

Cutaneous dendritic cells

Jenny Valladeau^{a,*}, Sem Saeland^b

^a *Université Claude Bernard Lyon I, Dermatologie-Pavillon R, EA 3732,
Hopital Ed Herriot, Pav R, 69437 Lyon cedex, France*

^b *INSERM U503, IFR128, Lyon, France*

Abstract

Cutaneous dendritic cells (DC) include epidermal Langerhans cells (LC), interstitial/dermal dendritic cells (DDC), as well as plasmacytoid DC (pDC) that occur under pathological conditions. These immune cells have a spectrum of different functions with implications that extend far beyond the skin. They have the potential to internalize particulate agents and macromolecules, and display migratory properties that endow them with the unique capacity to journey between skin and draining lymph nodes where they encounter antigen-specific T lymphocytes. Herein, we will review the features of human and mouse cutaneous DC, emphasizing characteristics representative of their life-cycle stages that occur within the skin.

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1. Introduction

The skin is the largest organ of the body and has many different functions. The most superficial layer of the skin, the epidermis, provides a first barrier of protection from invasion of foreign substances into the body. The dermis, separated from the epidermis by the basement membrane, assumes the important functions of thermoregulation and supports the vascular network to supply the avascular epidermis with nutrients. The hypodermis, also called subcutaneous connective tissue, stores adipose tissue.

Immune cells of the epidermis and dermis participate in the defense against pathogens, in particular through a network of cutaneous dendritic cells (DC), which are key for sensing danger and initiating both innate and adaptive protective responses. Two main populations of DC occur in normal skin: epidermal LC characterized by the expression of Langerin/CD207, and DDC in the dermis characterized in humans by DC-SIGN/CD208 and in the mouse, by expression of CD11c and exclusion of macrophage markers such as F4/80.

It is quite remarkable that, as early as 1868, Paul Langerhans discovered the stellate-shaped epidermal cells that now bear his name [1]. However, 100 years were subsequently needed to establish the fact that Langerhans cells (LC) are potent antigen-presenting cells belonging to the leukocyte system [2]. In parallel, the pioneering work of Steinman and Cohn leading to the identification of splenic DC [3] established the basis for connecting LC to the emerging DC system. One common feature of DC is their remarkable ability to present protein-derived peptide antigens (Ag) to naïve T lymphocytes [4]. For that reason, DC are referred to as “professional antigen-presenting cells” or “Nature’s adjuvant”.

Beyond their shared features, the great heterogeneity and complexity of the DC system soon became apparent. Skin DC are no exception, and while much knowledge has been gathered on these cells, many fundamental questions remain unanswered. Important limitations of the traditional view of epidermal LC life-history, known as the “Langerhans cell paradigm” have recently been pointed out [5,6]. Furthermore, there is increasing evidence that skin DC may be specialized in the presentation of non-peptide Ag, in the context of molecules of the CD1 family [7]. Finally, due to their anatomical proximity, heterogeneity and plasticity, it is important to note that only a very partial picture emerges as

* Corresponding author. Tel.: +33 4 72 11 02 82; fax: +33 4 72 11 02 90.
E-mail address: valladeau@lyon.inserm.fr (J. Valladeau).

to the relative contribution of the different skin DC subsets in shaping the immune response. This current limitation and future challenge will be discussed as concluding remarks of the present review.

Taken together, cutaneous DC are involved in a number of pathologies, including infectious and parasitic diseases transmitted through the skin, T lymphocyte-mediated dermatoses, and skin cancers. This will undoubtedly fuel future research to gain a more thorough understanding of their biology, in order to select skin DC as targets for rationale drug discovery.

