Antigen-Presenting Cells in the Skin

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Abstract

Professional antigen-presenting cells (APCs) in the skin include dendritic cells, monocytes, and macrophages. They are highly dynamic, with the capacity to enter skin from the peripheral circulation, patrol within tissue, and migrate through lymphatics to draining lymph nodes. Skin APCs are endowed with antigen-sensing, -processing, and -presenting machinery and play key roles in initiating, modulating, and resolving cutaneous inflammation. Skin APCs are a highly heterogeneous population with functionally specialized subsets that are developmentally imprinted and modulated by local tissue microenvironmental and inflammatory cues. This review explores recent advances that have allowed for a more accurate taxonomy of APC subsets found in both mouse and human skin. It also examines the functional specificity of individual APC subsets and their collaboration with other immune cell types that together promote adaptive T cell and regional cutaneous immune responses during homeostasis, inflammation, and disease.

INTRODUCTION

DC: dendritic cell
LC: Langerhans cell

Skin is composed of an outer epidermis anchored to the loose connective tissue of the dermis by the basement membrane. The epidermis contains a stratified layer of differentiated keratinocytes, dendritic epidermal γδ T cells (DETCs; only in mice), and melanocytes as well as epidermal appendages such as hair follicles (1). The dermis also contains numerous cell types that include innate lymphoid cells, γδ T cells, adaptive $CD4^+$ and $CD8^+$ T cells, and various stromal cells and appendages. Professional antigen-presenting cells [APCs; e.g., dendritic cells (DCs), monocytes, and macrophages] are another major leukocyte population in the skin. APCs are highly dynamic, with the capacity to enter the skin from the circulation, to patrol within the skin, and to migrate into regional lymph nodes via lymphatics. They are endowed with antigen-sensing, -processing, and -presenting machinery and play key roles in initiating, modulating, and resolving cutaneous inflammation. Skin APCs are a highly heterogeneous population with functionally specialized subsets that are developmentally imprinted and modulated by local tissue microenvironmental and inflammatory cues.

A major barrier to the study of skin APCs has been the absence of single functional markers that uniquely define individual subsets of cells. Whole-genome transcriptomics comparing mononuclear phagocyte (MP) populations between and across species combined with ontogeny studies has defined the developmental and functional relationships between DCs, monocytes, and macrophages. Recent technological refinements have also enabled sophisticated in vivo murine studies involving cell tracking and selective cell-type depletion to provide a picture of DCs in initiating and regulating immune responses.

This review focuses on current developments that have allowed for more accurate taxonomy of APC subsets found in the skin. It also examines the functional specificity of individual APC subsets and how they work in concert to orchestrate the complex interplay between many immune cell types within the skin under homeostatic and inflammatory contexts.

HISTORICAL PERSPECTIVE ON MONONUCLEAR PHAGOCYTES IN THE SKIN

In 1868, the German physician Paul Langerhans observed a network of cells with a dendritic morphology throughout the epidermis (2). These eponymous cells were thought to be part of the nervous system. Around the same time, Ilya Mechnikov discovered macrophages and phagocytosis, for which he was awarded a Nobel Prize in 1908 (3). Understanding the immune function of Langerhans cells (LCs) took another hundred years and was steered by the discovery of probing and branching (dendritic) cells in the mouse spleen by Ralph Steinman in 1973, a discovery recognized with a Nobel Prize in 2011. Steinman named these cells dendritic cells and showed that they are not as efficient at endocytosis as macrophages but express high levels of major histocompatibility (MHC) molecules and are potent inducers of naive T cell proliferation in mice (4, 5).

In the late 1970s, LCs were found at increased frequency in skin contact hypersensitivity reaction and shown to express MHC class II, Fc, and complement receptors, suggesting an immunological role (6–9). In the 1980s, Katz and Frelinger demonstrated LCs to be bone marrow derived and part of the hematopoietic system (10, 11). Balfour characterized cells resembling LCs in the afferent skin-draining lymphatics that promoted lymphocyte activation (12). Finally, in 1985, the Austrian dermatologist Schuler in the Steinman laboratory proposed LCs as members of the DC family (13). DCs in the dermis were discovered in 1993 (14, 15). The observation that LCs and DCs migrate spontaneously out of skin explants cultured ex vivo facilitated early experimentation on these cells from both mouse and human. As discussed in further detail below, more

recent advances in murine-depletion and antigen-targeting models have enabled fine functional dissection of LCs and the various subsets of dermal DCs (dDCs) in vivo. Logistical constraints of in vivo human studies resulted in the use of DCs derived from monocytes or hematopoietic stem cells (HSCs) as surrogate in vitro models of in vivo DCs. Although enormous insights have been amassed from HSCs and monocyte-derived DCs (mo-DCs), it remains unclear precisely how they resemble human DC subsets found in vivo. A further concept that shaped progress in the field is the intimate link between the ontogeny of DC subsets and their function (16). In contrast, macrophage function is determined by development (nature) as well as the microenvironment of their tissue of residence (nurture). The role of tissue environment on DC function has not been thoroughly explored.

cDC: conventional DC

LT: lymphoid tissue (i.e., lymph node, tonsil, and spleen in humans)

COMPOSITION OF MONONUCLEAR PHAGOCYTES IN THE SKIN

The two main families of skin MPs are migratory DCs and tissue-resident macrophages. Skin DCs can be further divided into LCs that are developmentally related to macrophages and self-renew in the epidermis and dDCs that are derived from HSCs and are closely related to conventional DC (cDC) subsets found in lymphoid tissues (LT) (**Figure 1**). Undifferentiated monocytes, typically

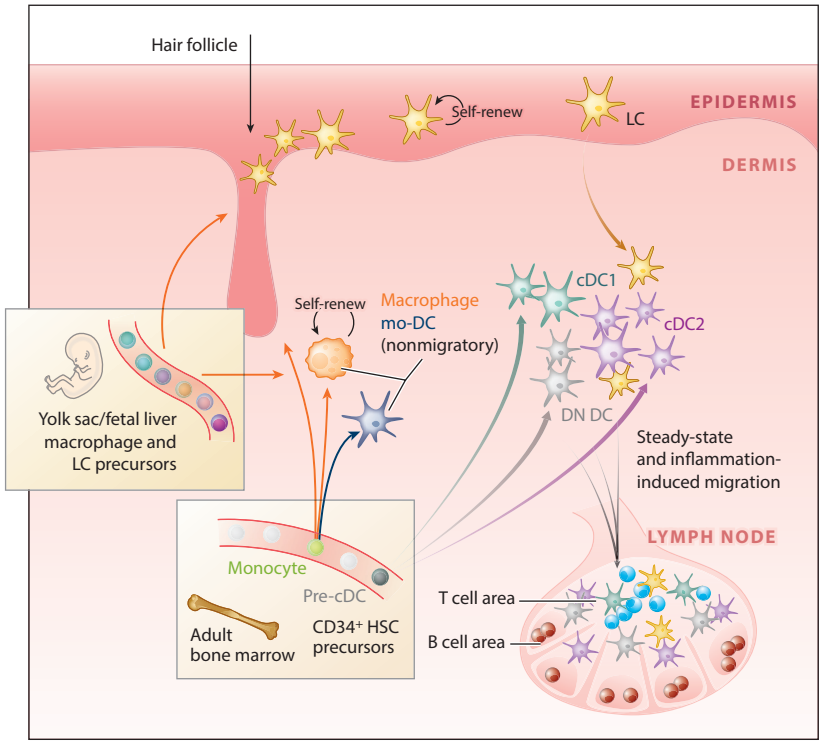

Figure 1

Current understanding of skin APC ontogeny. Skin APC subsets have distinct developmental origins, including yolk sac and fetal liver precursors and bone marrow HSCs. In steady state or under inflammation, specific DC subsets migrate into localized areas of skin-draining lymph nodes to present antigen to cognate $CD4^+$ and $CD8^+$ T cells. Abbreviations: cDC, conventional DC; DN, double negative; HSC, hematopoietic stem cell; LC, Langerhans cell; mo-DC, monocyte-derived DC.

found in the circulation and LT, have been observed in mouse but not human skin (17). MPs are phenotypically defined by the absence of lineage markers characteristic of neutrophils (CD66b), T cells (CD3), and B cells (CD19, CD20) combined with MHC-II expression (18, 19). In humans, expression of MHC-II is a defining feature of DCs, monocytes, and macrophages. In mice, all DCs but only some monocytes and macrophages express MHC-II. For many years, DCs and macrophages were thought to arise from bone marrow HSCs through an intermediate monocyte stage (20). However, murine adoptive-transfer, fate-mapping, and depletion models have shown convincing evidence that cDCs and dDCs arise from bone marrow HSC–derived, DC-restricted precursors that do not undergo a monocyte intermediate stage (16). In mice, commitment to the distinct cDC lineages is already evident within bone marrow common DC progenitors (21). In contrast, tissue macrophages and epidermal LCs are primarily derived from prenatally seeded yolk sac– or fetal liver–derived precursors that self-renew independent of bone marrow HSCs (**Figure 1**) (22). Monocytes can give rise to DC- and macrophage-like cells that are distinct from cDCs and prenatally derived macrophages. In humans, circulating DC precursors with the phenotype $CD123^{lo/-}CD135^{+}CD116^{+}CD117^{+}CD115^{-}$ are capable of differentiation into all cDC subtypes (23). A notable difference between mice and humans is the presence of DCs in human blood, which raises the possibility that they can seed and repopulate tissue cDCs (24). In addition, only human LCs and DCs express the MHC-I-related molecules CD1a, CD1b, and CD1c, which are involved in presentation of lipid antigens to T cells (25).

