

Multistep navigation of Langerhans/dendritic cells in and out of the skin

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Langerhans cells (LCs) are specialized antigen-presenting cells that reside in the epidermis as sentinels of the immune system. LCs constantly monitor the epidermal microenvironment by taking up antigen and processing it into fragments that can be recognized by cells of the adaptive immune response. Because of their unique migratory ability, LCs can transport antigen from the epidermis to regional lymph nodes, where they can initiate systemic immune responses. The mechanisms of LC trafficking thus seem to be of particular relevance for the induction and maintenance of cutaneous immunity. LCs or their putative precursors express surface molecules that allow them to home to skin and localize in the epidermis for prolonged periods of time. Tissue injury, microbial infection, and other perturbants of epidermal homeostasis (eg, contact allergens) provide danger signals, leading to a local production of proinflammatory cytokines that induce LC mobilization to the lymphoid tissue. At the same time, signals are generated that recruit LC precursors into the skin to maintain the epidermal LC population. Distinct pairs of chemokines and their receptors control the migration from blood to epidermis and from there to the regional lymphatics. In addition, trafficking is controlled at the level of cell adhesion, where LCs downregulate some adhesion molecules to exit the epidermis and upregulate others to migrate across the extracellular matrix and home to T-cell areas of regional lymphoid tissue. The improved understanding of mechanisms that regulate LC trafficking might offer new opportunities for therapeutic interventions to suppress, stimulate, or deviate cutaneous immune responses. (*J Allergy Clin Immunol* 2001;108:688-96.)

Key words: Langerhans cells, dendritic cells, migration-trafficking, adhesion, chemokines, skin, allergy, immune regulation

Epidermal Langerhans cells (LCs) are members of a family of highly specialized antigen-presenting cells termed dendritic cells (DCs). They are localized in the

Abbreviations used

ATP:	Adenosine triphosphate
CCR:	CC chemokine receptor
CLA:	Cutaneous lymphocyte-associated antigen
DC:	Dendritic cell
ECM:	Extracellular matrix
ELC:	EBV-induced molecule I ligand (CCL19)
LC:	Langerhans cell
MDR:	Multidrug resistance gene product
MIP-3 α :	Macrophage inflammatory protein 3 α (CCL20)
MMP:	Matrix metalloproteinase
SLC:	Secondary lymphoid tissue chemokine (CCL21)
TGF:	Transforming growth factor

basal and suprabasal layers of the epidermis (ie, at the interface between organism and environment) and represent important sentinels of the immune system. First described in 1868 by Paul Langerhans, a German medical student at the time, LCs were regarded as intraepidermal nerve cells because of their staining behavior, their dendritic morphology, and an apparent continuity with nerve fibers of the dermis. Since then, various concepts of LC origin and function emerged and disappeared again, until more than a century after their discovery, LCs were finally recognized as cells of the immune system.¹ In aggregate, epidermal LCs constitute a continuous network of cells (Fig 1) that are well equipped to ingest intruding microbes and other environmental components and to process complex antigen into small fragments that can be recognized by T cells. The unique migratory ability of LCs allows them to transport antigen from the epidermis to regional lymph nodes, where they can initiate systemic immune responses by presenting cell surface-bound processed antigen to resting unprimed T lymphocytes. LCs play a central role in a number of clinical conditions, ranging from allergic contact dermatitis and cutaneous infections to tumor rejection and HIV disease.² Because LCs act as initial gatekeepers in the cutaneous induction of systemic immune responses, the mobilization of epidermal LCs to regional lymph nodes, as well as the recruitment of LC precursors from the circulation into the skin, must be tightly regulated events. This review briefly outlines recent advances in understanding the mechanisms that control LC trafficking.

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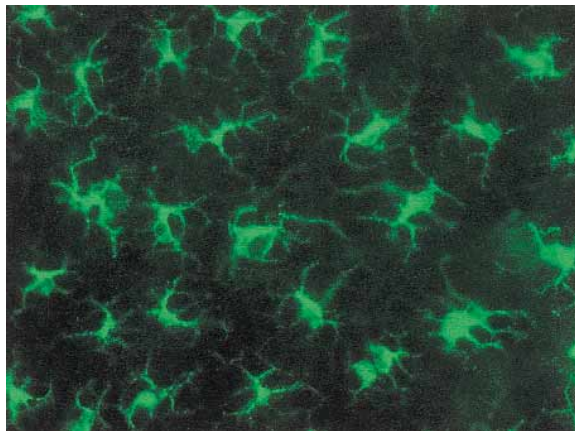


FIG 1. LCs form a network of antigen-presenting cells in the epidermis: en face view of an epidermal sheet prepared from murine ear skin and stained with anti-MHC class II mAb.

