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# Review

# The balance between immunity and tolerance: The role of Langerhans cells

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**Abstract.** Langerhans cells are immature skin-homing dendritic cells that furnish the epidermis with an immune surveillance system, and translate information between the internal and external milieu. Dendritic cells, in particular Langerhans cells, are gaining prominence as one of the potential principal players orchestrating the decision between immunity and tolerance. Langerhans cells capture aberrant self-antigen and pathogen-derived antigen for display to the efferent immune response. Recent evidence suggests redundancy in the antigen-presenting function of Langerhans cells, with dermal dendritic subsets capable of fulfilling an analogous role. There is mounting evidence that Langerhans cells can cross-prime T cells to recognize antigens. Langerhans cells are proposed to stimulate T regulatory cells, and are implicated in the pathogenesis of cutaneous T cell lymphoma.The phenotype of Langerhans cells, which may be tolerogenic or immunogenic, appears to depend on their state of maturity, inciting immunogen and cytokine environment, offering the potential for manipulation in immunotherapy.

**Keywords.** Langerhans cells, dendritic cells, immunity, tolerance, cutaneous T cell lymphoma, immunotherapy.

## Introduction

Langerhans cells (LCs) were first described by Paul Langerhan in 1868 as star-shaped epidermal nerve cells [1]. LCs are now recognized as an immature subset of skin-homing dendritic cells (DCs), whose primary role is classically described as recognition of foreign invaders at the skin barrier and transfer of this information to the adaptive immune system. LCs comprise 3–8% of epidermal cells, with representation in the oral and genital mucosa, and perform immune surveillance by sampling the external milieu through a combination of phagocytosis, macropinocytosis, and receptor-mediated endocytosis [2, 3]. Foreign antigens encountered are internalized by these endocytic processes, with the foreign peptides processed and ultimately displayed on either class I or II major histocompatibility complex (MHC) molecules, allowing presentation to cytotoxic T cells and T helper cells, respectively [4]. In the accepted paradigm, antigen presentation to $CD4^+$ T helper cells via MHC II molecules promotes expansion of antigen-specific $CD8^+$ cytotoxic T cell populations, and antigen-nonspecific natural killer cells (NK), macrophages and eosinophils [5]. DC and possibly LC mediated secretion of type I interferon and IL-12 cytokine stimulates NK and γδT cell activation, which can destroy targeted cells that lack self-identifying MHC class I molecules. NK and γδT provide positive

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feedback for DC and LC maturation, with propagation of both innate and adaptive immune responses [6].

Additionally, DCs are unique among antigen-presenting cells (APCs) in their ability to cross-prime exogenous antigens for presentation to T cells, and the LC subset appears to share this ability.

Thus, LC mediated antigen presentation to CD8 T cells via the class I MHC pathway results in CD8 T cell cytotoxic effector function enabling destruction of both infected cells and tumor cells carrying the relevant cell surface peptides [7, 8]. Furthermore, DCs – alone among APCs – can elicit primary immune responses, resulting in the establishment of immunologic memory [9]. To come in contact with high concentrations of naive T cells, activated epidermal LCs must reach draining lymph nodes, guided by a chemotactic cytokine gradient and maturing during the migration process [10].

LCs are distinguished by their expression of Birbeck granules, 'tennis-racket' shaped cytoplasmic granules that are thought to play a role in endocytic function [11]. Constitutively associated with Birbeck granules is the transmembrane C-type lectin Langerin, which is involved in ligand internalization [12]. Until recently, Langerin was thought to be specific to LCs. However, Langerin has now been identified in a murine population of dermal dendritic cells ($L^+$DDC) with no human correlate as yet. Their function is unclear, but they are proposed to provide a second line of defense against pathogens that gain access to the dermis via a disrupted epithelial barrier and to protect against pathogens that preferentially infect the dermis [13, 14]. In murine studies, it has been noted that LCs are slower to arrive at draining lymph nodes than Langerin-negative DCs, raising the possibility of an immune regulatory role for LCs when they enter the lymph nodes [15]. The fact that no exclusive marker associated with Langerin-negative DDC has been discovered, makes them difficult to track such that their role in skin immunity has largely been overlooked in preference of Langerin bearing LCs.

