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HIGHLIGHTS

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REVIEW

Langerhans cells in the skin and oral mucosa: Brothers in arms?

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The skin and the oral mucosa represent interfaces to the environment that are constantly exposed to pathogens and harmless foreign antigens such as commensal bacteria. Both barrier organs share the presence of Langerhans cells (LC), distinctive members of the heterogeneous family of antigen-presenting dendritic cells (DC) that have the unique ability to promote tolerogenic as well as inflammatory immune responses. While skin LC have been extensively studied in the past decades, less is known about the function of oral mucosal LC. Despite similar transcriptomic signatures, skin and oral mucosal LC differ greatly in their ontogeny and development. In this review article, we will summarize the current knowledge on LC subsets in the skin compared to the oral mucosa. We will discuss the similarities and differences in their development, homeostasis, and function in the two barrier tissues, including their interaction with the local microbiota. In addition, this review will update recent advances on the role of LC in inflammatory skin and oral mucosal diseases.

Keywords: Immune regulation · Langerhans cells · Ontogeny · Oral epithelium · Skin epidermis

Introduction

Langerhans cells (LC) are a special subset of antigen-presenting cells (APC) residing in stratified squamous epithelia like the skin and the oral mucosa [1]. They were originally identified in the skin in 1868 by Paul Langerhans as part of the peripheral nervous system due to their dendritic shape [2]. It took more than a century before epidermal LC were reassigned to the hematopoietic system as members of the dendritic cell (DC) family [3]. LC can be identified by the expression of Langerin/CD207, a membrane-bound C-type lectin receptor that is involved in antigen uptake and the formation of Birbeck granules. These racket-shaped organelles can be visualized by electron microscopy and are hallmarks of skin and oral LC [4–7]. It was initially assumed that Langerin is exclusively expressed by epidermal LC and was therefore used in various genetic approaches to specifically deplete LC [8, 9]. These experiments, together with Langerin-specific antibodies, led to the discovery of a Langerin-positive dermal DC subset, which differs from LC in that it lacks expression of epithelial cell adhesion molecule (Epcam/CD326) [10–13]. Langerin-specific antibodies were also instrumental in identifying LC in the oral mucosa. In addition to Epcam, the cell adhesion molecule E-cadherin is exclusively expressed by LC in the skin and oral mucosa [14, 15]. Thus, in both barrier tissues LC can be distinguished from other DC subsets by coexpression of Langerin, Epcam, and E-cadherin (Table 1).

Due to their location in the epidermis, respectively, oral epithelium, LC act as the first line of defense against invading pathogens. At the same time, in particular in the steady-state, LC are also

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Table 1. Overview of steady-state skin and oral mucosa LC.

	Skin LC	Oral mucosal LC		
	LC	CD103$^+$ LC1	CD11b$^+$ LC2	moLC
				
Location	Epidermis	Epithelium		
Origin	Yolk sac macrophages, fetal liver monocytes	Bone marrow		
		Pre-DC1	Pre-DC2	Monocytes
	Radioresistant	Radiosensitive		
	Self-renewal	Replenishment via circulation		
General phenotype	Immature	Immature, activated		
	CD11c$^+$, MHCII$^+$, CD24$^+$, CD11b$^+$, Langerin$^+$ (CD207)	CD11c$^+$, MHCII$^+$, CD24$^+$, EpCam$^+$ (CD326), E-cadherin$^+$, Langerin$^+$ (CD207)		
Subset specific phenotype	F4/80$^+$, EpCam$^+$ (CD326), E-cadherin$^+$	CD11b^{low}, CD103$^+$	CD11b$^+$	CD11b$^+$, CD64$^+$, CX$_3$CR1$^+$
Transcription factors	Id2, IRF2, IRF8, Runx3	Id2, IRF8, FLT3 (partially)	Id2, IRF8, FLT3 (partially)	
Cytokines (signaling)	TGF-β1 (ALK5), BMP7 (ALK3)	TGF-β1 (ALK5), BMP7 (ALK3)		
Chemokines (receptors)		CCL2 (CCR2), CCL20 (CCR6)		