As discussed in the following sections, DCs can be divided into several functionally distinct subsets, most with well-defined ontogeny primarily based on murine studies. All skin DC subsets transport cutaneous antigen to skin-draining lymph nodes and initiate T cell responses. Skin DCs also interact with skin-resident and -infiltrating immune cells during inflammation and steady state to shape the immune response. Resident dermal macrophages are functionally versatile, with critical roles in tissue development, homeostasis, and repair as well as varied immunological responses (26). Like DCs, macrophages display functional heterogeneity and specialization based on location and nature of their inflammatory environment (27). Unlike DCs, macrophage subsets remain relatively poorly characterized, and methods to selectively target or ablate macrophages are imprecise, thereby hindering a more comprehensive functional understanding of this cell type.

Epidermis

Under homeostatic conditions, LCs are the sole resident APCs in the epidermis and account for 3–5% of epidermal cells (28).

Langerhans cell phenotype. LCs in the epidermis occupy the interfollicular and follicular epithelium, where they acquire epidermal antigen. LCs have the capacity to protrude their dendrites through tight junctions to acquire antigen from the stratum corneum (29).

Both murine and human LCs express MHC-II; langerin (CD207); the epithelial cell adhesion molecule (EpCAM), which promotes LC migration by decreasing adhesion to keratinocytes (30); CD11b; CX3CR1; and SIRPα (CD172α) and possess the pathognomonic tennis racket–shaped Birbeck granules (**Tables 1** and **2**) (16). Unlike mouse LCs, which are $CD11c^{br}$, human LCs are $CD11c^{lo}$ and also $CD1a^{+}$ and $CD1c^{+}$ (31). Mouse, but not human, LCs express $F4/80^{+}$ and CD205.

Langerhans cell development and homeostasis. Evidence primarily from murine studies shows that LCs have unique ontogeny and homeostatic properties, in contrast to dermal MPs. LC precursors seed the epidermis first from hematopoietic precursors in the yolk sac and then from

Table 1 Mouse APC phenotypes and deletion models

	LC	cDC1	cDC2	DN dDC	mo-DC	Mφ	cDC1	cDC2
Other names		XCR1$^+$ dDC CD103$^+$ dDC IRF8$^+$ DC	CD11b$^+$ dDC IRF4 dDC				XCR1$^+$ cDC CD8$^+$ DC IRF8 DC CLEC9A DC	CD11b$^+$ cDC DCIR$^+$ DC IRF4 DC
Location	Epidermis	Dermis	Dermis	Dermis	Dermis	Dermis	LN/spleen	LN/spleen
Surface markers								
CD8α	−	−	−	−	−	−	+	−
CD103	−	+	−	−	−	−	−	−
XCR1	−	+	−	−	−	−	+	−
CD327 (Clec9A)	−	+	−	−	−	−	+	−
CD11b	+	−	+	−	+	−/+	−	+
CD207 (langerin)	+	+	−	−	−	−	−/+[a]	−
CD301b	−/+	−	+	−	+	+	−	−
CD172α (SIRPα)	+	−	+	+			−	+
CD64 (FcγR1)	−	−	−	−	+/low	+	−	−
MERTK	−	−	−		−/low	+	−	−
CCR2	−	−	+	+	+	−/low	−	−
F4/80	+	−	+	+	+	+	−	−
CD326 (EpCAM)	+	−	−	−	−	−	−	−
CX3CR1	+	−	+	+		+	−/+	−
CD24	+	+	−	−	−	−	+	+
CD205	+	+	+		+		+	+
Transcription factors								
Batf3	−	+	−		−	−	+	−
ID2	+	+	−		−	−	+	−
IRF4	−	−	+	+			−	+
IRF8	+	+	−		−	−	+	−
KLF4				+				

(*Continued*)

Table 1 (*Continued*)

	LC	cDC1	cDC2	DN dDC	mo-DC	Mφ	cDC1	cDC2
Soluble factors/receptors								
Flt3	−	+	+	+	−	−	+	+
Csf-1R	+	−	+		+	+	−	+
Csf-2R	−	+	+				+	+
Murine depletion models								
CD11c-DTR	+	+	+	+			+	+
Human-langerin DTR	+							
Human-langerin DTA	+							
Murine-langerin DTR	+	+					+	
Batf3$^{-/-}$		+					+[a]	
Zbtb46-DTR		+	+	+			+	+
CD11c cre-IRF4 fl			+[b]	+				+
CSF1-R DTR	+		+					
CSF2R-DTR		+	+	?				
CD11c cre Klf4 fl				+				
MGL2-DTR	+		+	?	+[c]	+[c]		

[a]Varies by background strain.
[b]Migration defective.
[c]Partial deletion.
Abbreviations: cDC, conventional DC; DC, dendritic cell; dDC, dermal DC; DN, double negative; LC, Langerhans cell; LN, lymph node; mo-DC, monocyte-derived DC; Mφ, macrophage.

Table 2 Human APC subsets

	LC	CD141$^+$ DC	CD1c$^+$ DC	pDC	CD14$^+$
Location	Epidermis	Dermis/lymph/blood			Dermis
Murine equivalent	LC	cDC1	cDC2	pDC	mo-Mφ
HLA-DR	+	+	+	+	+
CD11c	low	low	+	−	+
CD1a	++	−	+[a]	−	−
CD14	−	−	−	−	+
CD1c (BDCA1)	+	−	+	−	−/+
CD303 (BDCA2)	−	−	−	+	−
CD304 (BDCA3)	−	+	+/−	+	−
CD141 (BDCA4)	−	++	−/+	−/+	−
XCR1	−	+	−	−	−
CD370 (CLEC9A)	−	+	−	−	−
CD207 (langerin)	++	−	−/+[a]	−	−
CD326 (EpCAM)	+	−	−	−	−
CD324 (E-cadherin)	+	−	−	−	−
CD11b	low	−	+	−	+
CX3CR1	+	−	+	−	+
CD172α (SIRPα)	+	−	+	−	+

[a]Migratory skin DC.
Abbreviations: cDC, conventional DC; DC, dendritic cell; LC, Langerhans cell; Mφ, monocyte-derived macrophage; pDC, plasmacytoid DC.

the fetal liver (32, 33). In contrast to most cDCs, which are dependent on the growth factor receptor FLT3 and its ligand FLT3L, LCs require keratinocyte-derived IL-34 and inflammatory neutrophil-derived CSF1 signaling on CSF1R for their development, homeostasis, and regeneration during inflammation (34–36). In addition, LCs require autocrine transforming growth factor β (TGF-β) (37–42) and keratinocyte-derived integrins $\alpha_v\beta_6$ and $\alpha_v\beta_8$ as critical mediators of TGF-β activation to maintain the residence of LCs in the interfollicular and follicular epidermis, respectively (43). The receptor tyrosine kinase, AXL, and a member of the TGF-β superfamily, BMP7, are also required for LC development (44). Transcription factors implicated in LC development include RUNX3, PU.1 (SPI1), and ID2 (28). Human CD1c$^+$ DCs cultured in vitro with thymic stromal-derived lymphopoietin (TSLP) and TGF-β or GM-CSF and BMP7 can acquire a phenotype resembling LCs, but whether the cultured cells transcriptionally and functionally resemble in vivo LCs remains to be proven (45, 46).

LCs self-renew and remain of host origin in murine bone marrow transplantation models (10, 47, 48). In humans, LCs can proliferate in situ and remain donor in origin up to 10 years after limb transplant but are replaced by donor-derived cells after nonmyeloablative HSC transplant, which may be due to chemotherapy and conditioning prior to transplantation (49–52). LCs can repair DNA damage through the action of the cyclin-dependent kinase inhibitor, CDKN1A, which permits cell cycle arrest, providing protection against ionizing radiation (53). However, strong inflammatory stimuli such as UV light can deplete LCs (54). In this setting, LC replenishment occurs in two stages (55), a transient ID2-independent repopulation by Gr1hi monocytes (54) and a second ID2-dependent wave by bone marrow HSC–derived precursors (47–49, 54, 56, 57). Bone marrow HSC–derived LC precursors are recruited by hair follicle isthmus–derived CCL2 and infundibulum-derived CCL20 (58).