## Ontogeny of Langerhans cells

All DC subsets ultimately originate from hematopoetic stem cells (HSCs) in the bone marrow. In early embryonic life, LC precursors localize to the skin, and cell division maintains an autonomous epidermal LC population in the absence of active inflammation [16]. In humans, different subsets of monocytes with the capacity to generate LCs have been identified; $CD14^+$ monocytes predominate, while $16^+$ monocytes are relatively rare [10, 17]. Murine monocytes exhibiting high expression of the monocyte marker Gr-1, are recruited to inflamed skin, and differentiate into LCs [18]. Historically, DC subsets including LCs were thought to be of myeloid lineage, and can be generated *in vitro* by culturing monocytes with granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-4 [19]. In murine studies, the addition of transforming growth factor β (TGF-β) to such cultures results in high levels of expression of the phenotypic markers characteristic of LC populations, while TGF-β knockout mice are profoundly deficient in epidermal LCs [20, 21]. Therefore, LCs have an *in vivo* requirement for TGF-β, with keratinocytes providing a paracrine supply of TGF-β in the skin [22].

The transcription factors PU.1 and Id2, and the notch ligand δ-1 further mediate LC differentiation from monocytes [23]. In mice, lymphoid restricted precursors are able to differentiate into functional LCs, as determined by the isolation of pure populations of common lymphoid progenitors (CLPs) followed to terminal differentiation [24]. The identification of lymphoid lineage LCs indicates plasticity in LC development rather than lineage restriction, with the suggested evolutionary design of redundancy in maintaining an essential cell line [25].

The initial misconception that mouse-specific CD8a was a marker of lymphoid origin lead to the conclusion that $CD8a^+$ LCs were of lymphoid origin, however CD8a expression appears to be an activation marker of mobilized, antigen exposed epidermal LCs [26]. Human and murine LCs express the leukocyte surface antigens CD45 and CD11c, the cutaneous lymphocyte-associated protein (CLA), and the myeloid markers CD33, CD13, CD1a, and CD11c. Adhesion molecules expressed by LCs include ß1 integrins, CD44, CD54, E-cadherin, and the sialyl-Lewis X monocyte carbohydrate antigen CD15 s. FcγRII/CD32 and receptors for IgE and the IgE-binding protein play a role in allergen uptake, while the lectins dectin-1, DEC205 and Langerin are implicated in antigen internalization. LCs also express MHC I and II molecules, and the invariant chain Ii/CD74, a membrane localized MHC class II chaperone [1].

In humans, coexpression of Langerin, CD1a (which plays a role in presentation of lipid microbial antigens), E-cadherin (which mediates LCs attachment to keratinocytes), the membrane ATPase (CD39), the chemokine receptor CCR6, the adhesion molecule Ep-CAM, and the integrin CD11b, is highly specific for the LC population [14, 27, 28]. Originally, Langerin was thought to be an LC-specific marker, but it has now been identified in a population of DDC. The phenotype of murine $L^+$DDC is distinct from epidermal LCs, with murine $L^+$DDC expressing low/absent

**Table 1.** A comparison of three subsets of skin dendritic cells.

| Marker | Function | Human and murine | | Murine Langerin positive dermal dendritic cells | References |
|---|---|---|---|---|---|
| | | Epidermal Langerhans cells | Inflammatory dendritic epithelial cells | | |
| Birbeck granules | Endocytosis | + | – | – | [11,12] |
| Langerin | Ligand internalization | + | – | + | [12–14] |
| DC-SIGN | DC specific ICAM grabbing non integrin | – | + | ND | [29,30] |
| CD1a | Thymocyte antigen | + | – | – | [14, 27, 28] |
| CD103 | cell surface adhesion molecule (integrin) | – | – | + | [14] |

+, expressed; –, absent; ND, not defined.