critical for maintaining tolerance towards both self-antigens and harmless foreign antigens, including commensals. To this end, LC form a dense network and, like typical DC, sample the environment and capture antigens by extending and retracting their dendrites [16, 17]. Upon antigen (Ag)-recognition through specialized receptors of pathogen- and damage-associated molecular patterns, immature LC are activated and transformed into mature cells. This maturation is characterized by the upregulation of MHCII and costimulatory molecules like CD40, CD80, and CD86. Concurrently, mature LC downregulate E-cadherin and Epcam, which anchor LC to keratinocytes and epithelial cells, respectively, in the skin and oral mucosa. Downregulation of these adhesion molecules is a molecular hallmark of a genetic program called epithelial-to-mesenchymal-transition, which has been suggested to control epidermal LC development and migration [18–20]. Interestingly, Epcam-deficient LC are not able to migrate from the epidermis to draining lymph nodes (LN), while E-cadherin seems to be dispensable for LC migration [21, 22]. Although both adhesion molecules are also expressed on oral mucosa LC, whether they influence their development or migration has not been investigated yet. The C-C chemokine receptor 7 guides the migration of skin and oral LC from the barrier tissues to the draining LN, where they instruct naïve T cells to differentiate into appropriate effector or regulatory T (Treg) cells [23–25].

Subsets of LC and DC and their tissue localization

Murine DC in the skin and oral mucosa can be classified into conventional DC (cDC), LC, and plasmacytoid DC (pDC). Although healthy skin and oral mucosa contain very few pDC, they rapidly enter inflamed tissues and play pivotal roles during viral infections due to their secretion of high amounts of type-I interferons (IFN). Since pDC only play a subordinate role in terms of classic Ag presentation, they will not be discussed further here and the interested reader is referred to other excellent review articles [26–28]. In general, cDC are identified by the expression of CD11c and MHCII and can be divided into two main populations depending on their common phenotypic markers, developmental transcription factors and cytokines, and functions: conventional type 1 DC (cDC1) and type 2 DC (cDC2). While cDC1 express the X-C chemokine receptor 1 (XCR1), cDC2 are characterized by the expression of the signal regulatory protein alpha (SIRPα)/CD172a [29]. Notably, LC in the skin were long thought to be part of the cDC family; however, new molecular insights into their biology have led to reconsider this original classification and recognize them as a unique APC subset.

Steady-state LC and cDC subsets

In steady-state mouse skin, LC are the only CD11c$^+$MHCII$^+$ APC population in the epidermis and are characterized by the coexpression of Langerin, Epcam, and E-cadherin (Table 1). In the oral epithelium, this seems to be more complex and niche-dependent: in the tongue epithelium LC are the only residing APC [30], whereas in the gingiva, only 80–90% of CD11c$^+$MHCII$^+$ APC are LC, while the remaining 10–20% represent macrophages [1]. Moreover, contrary to the homogeneous population of steady-state epidermal LC, oral mucosal LC can be divided into LC1 (CD11b^{low}CD103$^+$), LC2 (CD11b$^+$CD103^{neg}), and monocyte-derived LC (CD11b$^+$CD64$^+$) (Table 1, Fig. 1) [31].

In the mouse skin, cDC1 and cDC2 are found exclusively in the dermis and can be further differentiated into subpopulations. While all dermal cDC1 (CD11c$^+$MHCII$^+$XCR1$^+$) express Langerin, only a minor fraction also expresses CD103. Dermal cDC2 (CD11c$^+$MHCII$^+$Sirpα$^+$) can be distinguished into CD11b^{high} and CD11b^{low} (also referred to as double negative) cells [32, 33].

In the mouse oral mucosa, cDC are found in the lamina propria and, similar to the dermis, can be divided into cDC1 and

