Review Article

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Dendritic Cell Subsets in the Skin and Their Functional Role in Contact Hypersensitivity

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Objectives: Skin is a primary epithelial organ, which protects our body from the surrounding environment. Besides physical barrier machineries, skin also contains a number of immune components that actively participate in the protective immune responses. Dendritic cells (DCs) are professional antigen-presenting cells and they are essentially required for mediating innate and adaptive immunity. Although emerging studies have demonstrated distinct resident DC subsets in the skin, their subset-specific functions still need to be elucidated. **Methods:** We reviewed recent works on the DC subset discrimination and specification in the mouse skin. To understand the DC subset-dependent functional diversity, we especially focused on the murine contact hypersensitivity (CHS), an experimental model of human allergic contact dermatitis. Furthermore, we discussed our recent work on the role of epidermal Langerhans cells (LCs) in CHS. **Results:** Murine skin harbors at least three DC subsets: (i) epidermal LCs, (ii) Langerin+ dermal DCs and (iii) Langerin– dermal DCs. Using more sophisticated cell markers, recent study has described monocyte-derived DCs in the skin. The role of each DC subset in CHS was somewhat inconsistent and redundant from study to study and needs further elaborative works for the general acceptance. **Conclusion:** Continuous efforts to understand the functional diversity among each cutaneous DC subset will be needed to develop the new anti-inflammatory and anti-tumor strategies by targeting relevant skin DC population *in vivo*.

Keywords: Hypersensitivity, Contact; Dendritic Cells; Skin

Introduction

Our body is constantly exposed to a number of daily antigens and should be properly protected from their challenge. For this purpose, we also need a set of refined machineries to discriminate extra harmful insults from frequently encountered innocuous exposures. Skin is the outermost organ covering our body, which belongs to the primary epithelial tissues. Although skin is originally considered to simply function as a physical barrier against external stresses [1], there are numerous immune cells found in the skin which constitute the cutaneous immune system along with epithelial keratinocytes, providing an additional immunological barrier between our body and surrounding environments [2]. As one example, skin harbors

two times greater number of resident T cells compared to that of circulating ones in blood, which simply represents the importance of the cutaneous immune sentinel [3, 4].

Among the skin-infiltrating immune cells, there is a discrete population of dendritic cells (DCs), the cells having distinct dendritic morphologies [5, 6]. DCs possess the specialized function for antigen uptake and processing, and they present peptide antigens in conjunction with major histocompatibility complex (MHC) (peptide-MHC complex) to T cells. Due to their highly efficient antigen-presenting capacity, DCs are known as professional antigen-presenting cells [7]. In the peripheral tissues, such as skin, DCs migrate toward the draining lymph nodes after acquiring antigens, and DC-mediated antigen presentation largely occurs within the T cell areas in the secondary lymphoid organs where DCs initiate antigen-specific T cell responses [8]. Thus, the cutaneous DC network is essentially involved in sensing pathogens and generating a productive immunity. However, on the other hand, DCs may also induce unnecessary and excessive immune responses against relatively harmless antigens and stimuli that eventually mediate several inflammatory diseases, such as allergic contact dermatitis, psoriasis, and atopic dermatitis [9, 10]. Recent elegant combinatorial approaches using (1) multi-color flow cytometry, (2) high throughput bioinformatics, and (3) lineage-tracing and subset-specific DC-depleting mouse strains have enabled us to deeply understand the ontogeny of the individual DC population and its functional implications in certain inflammatory conditions [11]. Here, we reviewed our current understanding of the cutaneous DC network of the skin. We also described their functional role in the context of murine contact hypersensitivity (CHS), a prototype of delayed-type hypersensitivity in the skin.

1. Dendritic cell subsets in the skin

There are two major anatomical layers in the skin: (1) outer layer epidermis composed of epithelial cell keratinocytes and (2) underlying layer dermis composed of stromal cells and extracellular matrixes such as collagens, elastic fibers, and glycoproteins [2, 12]. Those structural layers provide a physical protection for our body against mechanical stresses. As mentioned above, a number of immune cells also locate in the skin, including T cells, DCs, macrophages, and mast cells [13]. DCs are found both in the epidermis and dermis; however, the epidermis under steady-state only harbors Langerhans cells in contrast to the dermis, which contains heterogeneous dermal DC populations and LCs in transit to the regional lymph nodes. A certain fraction of cutaneous DCs shows a continuous migration toward the draining lymph nodes in part NF-kB-dependent manner [14, 15]. This homeostatic migration of peripheral DCs is thought to be critically involved in maintaining self-tolerance and immune homeostasis [15, 16].