**Table 2.** Differential phenotypes of Langerhans cells in mice and humans.

| Marker | Human | Mouse | References |
|---|---|---|---|
| CD86 | + | +/– | [31] |
| CD11b/CD18 | +/– | ++ | [1, 9] |
| CD11c/CD18 | + | ++ | [1, 8] |
| CD208/DCLAMP | + | – | [31] |
| CD44 | + | – | [1] |
| CD8α | ND | * | [26, 27] |
| CCR2 | – | + | [16] |
| CCR6 | + | +/– | [1, 28] |

++, highly expressed; +, expressed; +/–, low/absent; –, absent; ND, not defined; * expressed on maturation.

levels of CD1a, Ep-CAM, CD11b, and dectin-1 compared with the high levels of these molecules expressed on LCs.

Furthermore, murine $L^{+}$DDC express the integrin CD103, absent from epidermal LCs [14]. Lymphoid-derived plasmacytoid DC (pDC) expressing the lectin blood DC antigen 2 (BDCA2) also localize to the dermis in certain skin inflammatory conditions including contact dermatitis, atopic dermatitis and psoriasis [1]. Inflammatory dendritic epithelial cells (IDECs) are a population of epidermally localized DCs lacking Birbeck granules. They have been described in chronic inflammatory processes including atopic dermatitis, and have high expression of CD36, Fcε-RI, a potent IgE receptor, and low levels of CD1a [28]. Generally, DDC express C-type lectins mannose macrophage receptor (MMR), DC-specific ICAM grabbing nonintegrin (DC-SIGN), the scavenger receptor CD36, the chemokine receptor CCR5, the human coagulation factor XIIIa, and the monocytes/macrophage marker CD14; all of which are absent in LCs [29]. Thus, skin-homing DCs can be distinguished as epidermal LCs or DDCs on the basis of a phenotype that correlates with their function (as will be described in the next sections). The characteristic features of some of the DC subsets localized to the skin are shown in Table 1, and the phenotypic differences between human and murine LCs are summarized in Table 2.

## Langerhans cell precursor migration

LC precursors derived from the bone marrow must travel to the skin to fulfill their role as APC, with a pool of LC precursors seeding the skin during embryonic development [16]. Murine studies have shown that intravenously injected LCs specifically colonize the epidermis, and allogenic bone marrow transplantation (BMT) results in replacement of the host LC population by donor LC, indicating the migratory skin-homing capacity of the mobile pool of LC precursors [2, 32].

CLA has been implicated as the LC skin-homing receptor of the Langerhans precursors and is a ligand for endothelial E-selectin [33]. In the steady state, the turnover of epidermal LCs is low with infrequent recruitment of LC precursors to become epidermal LCs as exemplified by studies in parabiotic mice with shared blood supplies but distinct organs in which LCs did not mix. Furthermore, lethal irradiation of mice followed by BMT showed that host LCs remained for

a minimum of 18 months, while DCs present in other organs had been replaced by donor DCs within 2 months. Antigen-exposed LCs are thought to be replenished primarily by *in situ* proliferation of existing epidermal LCs under normal conditions. However, during proinflammatory states, LC precursors are actively recruited to the epidermis as shown by the initial rapid depletion of LCs on exposure to UV light, with replacement of epidermal LCs in murine studies within 2 weeks [10, 16].

Orchestrating the recruitment of LC precursors are the keratinocyte-derived monocyte chemoattractant protein-1 chemokines. In murine studies, UV light exposure resulted in a marked increase in the skin of the transcripts of the chemokine receptor CCR2 and the CCR2 ligands CCL2. BM chimeras of mice reconstituted with $CCL2^{-/-}$ had 75% fewer LCs compared with those reconstituted with wild-type BM, delineating the role of CCR2 in the rapid recruitment of LC precursors during inflammation [16]. Chemotaxis of LC precursors in response to these chemokines involves multiple steps. Rolling monocytes are arrested on endothelial cells, facilitated by CCL2 and the chemotactic chemokine CCL5 elaborated in inflammatory settings. CCL5, for example, is constitutively expressed in atopic skin [34]. Transmigration across the endothelial-dermal barrier involves interaction between monocytes and endothelial cells via adhesion molecules, in particular platelet/endothelial cell adhesion molecule-1 (PECAM-1) and the transmembrane protein CD99 [35]. The adhesion molecule P-selectin and its ligand P-selectin glycoprotein ligand-1 (PSGL-1) is constitutively expressed on resting endothelial cells and platelets with up-regulation in the inflammatory setting and mediates terminal differentiation of monocytes, in addition to adhesion and rolling [17]. Chemokines produced by LCs, epidermal keratinocytes, and dermal fibroblasts initiate signaling cascades resulting in enhanced integrin affinity and mobility. Specifically, interactions between the integrins CD11a/CD18, CD11b/CD18, ICAM-1, and ICAM-2 are implicated [36, 37]. Some of the cytokines attracting LC precursors to the epidermis require cleavage and activation by the matrix metalloproteinases MMP-2 and MMP-9 located on the LC surface and involved in degradation of the extracellular matrix [10]. The sum effect of the coordinated chemokine cascade is the migration of LC precursors to the epidermis, ready to capture and process antigen for presentation.