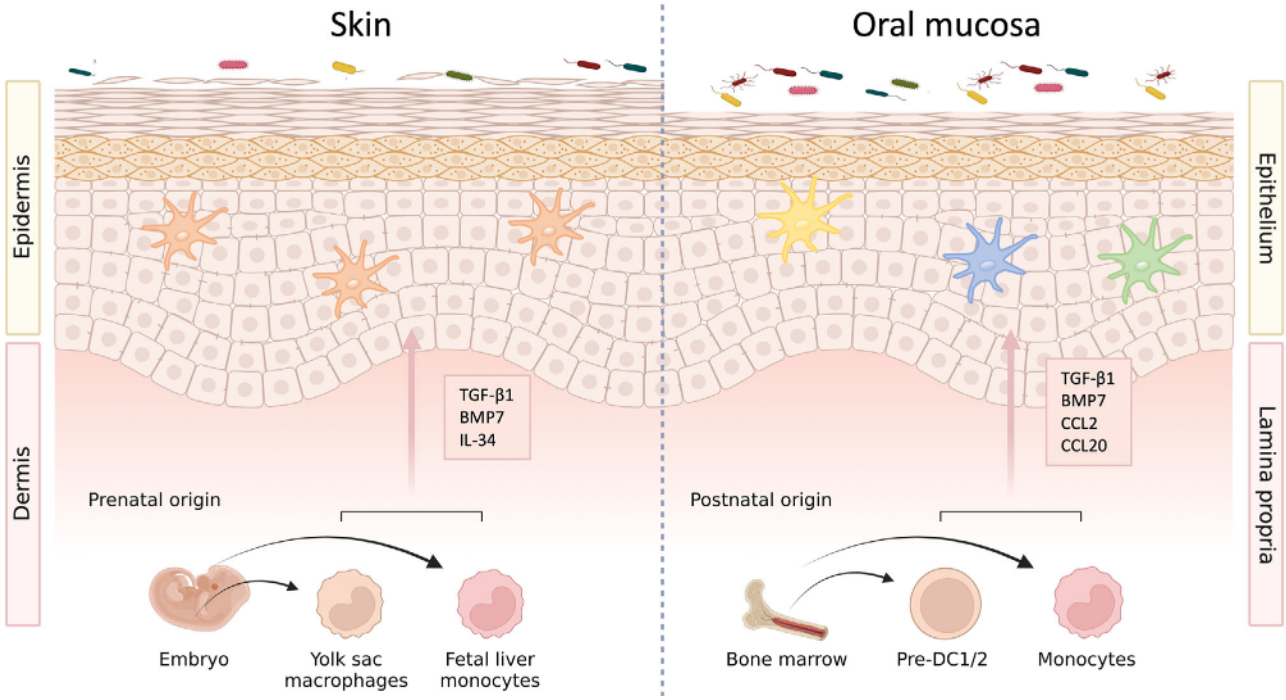

Figure 1. Homeostatic differentiation and functional specialization of LC in the skin and oral mucosa. LC reside in stratified squamous epithelia like the skin (left) and the oral mucosa (right) where they promote tolerogenic as well as inflammatory immune responses. Skin LC (orange) represent a homogenous population that originates prenatally from yolk sac macrophages and fetal liver monocytes. Subsequently, LC fill the epidermal niche by homeostatic proliferation during the first week after birth and self-renew in situ in the steady-state. On the other hand, oral mucosal LC comprise three distinct subsets: $CD103^+$ LC1 (yellow), $CD11b^+$ LC2 (blue) and moLC (green). They arise postnatally from BM-derived pre-DC and monocytes and are continuously replenished throughout life. In contrast to epidermal LC, the development of oral mucosal LC is regulated by the microbiota. Skin and oral mucosa LC exhibit high context-dependent functional plasticity. Figure created with BioRender.com (2022).

cDC2 according to their XCR1 and Sirpα expression, respectively. Within the cDC1 ($CD11c^+MHCII^+XCR1^+$) population, a small subset of cells expressing CD103 and in some tissues like the buccal mucosa, an even smaller subset of $Langerin^+$ cells can be detected. As in the skin, the major DC population in the oral tissue are cDC2 ($CD11c^+MHCII^+Sirp\alpha^+$) expressing CD11b [34–37].

Inflammatory LC and cDC turnover

Under inflammatory conditions, monocyte-derived LC (moLC) and monocyte-derived DC infiltrate mouse skin. moLC are recruited into the epidermis to replenish the loss of resident LC during inflammation and are replaced by resident LC once inflammation is resolved, demonstrating the existence of two types of inflammatory LC: short-term LC that develop from $GR\text{-}1^{hi}$ monocytes and long-term LC derived from LC precursors in situ [38]. In contrast to the skin, the oral mucosa harbors a moLC population already in the steady-state, which increases during inflammation. This is likely due to the fact that the epithelium of the oral mucosa presents a weaker barrier to the local microbiota compared to the robust keratinized epidermis. In addition, as a mucosal barrier, the oral epithelium interacts closely with and is continuously challenged by the microbiota (i.e., the dental biofilm on the tooth surface). As a result, at steady-state, the oral mucosa, like other mucosal barriers, is in a state of mild constitutive inflammation mediating low-level monocyte immigration (further discussed below).