NLT: nonlymphoid tissue (e.g., skin)

The similar developmental requirements between LCs and macrophages raised the question of whether LCs should be reclassified as macrophages (59–63). However, LCs function as the prototype migratory DCs, capable of migrating to the lymph node in steady state and under inflammation, reaching deep in the T cell zone of the draining lymph nodes within three to four days to drive T cell responses (64). An alternative view is that embryonic precursors are capable of generating cells with macrophage and DC function, but the only tissue compartment in early embryonic life where DC differentiation occurs is the epidermis.

Dermis

This compartment is populated by APCs found across all nonlymphoid tissues (NLT) in mouse and human. Two subsets of NLT-migratory DCs, XCR1$^+$ and CD11b$^+$ DCs, have equivalent populations in LT such as spleen, tonsil, and draining lymph node (31). A third population termed double-negative DCs is found exclusively in the skin (65). These DCs as well as mo-DCs are derived from bone marrow HSCs and express the transcription factor Zbtb46 in humans and mice (31).

Dermal cDC1 phenotype. In both human and mouse dermis, cDC1s are a relatively rare DC population characterized by expression of XCR1 [the chemokine receptor for XCL1, expressed by CD8 T cells (65, 66)], CLEC9A [a receptor for F-actin (67, 68)], CADM1, TLR3 (Toll-like receptor 3), CD141/BDCA3, and the transcription factor IRF8 (**Tables 1** and **2**) (69). In mice, this subset can also be referred to as dermal CD103$^+$ DCs or IRF8$^+$ DCs. Dermal cDC1s are developmentally closely related to LT-resident cDC1s (also referred to as CD8α^+ DCs or IRF8$^+$ DCs) (63, 70). Dermal cDC1s do not express CD14, CD11b, CD172α, or CX3CR1 (71). CD141/BDCA-3 was initially used to define this DC subset in human blood and skin, but it can be upregulated by other DCs and monocyte-derived cells in human tissue and is unreliable as a sole marker (19). Langerin is expressed on mouse dermal cDC1s but not their human homologs (19, 72). Murine dermal cDC1s in the skin express the integrin CD103, which enables binding to E-cadherin, in contrast to their cDC1 lymph node–resident counterparts, which lack CD103 but express CD8α (70).

Dermal cDC1 development and homeostasis. The differentiation of dermal cDC1s requires the growth factors FLT3L and CSF2 and their receptors, FLT3 and CSF2R (16, 70). Dermal cDC1s, like LT cDC1s, express high levels of FLT3, proliferate after administration of FLT3L, and are nearly absent in *Flt3l*$^{-/-}$ mice (73, 74). This subset is developmentally dependent on the transcription factors BATF3, IRF8, ID2, and NFIL3 (16, 70). Interestingly, *Batf3*-deficient C57BL/6 mice have a constitutive absence of dermal cDC1s and splenic cDC1s but not lymph node cDC1s (70, 75). Dermal cDC1s and LT cDC1s are absent on the 129 mouse background. The developmental requirement of human dermal cDC1s mirrors the needs of their murine counterparts. In vitro culture conditions permissive for their differentiation require the presence of FLT3L and CSF2 (23, 76, 77). Human biallelic K108E *IRF8* mutation presents with loss of all cDCs and monocytes (78). shRNA knockdown of *BATF3* in cord blood HSCs inhibits cDC1 differentiation. However, humanized mice reconstituted with *BATF3* knockdown CD34$^+$ HSCs still had detectable populations of cDC1s (76). This discrepancy may be due to compensation of function between members of the BATF family during conditions of inflammation (79).

In mice, commitment to the cDC1 lineage is already evident within bone marrow SiglecH$^-$Ly6C$^-$ and MHCII^{lo}CD117$^+$ cDC precursors (pre-DCs) (21, 80). Human blood cDC1s acquire CD1c and CD1a expression, adopting the phenotype of their dermal cDC1 counterparts,

upon culture with supernatant from skin digest (19). In addition, blood cDC1s possess a skin-homing (CLA^+CCR7^-) chemokine receptor profile, in contrast to skin cDC1s, which have a lymph node–homing profile (CLA^-CCR7^+). It is possible that in humans both circulating pre-DCs and blood cDC1s can seed the dermis.

Dermal cDC1s are a minor population in murine and human dermis with a high turnover rate (81). They migrate rapidly into the deep T cell zone of the lymph node (81, 64). In skin-draining lymph nodes, cDC1s represent 20–40% of lymph node–resident DCs (82–84). cDC1s in NLT and LT can be conditionally depleted in *Xcr1*-DTR and *Clec9A*-DTR mice (85). The functional importance of dermal cDC1s is underscored by their presence in teleost skin and their evolutionary conservation across species (86, 87).

Dermal cDC2 phenotype. Dermal cDC2s are the most numerous DC population in murine and human dermis and are best characterized by the expression of IRF4; CD11b; and, uniquely in humans, CD1c (**Tables 1** and **2**) (65). Dermal cDC2s are found in the dermis and throughout the NLT and LT and are developmentally related to LT-resident cDC2s, which are also called $IRF4^+$ DCs. In both mice and humans they are $CX3CR1^+$, $SIRP\alpha^+$, $CCR2^+$, and $CD11c^+$, but these markers can also be expressed by monocytes and macrophages, which can make it difficult to identify them phenotypically. In mice, additional markers that can distinguish them from macrophages include the absence of $Fc\gamma RI$ (CD64) and MERTK, and they are distinguished from monocytes by the absence of Ly6C expression (18). In humans, blood and dermal cDC2 have traditionally been distinguished from monocytes and macrophages by the lack of CD14 expression, although the identity of a distinct population that is $CD14^+$ and $CD1c^+$ in healthy dermis remains to be accurately characterized. In the mouse spleen, a subset of dermal cDC2s also express CD4, which is expressed by all human cDCs, plasmacytoid DCs (pDCs), and monocytes (88).

Dermal cDC2 development and homeostasis. Differentiation of dermal cDC2s and LT-resident cDC2s requires the growth factors FLT3L and CSF2 and their receptors, FLT3 and CSF2R (16, 70). They proliferate in response to FLT3L and are reduced in *Flt3*- and *Flt3L*-deficient mice, but to lower levels in comparison to cDC1s likely because of contamination of monocyte-derived cells within the flow cytometry parameter space of cDC2s (73, 74). Dermal cDC2s and LT cDC2s are developmentally dependent on PU.1, RELB, and RBPJ. IRF4 is expressed by both human and mouse cDC2s, though the IRF4 dependency is unclear. Murine dermal cDC2s develop in the absence of IRF4 but do not survive or migrate into skin-draining lymph nodes (89, 90). IRF8 is also implicated in the development of human dermal cDC2s. Human autosomal dominant IRF8 T108A mutation resulted in selective loss of blood cDC2s, contrary to mouse $Irf8^{-/-}$ and hypomorphic $Irf8^{R294C}$ mutation, where cDC2s are unaffected (78, 91). In mice, $Siglec\text{-}H^-Ly6C^+$ DC precursors are committed to the cDC2 lineage (21). Human dermal cDC2s turn over rapidly, becoming donor HSC–derived approximately 30 days after HSC transplantation (92).

Unlike dermal cDC1s, cDC2s coexpress many genes associated with mo-DCs at the bulk population level. Human blood cDC2s are transcriptionally less unique, being characterized by 170 genes, in contrast to 1,020 and 1,056 genes for cDC1s and pDCs, respectively (19). This may be due to unresolved heterogeneity within cDC2s resulting in averaging out of gene expression at bulk population level or their potential to respond flexibly according to environmental needs upon perturbation.

Dermal cDC2s migrate to the lymph node into the peripheral paracortex (64). Analysis of skin-draining lymph nodes by histocytometry showed localization of lymph node–resident cDC1s predominantly to the T cell zone and the localization of resident cDC2s principally within the

lymphatic sinus endothelium of the lymph node (64). Dermal cDC1s and cDC2s localize with their LT cDC1 and cDC2 counterparts. Dermal cDC2s can be conditionally ablated, alongside macrophages and LCs, in *Mgl2*-DTR mice (93). In addition, *Itgax*^{cre} *Irf4*^{fl} mice have defective migration of dermal cDC2s into the lymph node (90, 94).