## Antigen-presenting capacity of Langerhans cells

DCs, including LCs, are extremely plastic cells, thus associating phenotype to function has proved challenging. Conventionally, LCs have been considered the primary APCs of the skin. They are ideal for this function given their high representation in the epidermis. Their surface area for sampling is increased by the presence of long dendrites, which also allow for optimal interaction with T cells on migration to lymph nodes [38]. They are avidly phagocytic, ingesting antigen that has penetrated the skin barrier as well as the apoptotic bodies of damaged, malignant, or dying cells [4]. CD1a, which presents lipid antigens is highly represented on the LC surface, yet absent on DDC [27].

LCs are equipped with membrane spanning toll-like receptors (TLRs) which recognize pathogen-associated molecular patterns (PAMPs), conserved microbial motifs [39]. Interaction with TLR results in activation of the NF-κB pathway and subsequent LC maturation, with increased expression of co-stimulatory molecules and MHC II, thus enhancing the LC antigen-presenting capability. The coordinated release of proinflammatory chemokines including the T cell stimulating factor, IL-12, expression of adhesion molecules and migration to lymph nodes where interactions with naive T cells ensues, resulting in high T cell stimulatory capacity [3, 4]. In human LCs, TLR-2 acts in conjunction with its associated receptors TLR-1 and TLR-6 in detecting components of mycobacteria and gram-positive bacteria. Bacterial antigen exposure also results in phosphorylation of the MAP kinase ERK, resulting in LC survival which may contribute to the tolerogenic capacity of LCs [39, 40]. Of note, TLR-4 receptor specific for the LPS moiety on gram-negative bacterial cell walls is largely absent in both mice and human LCs. TLR-4 is present on human oral LCs, but ligation of TLR-4 results in production of the inhibitory cytokine IL-10 and mitigation of T cell stimulatory capacity, suggesting a role in tolerance for the absence of TLR-4 in LCs [41]. TLR-3, an intracellular receptor, plays a pivotal role in detecting viral antigens in mice and humans [40, 42].

Some of the key immune functions historically attributed to LCs are now recognized as being performed with greater efficiency by other DC populations, questioning the role of LCs as the primary APCs of the skin. Human pDC, for example, express a broader range of TLRs, making them more sensitive to microbial pathogens [43]. Murine $L^{+}$DDCs appear in lymph nodes long before LCs after immune stimulation with fluorescent contact sensitizers, suggesting a role for LCs in immuno-

modulation rather than as primary APCs for at least some skin antigens [14]. Additionally, the lower levels of proinflammatory cytokines IL-1, IL-6, and TNF activity by murine and human LCs compared with keratinocytes and dermal dendritic cells suggests a tolerogenic component to LC immune regulation [44, 45]. Fcε-RI activation on IDECS of atopic human skin leads to a proinflammatory cytokine cascade for example, yet Fcε-RI activation on LCs of oral human mucosa enables tolerogenic pathways [44]. IDECs appear to play a pivotal role in chronic cutaneous inflammatory diseases, where they mediate a switch from the humoral and anti-inflammatory Th2 response to a proinflammatory Th1 response [46].