Ontogeny and molecular control of LC differentiation

Despite the many phenotypic similarities described above, cutaneous and oral mucosal LC differ greatly in their origin and developmental pathways (Fig. 1, Table 1). LC in the skin first arise from yolk sac-derived myeloid progenitors around embryonic day 18 and are largely replaced by fetal liver monocytes during late embryogenesis [39, 40]. After birth, LC precursors acquire their dendritic morphology and phenotype in situ and undergo a massive proliferative burst, expanding their numbers by 10- to 20-fold to fill the epidermal niche [41]. While CD11c and MHCII are expressed immediately after birth, Langerin expression is only detectable 2–3 days later [42]. Skin LC form a radioresistant cellular network and are self-maintained locally throughout life [43]. As with macrophages, LC development and maintenance require the presence of IL-34, which is constitutively expressed by keratinocytes in the skin [44]. Due to their shared

ontogeny with macrophages, epidermal LC are often regarded as a tissue-specific macrophage subtype. Although functionally, they clearly represent cDC by migrating to LN, where they prime naïve T cells, rather than remaining in the tissue like macrophages [25, 45-47]. The fact that LC in the skin share features with both cDC and macrophages underscores their uniqueness among myeloid APC [48].

In contrast, LC in the oral mucosa are radiosensitive and unequivocally belong to the cDC family, with which they share both functional and developmental characteristics. They arise postnatally from bone marrow (BM)-derived pre-DC and monocytes and are continuously replenished throughout life (Fig. 1). These oral mucosal LC precursors enter the lamina propria via the circulation and differentiate into $CD11c^{+}MHCII^{+}$ cells in situ [31].

Differential control of LC development by TGF-β1 and BMP7

Although cutaneous and oral LC differ in their ontogeny, they share several cytokines and transcription factors required for their development and differentiation. One key molecule needed for the differentiation of both is the cytokine transforming growth factor-β 1 (TGF-β1). In the skin, it has been shown that mice deficient for TGF-β1 or the transcription factors Id2 or Runx3, which are controlled by TGF-β1, lack LC [49–51]. Further mouse studies with CD11c-specific deletion of the TGF-β receptor 1 (ALK5) revealed not only a crucial role for TGF-β1 in LC development but also in maintaining the pool of immature LC in the epidermis [52]. Moreover, a reduction of LC was also observed in mice lacking TGF-β receptor 2 or TGF-β1 specifically in LC, demonstrating that TGF-β1 produced by LC themselves acts directly on LC and promotes their own development through autocrine signaling [53, 54]. At the same time, the observation that deletion of components of the canonical TGF-β1 signaling cascade resulted in normal LC development called into question as to whether TGF-β1 is indeed required for LC development. Canonical TGF-β1 signaling is mediated via the type-I receptor ALK5, but is also dependent on type-II receptors, leading to downstream activation of the transcription factors Smad2 and Smad3 [55]. Impaired TGF-β1/Smad signaling was dispensable for LC development as demonstrated in Smad2- and Smad3-deficient mice [56, 57]. Simultaneously, bone morphogenetic protein 7 (BMP7), a member of the TGF-β superfamily, was identified as an inducer of human epidermal LC differentiation. BMP7 promotes LC differentiation and proliferation by activating the BMP type-I receptor ALK3 in the absence of canonical TGF-β1/ALK5 signaling. This led to the conclusion that BMP7 induces LC differentiation, whereas TGF-β1 promotes low-level cytokine production by differentiated LC, thereby maintaining the postnatal LC network and function [58].

Similar to the epidermis, the importance of TGF-β1/ALK5 and BMP7/ALK3 signaling for LC development and differentiation in the oral mucosa has recently been established [15]. Intriguingly, TGF-β1 expression is restricted to the suprabasal layer of the epithelium while BMP7 is expressed in the lamina propria, strongly suggesting that both cytokines play distinct roles in oral LC differentiation. Indeed, after entering the lamina propria, LC precursors are exposed to BMP7 and signaling via ALK3 leads to the expression of CCR2 and CCR6 on pre-DC, facilitating their chemokine-dependent translocation to the epithelium where CCL2 and CCL20 are expressed. Pre-DC and monocytes acquire their $CD11c^{+}MHCII^{+}$ phenotype before entering the epithelium, and blocking ALK3 signaling resulted in decreased numbers of these cells in the epithelium, underlining the importance of BMP7/ALK3 signaling for their entry into the epithelium. Once the pre-DC reach the epithelium, TGF-β1/ALK5 signaling takes over and drives their final differentiation into LC, including the expression of E-cadherin, Epcam, and later Langerin [1, 15]. In summary, TGF-β1 and BMP7 promote the translocation and differentiation of LC precursors into both the skin epidermis and the oral epithelium.