XCR1$^-$CD11b$^-$ DC phenotype. The mouse dermis and skin-draining lymph nodes contain a minor population of migratory DCs with the phenotype XCR1$^-$CD207$^-$CD11b^{lo} (referred to as double-negative dDCs). These double-negative DCs are CD11c$^{lo\text{-}int}$, CD103^{lo}, CD301b$^-$, CD326^{lo}, CD64$^-$, CD172α^+, and CX3CR1$^+$(**Tables 1** and **2**) (65). This population has not been delineated in other murine NLT or secondary LT, and there is no obvious human homolog.

XCR1$^-$CD11b$^-$ DC development and homeostasis. Double-negative DCs are Zbtb46$^+$ and depend on IRF4 and FLT3L for their optimal development from bone marrow precursors and CCR7 for migration into the draining lymph node (95). Recently, KLF4 has been shown to be important for the development of double-negative DCs that were consequently absent in the dermis and lymph nodes of *Itgax*^{cre} *Klf4*^{fl} mice (96). A parallel relationship between dermal double-negative DCs with splenic ESAM^{lo} DCs was recently proposed based on their dependencies on KLF4 and Th2 polarization potential upon *Schistosoma* and *Nippostrongylus* infection (97).

Upon TSLP production in skin through application of the contact sensitizer dibutyl phthalate, double-negative DCs rapidly migrated to skin-draining lymph nodes to become the most abundant migratory DCs at 24–48 h after stimulation (81).

mo-DC phenotype. mo-DCs are phenotypically similar to dermal cDC2s, sharing the expression of CD11c, CD11b, CX3CR1, SIRPα, and CCR2 and in the mouse being MERTK$^{-/lo}$ and CD64$^{-/lo}$ (**Tables 1** and **2**). mo-DCs have not been characterized in healthy human dermis but may be within the CD14$^+$CD1c$^+$ DC subset.

mo-DC development and homeostasis. mo-DCs can be distinguished from cDC2s/CD11b$^+$ dDCs based on the requirement for CCR2 signaling for their development in the skin. Evidence suggests that mo-DCs arise from Ly6C^{hi} monocytes that enter skin, acquire MHC-II, and gradually lose Ly6C expression (18). Interestingly, there were fewer mo-DCs in germfree mice, suggesting a role for skin commensals or an inflammatory trigger in their differentiation (18). Transcription factors such as PU.1, IRF8, and KLF4, which are critical for the development of monocytes, will also affect mo-DC development in the dermis. IRF4 was also implicated in the differentiation of human in vitro mo-DCs (98).

mo-DCs are inferior to cDC2s in activating CD4$^+$ and CD8$^+$ T cells and migrating to lymph nodes (18). Their shared transcriptional similarity to cDC2s and rapid expansion upon inflammatory stimulus also suggest responsive mode capabilities dictated by microenvironment requirements.

Macrophage phenotype. Murine dermal macrophages are MHC-II^{int}, CD115$^+$, F4/80$^+$, CD11b$^+$, CSF1R$^+$, Ly6C$^{\pm}$, CCR2$^-$, CD64$^+$, and MERTK$^+$ (**Tables 1** and **2**) (26). Human dermal macrophages are MHC-II^{br}, CD14$^+$, and FXIIIa$^+$ and are highly autofluorescent, partly owing to their cytoplasmic content including melanin granules (92). Dermal macrophages are vastly superior at phagocytosis but inferior at T cell activation compared to LCs, cDCs, and mo-DCs (18). In addition, a subset of dermal macrophages expressing CD4 in mice was shown to be in close apposition to postcapillary venules and to drive the recruitment of leukocytes during inflammation (27).

Macrophage development and homeostasis. CSF1 and CSF1R signaling are required for dermal macrophage development (22). The transcription factors PU.1, IRF8, NFATc1, and SPI-C have been implicated in macrophage development. In both mice and humans, there are likely two developmental origins of dermal macrophages. The first and main source of macrophages in the mouse dermis is circulating monocytes, as evidenced by a dramatic reduction of dermal macrophages in $Ccr2^{-/-}$ mice and a high frequency of macrophages derived from bone marrow HSCs in a fate-mapping mouse model (99). A minor contribution from prenatal embryo-derived macrophages has been speculated. In humans, there are two populations of $CD14^+$ macrophages: (*a*) Non-autofluorescent macrophages are $CD14^+$ blood monocyte derived, absent in patients lacking peripheral blood monocytes owing to autosomal dominant GATA2 or autosomal recessive IRF8 deficiencies, and turn over rapidly—within 30 days—into donor HSC–derived cells following HSC transplantation; and (*b*) autofluorescent melanin-granule-containing macrophages, which are partially reduced in GATA2- and IRF8-deficient patients, are long-lived, and turn over slowly—remaining recipient HSC–derived in origin up to one year after HSC transplantation (19, 78, 99, 100). The presence of dermal macrophages in the absence of circulating blood monocytes in a six-month-old, IRF8-deficient patient is strongly suggestive of prenatal embryonic–precursor contribution to human dermal macrophages (78).

Dermal macrophages in mice have a half-life of four to six weeks, and the contribution of Ly6Chi monocytes to the dermal macrophage pool increases over repeated episodes of inflammation (101). How the dermal niche availability and nature of inflammatory stimulus modulate macrophage homeostasis by self-renewal of resident cells and recruitment of circulating monocytes is not well understood (101). The longevity and tissue-resident nature of macrophages in contrast to DCs permit the local microenvironment to shape the enhancer landscape and transcriptome in addition to developmental imprinting to fine-tune macrophage function (102). Transcriptomic analysis of macrophages has demonstrated that they have genes supporting scavenging and microorganism killing as well as tissue-specific genes involved in residence and maintenance (102–104).

Inflammatory DC phenotype. Inflammatory DCs are a transient population that appear in response to an inflammatory stimulus and disappear when the stimulus is resolved. They share many phenotypic markers with dermal cDC2s and mo-DCs. Their transient nature and lack of unique surface markers pose two problems: first, how to distinguish inflammatory DCs from steady-state cDCs and mo-DCs, and second, how best to study their mobilization kinetics and function in humans simply based on snapshot analysis during disease without adequate recourse to the evolution of the inflammation/disease process.

Inflammatory DCs are MHC-II^+ and $CD11c^+$. The nature of the inflammatory stimuli influences the reported phenotype of inflammatory DCs. In response to lipopolysaccharide, inflammatory DCs express Zbtb46 and accumulate in the lymph node in a FLT3L-dependent manner (105, 106). However, *Listeria monocytogenes* stimulation in mice induces TNF-α/iNOS-producing DC (TipDC) differentiation (107). Unlike lipopolysaccharide-induced inflammatory DCs, TipDCs lack expression of Zbtb46, raising the question of whether they are DCs (105). TipDCs have also been observed in human psoriatic skin (108). Other inflammatory populations previously described in human skin include inflammatory dendritic epidermal cells (IDECs) observed in atopic dermatitis (109) and slan DCs in psoriasis (110). The interrelationships between TipDCs, IDECs, and slan DCs are unclear.

Inflammatory DC development and homeostasis. Inflammatory DCs are assumed to be monocyte derived because of their dependency on CCR2 signaling. The phenotype, origin, homeostasis, and function of inflammatory DCs are the subject of ongoing studies.

Plasmacytoid DC phenotype. Plasmacytoid DCs (pDCs) are a highly specialized IFN-α-producing population that have been shown to have a similar origin as cDCs. pDCs circulate in blood and LT in steady state and can be found in skin and peripheral tissues under inflammatory conditions (111).

Mouse pDCs share many phenotypic markers of the B cell family, including B220 and CD317 (BST2), but are $CD11c^{int}$. In humans, pDCs are $CD11c^{-}$, $CD123/IL\text{-}3R^{+}$, CD303 $(BDCA\text{-}2)^{+}$, and CD304 $(BDCA\text{-}4)^{+}$. pDCs also have well-developed secretory machinery and morphologically resemble B cells/plasma cells. Whether this reflects shared ancestry with B cells is unclear. In both mice and humans, a cell subset that possesses pDC and cDC phenotype and function has been reported (112–114), but the biological significance of this is still unclear.

Plasmacytoid DC development and homeostasis. In mice and humans, pDCs have been shown to arise from a DC-restricted progenitor population in the bone marrow. E2-2 is the master transcription factor regulating pDC development and controls the expression of other transcription factors, including SPIB, IRF7, and IRF8 (115). The fate of pDCs upon inflammatory tissue entry is unclear. The transcription profiles of pDCs in humans and mice reveal a shared specialized program dedicated to type I IFN production critical for protection against viral infections. This functional specialization is exemplified by the developed endoplasmic reticulum–Golgi apparatus and the narrow range of PRR expression that includes TLR7 and TLR9 tailored for viral sensing. pDCs have been implicated in the development of autoimmune conditions such as psoriasis and systemic lupus erythematosus (116–119).