2. Epidermal Langerhans cells

2.1. Phenotype of epidermal Langerhans cells

The phenotype of LC is consistent with their origin and function. First, LC represent a leukocyte population expressing CD45 and the myeloid markers CD33 and CD13, while lacking CD3, CD19, CD20, CD14, CD16 and CD56. Although not restricted to the DC lineage, human LC display CD1a, a molecule also found on cortical thymocytes (Table 1). LC express CD11c, adhesion molecules such as β 1 integrins, CD44, CD54, CD15s, CLA (cutaneous lymphocyte associated protein), and E-cadherin which is also found on keratinocytes and mediates homotypic adhesion between these two cell types [8]. To internalize Ag, LC feature a number of C-type lectins which are able to bind sugar moieties present on micro-organisms. The type-II lectin Langerin/CD207 unequivocally identifies both human and mouse LC in epidermis [9,10]. LC also express dectin-1, a C-type lectin capable of glucan and zymozan uptake [11]. Mouse LC express the type-I lectin DEC205, in contrast to human LC that only exhibit low levels of this molecule which is up-regulated during further maturation [12] (Table 1). Neither human nor mouse LC express the macrophage mannose receptor (MMR) [13]. LC express Fc γ RII/CD32, high and low-affinity receptors for IgE, and IgE binding protein (EBP) implicated in allergen uptake. Finally, LC are potent antigen-presenting cells (APC) and, as components of this machinery, express MHC class I and class II molecules, and the invariant chain Ii/CD74.

In mice, many studies have characterized LC following skin application (“painting”) of FITC as a tracer. Such studies have analyzed the phenotype of FITC⁺ cells subsequently reaching draining lymph nodes (LN), but most often without discriminating their LC or DDC nature. In this context, differential expression of DEC205/CD205, CD11b, and CD4, have been used to define different DC subpopulations in LN draining the skin [14–17]. It has also been shown that LC up-regulate the CD8 α chain after migration to the LN [18]. The identification of mouse Langerin/CD207 has now made it possible to unequivocally define skin LC-derived cells [19,20] (Table 1).

Table 1
Useful markers of human and murine cutaneous DC

	Human		Mouse	
	LC	DDC	LC	DDC
MHC-class II	++	++	++	++
CD1a	++	ss	nd	nd
CD1c	+/-	+	nd	nd
CD1d	-	+	-	+
CD45	+	+	+	+
T cell				
CD4	+	+/-	nd	nd
CD8 $\alpha\beta$	-	-	nd	nd
CD8 $\alpha\alpha$	nd	nd	*	nd
Adhesion				
CD11b/CD18	+/-	+	+	-
CD11c/CD18	+	+	++	++
E-cadherin	+	-	+	-
Lectins/scavenger receptors				
CD205/DEC205	+	+	+	+
CD206/MMR	-	+	-	+
CD207/Langerin	++	-	++	-
CD209/DC-SIGN	-	++	nd	nd
DC-ASGPR/mMGL	-	+	-	+
CD36	-	+	nd	nd
Fc receptors				
CD16/Fc γ RIII	-	-	+	+
CD32/Fc γ RII	+	+	+	+
CD64/Fc γ RI	-	+	+	+
Intracytoplasmic				
Factor XIIIa	-	+	nd	nd
CD208/DC-LAMP	*	*	-	-
hCD68/mMacrosialin	+/-	+/-	-	-
ATPase	++	-	++	-
Chemokine R				
CCR6	+	-	+/-	+
CCR7	*	*	*	*
CCR2	-	+	+	nd

(* up-regulated during maturation; (nd) not defined; (ss) defines two subsets. (LC) Langerhans cells; and (DDC) dermal dendritic cells.

2.2. Langerhans cells and lessons from genetically modified mice

LC constitute a dense network located in the basal and supra-basal layers of the epidermis [21]. They represent 2–4% of the total epidermal cell population and their density varies from 200 to 1000 cells/mm² depending on the anatomical area. At the ultra-structural level, LC feature a unique cytoplasmic organelle as their hallmark. This organelle, known as the Birbeck granule (BG), presents as a linear or curved rod, often with a so-called “tennis racket” shape when one of its ends is in continuity with a vesicle. In mice and humans, the C-type lectin Langerin/CD207 is responsible for BG formation and thus a key marker of the LC lineage [22]. In addition to LC, a population of BG-negative/Langerin⁻ DC, known as IDEC (inflammatory dendritic epithelial cells) has been described in epidermis of patients with chronic inflammatory diseases [23].