LC replacement during inflammatory conditions

Severe skin injury, such as exposure to ultraviolet (UV) light or cutaneous graft-versus-host disease (GVHD), leads to almost complete emigration of LC from the epidermis. In this context, LC repopulate the epidermis from circulating $Gr1^{hi}$ blood monocytes dependent on the expression of CCR2 and CCR6 [46, 59]. While CCR2 controls the recruitment of monocytes to the dermis, CCR6 guides their further migration into the epidermis. Consistent with these data, in vitro studies demonstrated that human $CD34^{+}$ hematopoietic progenitors give rise to LC through a monocytic differentiation pathway in response to the CCR2 and CCR6 ligands CCL2 and CCL20 [60]. Recent mouse experiments indicate that the initial wave of moLC reconstitution after UV radiation generates short-term LC that are transient and are replaced by a second wave of long-term LC derived from steady-state precursors in situ. Interestingly, short-term LC develop independently of Id2, while long-term LC are critically dependent on Id2 [38, 61]. Repopulation of the epidermis by LC of mixed origin, that is the circulation and local precursors, was previously observed upon resolution of skin disease following cutaneous infection with *Herpes simplex virus* (HSV) [62]. On the other hand, Ferrer et al. have demonstrated that resident LC can be exclusively replaced by circulating monocytes using a hematopoietic stem cell transplantation model in which allogeneic T cells are recruited into the epidermis and directly target embryo-derived LC. Under these conditions monocytes become long-term LC that engraft in the epidermis and acquire the capacity of self-renewal and proliferation. In contrast to the transient exposure to UV light, local LC precursors do not contribute to the emerging LC network during prolonged immune injury [61]. These findings underscore the plasticity of LC development in the skin and reveal different origins and pathways under steady-state and inflammatory conditions. Oral mucosal LC, on the other hand, are constantly challenged by the microbiota, resulting in a constitutively activated state resembling mild inflammation. This could explain the

Table 2. Context-dependent functional specialization of LC in the skin and oral mucosa. See text for details (↑ induction, ↓ suppression).

Context	Function	Reference
Skin LC		
CHS	Redundant	[64–68]
L. major	Regulatory: ↑Treg, ↓Th1	[71]
C. albicans	↑Th1, ↑Th17	[93, 94]
Malassezia ssp.	↑Th17	[95]
S. aureus	↑Th17	[97]
CSCC	Protective, ↑NK cells	[116]
Oral mucosa LC		
P. gingivalis	↑Treg, ↓Th1, ↑Th17	[35, 72]
OSCC	Protective	[115]

presence of moLC in oral tissues, which are not found in steady-state skin. Novel technologies such as unbiased single cell RNA-sequencing of steady-state oral moLC and inflammatory short-term epidermal LC will allow a detailed comparison to determine whether they represent the same cell type.

Functional specialization of skin and oral mucosa LC and cDC subsets

The identification of phenotypically distinct LC and cDC subsets in the skin and oral mucosa led to the hypothesis that each subpopulation exerts a particular function ("division of labor"). Of note, this concept is not mutually exclusive with the original idea that any given cDC is capable of responding appropriately to any pathogen challenge ("multitasking") [39, 63]. Rather, LC and cDC subsets exhibit a high level of functional plasticity along with a context-dependent functional specialization, which are the subject of much published and active ongoing research (Table 2).

Early studies demonstrated that epidermal LC are involved in both regulating immunity as well as tolerance. In mouse skin, the inducible diphtheria toxin–mediated depletion of LC in Langerin-DTR mice has been shown to ameliorate or leave unchanged the inflammatory response in contact hypersensitivity (CHS), depending on the concentration of the topically applied antigen (hapten) [64–68]. In contrast, Langerin-DTA mice, which constitutively lack LC from birth, exhibit an increased disease severity, suggesting a tolerogenic function of LC [69]. These conflicting results were attributed to the use of different transgenic mouse models, that is inducible versus constitutive ablation of LC, without understanding the underlying mechanism [8, 25, 39]. Recently, Kaplan and colleagues proved that the lifelong absence of LC in Langerin-DTA mice leads to a reduction of epidermal nerve endings from a subset of sensory nonpeptidergic neurons, resulting in hyperresponsive mast cells and cutaneous inflammation [70]. Of note, this was observed only with constitutive long term and not with inducible short-term LC ablation, which still supports functional redundancy of the different skin DC in CHS. On the other hand, a dominant negative regulatory function of LC was demonstrated after their selective short-term depletion in a physiological low-dose cutaneous *Leishmania (L.) major* infection model, which led to decreased Treg cell immigration and enhanced Th1 reactivity, resulting in improved parasite clearance and attenuated disease [71].