FUNCTION OF SKIN MONONUCLEAR PHAGOCYTES

Priming Adaptive Immune Responses

DC paradigm. Migratory DCs are adept at transporting captured antigen from the periphery to regional lymph nodes and subsequently activating naive T cells (**Figure 1**). DCs provide three signals to T cells during immune priming: (*a*) T cell receptor (TCR) activation, (*b*) costimulatory molecule activation, and (*c*) specific combinations of cytokines (120). These signals can be modulated by the environment, nature of stimulus, and the adjuvant used to activate DCs. A two-stage model for DC immune licensing was initially proposed: DCs in the tissue are immature with low levels of migration and low expression levels of costimulatory molecules, thereby promoting T cell tolerance to benign environmental antigens and self-antigens. In the presence of inflammation, DCs undergo a maturation process, increase their rate of migration, and become highly immune stimulatory (121). Peripheral tissue DC maturation following inflammation or pathogen challenge results in upregulation of costimulatory molecules as well as MHC-II and CCR7. Mature DCs migrate to the lymph nodes, where they promote differentiation of various distinct T cell lineages through direct cell contact and cytokine secretion (**Figure 2**) (65). Murine total DC depletion models demonstrate their importance in initiating protective immune responses (122). As discussed below, a collective body of evidence demonstrates functional specializations of DC subsets to induce the appropriate immune response to a specific pathogen challenge. Selective DC deficiency has not been described in humans, but severe immune defects, particularly against mycobacteria, and autoimmunity occur in patients lacking peripheral blood DCs and monocytes

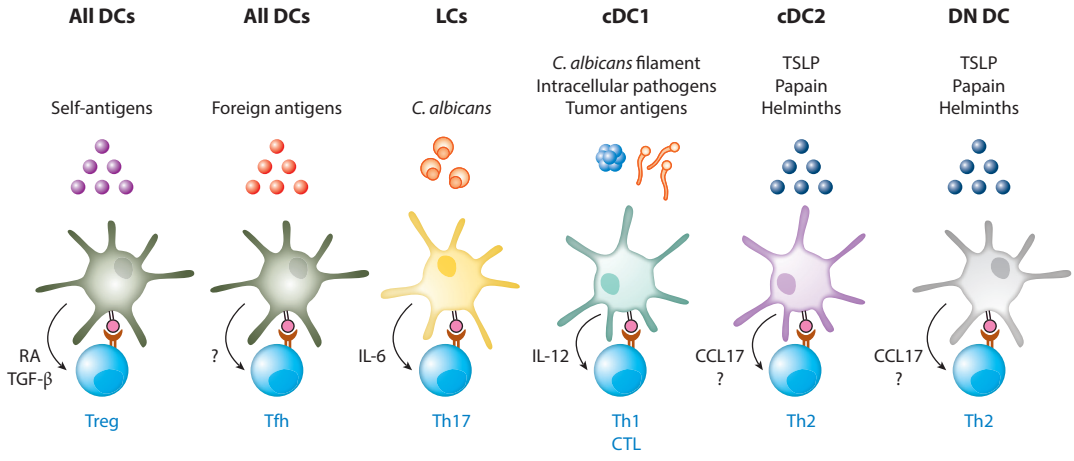

Figure 2

APCs in adaptive immunity. APC subsets induce common and distinct T cell activation and differentiation pathways. This illustration demonstrates the role of each DC subset in $CD8^+$ and $CD4^+$ T cell differentiation into various effector and memory T cell types. Differentiation is determined by the type of antigen, inflammatory cues, recognition and processing by a specific DC subset, and the subsequent MHC-peptide and cytokine signals from the DC and local microenvironment to the T cell. Abbreviations: cDC, conventional DC; CTL, cytotoxic T lymphocyte; DN, double negative; LC, Langerhans cell; RA, retinoic acid; Tfh, T follicular helper cell; TSLP, thymic stromal-derived lymphopoietin.

owing to *GATA2* and *IRF8* mutations, although these patients also have variable B cell and natural killer cell loss (78, 123).

Tolerance. Antigen presentation in the absence of costimulatory signals or appropriate cytokines can lead to induction of anergic or immunosuppressive T cells. Classic experiments targeting antigen via anti-DEC205 or, more recently, via anti-CLEC9A to cDC1s in the absence of an activating stimulus result in $CD4^+$ and $CD8^+$ T cell tolerance (124–126). More recently, FLT3L-dependent migratory skin DCs have been shown to be required for tolerance (127). DC-mediated immune tolerance is not a default outcome due to absence of inflammatory stimuli but an active process involving upregulation of MHC-II and CCR7 and is mediated by several mechanisms, such as activation through E-cadherin-mediated DC adhesion involving the β-catenin pathway (128, 129). Active NF-κB signaling has been shown to be critical for steady-state cutaneous DC migration to skin-draining lymph nodes (130).

In addition to inducing T cell deletion or anergy, DCs have the capacity to induce formation of regulatory T cells (Tregs) through the secretion of specific cytokines and factors such as TGF-β and retinoic acid (131–133). Steady-state targeting of self-antigens, but not foreign antigens, to LCs can induce Treg differentiation (134, 135). Notably, targeting many DC subsets in the absence of adjuvant also promotes development of protective humoral responses (135, 136). In humans, resting LCs have been shown to activate and induce proliferation of skin-resident Tregs (137). However, in the presence of a pathogen, LCs preferentially induced proliferation of effector memory T cells. In contrast, a recent study of Treg clusters in mouse skin-draining lymph nodes demonstrated close approximation of Tregs with dermal migratory cDC2s (138). Furthermore, dermal cDC2s in mice constitutively express aldehyde dehydrogenase, an enzyme involved in retinoic acid production, which is required for Treg differentiation from naive $CD4^+$ T cells (139). Dermal cDC2s also express the C-type lectin receptor for N-GalNac, macrophage

galactose C-type lectin type 2 receptor (Mgl2; CD301b) (93). Targeting of antigen via the human homolog of Mgl2, DC-ASGPR, in mice and in nonhuman primates induced IL-10-producing FoxP3−CD4$^+$ T cells (140). Despite strong evidence for the tolerogenic potential for individual DC subsets, no single constitutive DC subset deficiency model has been reported to result in autoimmunity. Thus, it is likely that individual skin migratory DC subsets are sufficient to promote peripheral tolerance, but with a high degree of redundancy between subsets.

Cross-presentation. Most cells can present intracellularly derived antigens to CD8$^+$ T cells, but presentation of exogenous antigen requires specialized processing and cross-presentation, a function critical for cytotoxic T cell responses against viruses, intracellular pathogens, and tumors. Identifying and targeting the appropriate DC subset for cross-presentation presents an enormous immunotherapeutic potential for effective vaccine design. Whereas all DC subsets may be able to present soluble antigens loaded in vitro to CD8$^+$ T cells, mouse and human dermal cDC1s have a superior ability to present particulate or necrotic cell–derived antigens for cross-priming to CD8$^+$ T cells, particularly in the context of TLR3 costimulation (16, 19, 141).

Batf3$^{-/-}$ mice deficient in cDC1s in dermis and secondary LT are unable to mount a CD8$^+$ T cell response against subcutaneous infection with West Nile virus, epicutaneous infection with *Candida albicans* or herpes simplex virus (HSV), or prime commensal-specific IL-17-secreting CD8$^+$ T cells and reject syngeneic tumors (75, 142–145). Recent studies have revealed the spatiotemporal dynamics of CD4$^+$ and CD8$^+$ T cell activation in lymph nodes. In a cutaneous HSV-1 infection model, migratory DCs first interacted with and presented antigen to CD4$^+$ T cells prior to antigen transfer to lymph node–resident cDC1s, which interacted with CD8$^+$ T cells (146). In a vaccinia virus infection model, infected DCs and uninfected DCs first interacted with CD8$^+$ T cells and CD4$^+$ T cells in two distinct areas of the lymph nodes. At a later stage of infection, cDC1s clustered with both CD4$^+$ and CD8$^+$ T cells to coordinate their activation. Depletion of cDC1s or MHC-II in cDC1s ablated the dual CD4$^+$/CD8$^+$ T cell clusters, but not single CD4$^+$ or CD8$^+$ T cell clusters, which adversely affected subsequent CD8$^+$ T cell memory response (147). Intravital imaging study of a photoconvertible reporter mouse strain capable of distinguishing migratory from resident DCs demonstrated that dermal cDC1s formed more sustained conjugates with CD8$^+$ T cells compared to resident cDC1s in lymph nodes (148). cDC1s also cross-present keratinocyte-derived antigens to CD8$^+$ T cells, suggesting a role in cross-tolerance (81, 145).