1.1 Langerhans cells

LCs are migratory DCs that reside in the epidermis. Although LCs were first described as neuronal cells, it is now clear that they are a member of the antigen-presenting cell family [17]. The outermost residence of LCs among the cutaneous DC subsets may be evolutionally associated with their primary immune-surveillance of the skin surface where they directly contact with the environmental phase [18]. Accordingly, LCs extend their dendrites toward the breaches of epidermal barrier and actively uptake exterior antigens [19]. By doing this, LCs can induce a strong humoral immunity which confers our body to be protected against certain pathogenic microbial challenges [20]. However, this immunological process is also involved in particular allergic inflammations of the skin including atopic eczema [21]. Thus, further molecular and genetic differences in LCs between healthy and inflammatory conditions need to be addressed to explain their molecular mechanistic role in atopic dermatitis patients [22].

Unlike other cutaneous DC populations, which are continuously replaced by bone marrow-derived precursors entering the skin (see below), LCs have distinct developmental and homeostatic properties. LCs are derived from skin-infiltrating myeloid precursors that seed fetal epidermis at the late stage of embryogenesis [23]. After birth, LC precursors actively proliferate and start to gain mature LC markers such as MHC-II and Langerin within the first week of postnatal period [23, 24]. Subsequent elegant lineage-tracing study showed that those LC precursors derive from at least two cellular origins, embryonic fetal liver monocytes and yolk-sac macrophages [25]. After completion of epidermal LC network formation, LCs maintain their cellular pool by slow self-turnover without any blood precursor input [26, 27]. LCs also demonstrate a unique cytokine dependency for their development and homeostasis different from those of other conventional DCs. Although most of conventional DC homeostasis largely depends on Fms-like tyrosine kinase 3 (Flt3)

ligand cytokine signaling, LCs are independent of Flt3 ligand for their homeostasis and development [28]. Rather, similar to tissue-resident macrophages, LC development strictly depends on colony-stimulating factor 1 (CSF-1) signaling [29]. Recent studies have revealed that IL-34 was specifically expressed in the stromal components of skin and brain, and IL-34 was an actual ligand for CSF-1 receptor that supports LC and brain microglia development *in situ* [30, 31]. Furthermore it has been shown that circulating monocytes could transiently differentiate into short-lived LCs in the inflamed skin; however, LCs found in the long-term steady state after inflammation would be reconstituted by bone marrow-derived progenitors of unknown origin [29, 32]. Another major cytokine that supports LC development and homeostasis is transforming growth factor beta 1 (TGF-β1). TGF-β1 null mice showed a lack of epidermal LC, and both LC autocrine and paracrine sources of TGF-β1 were essential for LC homeostasis [33, 34]. Interestingly, TGF-β1 signaling on LCs is not only involved in LC development but also in actively suppressing homeostatic LC migration out of the epidermis [35]. TGF-β1 dependency in LCs has further been supported as a result of defective LC development in Id2, Runx3, and PU.1 knockout mouse strains, the genes that are TGF-β1-associated signaling transcription factors [36-38].

We have recently described that one of the genome structure-organizing DNA-binding proteins CCCTC-binding factor (CTCF) has a critical role for the quantitative maintenance of LCs by positively regulating their homeostatic proliferation [39]. Functionally, CTCF-depleted LCs revealed migration defects both in the steady and inflammatory state due to increased cell adhesion genes expression. Our results suggest that epigenetic regulation programs may tightly control the LC homeostasis and function, which would be an exciting area of DC biology study in the future.