Similar to epidermal LC, a protective immunoregulatory role was also established for oral LC in controlling inflammation-driven alveolar bone loss following *Porphyromonas (P.) gingivalis* infection. Depletion of LC using Langerin-DTR mice resulted in reduced numbers of Treg cells, elevated levels of IFN-γ, and high numbers of $RANKL^+CD4^+$ T cells, which promote osteoclastogenesis, leading to increased alveolar bone resorption [35]. In contrast, another study showed that LC are not required for *P. gingivalis* driven alveolar bone destruction, but rather were responsible for promoting Th17 cell differentiation [72]. However, similar to the discrepancies in CHS in the skin this might be explained by the distinct mouse models used in both studies to ablate LC in vivo [8, 25, 39]. On the other hand, not much is known yet about the distinct functions of the different LC subsets in the oral mucosa, and further experiments are needed to understand their functional specialization, including a possible role of niche-specific localization within the epithelium. The finding that germ-free mice harbor less $CD103^+$ LC1 compared to SPF mice, while $CD11b^+$ LC2 numbers are hardly changed, suggests that mainly $CD103^+$ LC1 interact with the microbiota [15].

In murine skin, $Langerin^+$ cDC1 capture dead cell debris and express various receptors for the recognition of intracellular pathogens, including viruses. The most prominent function of this dermal cDC subset is the cross-presentation of pathogen-derived or self-Ag via MHCI molecules to $CD8^+$ T cells [73]. However, cDC1 also ensure peripheral tolerance to cutaneous Ag by deletion of self-reactive T cells [74]. Similarly, in oral mucosal tissues mice generated robust local and systemic $CD8^+$ T-cell responses after plasmid DNA immunization and, in particular, $CD103^+$ cDC1 were able to cross-present viral and self-antigens critical for the initiation of $CD8^+$ T-cell responses [75].

In mice, dermal cDC2 have been reported to preferentially present Ag to $CD4^+$ T cells and can induce the activation of memory T cells in nonlymphoid tissues [76]. $CD11b^{low}$ cDC2 are highly responsive to thymic stromal lymphopoietin, a cytokine produced by keratinocytes during contact sensitization. Thymic stromal lymphopoietin stimulates cDC2 and increases their C-C chemokine receptor 7–mediated homing to cutaneous LN where they initiate Th2 cell differentiation leading to allergic inflammation [77–79]. $CD11b^{high}$ cDC2 induce anti-microbial immune responses in mice colonized with *Staphylococcus epidermidis*. Here, the secretion of IL-1 by $CD11b^{high}$ cDC2 promotes the induction of IL-17A expression by $CD8^+$ T cells that home to the epidermis and limit pathogen invasion [80–82]. However, $CD11b^+$ cDC2 can also induce the formation of $Foxp3^+$ Treg cells by constitutive production of retinoid acid (RA) [83]. In the oral mucosa, cDC2 represent the most abundant cDC subset as well and appear to be the main cDC population inducing $CD4^+$ T-cell responses. As in the skin, oral $CD11b^+$ cDC2 present sublingual antigens and induce $Foxp3^+$ Treg cells in the draining LN after application of ovalbumin. These $CD11b^+$ cDC2 also produce RA and were able

to convert naïve CD4$^+$ T cells into Foxp3$^+$ Treg cells in a RA-dependent manner in vitro [36].

Similar to other LC and cDC subsets, moLC and monocyte-derived DC present Ag and are even able to cross-present Ag via MHCI to CD8$^+$ T cells in mice [84]. Therefore, they serve as a reservoir to supplement the LC and DC network during inflammatory conditions to ensure efficient Ag presentation and T-cell priming during ongoing inflammation. On the other hand, monocyte-derived cells may contribute significantly to chronic inflammatory diseases by producing inflammatory cytokines that augment the activation of skin T cells [85]. However, the complementarity and functional integration of cDC and monocyte-derived cells is actively explored and only beginning to emerge [86].

LC interaction with the microbiota

The microbiota plays critical roles in modulating tissue-specific immune responses particularly at barrier sites such as the skin and oral mucosa [87]. Both barriers host a plethora of microorganisms including bacteria, fungi, and viruses. Hence, the epithelium is well adapted to maintain homeostasis between the microbiota and host. This homeostasis is established and shaped by host–microbiota interactions early in life and has been recently reviewed in detail by Zubeidat et al. [88]. In addition, the immune system, that is, sentinel LC and cDC, faces the challenging task of maintaining homeostasis with beneficial commensals while fighting pathogen threats.