Platelet-activating factor (PAF) which acts as a phospholipid inducer of the immune response to microbes and contact hypersensitivity has been shown to induce LC migration although it has no effect on DDC migration to draining lymph nodes [47]. In PAF receptor-deficient mice where LCs migration is by design not possible, T cell mediated contact hypersensitivity still occurs, suggesting that LCs are dispensable as skin APCs, and that DDCs are able to serve this function [48]. Much of our insight into the interactions between LCs and parasites has been gained from the experimental model of *Leishmania major* infection. The observation that LC phagocytosed *Leishmania* parasites and triggered *L. major* specific lymphokine and human T cell expansion *in vitro*, has resulted in the initial hypothesis that LCs are central to the immune response to *Leishmania* [49]. Recent evidence gained from murine studies show that DDCs also phagocytose and transport *Leishmania* antigen to lymph nodes, and are sufficient to elicit a T cell mediated immune response [50]. Rather than being the primary APCs, LCs may play a role in containing the ongoing immune response to the parasite.

Similarly, LCs have been shown to contravene the immune response in contact hypersensitivity thereby limiting the pathologic potential of unrestrained innate immune system activation. This occurs by LC mediated presentation of self-antigen to $CD4^+$ T cells via MHC II, which may then go on to differentiate into regulatory T (Treg) cells [51, 52]. LC responses to viruses are complicated by the capacity of some viruses to subvert the immune response on internalization and propagate within the LCs, escaping immune surveillance. Given their distribution in the epidermis and mucosal epithelia, LCs are often the viral point of first entry, enabling viral access to target cells. Some viruses, such as the HIV and measles viruses, have cytopathic effects on DCs, whereas the double stranded DNA of other viruses can induce DC maturation or resistance to viral cytopathic effects as occurs with influenza. Internalization of viral particles is mediated by several mechanisms including: phagocytosis of virus infected apoptotic bodies in influenza infection; internalization via clathrin coated caveolae in respiratory syncytial virus infection; and expression of CD4 receptor and the CCR5 coreceptor on LCs with resultant HIV tropism for these cells [4]. DDC transmission of HIV-1 to T cells occurs via the lectin DC-SIGN.

The working assumption has been that LCs facilitate HIV-1 transmission in a similar manner through the lectin Langerin. Novel data derived from skin explant studies indicate that Langerin is an HIV-1 receptor, and Langerin-mediated HIV-1 internalization into Birbeck granules results in HIV-1 degradation and clearance. Of note, antigen naive LCs prevent HIV infection in this manner, however mature LCs are efficiently infected by HIV-1 with subsequent transmission to T cells, likely due to the down-regulation of Langerin that occurs on maturation [53]. The observation that activated LCs mediate transmission of HIV-1 to target cells is consistent with the higher rate of HIV infection in the setting of sexually transmitted diseases causing inflammation and ulceration of the genital mucosa [54].

In the classical paradigm, murine LCs exposed to foreign antigen transmigrate from the epidermis, through gaps in the basement membrane where the *lamina densa* is absent, clinging to collagen fibrils as they advance their cytoplasmic processes along dense networks of fibrils, accessing dermal lymphatics via intercellular spaces between endothelial cells [55]. Human studies have shown that LCs localize to the T cell-rich inner paracortex on arrival at draining lymph nodes [31]. Contributing to the controversy regarding the role of LCs as primary APCs is the possibility that murine epidermal LCs can transfer their viral antigen cargo to a $CD8\alpha+$ DC population, which then prime $CD8+$ T cells during herpes simplex virus I (HSV-1) infection. It remains unclear whether this finding identifies redundancy in routes of APC presentation to effector T cell populations, or suggests a diminished role for LCs as the primary APCs of the skin [56].