LC MIGRATION FROM THE EPIDERMIS TO REGIONAL LYMPH NODES

LC kinetics

The rate of LC turnover in normal epidermis is still somewhat uncertain. Studies of LCs in full-thickness grafts of murine skin demonstrated that LCs of graft origin can persist for as long as 5 to 8 months, suggesting either that LCs are very long lived or that they develop from dermal or epidermal precursors.³ After depletion by repeated tape stripping of murine skin, LCs (perhaps derived from local precursors) reappeared within 24 hours.⁴ Studies of skin grafts in mice demonstrated that blood-borne recipient LC progenitors can repopulate donor skin within 2 weeks.^{3,5} By using a radiation chimera model, the turnover (half-life) of epidermal LCs under steady-state conditions was calculated to be in the order of 15 days.⁶ Although LCs appeared almost stationary compared with DCs in other epithelial tissues (eg, airway epithelium; half-life approximately 2 days), there still seemed to be a slow but consistent turnover of LCs. Studies analyzing the numbers of LCs in the afferent lymph-draining normal human epidermis demonstrated that considerable numbers of LCs were continuously leaving the epidermis, even in the absence of inflammatory stimuli.⁷ Local activation by inflammatory stimuli, such as contact allergens or irritants, led to a dramatic increase of LCs in the afferent lymphatics, suggesting that under these conditions, LCs are actively mobilized from the skin.

What makes them stay?

While attempting to identify adhesion molecules that might retain LCs in their epidermal environment, Tang et al⁸ determined that LCs expressed high levels of E-cadherin and demonstrated E-cadherin-mediated adhesion of LCs to keratinocytes in vitro. E-cadherin is a member of a family of homophilic adhesion molecules that mediate intercellular adhesion and play an important role in maintaining tissue integrity.⁹ Classical cadherins (including E-cadherin) constitute a major component of ker-

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FIG 2. E-cadherin-containing cell junctions at sites of contact between immature skin-derived DCs and keratinocytes. Confocal image of in vitro-generated, immature LC-like DCs sitting on top of keratinocytes stained for F-actin (red), MHC class II (blue), and E-cadherin (green). Colocalization of MHC class II and F-actin is displayed in pink, and colocalization of E-cadherin and F-actin is displayed in yellow. Immature DCs can be identified by intracellular MHC class II expression. Note the focal accumulation of E-cadherin (green) at end of actin (red) containing spikes of DC processes. For details, see text.¹¹ Reproduced by permission of Blackwell Science, Inc.

atinocyte adherens junctions, suggesting that LCs may be anchored in the epidermis by forming similar junctions with keratinocytes. In coculture studies using in vitro-generated murine LCs¹⁰ and primary keratinocytes, we demonstrated that LCs indeed formed focal accumulations of E-cadherin at sites of cell contact with keratinocytes that closely resembled adherens junctions (Fig 2).¹¹

Given the slow turnover of LCs (compared with that of DCs in other epithelia), it seems likely that the epidermal microenvironment provides additional signals that retain LCs in their epithelial location. A number of studies indicate that the pleiotropic cytokine transforming growth factor β (TGF- β) may represent such a signal. Interest in the role of TGF- β was initiated by the observation that TGF- β 1 knockout (TGF- β 1 $-/-$) mice did not have epidermal LCs.¹² Skin-draining lymph nodes also lacked glycoprotein 40-positive DCs, which were presumably derived from epidermal LCs, whereas other DC populations could be readily detected. In bone marrow transplant studies it was demonstrated that LC precursors were present in the bone marrow of TGF- β 1 $-/-$ mice, suggesting that the LC requirement for TGF- β 1 could be satisfied by exogenous cytokine.¹³ On the basis of these results, it was tempting to speculate that TGF- β locally produced by keratinocytes plays a major role in retaining LCs in the epidermis. Even though skin from TGF- β 1 $-/-$ mice, when transplanted onto nude mice, was normally popu-

