REAGENTS AND SOLUTIONS

- Cell media

- RPMI-1640 (Lonza-12 167F)
- 10% Fetal Bovine Serum (FBS, Hyclone-SH10910)
- 1:100 HEPES (Sigma H0887)

- FACS staining media
  - 3% calf serum
  - 2 mM EDTA
  - 0.04% Azide in PBS
  - This should be stored at 4C.
- Trypsin/GNK solution for epidermal separation
  - 2.94 g NaCl (Fisher Scientific-BP358)
  - 0.14g KCl (Sigma P9541)
  - 0.34g Glucose (Sigma G5767)
  - 340 mL of PBS
  - 1g of Trypsin (Sigma T1005) added last
  - pH titrated to 7.6 with 1 M NAHCO3, filter sterilized, aliquot and store at −20°C.
- DNAse solution for epidermal and dermal isolation
  - Reconstitute DNAse powder (Sigma D5025-150KU) in PBS up to 1mg/ml.
  - Aliquot and store in −80°C.
- Dispase solution for epidermal separation and digestion
  - Dispase II from *Bacillus polymyxa* (ThermoFisher 17105041)
  - Prepare 50× solution in HBSS, sterile filtered and stored at −20°C
  - Use a working solution of dispase in cell media (2U/ml) on the day of the experiment
- Collagenase XI/hyaluronidase solution for dermal digestion (amount described is for entire dorsal skin from one individual mouse)
  - To be made fresh day of digestion:
  - 27 mg collagenase XI (Sigma C7657)
  - 2.5 mg hyaluronidase (Sigma H3884)
  - Pre-warmed 9 mL cell medium at 37C (see above)
  - 1 mL DNAse solution (see above)
- Collagenase D solution for lymph node digestion

- The specific activity of collagenase D varies by the lot and is listed by manufacturer. Dissolve the lyophilized collagenase D (Roche #11 088 882 001) to 4000 Mandl U/ml in Hanks Balanced Salt Solution (HBSS, Gibco #14170) containing Ca^{++} and Mg^{++} to a final volume calculated by:
  - ml/vial = ((specific activity given by manufacturer in Wünch U/mg) × (750 Mandl U/Wünch U) × (weight of collagenase D in mg/vial))/4000 Mandl U/ml
- Filter sterilize the clear, light-brown to pink solution and divide into 1 mL aliquots and store at −20°C.
- Ca^{++} containing HBSS is essential as collagenase D is calcium dependent enzyme.
- The amount of enzyme used may be titrated to the amount of tissue used empirically.

COMMENTARY

Background Information

Dendritic cells (DCs) are highly specialized antigen-presenting cells (APCs) that are highly dynamic, with the capacity to circulate the skin, to patrol the skin, and to migrate into regional lymph nodes (Merad et al., 2013). They are capable of sensing, processing and presenting antigens and play key roles in initiating, modulating, and resolving cutaneous inflammation. Skin DCs are a highly heterogeneous population with functionally distinct subsets that are imprinted by its development and the tissue microenvironment (Kashem et al., 2017).

One of the barriers to analysis of DC subsets is thorough and consistent digestion and isolation of tissue resident and migratory APCs. Preliminary attempts at establishing dendritic cell lines have been performed with limited success that requires specific conditions of culture (van Helden et al., 2008). Further studies of generating DCs from human and mouse bone marrow progenitors have been describes extensively. These progenitor differentiation studies utilize skewing DCs to various conventional DC lineages with cytokines such as Flt3 ligand or GM-CSF and IL-4 (Caux et al., 1992; Inaba et al., 1992; Bruno, 2007). They have allowed for studying DC differentiation as well as for generation of DCs in larger numbers for functional studies. Recently, we have appreciated that APCs have transcriptional landscapes, as well as homeostatic and immune functions, that are dictated by their tissue microenvironment(Gosselin et al., 2014; Lavin et al., 2014). Thus, analysis of DCs directly from tissue allows for appropriate delineation of these tissue specific DC function.

DCs have been isolated from nearly every mouse and human tissue (Inaba et al., 2009). First methods for isolation of skin DCs include culturing of skin explants (Schuler and Steinman, 1985). These simple methods allow for migration of Langerhans cells (LCs) and DCs into the culture, with the isolated DCs from culture undertaking a mature APC phenotype.

Enzymatic digestion allows for fast and efficient isolation and analysis of skin resident DCs that are still in its immature state from the steady state skin or mature DCs from the inflamed skin. Unlike skin explant methods, enzymatic digestion is complicated by concomitant isolation of keratinocytes, stromal cells and other leukocytes. Further purification of DCs can be achieved through column selection of CD11c positive cells through magnetic cell isolation or cell sorting.