An additional complexity in evaluating DC function is comparing in vivo with in vitro studies, which is particularly pertinent in the assessment of human DC functions. LCs isolated from human or mouse skin explants efficiently cross-present antigens to CD8$^+$ T cells in vitro. However, in vivo, LCs were unable to generate CD8$^+$ T cell expansion in response to skin infection with HSV-1 or *C. albicans* (81, 143, 149). Furthermore, targeting LCs with foreign antigen in vivo in the presence of certain adjuvants resulted in cross-tolerance instead of cross-priming (150). Interestingly, all DC subsets isolated from human skin explants can prime CD8$^+$ T cells in vitro, but cDC1s are more efficient (19, 141, 142, 151, 152). Human skin cDC1s are also superior at cross-presentation (19).

Th1 cell differentiation. CD4$^+$ T helper 1 (Th1) cells provide immunity against intracellular microbes, such as bacteria and viruses, as well as tumors and require polarizing cytokine signals, such as IL-12 and IL-27. IL-12 is primarily secreted by cDC1s in the spleen in response to intraperitoneal *Toxoplasma gondii* (153). *Batf3*$^{-/-}$ mice lacking cDC1s failed to generate adequate Th1 cells, which together with impaired cross-presentation function rendered them unable to mount protective immunity against *T. gondii*. Similarly, intradermal infection of *Leishmania major* in dermal cDC1–deficient *Batf3*$^{-/-}$ mice led to unresolved and exacerbated pathology, with

increased parasite load, decreased IL-12, severe impairment of Th1 immunity, and concomitant skewing toward nonprotective Treg and Th2 responses (154). In a mouse model of epicutaneous *C. albicans* infection, *C. albicans* pseudohyphae, but not yeast, invaded into the dermis of mouse skin, leading to differentiation of Th1 cells via dermal cDC1s, but not LCs or cDC2s. These *C. albicans* Th1 cells protected against secondary systemic, but not skin, *C. albicans* infections (155).

Th1 polarization is not an exclusive property of migratory and LT cDC1s. $CD11b^+$ submucosal DCs were demonstrated to be critical mediators of Th1 cells in a vaginal infection of HSV-2 (156). Upon skin inoculation with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG), dermal cDC2s migrated to lymph nodes in an IL-1R-Myd88-dependent manner to promote BCG-specific Th1 responses (157). mo-DCs have also been shown to have a Th1 polarization property. During *L. major* infection, monocytes were recruited to the dermis and differentiated into mo-DCs, which subsequently migrated into the lymph nodes and controlled induction of a Th1 response (158). mo-DCs were potent IL-12 producers in vitro in response to *L. monocytogenes* (158, 159). For humans, dermal cDC1s, cDC2s, and peripheral blood mo-DCs were potent at inducing Th1 proliferation in vitro (31).

Th2 cell differentiation. Th2 cells participate in immunity against parasites and helminthes and play a pathogenic role in allergic and atopic diseases. Th2 polarization is associated with the cytokine and alarmin signals IL-4, TSLP, IL-25, IL-33, and CCL17. Recently, tremendous progress has been made toward identification of a Th2-driving DC subset. Transfer of $CD301b^+$ cDC2s but not dermal cDC1s isolated from FITC-sensitized mice induced a Th2-type immune response in a mouse model of contact hypersensitivity (160). Sensitization of skin with FITC-dibutyl phthalate (DBP) induced TSLP-mediated activation of dermal cDC2s to upregulate STAT5 and CCR7 and produce CCL17 to initiate Th2 differentiation (161, 162). Although all DC subsets express the receptor for TSLP, deficiency of TSLPR preferentially affected STAT5 phosphorylation in cDC2 and Th2 generation (162). STAT5 deficiency in dermal DCs, but not LCs, was required for Th2 type contact hypersensitivity response, suggesting that cDC2s possess a unique capability to promote type 2 responses (162).

Depletion of cDC2s as well as some macrophages and monocytes in *Mgl2*-DTR mice blunted Th2 response to ovalbumin (OVA) and papain immunization as well as *Nippostrongylus brasiliensis* infection without affecting Th1 and T follicular helper (Tfh) cell responses (93). Similar studies using *Itgax*-Cre *Irf4*-floxed mice in which dermal cDC2s and double-negative DCs have impaired DC migration to lymph nodes also showed defective Th2 responses to papain, *N. brasiliensis*, *Schistosoma mansoni*, and house dust mite–induced allergic inflammation (94, 96). In contrast, dermal cDC1s suppressed development of helminth-driven Th2 responses through steady-state production of IL-12 that was independent of TLR signals or commensal microbes (164). Notably, coculture of stimulated migratory cDC2s with T cells in vitro did not lead to Th2 differentiation, suggesting that dDCs are not sufficient inducers of Th2 cells. Targeted depletion of group 2 innate lymphoid cells (ILC2s) led to an impairment of memory Th2 cell localization in the skin and lungs after allergen rechallenge owing to the absence of ILC-derived IL-13 that induced $CCL17^+$ cDC2s (165). Thus, there is a coordinated feedback loop between ILCs and DCs in the skin that controls recall and likely priming of Th2 cells. Human LCs, dermal cDC2s, and cDC1s can all induce Th2 cells in vitro (31).

Th17 cell differentiation. Th17 cells protect against extracellular fungal and bacterial pathogens and can be pathogenic in autoimmune diseases such as psoriasis. Polarization of Th17 cells requires combinations of TGF-β, IL-1β, IL-6, and IL-23 (166). In humans and mice, immunity against

C. albicans requires Th17 cells. DOCK8 mutations in humans that result in defective DC migration as well as many mutations in fungal recognition or Th17 differentiation all result in chronic mucocutaneous candidiasis and hyper-IgE syndrome (167–169).

In mice, *C. albicans* epicutaneous skin infection promotes LC migration to lymph nodes, where they express high amounts of the Th17-differentiating cytokines IL-1β, IL-6, and TGF-β (143). Constitutive or conditional depletion of LCs leads to intact *C. albicans*–specific T cell expansion but significantly decreased Th17 cell differentiation (143, 155). LC-derived IL-6 was necessary for Th17 cell differentiation, whereas LC-extrinsic IL-6 was dispensable (155). Engagement of dectin-1 on LCs by budding yeast in the epidermis but not by filamentous forms of *C. albicans* in combination with MyD88 signaling was required for IL-6 production (170). Differentiation of Th17 cells did not provide protection from an initial encounter with *C. albicans*; it provided protection against secondary skin but not systemic infections (143, 155). Notably, dermal cDC1s were required for Th1 differentiation and provided protection from secondary systemic *C. albicans* infection. In a mouse model of *Staphylococcus aureus* dysbiosis, LCs were required for the generation of IL-17-producing $CD4^+$ TCR αβ and TCR γδ T cells in the skin (171). In humans, skin migratory LCs are potent inducers of Th17 differentiation through the combined effects of IL-6 and IL-15 (172).

Migratory cDC2s are required for Th17 cell generation against bacterial and fungal pathogens other than *C. albicans* (89, 173). *C. albicans* yeast do not extend sufficiently deep to interact with dectin-1 on cDC2s (155). Although dermal cDC1s and cDC2s are not required for Th17 response against *C. albicans* (155), they play a role in activating and differentiating IL-17-secreting $CD8^+$ T cell (Tc17) responses to the skin commensal *Staphylococcus epidermidis* and provide heterologous protection to *C. albicans* (144). Th17 polarization propensity in mice by skin DC subsets is thus determined by the infecting pathogen.

Tfh differentiation and humoral immunity. DCs and LCs have been shown to be important for B cell–mediated antibody responses through induction of Tfh cells, which support plasma cell and germinal center responses. Antigen capture through the epidermis by LCs was critical for protective IgG1 production in a mouse model of *Staphylococcus* scalded skin syndrome (29). LC depletion leads to low serum IgE levels and decreased germinal center–Tfh cell interactions after *L. major* infection (174, 175). Epicutaneous application of OVA induced OVA-specific IgE increase, OVA-specific T cell proliferation, and IL-4 mRNA expression in the draining lymph nodes that were dependent on presence of LCs. Mice deficient in TSLPR on LCs demonstrated abrogated antigen-specific IgE levels in response to sensitization (174). Unlike epicutaneous protein immunization, germinal center formation in response to a hapten that can penetrate deeper into the skin was found to be independent of LCs (175). Antigen-specific IgE and IgG1 are reduced after dermal injection of antigen in *Itgax*-Cre *Irf4*^{fl} mice, indicating a requirement for dermal $IRF4^+$ DCs (i.e., cDC2s) (94). Interestingly, Tfh and humoral responses are unaffected in *Mgl2*-DTR mice after dermal infection with *N. brasiliensis* (176) but are exaggerated after epicutaneous protein immunization (177). This discrepancy may result from differences between *Itgax*-Cre *Irf4*^{fl} mice in which cDC2s are present but cannot migrate from the skin and *Mgl2*-DTR mice in which cDC2s as well as some monocytes and macrophages are ablated.