The generation of genetically modified mice has provided useful information about LC organization, ontogeny, and function. For example, the LC network can be visualized

in live tissue in transgenic mice expressing the fluorescent protein GFP under control of an MHC class II promoter [24]. Moreover, TGF- β knock-out (KO) mice have demonstrated the key role of TGF- β in LC ontogeny as those mice are devoid of LC [25]. In line with this finding, no LC are observed in mice lacking Id2, a transcription factor induced by TGF- β , and whose invalidation also results in NK deficiencies and abnormal development of LN and Peyer's patches [26]. Analysis of mice genetically disrupted for IRF2, a member of the IRF family, have shown that this transcription factor is partly involved in LC differentiation as the number of LC is decreased in such mice, which also present defective NK cell development [27]. It has also been shown that a murine thymic progenitor population can give rise to LC in vivo [18,28], and that human LC-like DC can be obtained from CD34⁺CD7⁺CD45RA⁺ cells which have lymphoid potential [29]. Taken together, these studies demonstrate that LC can originate from a lymphoid progenitor. However, PU1 which is implicated in myeloid differentiation, positively regulates human LC differentiation, and emphasizes the complex interplay between lymphoid and myeloid LC differentiation [30].

Finally, Langerin/CD207 KO mice are devoid of Birbeck granules, demonstrating its indispensable role for the formation of these LC organelles. Yet, a number of LC functions appear to be unaltered in those mice [31].

2.3. Origin of Langerhans cells: the question of a resident skin precursor

The haematopoietic origin of LC was clearly established using bone marrow transplantation [32,33]. Nevertheless, for ethical reasons most of our knowledge on LC differentiation in humans is derived from in vitro studies. In 1992, Caux et al. demonstrated that CD34⁺ progenitors cultured with GM-CSF and TNF- α differentiate into DC with one subset related to LC [34]. Furthermore, several precursors that can differentiate into LC in vitro have been described in adult human peripheral blood: (i) CD34⁺ cells expressing the skin-homing receptor CLA, cultured with GM-CSF/TNF- α [35]; (ii) the CD1a⁺/CD11c⁺/CD14⁻ subset of human blood DC, cultured with GM-CSF/TGF- β /IL-4 [36]; and (iii) CD14⁺ monocytes, cultured with either GM-CSF/TGF- β /IL-4 [37] or GM-CSF/TGF- β /IL-13 [38].

The existence of a skin-resident LC precursor was brought up by a study of Larregina et al., who described dermal CD14⁺ cells that already express Langerin and are able to acquire features of LC when cultured with TGF- β [39]. Although previous studies in vitro had shown that commitment to LC differentiation was already established at the level of circulating DC precursors early in ontogeny [40], later reports strongly suggested that LC differentiation could depend on the cytokine environment once LC precursor cells had entered the conjunctive tissue of the skin. Transcriptional processes involved in the regulation of lineage fate decisions of putative common DC progenitors remain poorly defined, as is our knowledge of the identities of human DC precursors in vivo.

Mechanisms of LC colonization of the epidermis have been studied in the mouse. In particular, Merad et al. have shown that in lethally irradiated mice that had received bone marrow transplants, LC of host origin remained for at least 18 months, whereas DC in other organs were almost completely replaced by donor cells within 2 months. In parabiotic mice with separate organs, but a shared blood circulation, there was no mixing of LC. However, in skin exposed to ultraviolet light, LC rapidly disappeared and were replaced by circulating LC precursors within 2 weeks. These results indicate that under steady-state conditions, LC are maintained locally and may replicate in the epidermis, but that inflammatory changes in the skin result in their replacement by blood-borne LC progenitors [41].

3. Dendritic cells in the dermis

3.1. Interstitial/dermal DC: are they very different from macrophages?

The dermis is primarily composed of extracellular matrix (ECM), and contains fibroblasts, dendritic cells, macrophages, mastocytes, and infiltrating T lymphocytes. Like their LC counterparts in the epidermis, dermal DC (DDC) present in the ECM are able to alert the immune system when receiving a danger signal. Located at the level of the capillaries and in the higher part of the reticular human dermis, DDC also called type-I dendrocytes, are divided into various subpopulations according to their expression of CD1a and CD14 [42]. In addition to their dendritic morphology, one of their common characteristics is the expression of MHC class II molecules, the scavenger receptor CD36, and the coagulation factor XIIIa (FXIIIa). However, these three markers are not DC-specific because they are also a feature of macrophages. In humans, expression of the lectin DC-SIGN/CD209 distinguishes DDC from other dermal cellular populations [43,44], although it should be pointed out that DC-SIGN may also be observed on macrophages under particular circumstances [45]. Two subpopulations of human DDC can be characterized by their phenotype: one is CD1a^{high}/CD4⁺/DC-SIGN⁻/macrophage mannose receptor (MMR, CD206)⁺⁺/CD14⁻/CD16⁻, while the other is CD1a^{low}/CD4⁺/DC-SIGN⁺/MMR⁺/CD14⁺/CD16⁻ [44]. In this context, Nestle et al. have showed that CD14⁺ DDC are weaker allogeneic stimulators in a manner comparable to macrophages, suggesting a common differentiation pathway for these subpopulations [42]. Notably, Langerin/CD207 is not expressed by DDC [44].