1.2 Langerin+ and Langerin– dermal dendritic cells

Dermal DCs are composed of the heterogeneous populations that can be classified into two major groups according to the differential cellular marker Langerin expression, Langerin+ and Langerin– dermal DCs [40]. Langerin/CD207 is the C-type lectin receptor that had been previously considered as a hallmark of epidermal LCs [40]. However, a recent elegant series of study have demonstrated a distinct conventional DC population expressing Langerin other than LCs in murine dermis [41-43]. Dermal Langerin+ DCs differ from epidermal LCs as Langerin+ dermal DCs are radiosensitive, dependent on Flt3 ligand for development and homeostasis, and continuously replenished by bone marrow-derived committed DC progenitors. Langerin+ dermal DCs are also known as one of the CD103+ peripheral DC population in non-lymphoid organs, which shows close developmental relationship with lymphoid CD8α+ DCs. Both CD103+ non-lymphoid and CD8α+ splenic DC development is regulated by interferon regulatory factor 8 (IRF8) and basic leucine zipper transcription factor ATF-like 3 (Batf3) [44, 45]. Langerin+ dermal DCs specifically express XC-chemokine receptor 1 (XCR1), and recent study showed that XCR1+Langerin+ dermal DCs could be divided into CD103+ and CD103– subsets [46, 47]. Langerin+CD103+ dermal DCs possess a highly efficient cross-presentation capacity of viral, fungal and self-antigens compared to other DC subsets in the skin [46, 48, 49]. In line with this, recent study has demonstrated that tumor-infiltrating CD103+ DCs are a superior cytotoxic T cell-stimulating subset [50].

Langerin– dermal DCs can be divided into the most abundant CD11b+ DC subset and small population of CD11b– subset. The precise role for Langerin– dermal DCs has not been well understood because of much greater heterogeneity among CD11b+ dermal DC populations and a lack of population-depleting mouse system until recently. However, recent study has demonstrated that a selective expression of macrophage galactose-type C type lectin 2 (Mgl2/CD301b) within Langerin– dermal DCs, and depletion of CD301b+ dermal migratory DCs resulted in reduced Th2-type immune responses [51]. In addition, CD301b+ DCs require IRF4 transcription factor for inducing Th2 cell responses *in vivo* [52]. However, IRF4 is dispensable for dermal CD301b+ DC development, rather it seems to regulate the existence of CD301b+ DCs in the skin draining lymph nodes, suggesting the possible importance of IRF4-dependent CD301b+ DC migration in Th2-type immunity [52, 53]. Furthermore, CD301b+ DCs need extra requirements for inducing Th2 immune responses, which have not been elucidated so far [51]. In other barrier tissue such as lung, it has been shown that IRF4-dependent CD11b+ DCs control lung mucosal IL-17 immune responses [54]. In line with this finding, recent study demonstrated that CD301b+ dermal DCs are a critical dermal DC subset that mediates resistance to cutaneous *Candida albicans* infection by releasing IL-23, which in turn activates γδ T cells to produce protective cytokine IL-17A [55]. Thus

CD301b+ dermal DCs are differentially involved in the different types of cutaneous immune responses in a context-dependent manner. A minor CD11b− dermal DC population has not been well investigated so far compared to other DC subsets. Although recent study has demonstrated the role of transcription factor Kruppel-like factor 4 (KLF4) in CD11b− population development or homeostasis in skin-draining lymph nodes, CD11b− DC development was relatively intact in the dermal skin [56]. KLF4-dependent migratory DC subsets were required for various Th2 cell responses, and interestingly, KLF4 regulated IRF4-expressing classical DC populations, indicating a close relationship between IRF4- and KLF4-dependent classical DC development pathways. The precise role of dermal CD11b− DCs in cutaneous immune responses needs to be further elucidated.

1.3 Monocyte-derived dendritic cells

It has long been believed that monocytes continuously differentiate into DCs in the peripheral tissues like DC progenitors [57]. In the inflammatory conditions, monocytes can be converted into CD209a+ DCs, which localize to T cell areas and induce T cell activation [58]. However, until recently monocyte-to-DC conversion in the steady-state skin has not been that clear [59]. Recent elegant study has dissected a complex mononuclear phagocyte system in the skin using sophisticated flow cytometry and systems biology approaches. The researchers showed that dermal skin contains a discrete population of monocytes, monocyte-derived DCs, and macrophages based on their different CD64 and CCR2 expression [11, 60]. Interestingly, monocyte-derived DCs revealed an intermediate gene expression signature between that of dermal monocytes and CD11b+ dermal DCs, suggesting a monocyte to DC transition in the skin. Although dermal monocyte-derived DCs are capable of migrating to local lymph nodes and T cell stimulation, those essential immunological features defining 'True' DC lineage are less prominent than those of classical CD11b+ dermal DCs [60].