In terms of assessing DC function, it is imperative to isolate and assess distinct DC subsets separately as they have differential roles in innate and adaptive immunity. All DCs express the markers CD45, MHC-II and CD11c. Epidermis contains Langerhans cells, which express the markers CD207, CD11b but not CD103 (Kaplan, 2010). They are involved in mediating Th17 responses to *Candida albicans* yeast and controversially play suppressive roles in the context of contact hypersensitivity and delayed type hypersensitivity(Kaplan et al., 2005; Bobr et al., 2010; Igyártó et al., 2011; Kashem et al., 2015a). The dermis contains a more heterogeneous population of DCs including migratory LCs. CD11b +CD207−CD103− conventional DC (referred as dermal cDC2) subset that make up for majority on the DC network and induce adaptive T helper 2 (Th2) responses and innate IL-17 responses in tissue(Gao et al., 2013; Kashem et al., 2015b; Kumamoto et al., 2013). CD11b−CD207−CD103− cells (referred to as DN DCs) are a smaller population of DCs that similarly induce Th2 responses while CD207+CD103+CD11b- conventional dermal cDC1 subset induce potent Th1 responses to infection and neoplasia(Tussiwand et al., 2015; Edelson et al., 2010). These cutaneous DC subsets migrate to the lymph node in distinct anatomical compartments. Alongside lymph node resident DCs, they induce T cell responses through antigen-presentation, co-stimulation and cytokine secretion. Of note, dermal conventional DCs may be distinguished from their monocyte derived counterparts and tissue resident macrophages by the lack of expression of CD64(Tamoutounour et al., 2012; McGovern et al., 2014; Tamoutounour et al., 2013) Further discussions of DC markers, transcriptional factors, function and murine models of depletion are reviewed in detail elsewhere(Merad et al., 2013; Kashem et al., 2017).

Critical Parameters

Given that this unit covers the isolation and analysis of murine dendritic cells, a few general guidelines should be followed for achievement of consistent, reproducible analysis of mouse tissue resident DCs. As corticosteroids and dehydration reduces yield, mice should not be stressed prior to and be allowed to rest for 1 week after shipment(Inaba et al., 2009). We generally only use age-matched female mice to avoid violent skin wounds that are frequently found in the male counterparts. Increased skin wounding increases the inflammatory cell response as well as augment any ongoing infection. If male mice must be used, ear and ventral skin are sites may be less likely to be wounded through fighting. Distinct skin sites may have differential leukocyte compositions at steady state and, thus, samples should be only compared to skin from the same anatomical sites. We prefer to use 7–10 week old mice as synchronous hair growth takes place during the age of life 5–7 weeks after birth(Lin et al., 2004). Hair growth may have differential recruitment of leukocyte subsets into the skin, as well as increased melanin deposition into the hair follicles(Nagao et al., 2012). Digestion of the skin during skin pigmentation leads to deposition of melanin into the cell suspension,

which could further affect instrumentation and analysis by flow cytometry. Intracellular fixation, staining and thorough filtering can effectively remove the contamination of these particles.

Enzymatic digestion of tissue has specific critical parameters of consideration. As with all enzymatic digestion, there is lot to lot variability of the enzyme activity and cell viability decreases with over-digestion. Proper enzyme concentration and digestion timing can be titrated empirically to control for the highest yield to lowest cell death. Epidermal separation and digestion requires trypsin and DNAse. It is critical for skin samples to be floated gently on the trypsin solution with the dermis side down to achieve maximal separation of the epidermis. Trypsin is a potent proteolytic enzyme that will cleave certain cell surface markers such as CD11c and CD3. While these markers may be dispensable for analysis of epidermal LCs and T cells, both of these markers are imperative for proper analysis of dermal leukocytes. Proteolytic cleavage still occurs with dispase separation and digestion but is improved in comparison to trypsinization. An alternative possibility is to harvest the skin at one site specifically for epidermal digestion and an adjacent or distinct site for whole skill collagenase digestion, avoiding any cleavage of cell surface markers on dermal leukocytes. Mechanical disruption of the dermis by mincing and of the lymph nodes by crushing before digestion will lead to increased yield, decreased digestion times and cell death.

All of the above mentioned enzymatic digestion processes will lead to isolation of non-DC components in addition to DCs. Magnetic cell separation of CD11c positive cells by MACS (Miltenyi Biotec) or EasySep (Stem Cell) kits will generate enrichment of DCs that can be further purified through cell sorting. Skin explant models can generate a purified population of migrated DCs that take on a mature phenotype(Inaba et al., 2009).