In mice, Cooper et al. have shown that a population of DC, which is MHC class II⁺/CD11b⁻, and distinct from macrophages (lacking phagolysosomes) is able to induce T cell activation [46,47]. However, Dupasquier et al. have recently shown that this DC subset only accounts for a small fraction of the total dermal CD45⁺ cells, and that the vast majority of the dermal CD45⁺ leukocytes represent

macrophages, as identified by mMGL, F4/80, and CD11b expression [48].

3.2. *Plasmacytoid DC: a new dermal DC subset in inflammatory conditions*

Plasmacytoid DC (pDC) are of lymphoid origin, and also known as natural interferon-alpha/beta-producing cells [49]. Recently, pDC were described to occur in a cutaneous localization. Indeed, pDC are present in skin in atopic dermatitis, contact dermatitis, and psoriasis [50,51]. pDC are also found in SLE1-associated skin lesions [52], and immature pDC have been described in situ in primary melanoma [53]. The existence of a rare leukemic counterpart of pDC is established, and such patients suffer from many cutaneous lesions [54]. Finally, two studies in mice and humans [12,55] have described pDC in normal skin, although at very low frequency. Interestingly, pDC appear more sensitive than myeloid skin DC to some danger signals, leading to the production of type-I interferons via ligation of toll-like receptors (TLRs) [56].

Whether pDC can migrate to skin to encounter antigens remains to be investigated. Little is known about pDC trafficking in vivo. Yet, some clues into the mechanisms accounting for their recruitment into skin may be provided by the facts that: (i) pDC can express CCR6, the receptor for CCL20 responsible for homing of Langerhans cells to the skin [54,57]; (ii) pDC express CXCR3 whose ligands are highly expressed in inflammatory skin [58,59]; and (iii) pDC express ChemR23 which binds chemerin, expressed on dermal inflamed vessels [60].

4. Antigen recognition and capture by cutaneous dendritic cells

As a consequence of their physical location at the interface of the organism and its external environment, cutaneous DC are well endowed with mechanisms enabling recognition and capture of antigens.

4.1. *Pathogen recognition by C-type lectins*

The numerous cell-surface Ca^{++} -dependent (C-type) lectins expressed by DC represent pattern recognition receptors (PRR) by which they decipher particular glycosylation patterns enabling their uptake of micro-organisms, cell fragments, or individual molecules [61]. Dermal DC express the lectins MMR/CD206 and the DC-asialoglycoprotein receptor (ASGPR)/mMGL for antigen capture [62]. DDC also express the C-type lectin DC-SIGN which binds a large variety of microorganisms such as HIV, Dengue virus, hepatitis C virus, CMV, Ebola virus, *Helicobacter pilori*, *Leishmania* amastigotes, and *Schistosoma mansoni* egg antigens [63]. The C-type lectin Langerin/CD207, a hallmark of epidermal LC, captures glycolipids derived from *Mycobacterium leprae*,

bind HIV gp120 and a LewisX-related sequence [7,64,65]. Human and mouse LC express Dectin1, the β -glucan receptor [66,67], which may have a role in Toll-like receptor (TLR)-independent activation of LC [68]. Finally, although the C-type lectin DEC205/CD205 is extensively used as a marker of mouse DC subsets and a target receptor to induce T cell immunity or tolerance, its function as a Pathogen Recognition Receptor is not fully understood [69–71].

4.2. *Pathogen recognition by Fc, complement, and scavenger receptors*

Mouse LC express $\text{Fc}\gamma\text{RI}$ (CD64), $\text{Fc}\gamma\text{RIII}$ (CD16) and $\text{Fc}\gamma\text{RII}$ (CD32) receptors for the Fc portion of immunoglobulins [72–74]. Human LC express $\text{Fc}\gamma\text{RI}$ and $\text{Fc}\gamma\text{RII}$ although no calcium influx is induced after triggering those receptors [75]. Human LC express $\text{Fc}\epsilon\text{RI}$ (CD23) which is involved in atopic disease [76,77]. Human cutaneous DC express the complement receptor CR3 [78], but neither CR1 nor CR2 [42,79,80].