2. Functional role of cutaneous dendritic cell subsets in murine contact hypersensitivity

CHS is an experimental model of human allergic contact dermatitis [61]. CHS is induced by haptens, which are small molecules that bind to host carrier proteins to become antigenic [62]. Haptens are recognized by several innate molecular immune components such as toll-like receptors, NOD-like receptors, ATP receptors, and reactive oxygen-species [62, 63]. Those activated innate immune systems can activate the inflammatory cascades, which contribute to DC-mediated adaptive immune responses against haptens. Cutaneous DCs acquire haptenated proteins and migrate to the regional lymph nodes where they present peptides to antigen-specific naïve T cells. Priming and activation of antigen-specific T cells leads to their differentiation into CD4+ Th1/Th17 cells and CD8+ cytotoxic T cells. Subsequent introduction of the same hapten results in skin-homing of the differentiated effector T cells, and they produce effector cytokines such as interferon-gamma (IFN-γ) and IL-17A by interacting with cutaneous DCs in situ [64], which stimulate keratinocytes or other surrounding cells to develop skin inflammation [65].

2.1 Langerhans cells and Langerin+ dermal dendritic cells in contact hypersensitivity

LCs had been the most extensively studied cutaneous DC subset until dermal Langerin+ DCs were characterized [66]. LCs are strong antigen-presenting and T cell-stimulating DCs in the *in vitro* system, and these cardinal properties led us to consider that, LCs were solely immunogenic [67, 68]. However, the resultant CHS studies using genetically engineered LC-ablating mouse strains have dramatically changed our longstanding concepts on LCs [69, 70]. The use of diphtheria toxin-induced LC-ablating mouse strains (diphtheria toxin receptor expression driven by murine langerin promoter; muLangerin-DTR) has demonstrated that CHS responses were comparable or only slightly reduced after LC depletion [71, 72]. At that moment, these surprising results suggested that LCs were dispensable for CHS and rather that other DC subsets in the skin would contribute to T cell activation. As mentioned above, subsequent discovery of Langerin+ dermal DC population has provided a relevant explanation on these findings. Diphtheria toxin administration 1 to 3 days before hapten sensitization led to a depletion of all cutaneous Langerin+ DC subsets including LCs and dermal Langerin+ DCs [41, 73]. In this condition, one study demonstrated that CHS responses were reduced both in a low-dose and high-dose hapten sensitization protocol [73], but another group reported that Langerin+ subsets were required only in a low-dose hapten concentration [74]. The timed diphtheria toxin injection 7 to 13 days before sensitization resulted in muLangerin-DTR mice only lacking LCs while some degree of Langerin+ dermal DCs reappeared due to different

repopulation kinetics between LCs and Langerin+ dermal DCs after acute depletion [41, 73]. However, there were also inconsistent CHS outcomes in this experimental setting, demonstrating the differential requirement of LCs between low-dose and high-dose hapten protocols [73, 74]. By using an elegant bone marrow chimera approach, it has been shown that Langerin+ dermal DCs were not essentially involved in CHS sensitization [74]. In line with this, Batf3 knockout mice, which constitutively lack Langerin+ dermal DCs, revealed similar CHS responses compared to wild-type mice [45]. Other studies using constitutive LC-deficient mouse strains mediated by a murine Langerin promoter-driven conditional gene knockout system have demonstrated that LCs are actually required for optimal CHS induction [75, 76]. Taken together, those key studies indicate that there is a functional compensation and/or redundancy between LCs and Langerin+ dermal DCs in CHS settings, and both LCs and Langerin+ dermal DCs are somehow involved in CHS induction.