Here, we will focus on the role of LC and their crosstalk with the microbiota in the skin and oral mucosal tissues. Due to their strategic positioning in the epithelium, LC are in close contact with commensals and form the first line of defense against invading pathogens [89, 90]. In the skin, LC sample the environment and capture antigens in a dynamic process by extension and retraction of their dendrites through epidermal tight junctions to the stratum corneum [16]. Thereby, LC survey the microorganisms not only within the epidermis but also on the skin surface without disturbing barrier integrity [17]. The latter has been shown to confer preemptive immunity in experimental staphylococcal scaled skin syndrome [91]. In turn, the cutaneous microbiota does not influence the development and homeostasis of LC as they were neither affected in germ-free nor in antibiotic-treated mice [15]. This is likely due to the prenatal seeding of the epidermal niche and the strong mechanical barrier of the dry, keratinized skin epithelium that effectively shields LC from the microbiota [92].

In contrast, the microbiota in the oral tissues with their thin, moist epithelium shapes the postnatal differentiation of mucosal LC. Adult germ-free animals that lack microorganisms from birth harbor 50% fewer LC compared to SPF mice and cohousing of germ-free and SPF mice partially restored LC numbers [15]. This reduction was mainly due to a decrease in CD103$^+$ LC1, indicating that the development is microbiota-dependent specifically for this LC subpopulation. A similar reduction of oral LC was also observed after a 2-week antibiotic treatment of SPF mice. Although these microbiota-mediated changes in LC homeostasis were observed in gingival as well as buccal mucosal tissues, the effect was stronger in the gingiva likely due to the high microbial load in the dental biofilm [15].

Conversely, oral LC regulate microbial homeostasis since their prolonged ablation induces oral microbial dysbiosis, leading to inflammation and tissue destruction [15]. This was demonstrated in adult Langerin-DTR mice that were treated with diphtheria toxin for 4 months, resulting in the long-term ablation of LC [9]. Long-term LC-depleted mice exhibited an increased bacterial load, reduced frequencies of Treg cells, and exacerbated alveolar bone loss establishing that LC are indeed crucial for maintaining microbial homeostasis [15]. Consequently, alterations in the intricate crosstalk between the immune system and the microbiota at the oral mucosal barrier can drive inflammatory diseases like gingivitis and periodontitis. Moreover, this local chronic inflammation is linked to severe systemic comorbidities like cardiovascular disease. Thus, re-establishing microbial homeostasis not only has the potential to restore barrier tissue integrity but also to attenuate life-threatening systemic immune-mediated diseases.

Role of LC in skin and oral infectious diseases

Despite rapid progress in the field, the exact role of LC in different skin and oral mucosal diseases remains incompletely understood. LC are essential to induce antigen-specific Th17 responses after epicutaneous *Candida (C.) albicans* infection. Notably, only budding yeast triggered Th17 cell responses through LC-derived IL-6, while the filamentous form induced Th1 without Th17 cells. Intriguingly, Th17 responses provided protection against cutaneous infection, whereas Th1 responses provided protection against systemic infection, demonstrating that *C. albicans* morphology drives distinct T helper cell responses [93, 94]. Similarly, LC are crucial for *Malassezia*-induced Th17 responses, a commensal yeast that is a prominent part of the skin microbiota, and its dysbiosis is associated with atopic dermatitis and other common inflammatory skin disorders. Specifically, LC appear to be important for effective antifungal immunity by the early production of high levels of IL-23, which then activate T cells and innate lymphocytes to produce IL-17 [95, 96]. Infections with *Staphylococcus aureus* are associated with atopic dermatitis. Upon epicutaneous exposure, LC sense *S. aureus* cell wall teichoic acid through Langerin to induce protective Th17 responses [97]. A protective role of LC was also observed during HIV transmission. Here, Langerin facilitates the capture of HIV and prevents infection by delivering HIV into Birbeck granules for virus degradation [98]. On the other hand, following localized infection with skin tropic HSV, LC were dispensable and transferred their cargo Ag to LN-resident cDC1 to induce anti-viral immunity [99].