Scavenger receptors (SR) are cell surface glycoproteins defined by their potential to bind chemically modified low-density lipoproteins. Recently, SR were found to play an important role in host defense as they are implicated in the internalization of various bacteria. Human DDC express CD36 (a class B-SR), involved in the uptake of apoptotic bodies and diacylglycerides [81,82], and LOX-1, which binds heat-shock protein [83].

4.3. *Endocytosis of macromolecules, latex beads, and microorganisms*

Epidermal LC were long believed to display low endocytic activities. Because of their low ability to take up antigens, and despite their high MHC class II expression, LC were not considered APC. This idea lasted until phagocytic LC were shown to be precursors of some DC found in lymphoid organs, and bone marrow-derived DC at an early stage of their development were demonstrated to internalize particulate antigens [84]. However, most studies on the endocytic capacity of DC have been performed using monocyte-derived DC in humans, or bone-marrow (BM) derived DC in mice, and very few reports have demonstrated endocytosis by freshly isolated skin DC.

Both LC and DDC efficiently perform receptor-mediated endocytosis and macropinocytosis as assessed by uptake of FITC-OVA/dextran or Luciferase Yellow [85–88]. Freshly isolated human and mouse LC also phagocytose 0.5 to 1 μm latex beads, but in contrast to macrophages, LC have a lower capacity to internalize larger particles such as beads of 3.5 μm . Reis e Sousa et al. have shown that isolated mouse LC internalize *Saccharomyces cerevisiae*, *Staphylococcus aureus*, *Cryptosporidium parvum*, and the yeast wall compound zymosan [89]. In addition, human DDC but not LC are able to internalize and process *Borrelia burgdorferi*, a spirochete responsible for Lyme disease

[90]. Mouse LC internalize parasites such as *Leishmania major*, but do not appear to be the DC subset inducing a protective immune response, a function rather associated with DDC [19,91,92]. In this context, the C-type lectin DC-SIGN/CD209, expressed by dermal DC, discriminates between species and life cycle forms of *Leishmania* [93].

Taken together, many micro-organisms are recognized and internalized by cutaneous DC but whether LC or DDC are responsible for the subsequent immune response is largely unknown.

4.4. Internalization of cellular apoptotic bodies

It has been suggested that LC induce a state of tolerance by presenting self antigens of apoptotic bodies from cells dying within the epidermis. Indeed, mouse LC that have migrated out of skin explants, contain melanosomes and apoptotic bodies as assessed by electron microscopy analysis [94]. Furthermore, vaginal epithelial cells undergoing apoptosis during the estrous cycle have been observed within murine LC [95]. Similarly, in the rat, an immature population of DC in mesenteric LN contains epithelial apoptotic corpses and may represent LC which have recently migrated from the intestine [96].

In humans, LC have been found to contain cellular fragments as a consequence of UV-induced skin erythema [97]. In vitro, CD34-derived LC have been shown to internalize an apoptotic melanoma cell line, but with lower capacity than monocyte-derived DC [98]. Indeed, human monocyte-derived DC which represent the closest in vitro counterpart to DDC, efficiently internalize apoptotic and necrotic bodies derived from T and B cell lines [99], virus-infected apoptotic monocytes [100,101], or tumor cell lines, including melanoma [102–104], squamous cell carcinoma [105] or kidney adenocarcinoma cells [106]. Finally, mouse BM-derived DC engulf apoptotic bodies derived from fibroblasts [107], allogeneic B cells [108] or macrophages infected with *Salmonella typhimurium* [109].

5. Migration of cutaneous dendritic cells

LCs constantly monitor the epidermal microenvironment by taking up antigen and processing it into fragments that can be recognized by effector cells of the adaptive immune response. Because of their migratory ability, LC can transport antigen from the epidermis to regional lymph nodes, where they enter *via* the afferent lymphatic vessels to initiate systemic immune responses. The mechanisms of LC trafficking thus seem to be of particular relevance for the induction and maintenance of cutaneous immunity. In this context, the use of gene-deficient mice is very useful to decipher the migration process of LC, although experiments using FITC painting or crawl-out cells from total skin explants may not elucidate whether migratory cells represent LC or DDC.