lated by LCs of recipient origin, we could not rule out the possibility that this was due to the presence of sufficient paracrine TGF- β produced by the recipient.¹³ Additional indirect evidence supports the concept that TGF- β may be involved in retaining LCs in their epidermal location. TGF- β has been shown to upregulate E-cadherin (see above) expression on human DC precursors and induce morphologic changes characteristic of LCs.^{14,15} In addition, TGF- β was found to inhibit the maturation of LC-like DCs induced by proinflammatory cytokines, such as TNF- α and IL-1,¹⁶ which are well documented to be key mediators in inducing mobilization of LCs (see below). Finally, by using *in vitro*-generated DCs, it was demonstrated that TGF- β blocks the TNF- α -induced upregulation of the CC chemokine receptor 7 (CCR7),¹⁷ which is critically involved in the emigration of LCs from the epidermis (see below). Other mediators constitutively produced in the epidermis, such as IL-10 or lactoferrin, have been reported to inhibit the maturation and mobilization of LCs, respectively,^{18,19} and may in conjunction with TGF- β be responsible for a microenvironment-driven arrest of immature LCs in the epidermis.

What makes them leave?

Much of our knowledge about the mobilization of LCs has been obtained while studying the initial events in the sensitization phase of allergic contact dermatitis. After it had been determined that epicutaneously applied allergens were taken up by murine LCs and transported to regional lymph nodes,² research efforts focused on dissecting the initial events of this process. Topical application of contact allergens induced focal activation of LCs, resulting in increased expression of MHC class II antigen and decreased LC density in the epidermis.²⁰ Subsequent studies demonstrated that within minutes of allergen application, intraepidermal levels of mRNAs encoding the proinflammatory cytokines IL-1 β and TNF- α were upregulated.²¹ Blocking the activity of these cytokines with neutralizing antibodies or receptor antagonists prevented the induction of contact hypersensitivity^{22,23} and reduced the accumulation of DCs in the regional lymph nodes. In addition, intradermal injection of either cytokine mobilized LCs from epidermis and led to an accumulation of DCs in draining lymph nodes.^{22,24} Studies performed with cytokine receptor knockout mice demonstrated that signaling through the type II TNF- α receptor and through the type I IL-1 receptor is required for normal migration of LCs induced by the respective cytokine.^{25,26} Additional evidence for the involvement of IL-1 β has been provided by recent studies that analyzed LC migration in mice deficient in caspase 1, a cysteine protease formerly known as IL-1 β -converting enzyme that is required for the release of active mature IL-1 β . Caspase 1 knockout mice displayed reduced LC migration in response to promigratory stimuli, such as contact allergen or TNF- α , and showed an impaired contact hypersensitivity response.²⁷ In aggregate, these results suggested that the local production of the proinflammatory cytokines IL-1 and TNF- α was directly involved in

the induction of LC migration. Cross-inhibition studies with their respective neutralizing antibodies implied that both cytokines may act in concert to mobilize LCs from their epidermal environment.²⁴

Interestingly, there seems to exist a functional heterogeneity within the epidermal LC population²⁸ in response to these migration-promoting signals because, regardless of the nature of the stimulus, only a proportion of LCs (approximately 30%) leaves the epidermis. Differences in responsiveness to migratory signals might be due to variable expression of membrane receptors, such as the type II TNF- α receptor or the type I IL-1 receptor, and/or differential autocrine production of promigratory mediators, such as bioactive IL-1 β , possibly as a consequence of variable expression of the IL-1 β -converting enzyme caspase 1 in LCs. Recently, it was demonstrated that p-glycoprotein (multidrug resistance gene product 1 [MDR-1]) plays a functional role in LC migration. Blocking MDR-1 activity with inhibitory antibodies or MDR-1 antagonists inhibited the migration of LCs from skin explants and caused the retention of LCs in the epidermis.²⁹ MDR-1 is an adenosine triphosphate (ATP)-dependent membrane transporter protein that mediates the efflux of chemotherapeutic agents from the intracellular space and confers multidrug resistance to cells. In addition, MDR-1 may also transport endogenous polypeptide cytokines to the extracellular milieu, and it was suggested that MDR-1 activity is required for the secretion of the promigratory cytokines IL-1 β and TNF- α .²⁹ Most recently, another member of the MDR family of transporter proteins has been demonstrated to be involved in the chemokine-driven mobilization of LCs from the epidermis.³⁰ Because activity of MDR-1 and related proteins is ATP-dependent and epidermal LCs are heterogeneous in their expression of surface ATPase, it can be speculated that cells with high ATPase expression are relatively resistant to the effects of extracellular ATP (released during epidermal injury), whereas LCs that lack ATPase activity will respond to extracellular ATP by the release of promigratory cytokines. The observation that the fraction of LCs lacking ATPase expression is similar to the fraction of LCs that become activated to leave the epidermis³¹ is consistent with this theory; however, direct experimental evidence is still lacking.