Anticipated Results

Results will vary significantly depending on the digestion method and anatomical site used. Inflammation will also alter yield as well as composition of the DC subsets. Epidermal isolation and digestion will can efficiently yield $0.5 - 1\times10^6$ total cells per mouse ear or patch of 1 cm^2 skin with 1–3% of which being epidermal LCs. The mouse dermis will hold $1–10 \times 10^6$ cells per cm^2 with 0.5–10% of cells being antigen-presenting cells depending on stromal contamination, inflammation and flow cytometry gating. Of the dermal DC network, migrating epidermal LCs account for ~6%, CD207+CD103+CD11b- dermal cDC1 account for ~3%, CD11b+CD103−CD207− dermal cDC2 account for 66% and CD207−CD103−CD11b− double negative dermal cDC account for approximately 16%. In the lymph node, these subsets account for approximately 10%, 14%, 35% and 21% respectively(Henri et al., 2010).

Time Considerations

Epidermal separation and digestion will take approximately 1.5–2 hours from euthanasia to single cell suspension for harvesting a single anatomical site per 5 mice. Dermal digestion will require 1–2 hours from euthanasia to single cell suspension per 5 mice. Lymph node digestion will take 1 hour from harvest to suspension per 5 mice.

The dermis may be digested after epidermal separation and digestion. This allows for analysis of the same anatomical site as the epidermis and separation of epidermal components for distinct analysis. However, sequential analysis of epidermal and dermal components will increase the time of experiment and cleave cellular markers such as CD11c. Alternatively, one could isolate the epidermis from the mouse ears and perform whole skin collagenase digestion concurrently from a distinct anatomical site such as the flank skin to minimize time requirements and preserve cell surface markers on the dermal DCs. Analysis of the lymph node migratory and resident cells can occur concurrently with either tissue enzymatic digestion.

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Significance Statement

Dendritic cells (DCs) are a small, but highly specialized subset of professional antigen-presenting cells (APCs) that reside in peripheral and lymphoid tissues. Skin DCs are a highly heterogeneous population with subset specific functional distinctions that are imprinted through their development and by their tissue microenvironment. Thus, appropriate investigation of DCs requires the purification of the trace amounts of APCs from tissues directly. Variety of methods have been developed for analysis of DC *in vitro* and *in vivo*. Here, we discuss methods of enzymatic digestion to isolate and analyze DCs from the epidermis, dermis and skin draining lymph nodes of mice.

Table I

Antibodies for the characterization of murine dendritic cells

	Manufacturer	Clone	Notes
CD45	Biolegend	30-F11	
CD11c	Tonbo	N418	Cleaved with trypsin
I-Ab	Biolegend	KH74	
CD207	Dendritics	929F3.01	Intracellular
CD11b	Tonbo	M1/70	
CD103	Biolegend	2E7	
CD64	Biolegend	X54-5/7.1	Identifies macrophages
CD370	Biolegend	7H11	
CD301b	Biolegend	URA-1	
CD326	Biolegend	G8.8	
CX3CR1	Biolegend	SA011F11	
CD24	Tonbo	M1/69	
CD205	Biolegend	NLDC-145	
CD172a	Biolegend	P84	
XCR1	Biolegend	ZET	
CD3e	Tonbo	145-2C11	Cleaved with trypsin

Table II

Markers for the characterization of epidermal Langerhans cells

	LC	DETC	KC
CD45	+	+	−
CD11c	+	−	−
MHC-II	+	−	−
CD207	+	−	−
CD11b	+	−	−
CD103	−	+	−
CD3e	−	+	−

Abbreviations: Langerhans cell (LC), dermal epidermal T cell (DETC), keratinocyte (KC)

Table III

Markers for the characterization of dermal dendritic cells

	mLC	CD103+ cDC1	CD11b+ cDC2	DN cDC
CD45	+	+	+	+
CD11c	+	+	+	+
MHC−II	+	+	+	+
CD8a	−	−	−	−
CD207	+	+	−	−
CD11b	+	−	+	−
CD103	−	+	−	−
CD64	−	−	−	−
CD370	−	+	−	−
CD301b	−/+	−	+	−
CD326	+	−	−	−
CX3CR1	+	−	+	+
CD24	+	+	−	−
CD205	+	+	+	
CD172a	+	−	+	+
XCR1	−	+	−	−

Abbreviations: migratory Langerhans cells (mLC), conventional dendritic cells (cDC), double negative conventional DC (DN dDC)

Table IV

Markers for the characterization of skin draining lymph node resident dendritic cells

	cDC1	cDC2
CD45	+	+
CD11c	+	+
MHC−II	+	+
CD207	+*	−
CD11b	−	+
CD103	−	−
CD64	−	−
CD370	+	+
CD301b	−	−
CD326	−	−
CX3CR1	+/−	−
CD24	+	+
CD205	+	+
CD172a	−	+
XCR1	+	−

Abbreviation: conventional dendritic cell (cDC)

Notes:

*mouse strain dependent (positive in C57BL/6 mice). Markers for migratory DCs listed in Table III.