Other constitutive and inducible LC-depleting mouse strains, which utilize human Langerin promoter to express transgenes (huLangerin-DTA and huLangerin-DTR), have produced an opposite result for the role of LCs in the CHS model [69, 70]. CHS responses were enhanced in those mouse strains, indicating a tolerogenic role of LCs [77, 78]. LC-mediated suppressive effect in CHS depends on the LC-driven IL-10 and interaction with cognate CD4+ T cells [79]. Another research group generated LC-specific EpCAM-deficient mice using human Langerin promoter-driven Cre transgenic mice [34], which revealed a defective LC migration *in vivo*. Interestingly, LC migration defect led to enhanced CHS responses, indicating their suppressive role in the priming phase [80]. Our group employed the same Cre-expressing transgenic mice to generate LC-specific CTCF gene knockout mice [39]. LC-specific CTCF-ablated mice showed several features of the reduced number of epidermal LCs and defective migration, which led us to test the role of LCs in the CHS model. In this mouse stain, we found more sustained and exaggerated ear swelling responses in line with higher IFN-γ-producing CD8+ T cell priming in the regional lymph nodes. These data suggest that proper and sufficient LC migration may be an important factor for restraining an excessive cross-priming in the CHS model. However, CTCF-ablated LCs also differentially expressed a large number of inflammation-associated genes compared to wild-type LCs, which possibly affected the enhanced CHS phenotype in this tested strain [39].

In conclusion, the exact role of LCs and Langerin+ dermal DCs in CHS is still controversial probably due to: (1) different mouse strains tested, (2) different type and concentration of haptens for inducing each CHS model and (3) a relatively high variance in ear thickness measurement. More unified or reliable readouts will be needed to directly compare the different CHS studies to draw a general acceptance in the near future.

2.2 Langerin– dermal dendritic cells in contact hypersensitivity

The functional role of Langerin– dermal DCs in CHS has not been much appreciated so far since they are a heterogeneous population. Recent study has described that CD301b was selectively expressed within the Langerin– DC compartment in the dermis and skin-draining lymph nodes [51, 81, 82]. The CD301b+ dermal DC population showed relatively fast migration kinetics after hapten painting compared to other DC subsets, which are also demonstrated in the Langerin–CD103– dermal DC population [39, 81]. Interestingly, CD301b+ dermal DCs are functionally sufficient to elicit Th2-type CHS responses [82]. Selective depletion of CD301b+ dermal DCs led to an impaired Th2 cell development under Th2-skewing adjuvant injection and hookworm-mediated Th2 immunity models, indicating that this unique population is a critical mediator of allergic inflammation *in vivo* [51]. Thymic stromal lymphopoietin (TSLP) is a pro-allergic cytokine largely secreted by epithelial tissues and it activates local DCs to elicit strong Th2 differentiation [83]. Recent study has described a TSLP-responsive CD11b+ DC subset, which concurrently expresses CCL17 [84]. This finding indicates that a certain population within CD11b+ DCs actively mediates TSLP-dependent Th2-type immune responses, however, the association between CD301b+ DCs and TSLP-responsive CD11b+ DCs has not been examined so far.

2.3 Monocyte-derived dendritic cells in contact hypersensitivity

Currently, the role of monocyte-derived DCs in CHS has not been properly examined. Although recent elegant study demonstrated that antigen-acquiring dermal monocyte-derived DCs can migrate to local lymph nodes under the inflammatory state, CD11b+ dermal DCs have much greater migratory potential [60]. Furthermore, migratory monocyte-derived DCs were less efficient in hapten-specific T cell stimulation, suggesting their

minor contribution in CHS induction. However, future studies are needed to evaluate whether monocyte-derived DCs are virtually immunogenic or tolerogenic in CHS and other allergic skin inflammation models [85].

Conclusion

In this review, we have introduced our current knowledge on the cutaneous DC network and have provided its functional role under the CHS model. However, each cutaneous DC subset displays a different immunological property according to the applied model systems. Therefore, continuous effort is certainly needed to understand the underlying functional diversity of cutaneous DCs. This is not only important for understanding the pathogenesis of particular inflammatory skin disorders, but also vital for developing future anti-tumor and vaccination strategies by modulating or targeting appropriate DC subsets *in vivo*.

Conflict of Interest

The authors state no conflict of interest.

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