- First, it is now clear that TNF- α and IL-1 β induce migration of LC out of the epidermis. In this context, many compounds which induce TNF- α release by surrounding epidermal keratinocytes have a similar effect on LC migration [110–112].
- Second, distinct pairs of chemokine receptors (CCR6 and CCR7) control the migration from blood/dermis to epidermis and from there to the regional lymphatics respectively. Indeed, CCR7-null mice show impaired migration of LC to the draining LN [113,114]. Nevertheless, the relative contribution of CCL21/6CKine/SLC and CCL19/MIP3 β /ELC, the two ligands of CCR7, is not precisely known [115], although Robbiani et al. have shown that CCL19 is a key chemokine for LC migration [116]. As it was demonstrated for CCR7 which also permits steady state migration of LC, CCR6-EGFP mice display very low levels of this chemokine receptor on LC compared to myeloid DC in lymphoid organs [117]. However, the role of CCR6 in LC recruitment was indirectly proven in humans by LC precursor chemotaxis experiments [118,119]. In this context, Randolph et al. have shown that lipids such as leukotriene C(4), also modulate the chemokine response [120]. CCR2 also plays a role in recruitment and migration of LC as demonstrated by CCR2 KO mice [41,121]. In this context, the balance of chemokine expression is strictly regulated between endothelial cells in blood and dermis [122], and is modified by inflammatory signals [123].
- Third, trafficking is controlled at the level of cell adhesion, as LC down-regulate some adhesion molecules to exit the epidermis [124], and up-regulate others to migrate across the dermal extracellular matrix and home to T cell areas of regional lymphoid tissue. Indeed, the key role of CD44 and α 6 integrins in LC migration was demonstrated on human skin-explants and in KO mice [124–126]. Down-regulation of E-cadherin is also a key event in LC migration [127]. Furthermore, it was recently shown that the junctional adhesion molecule-A may negatively regulate DC migration to LN in mice, suggesting a more complex view of DC migration [128].
- Finally, expression of the matrix metalloproteinases (MMP) -9 and -2 are necessary both for the migration of LC and DDC [129].

6. Maturation of cutaneous dendritic cells

Tissue injury, microbial infection, and other changes of homeostasis (e.g., contact allergens) provide danger signals, leading to a local production of pro-inflammatory cytokines that induce cutaneous DC mobilization to lymphoid tissue. At the same time, signals are generated that recruit precursors into the skin to maintain the resident DC population. Diverse stimuli which engage the following types of surface receptors have been reported to mediate DC maturation:

6.1. *TNF Receptor family and cytokine receptors*

CD4⁺ T cells induce DC maturation both in vivo and in vitro and triggering of CD40 by CD40L on T cells induces DC maturation [130]. LC sense danger and infections indirectly through inflammatory mediators, such as TNF- α and IL-1 β whose secretion is triggered by pathogens [131].

6.2. *Toll-like receptors (TLR)*

DC undergo maturation in response to various pathogen compounds which are mainly recognized by a large (10 members) family of Pattern Recognition Receptors (PRR), the TLRs [132]. Cells of the immune system specifically recognize different pathogen-associated pattern molecules through differential expression of TLRs.

In humans, monocyte-derived DC, the in vitro counterpart of DDC, display TLR1-6, TLR8, TLR10, and low levels of TLR7 [133,134], but the expression pattern on freshly isolated DDC is not known. Human monocyte-derived DC further express TLR3 and respond to Poly I:C [133,135,136]. In contrast to murine LC, human LC respond to Poly I:C (our unpublished observations and [137]).

Freshly isolated LC, or LC derived from monocytes do not express detectable levels of TLR4, and do not mature significantly in response to its ligand LPS ([138–140] and our unpublished results). In this context, injection of LPS in skin has only minor or indirect effect on LC migration and activation [110]. On the other hand, keratinocytes express TLR4 and its triggering induces the release of inflammatory cytokines [141].