Collectively, the homeostasis of LCs seems to be tightly controlled by a delicate balance of mediators, some of which retain the cells in an immature state within the epidermis (see above) and others that promote the activation and mobilization. Minor perturbations of this homeostasis by chemical (eg, contact allergens and irritants), physical (eg, UV radiation), or biologic factors (eg, microbial products: LPS, bacterial DNA, and viral RNA) may signal danger to shift this balance in favor of an increased migration of LCs from the epidermis to the regional lymph node.

Detachment from keratinocytes

Because overcoming the adhesion to keratinocytes seemed the initial event in LC mobilization, we studied the regulation of E-cadherin-mediated adhesion in LCs.

The *in vitro* observation that LCs that had migrated from skin explants displayed a strongly reduced E-cadherin surface expression³² and the *in vivo* finding that in skin-draining lymph nodes DCs, which were presumably derived from epidermal LCs, also only displayed low E-cadherin surface reactivity³³ indicated that E-cadherin is selectively regulated during the process of LC mobilization. To further address this question, we established a culture system that allowed the routine propagation of skin-derived DCs that model multiple aspects of LC biology and are well suited to study E-cadherin-mediated adhesion.¹⁰ Interestingly, mediators that were known to induce LC migration *in vivo* (proinflammatory cytokines such as IL-1 or TNF- α) induced a time- and dose-dependent downregulation of E-cadherin mRNA, followed by a reduction in E-cadherin surface expression, which ultimately led to a loss of E-cadherin-mediated adhesion.³⁴ Stimulation with microbial products, such as LPS or bacterial DNA, resulted in a similar reduction in E-cadherin surface expression and loss of cellular adhesion.³⁴⁻³⁶ Cross-inhibition studies with neutralizing or receptor-blocking antibodies demonstrated that the proinflammatory cytokines seemed to act directly, whereas the effect of LPS was mediated by induction of TNF- α .³⁴ These results suggested that similar mechanisms seemed to attenuate the LC-keratinocyte adhesion *in vivo* and enabled LCs to leave the epidermis. In line with this, local application of contact sensitizers or proinflammatory cytokines was found to induce a marked downregulation of E-cadherin on activated LCs *in situ*.³²

Migration through the extracellular matrix

After leaving the epidermis, LCs must traverse the basement membrane and migrate through the dermal extracellular matrix (ECM) to gain access to the afferent lymphatics and ultimately localize in the T cell-rich paracortical areas of regional lymph nodes. Recent advances in the understanding of leukocyte migration point to a potential role of integrins in LC migration. Integrins comprise a large family of heterodimeric adhesion molecules that mediate cell-matrix interactions and, in the case of the $\beta 2$ integrins, cell-cell interactions. More than 20 combinations of different α and β chains form integrins that bind selectively to various ECM proteins, including collagen, fibronectin, and laminin. Several members of the $\beta 1$ integrin family (very late antigen 1-6) were found to be expressed on LCs, and it has been suggested that they represent ECM receptors that were required for migration and homing of LCs to T-cell areas of lymphoid tissue. The selective upregulation of the $\alpha 4 \beta 1$ integrins during LC maturation was consistent with this concept,³⁷ but direct functional evidence showing that this was the case was not provided. Recently, Price et al³⁸ reported that $\alpha 6$ integrins are functionally involved in LC migration. $\alpha 6$ Integrins were detected at high levels on freshly isolated murine LCs but were absent on DCs from skin-draining lymph nodes. Blocking antibodies that interfered with the binding of $\alpha 6 \beta 4$ to laminin inhibited spontaneous, cytokine-induced, and

contact allergen-induced migration of LCs from the epidermis.³⁸ These results suggest that $\alpha 6 \beta 4$ integrins may well be involved in the migration of activated LCs through laminin-rich epidermal basement membranes.