Steady-state antigen targeting to DCs via various antigen uptake receptors has induced robust humoral immunity. Specifically, targeting foreign antigen but not self-antigen in the absence of exogenous adjuvant to LCs and cDC1s through langerin and CLEC9A, respectively, induced a large expansion of Tfh cells that was accompanied by B cell activation, germinal center formation, and protective antibody responses against influenza infection (135, 178, 179). The mechanism of Tfh induction by antigen targeting to DCs was independent of IL-6 and type I interferons but

required B cells for sustained Bcl-6 expression (135). Thus, most skin DCs have the capacity to expand Tfh cells and promote humoral responses, but the precise role of individual DC subsets remains to be fully elucidated.

TISSUE IMMUNE REGULATION

Recall and Memory T Cell Response

Regulation of recall and effector memory responses occurs in the lymph nodes and regionally in the skin. In the past, the major focus of study had been the role of skin APCs in recruiting and regulating the fate of peripheral blood circulating memory and effector memory T cells through chemokine and cytokine secretion. However, the paradigm change in T cell biology of long-lived noncirculating resident memory T cells in peripheral tissues underscores the importance of regional immune regulation in the skin. Both APCs and T cells are abundant in human skin. There are twice as many T cells and ten times as many DCs present in the average adult skin surface area of 1.7 m^2 compared to 5 L of peripheral blood (180), in keeping with the role of skin as an immune surveillance and regulatory organ.

The importance the APC–T cell interaction in the skin is evidenced by the development of clusters of APCs with T cells within dermis in various animal models of infection and contact hypersensitivity (**Figure 3*a***). In a murine model of contact dermatitis, dermal DCs and T cells are in direct contact as clusters around postcapillary venules during the elicitation phase of contact hypersensitivity (181). Interestingly, IL-1 from keratinocytes driving expression of CXCL2 by macrophages was important for dDC–T cell clustering and effector responses. Perhaps analogously, after HSV-2 infection of genital mucosa, IFN-γ-producing $CD4^+$ resident memory T cells activated macrophages to produce CCL5, thereby promoting memory lymphoid clusters that were required for protection to subsequent challenge (182). In the skin, CCL5 from $CD11b^+$ cells and $CD8^+$ T cells promotes T cell clusters around hair follicles (183). Clustering of DCs and T cells has also been demonstrated in psoriasis and allergic contact dermatitis patients (181, 184).

The maintenance of resident memory T cells in the epidermis does not require LCs (43, 185), but when cultured ex vivo with skin-resident memory cells, LCs have the capacity to expand Tregs or effector memory T cells, depending on the context (**Figure 3*b***) (137). After HSV-1 infection, reactivation of antigen-specific $CD8^+$ resident memory T cells required $CD4^+$ T cells as well as bone marrow–derived APCs and mo-DCs (186). In the dermis, antigen presentation by dermal cDC2s or mo-DCs after immunization with foreign peptide and CFA induced the recruitment of Tregs into the site of antigen, where they produced IL-10 and decreased the proportion of effector Th1 cells (187). Interestingly, IL-10 also signals on DCs to suppress contact hypersensitivity at a delayed time point owing to diminished T cell reactivation (188).

LCs and dDCs in humans also express CD1 molecules that bind and present lipids from mammalian and microbial cells, as well as natural skin oil components (25, 189, 190). T cells in the skin survey APCs for CD1-expressing antigens for changes in lipid content caused by infection, inflammation, or malignancy. T cells responded at higher rates to APCs expressing CD1a compared to other members of the CD1 family of molecules. These CD1a-autoreactive T cells home to skin and produce IL-22 in response to CD1a on LCs (**Figure 3*b***) (25). In the context of *Mycobacterium leprae*, CD1a-expressing LCs efficiently present a subset of nonpeptide antigens to T cells. LCs isolated from skin presented antigen to T cell clones derived from patients with leprosy in a CD1a-restricted manner more efficiently than mo-DCs (191). Recent studies reveal further subtleties relating to CD1a-mediated antigen presentation. Bee venom and house dust mites can generate neolipid antigens recognized by pathogenic CD1a-restricted T cells

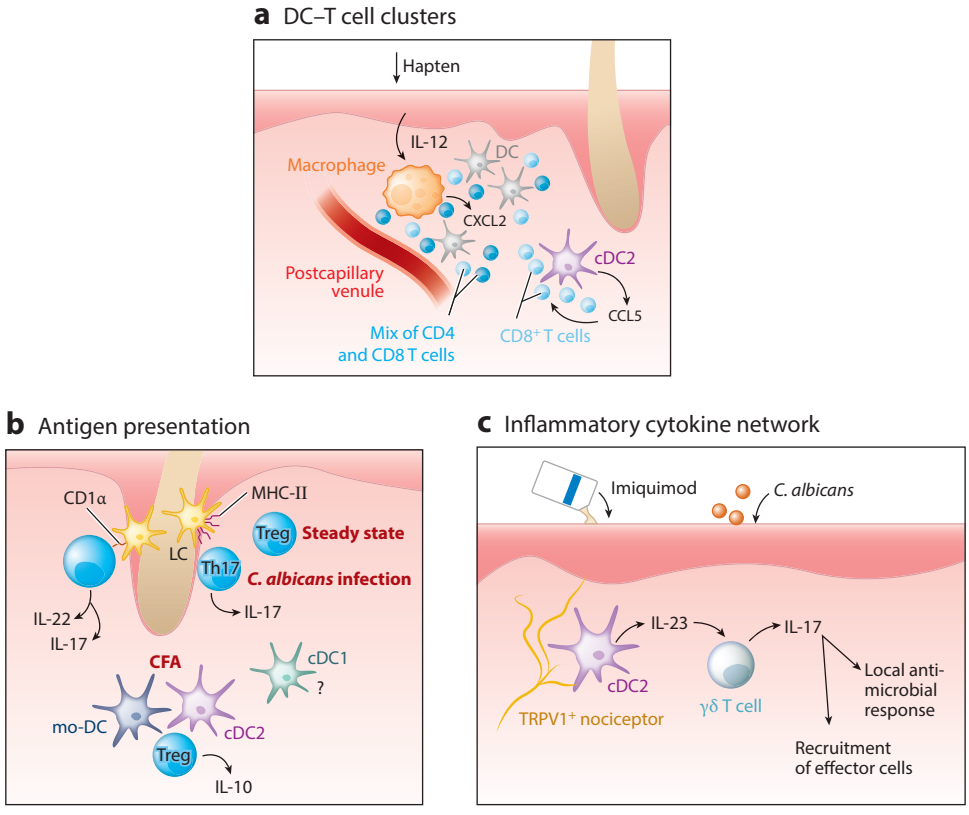

Figure 3

Regional immune regulation by APCs in the skin. Skin APC subsets collaborate with leukocytes and nonleukocytes in their microenvironment to maintain homeostasis and regulate immunity regionally. (*a*) Formation of DC–T cell clusters. (*b*) Antigen presentation to T cell subsets in the skin. (*c*) A local inflammatory cytokine network. Abbreviations: cDC, conventional DC; LC, Langerhans cell; mo-DC, monocyte-derived DC.

(192, 193). Interestingly, the skin barrier protein filaggrin, insufficiency of which is associated with atopic skin disease, inhibited house dust mite–derived phospholipase A_2 activity, which is essential for the generation of the neolipid antigens (193). Finally, presentation of urushiol antigen by CD1a on LCs in the context of poison ivy dermatitis activated IL-17-producing $CD4^+$ T cells. Similarly, presentation of self–lipid antigen by CD1a increased IL-17 expression by $CD4^+$ T cells in the imiquimod mouse model of psoriasis-like inflammation and in T cells isolated from psoriasis patients (194). Interestingly, CD1a deficiency in human LCs was reported but did not result in any obvious immune defect in the affected individuals (195).

Activation of TCR γδ T Cells

TCR γδ T cells mediate many aspects of cutaneous Type 17 immunity and are both resident and recruited into human and murine dermis. These cells produce protective IL-17 in response to infection with BCG, *S. aureus*, and *C. albicans* (196–199). Dermal TCR γδ T cells express the receptor for IL-23, which is required for their proliferation and production of IL-17

(199, 200). In the setting of *C. albicans* skin infection, IL-23 from dermal CD301b$^+$ cDC2s but not LCs or cDC1s was required for expansion and production of IL-17A by dermal TCR γδ T cells for optimal host defense (**Figure 3*c***) (201). Interestingly, production of IL-23 by CD301b$^+$ cDC2s required intact nociception (pain sensation) and the neuropeptide CGRP acting upstream of CD301b$^+$ cDC2. In a filaggrin mutation mouse model of atopic dermatitis, LCs were required for *S. aureus* dysbiosis and expansion of IL-17-secreting CD4$^+$ T cells and TCR γδ T cells that mediate immune pathology (171).