Following exposure to exogenous antigen, all DC subsets including LC undergo a similar pattern of phenotypic changes as they mature. In addition to interactions with the TLR ligands and NK cells of the innate immune system, heat shock proteins, chemotaxins, chemokines, proinflammatory cytokines, and costimulatory molecules effect DC maturation. Increased expression of the costimulatory molecules CD40, CD80, CD86 and the adhesion molecules VLA-4 and ICAM-1 occurs (as reviewed in [57, 58]). MHC-peptide complexes are mobilized from MHC II-rich intracellular compartments with exportation to

**Table 3.** Phenotypic changes on maturation of Langerhans cells.

| | Function | References |
|---|---|---|
| Up-regulated | | |
| MHC-I | Self-antigen presentation | [9, 30] |
| MHC-II | Exogenous antigen presentation | [9, 31] |
| VLA-4 | Integrin | [31] |
| ICAM-1 | Cell adhesion marker | [31] |
| CD40 | Costimulation on antigen presentation | [31] |
| CD80 | Costimulation on antigen presentation | [30] |
| CD83 | Maturation marker | [30] |
| CD86 | Costimulation on antigen presentation | [31] |
| CCR7 | Lymph node homing molecule | [55] |
| DC-LAMP | Lysosomal marker linked to antigen processing | [31] |
| Down-regulated | | |
| CD34 | Myeloid marker | [53] |
| CD14 | Lipopolysaccharide receptor | [28] |
| CD44 | Leukocyte adhesion/migration | [1] |
| CD11c | Integrin/cell adhesion/migration | [1] |
| CD36 | Scavenger receptor | [27, 28] |
| Langerin | Ligand internalization | [11–13] |

the cell surface for antigen coupled presentation to $CD4^+$ T cells. Degraded intracellular proteins are released into the cytosol for processing through the endoplasmic reticulum, complexed to MHC I molecules, and are subsequently delivered to the cell surface [4]. LCs have been noted to internalize antigen-loaded surface MHC-II into acidic compartments with an efficiency dependent on the degree of antibody cross-linking [59].

As LCs undergo maturation, DC-lysosomal associated membrane protein (DC-LAMP), a lysosomal marker linked to antigen processing is expressed in lysosomes; while CD83, a maturation marker also found to influence the turnover of cell surface MHC II, appears on the cell surface [60, 61]. Proinflammatory chemokine receptors, phagocytic and endocytic receptors are down-regulated, while lymphokine and chemokine receptors are up-regulated, notably CCR7 which orchestrates DC migration to the lymph nodes [62]. Priming of T cells occurs upon arrival at the T cell zones of lymphoid tissue. MHC-peptide complex association with T cell receptors (TCR) results in an antigen-specific interaction and recognition by CD28 on T cells of the costimulatory CD80 and CD86 molecules present on mature DC surfaces. LCs express high levels of CD1a allowing efficient presentation of microbial lipid antigen to T cells via the CD1 pathway [30]. The phenotypes of mature and immature LCs are shown in Table 3.

Cross-presentation is recognized as a key mechanism in the generation of tolerance to self antigens and induction of immunity to viral infection and tumors. There has been some controversy regarding the ability of LCs to engage in cross-presentation, with multiple studies now providing supporting evidence for this role [63–65]. Cross-presentation of antigens to cytotoxic CD8 T cells by LCs and DCs enables extracellular pathogens to access MHC I mechanisms, with cross-priming of $CD8^+$ cells effecting a cytotoxic response to most tumors and to viruses that do not infect APCs [63, 64]. Murine LCs have been shown to cross-present ovalbumin as an exogenous antigen to antigen-specific CD8+ T cells, notably with lower potency than C8+ splenic DC subsets [8]. Human papilloma virus-like particles (HPV-VLP) processed by human LC prime CD8+ T cells *in vitro*, while antigens captured from necrotic melanoma cells by LCs are efficiently cross-presented with subsequent priming of CD8+ T cells [64, 65]. The design of DC-based vaccines potentially inclusive of LC is a logical progression of the observation that tumor antigen-loaded DC can induce antitumor immunity in mouse studies [66]. The design of such vaccines has thus far been limited by incomplete information on the ideal tumor antigen delivery routes for DC to direct immune responses *in vivo*, and the appropriate DC subset to activate. Unactivated DC or activation of the wrong

DC subset may result in the silencing of the immune response [4].