Human LC respond to PGN and LTA, two potent agonists of TLR2, by an up-regulation of maturation markers, and strong expression of TLR6, a possible co-receptor for PGN [142,143] is detected in LC, but not in keratinocytes. Furthermore, LC-like cells generated in vitro from CD34⁺ precursors up-regulate TLR2 expression following TNF- α treatment [144]. Considering that TLR2 levels may not be functional in resting LC, PGN could still trigger alternative receptors, such as NOD family proteins [145]. In contrast to TLR2 agonists, human LC do not phenotypically mature in response to imiquimod and R-848, which represent synthetic ligands of TLR7 and TLR8 [146]. Finally, administration of CpG motifs (TLR9 ligands) to mice results in enhanced contact hypersensitivity that may indirectly implicate LC, via an effect similar to that occurring in response to LPS activation, or via triggering of TLR9 on monocytes [147–151]. In our hands, we have never observed activation of fresh human LC in response to CpG motifs (our unpublished observations).

6.3. *Fc receptors and “cell death” receptors*

Engagement of most FcRs, including Fc γ RI, Fc γ RIII, Fc ϵ RI and Fc α R, by immune complexes or specific antibodies, induces DC maturation in mice and humans, but this

has not been demonstrated on freshly isolated cutaneous DC [152,153].

Various studies have shown that necrotic, but not apoptotic, cell death induces mouse and human DC maturation in vitro [106,107], although other reports indicate that apoptotic bodies may induce DC maturation [154,155]. One should be careful in interpreting these results, however, since LPS [156] or mycoplasma contamination [157], CD40L expression by apoptotic bodies [158], or compounds released by dying cells could be responsible for DC maturation.

The nature of endogenous activating compounds is still unclear although hyaluronate [159], soluble heparan sulfate [160], oxidatively modified lipids [161] and crystals of uric acid have been described to activate BM-derived DC [162,163]. To date, very few results have been reported using freshly isolated cutaneous DC.

7. **Conclusions and future directions**

Dendritic cells form a complex cell population within the skin. Yet, despite their heterogeneity, they share a number of features (anatomical location at the interface of the organism, antigen recognition/processing machinery, and migratory capacity) that make them privileged “sentinels” to survey intruding agents and transmit information that will shape the immune response.

Our present review has focused on characterizing the various cutaneous DC subsets, and on the physiological events that these cells undergo within the skin and as they depart on their way to the draining lymph nodes. A considerable future challenge will be to establish the relative antigen-presenting contributions of each of the highly plastic skin DC subpopulations once they reach draining lymph nodes. Because of the broad uncertainties currently existing within this area, we have not here attempted a dissection of these functional aspects. Indeed, recent studies have pointed to the need to revisit the “paradigm” that, upon reaching draining LN, immigrant LC systematically present skin-acquired Ag to T cells. For instance, infection of mouse epidermis by herpes simplex virus does result in virus-specific T cell priming, but this is strikingly accomplished by a lymph node DC population distinct from LC [164]. In addition, distinct DC populations may sequentially present Ag acquired in the skin and thus regulate cell-mediated immunity, a notion that adds another level of complexity, and warrants reconsideration of several “established” concepts [165]. Another challenge will be to further understand the role played by the various cutaneous DC subpopulations in regulating the balance between immunity and peripheral tolerance. It is widely accepted that DC have tolerogenic properties while in an immature state. Yet, data recently reported by Mayerova et al., using a transgenic mouse model expressing self-Ag in epidermis, suggest that LC may not have tolerogenic properties in the steady-state [166]. Previously, Geissmann et al. had shown that LC present in lymph nodes draining

human chronically inflamed skin lack maturation markers [140]. The potential lack of tolerogenic capacity of these LC, and its consequent adverse role in maintaining an inflammatory condition should be considered. A related and important issue is how systematic the functional maturation of DC is linked to their migration from skin to LN.

Irrespective of their subset affiliation, cutaneous DC are involved in a number of pathologies, including skin-transmitted viral, bacterial, and parasitic infections, dermatoses mediated by T lymphocytes, and skin cancers. Since the discovery of a novel splenic cell type by Ralph Steinman in 1973, our understanding of DC and their pivotal role in immune regulation has grown exponentially. DC are truly Nature's most potent adjuvant for initiating immunity. Therefore, the manipulation of skin-derived DC has vast potential for generating productive immunity or for inducing tolerance in a number of disease conditions. In the foreseeable future, biomedical researchers and vaccine developers will undoubtedly seek to translate further progress in the biology of cutaneous DC into therapeutic indications.

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