Oral pathologies are often induced by microbial dysbiosis. The availability of transgenic mouse models has established a strong link of LC and periodontal diseases, which has also been

made in humans. Similar to epidermal LC, oral mucosal LC are responsible for promoting Th17 cell differentiation in a mouse model of *P. gingivalis* induced periodontitis. Here, LC mainly drive Th17 responses while dampening Th1 responses without inducing alveolar bone destruction [72]. Consistently, in gingival tissue of patients suffering from chronic periodontitis an increased number of LC was observed compared to healthy controls [100, 101]. Likewise, higher LC numbers were detected in patients with chronic gingivitis [102]. Immigration of LC into the gingiva was induced in response to bacterial plaque accumulation, while periodontal treatment including intensive oral hygiene and mechanical removal of the bacterial plaque resulted in decreased LC counts in the gingiva [89, 103]. On the other hand, in line with a regulatory function of LC, in mice the ablation of LC resulted in reduced numbers of Treg cells, elevated inflammation, and alveolar bone loss in a model of *P. gingivalis* induced periodontitis [35]. Moreover, aged mice develop naturally occurring periodontal bone loss. Interestingly, the frequencies of gingival LC in both humans and mice decrease with age, suggesting a potential protective role for LC in age-related oral bone loss [92, 104, 105]. The reason for these incongruous findings that LC either promote or attenuate *P. gingivalis* mediated oral pathology remains elusive.

LC in squamous cell carcinoma

Recently, major progress has been made in uncovering a significant role of LC in cancer, particularly squamous cell carcinoma (SCC), with diverging data being generated for cutaneous and oral LC. Due to their epithelial localization, LC are essential in cancer immune surveillance as the first APC to encounter, take up, and cross-present tumor-associated Ag via MHCI to naïve $CD8^+$ T cells. This drives the generation of tumor-specific effector T cells capable of recognizing and eliminating cancer cells [106]. In cutaneous SCC (CSCC), LC numbers are decreased as compared to healthy epidermis, however, these tumor-infiltrating LC were more activated and induced higher $CD4^+$ and $CD8^+$ T-cell proliferation as well as IFN-γ production than LC from healthy human skin [107, 108]. Other studies reported increased or comparable LC numbers in the skin of CSCC patients [109, 110] and that cDC from CSCC are poor stimulators of T-cell proliferation [111]. Similar to the inconsistent observations in the skin, oral LC numbers were reported to be reduced [112, 113] or elevated [114] in human oral SCC (OSCC) compared to healthy tissue. A recent study in mice demonstrated that oral LC protect the epithelium from developing carcinogen-induced OSCC by rapidly priming αβ T cells capable to eliminate epithelial cells that have suffered DNA damage from the carcinogen. This indicates early antitumor activity of LC in OSCC [115]. Such an early protective function was also shown for epidermal LC during carcinogen-induced CSCC. Here, the production of TNFα by LC leads to the rapid recruitment of NK cells, which are crucial for the elimination of DNA-damaged keratinocytes [116]. In conclusion, despite the existing inconsistent data on the role of LC in SCC, numerous reports suggest that the presence of LC is associated with an improved prognosis and therefore the number of LC infiltrating the tumor is considered a prognostic parameter in patients with SCC [117, 118].

Conclusion

LC in the skin and oral mucosa are the first immune cells to encounter environmental Ag. Thus, they have to decide whether to induce a protective immune response against invading pathogens or to tolerate self- or harmless foreign Ag. In recent years, the availability of cell type-specific transgenic mouse models and major advances in high-dimensional screening techniques combined with unbiased analysis, have led to the identification and characterization of different LC subsets in the skin and particularly oral tissue. Thereby, it has become clear that skin and oral mucosa LC differ in their ontogeny. While epidermal LC share an embryonic origin with macrophages and are self-renewing throughout life, they can migrate and mature into functional DC. Oral mucosa LC, on the other hand, represent typical sentinel cDC that are continuously replenished from the BM, that is, the circulation. The fact that under inflammatory conditions cutaneous LC can also arise from BM-derived monocytes highlights their developmental plasticity, suggesting that to a large extent the local tissue environment shapes LC differentiation. Likewise, skin and oral LC exhibit high functional plasticity and become tolerogenic or immunogenic depending on the nature of the encountered pathogen. Thus, context rather than genetic programming defines LC development, homeostasis, and function. Dissecting the mechanisms for this dynamic heterogeneity will be challenging, yet, this knowledge holds great potential to target LC in both skin and oral tissues for future therapies of chronic inflammatory diseases or cancer.

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Abbreviations: **cDC**: conventional DC · **CHS**: contact hypersensitivity · **CSCC**: cutaneous SCC · **pDC**: plasmacytoid DC · **OSCC**: oral SCC · **RA**: retinoid acid · **SCC**: squamous cell carcinoma

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