## Tolerogenic role of Langerhans cells

While a tolerogenic role for LCs may initially seem contradictory given their antigen-presenting capacity, the need to limit the intensity of immune and inflammatory responses below pathologic levels, and to maintain self-tolerance and homeostasis of the immune system is clear [38]. The evidence for a tolerogenic function for LCs was initially based on the observation that during the steady state, LCs internalize self-antigens, namely constituents of apoptotic keratinocytes and melanocytes including melanin, yet this does not result in cutaneous autoimmune responses, leading to the conclusion that LCs mediate self-tolerance to skin antigens [67]. Constitutive expression of TGF-β and IL-10 mediate physiologic migration of LCs to lymph nodes for presentation of self-antigens. Notably, IL-10 produced by melanomas stimulates migration of tumor-resident LC, and is thought to contribute to the capacity for immune evasion seen in melanoma [38, 68].

While the conventional model for LC migration involves LC maturation, LC transporting self-antigen can migrate to lymph nodes in an immature state, consistent with a tolerogenic function for LC in the steady state. In human dermatopathic lymphadenitis, a disease characterized by an expanded LC population in a lymph node draining an inflammatory tributary area of skin, migrated LCs are primarily immature as indicated by their phenotypic markers [31]. Furthermore, the transportation of self-antigens to lymph nodes is independent of the CCR7 dependent mechanism that occurs during inflammation. In both mice and humans, epidermal keratinocytes and LCs can produce pro-opiomelanocortin (POMC)-derived neuroendocrine hormones such as α-melanoctye stimulating hormone (α-MSH) [69, 70]. α-MSH has been shown to promote secretion of the anti-inflammatory cytokine IL-10 and inhibit IFN-γ production by lymphocytes, as well as stimulate immature DC conversion to tolerizing DCs that can induce Tregs, although this effect has yet to be clarified in the LC subset [31]. LCs bear receptors for calcitonin gene-related peptide (CGRP) and are closely associated with CGRP producing nerves in human epidermis. Cultured human LCs have reduced antigen-presenting function in the presence of CGRP, suggesting a neuroendocrine component to tolerance and specifically a role for CGRP in inducing the tolerogenic potential of LCs [71].

On arrival at the lymph node, tolerogenic DCs inclusive of LCs are thought to proceed with antigen presentation to antigen specific T cells, but do not affect T cell activation and clonal expansion due to inadequate costimulatory signaling, or a net coinhibitory signaling. The inhibitory molecules IL-10 and the catabolizing enzyme indeolamine 2,3-dioxygenase (IDO) are key mediators of tolerance by enabling T cell anergy, T cell death, and Treg proliferation [72]. Interestingly, in both mice and humans mature DC down-regulate IDO on stimulation with IFN-γ, thus associating immature or semi-mature DC with the tolerogenic profile. Tregs can, however, produce IL-10 and TGF-β, which prevent the IFN-γ inhibitory effect on IDO production, such that induction of a Treg response consistently results in an immunosuppressive profile [73, 74]. Furthermore, tolerogenic DCs are resistant to maturation in response to the usual maturation signals including TLR ligands, and are refractory to T cell or NK cell-mediated killing [72]. While there is mounting evidence for the role of DCs in immune tolerance, the tolerogenic role of the LC subset remains inconclusive [56]. Many of the murine studies probing DC tolerance involve antigen delivery intradermally and subcutaneously, and are ultimately unable to distinguish between LCs and DDCs as mediators of the tolerogenic outcome [75, 76]. It is, for example, conceivable that LCs may 'hand-off' encountered antigen to DDCs, which may then orchestrate tolerance between epidermal LCs and DDCs [56]. Further work is needed to fully delineate the role of LCs in immune tolerance.

## Conclusion

The portfolio of DCs and in particular LCs is highly plastic. Depending on their maturation status, captured immunogen and extent of apoptotic cell death, environmental danger signals and cytokine mileu, LCs have been implicated in promoting adaptive T cell immunity, innate immunity, or tolerance to self-antigens. These diverse capabilities potentially allow LCs to affect immunostimulatory pathways in response to infections and malignancy, while LC mediated stimulation of Tregs may orchestrate immunosuppressive responses that inhibit autoimmunity, or deleteriously perpetuate tumor cell growth. Research into the LC mechanisms leading to either immunostimulatory or tolerogenic responses of LCs is intensifying, with the goal of manipulating either pathway for immunotherapy in a range of disease processes.

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