The psoriasis-like skin inflammation that results from repeated application of imiquimod requires IL-17 from TCR γδ T cells (196, 200, 202). The source of IL-23 in this model is unclear, with reports that dermal cDC2s and/or double-negative DCs were the critical producers or IL-23, whereas others found that LCs produced IL-23 and were required for IL-17 and resultant inflammation (202, 203). In an elegant study, dermal DCs were shown to be in proximal contact with sensory nerves in the dermis (200). Ablation of nociceptive responses in mice led to decreased production of IL-23, IL-17, and imiquimod-induced inflammation.

Epithelium–APC Interactions

Keratinocytes do not express MHC-II in steady state but can be induced to express MHC-II under inflammatory conditions such as graft-versus-host disease, autoimmunity, allergic contact dermatitis, and tuberculin reactions through signaling via IFN-γ (204, 205). Cocultures of human keratinocytes stimulated with IFN-γ showed increased expression of ICAM-1, but not CD80/CD86, and a potent ability to induce stimulation of functional CD4$^+$ and CD8$^+$ T cells in response to peptide (206). Thus, antigen presentation by keratinocytes likely participates in local activation of effector memory T cells or resident memory T cells, but their precise role as APCs during adaptive cutaneous responses remains to be determined.

Keratinocytes play a critical role in the recruitment and maintenance of LCs within the epidermis. During inflammation a subset of Gr1^{hi} monocytes rapidly accumulated near hair follicles and migrated to the interfollicular epidermis via a CCR2- and CCR6-dependent mechanism (58). Ligands for CCR2 and CCR6 were shown to be expressed by the hair follicle isthmus, whereas the infundibulum expressed only CCL20, the ligand for CCR6. CCR8 signaling inhibited LC recruitment, and its ligand, CCL8, was expressed by keratinocytes of the bulge, thereby excluding LCs from this region. Under steady-state conditions, LCs require autocrine/paracrine TGF-β1 for epidermal maintenance (40, 207, 208). Loss of TGF-β signaling leads to spontaneous migration of LCs to skin-draining lymph nodes. TGF-β is secreted as an inactive, latent form that requires activation by the integrins $a_v\beta_6$ or $a_v\beta_8$, which are expressed in nonoverlapping patterns by keratinocytes (43). Steady-state and UV-induced LC migration requires loss of TGF-β signaling in LCs and was associated with reduced expression of $a_v\beta_6$ and $a_v\beta_8$ by keratinocytes.

SKIN APCs AND CANCER IMMUNITY

Analysis of APCs in tumor models has demonstrated their role in tumor rejection and enhanced carcinogenesis. The importance of LCs for the development of epidermal malignancy has been demonstrated using a polyaromatic hydrocarbon (PAH)-induced, *Hras*-mediated chemical tumorigenesis model of squamous cell carcinoma. Mice lacking LCs, but not TCR αβ or TCR γδ T cells, were protected from tumor development. LCs express CYP1B1, which metabolizes DMBA to an intermediate that induced *Hras* mutations in keratinocytes (209). CYP1B1 expression by LCs is required for DNA damage and neoplasia but not tumor progression, suggesting that LCs promote tumorigenesis through additional but still uncharacterized mechanisms in this

model (210). This is supported by the observation that LC-deficient mice developed expanded chronic UVB-induced p53 mutant clonal islands and enhanced UVB-induced carcinogenesis. Enhanced UVB carcinogenesis in LC-deficient mice was independent of all T cells but was associated with increased LC-dependent IL-22 production by epidermal ILCs (211).

The function of DCs and macrophages in human and mouse models of melanoma has been studied extensively. In human metastatic melanoma tissue, type I interferon transcriptional profile correlated with T cell markers (212). Murine tumor studies demonstrated that IFN-β was produced by $CD11c^+$ APCs after implantation, and type I interferon signaling on APCs was required for intratumor accumulation of dermal cDC1s. *Batf3*$^{-/-}$ mice lacking LT and dermal cDC1s had diminished tumor-specific $CD8^+$ T cell response and tumor resistance. Further studies in mouse melanoma models demonstrated that prominent tumor-associated macrophages had the most potent uptake of the tumor particles, whereas dermal cDC1s and cDC2s were sparse and took up little tumor antigen (213). However, cDC1s were superior at activating naive $CD8^+$ T cells and were required for efficient antitumor adoptive cytotoxic T cell therapy. Finally, analysis of quantitative relative gene expression data from human cancers matched with survival outcomes demonstrated that the gene signatures of dermal cDC1s correlated with better outcome in human cancers (213). More recent studies have further shown that although cDC1s were sparse within B16 melanomas, they were the only tumor myeloid cell subset capable of activating tumor-specific $CD8^+$ T cells (214). Checkpoint blockade with PD-L1 has been demonstrated to be effective in tumor immunotherapy in mouse models and clinical trials. Notably, mice that had a constitutive absence of cDC1s did not experience benefits of delays in tumor rejection associated with PD-L1 blockade. Conversely, administration of FLT3L and the TLR3 adjuvant, poly I:C, dramatically improved tumor clearance mediated by cDC1s and type I interferons. Although PD-L1 blockade was effective in delaying tumor growth, it did not lead to an increase in tumor-infiltrating $CD8^+$ T cell numbers. When combined with FLT3L and poly I:C, PD-L1 blockade became significantly effective at blunting tumor growth as well as inducing the recruitment of tumor-infiltrating T cells (214). Thus, tumor-associated cDC1s are critical drivers of antitumor $CD8^+$ T cell immunity and can be exploited in tumor immunotherapeutic strategies. Recent studies have also exploited human blood DCs for use in melanoma vaccines, which appear to be more potent than mo-DC-based vaccines (215). Identifying the relevant tumor antigens enables additional refinements toward personalized DC-based cancer immunotherapy.

The tumor microenvironment can strongly impair APC function and antitumor immunity. The critical role of cutaneous immune regulation in the development of skin cancer was elegantly demonstrated by the findings of similar burden of somatic driver mutations between normal skin and skin cancer (216, 217). This is also supported by the well-recognized susceptibility of solid organ transplant recipients to developing squamous cell carcinomas while on immunosuppressive medication. In mice injected with syngeneic tumors subcutaneously, tumor cell–derived lactic acid induced the production of VEGF and arginase 1 by tumor-associated macrophages through HIF1α (218). Lactic acid polarizes macrophages toward an M2 phonotype, leading to a delay in tumor progression (218). Thus, metabolic products of tumors polarize macrophages toward a tumor permissive phenotype. Gain-of-function mutations and increased expression of β-catenin in melanoma lead to enhanced tumor growth and reduced recruitment of cDC1s into tumors (219). Genes associated with cDC1s, such as *CD141*, *BATF3*, and *IRF8*, were negatively associated with active β-catenin signaling in human melanoma metastases. Immunotherapy with anti-CTLA4 and anti-PD-L1 had no effect in tumor progression of active β-catenin tumors, but reconstitution with FLT3L-induced DCs had a significant delay in tumor progression and improvement in reducing tumor burden when combined with immunotherapy (219).

FUTURE PERSPECTIVES

The last several years have seen tremendous progress in accurately defining APC subsets incorporating ontogeny along with surface marker expression. This has allowed for a more comprehensive and accurate description of APCs in all tissues, including skin. Despite this progress, many currently defined subsets, including cDC2s, double-negative DCs, mo-DCs, and macrophages, still likely contain substantial heterogeneity. The application of single-cell RNA-sequencing and mass cytometry or proteomics-based high-dimensional techniques can provide unbiased approaches for precise taxonomy and nomenclature. This is of particular importance for the continued refinement of human and mouse APC subsets.

The development of increasingly specific mouse models with absent or defective APC subsets has gone hand in hand with the refinement of DC subsets. Despite the great utility of these models for the study of APC function in vivo, they can have incomplete or off-target effects and have the potential for unexpected compensatory consequences. Future models that can more selectively and efficiently target LT and NLT cDC2s as well as macrophages will be of great utility in dissecting the functional contributions of these populations.

Finally, our understanding of the effects of microenvironment on APC function remains nascent. It has become clear APCs can be functionally programmed by tissue-derived factors under steady-state conditions. The mechanistic details of this programming, the effect of the microenvironment on the steady state (e.g., microbiome) and inflammatory settings (e.g., pathogen infection, autoimmunity, tumors), the precise molecular circuits driving specific functional states, and tissue microanatomical features influencing APC function remain major challenges for future exploration.

DISCLOSURE STATEMENT

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