A number of additional ECM receptors seem to be relevant. Several isoforms of the hyaluronate receptor CD44 are expressed by LCs, and CD44 has been implicated in LC migration and localization in lymph nodes.³⁹ Some, but not all, anti-CD44 antibodies inhibited spontaneous migration of LCs from the epidermis in a skin explant model. Other anti-CD44 antibodies (which were known not to interfere with the binding of CD44 to hyaluronate) also inhibited the migration of LCs, suggesting that other unidentified ligands were involved.³⁹ During LC maturation, expression of selected CD44 isoforms was upregulated, and mature (but not freshly obtained) LCs adhered to lymph node sections in a CD44-dependent fashion.³⁹ In addition, selected anti-CD44 antibodies inhibited the sensitization and elicitation phases of contact hypersensitivity *in vivo*.³⁹ In aggregate, these results suggest that CD44 isoforms expressed by LCs *in situ* may be involved in migration of LCs through hyaluronate-rich epidermis and dermis, whereas other isoforms may play a role in the localization of mature DCs in the T-cell areas of lymph nodes.

It seems likely that LCs also use proteolytic enzymes to make their way through the ECM. Matrix metalloproteinases (MMPs), such as MMP-9, have been detected in LCs *in situ* under conditions that induce LC emigration,⁴⁰ suggesting their involvement in the process. This was supported by our recent observation that spontaneous emigration of LCs from skin explants can be blocked almost completely by the addition of a synthetic broad-spectrum inhibitor of metalloproteinases (T. Jakob, unpublished observation). More recently it was reported that blocking MMP-9 activity by selective antibodies inhibited hapten-induced decreases in LC numbers in the epidermis and the accumulation of DCs in the regional lymph node.⁴¹ Additional evidence for the role of MMPs comes from a recent study that demonstrated altered contact hypersensitivity responses to chemical haptens in MMP-3- (stomelysin 1) and in MMP-9-deficient mice.⁴² Given the redundant expression of various MMPs with overlapping substrate specificity in other cell systems, it seems likely that additional MMP family members are involved in the movement of LCs through the ECM.

Chemokines and chemokine receptors

The same inflammatory stimuli that allow LCs to detach from keratinocytes (see above) concomitantly induce LC maturation. Maturation of DCs is associated with a selective change in chemokine receptor profile. Immature DCs express a number of chemokine receptors for inducible chemokines, such as IL-8 (CXCL8), RANTES (CCL5), macrophage inflammatory protein 1 α (MIP-1 α ; CCL3), or monocyte chemoattractant protein 3 (MCP-3; CCL7), by which immature DCs are attracted to the site of inflammation (see below). On maturation, DCs downregulate these receptors, which may allow them to

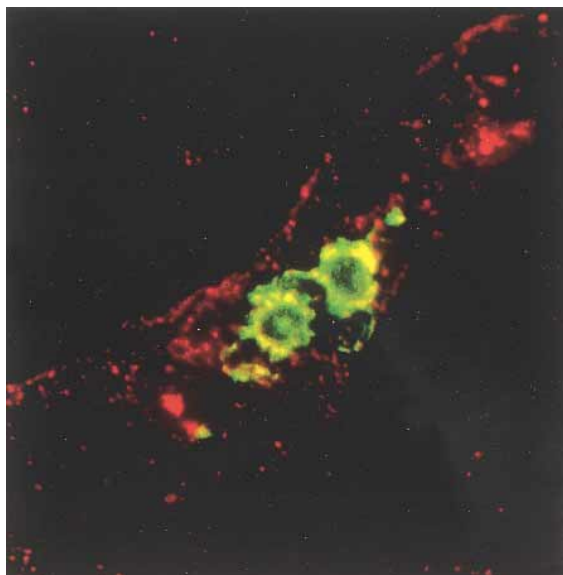


FIG 3. Mature DCs leave the skin through afferent lymphatics that constitutively express SLC. Confocal image of a dermal skin preparation stained for MHC class II (green) and SLC (red) is shown. Note 2 mature DCs with high MHC class II surface expression inside a lymphatic vessel expressing SLC. For details, see text.⁴⁶ Courtesy of H. Saeki and S. Hwang, National Cancer Institute, Bethesda, Md.

leave the inflammatory site (ie, the site with the highest chemokine concentration). At the same time, maturing DCs upregulate receptors for constitutively expressed chemokines, such as the CXC chemokine receptor 4 and CCR7.^{43,44} Consistent with this, CCR7 expression in epidermal LCs can only be observed after *in vitro* or *in vivo* induction of LC maturation.^{45,46} Interestingly, the CCR7 ligand secondary lymphoid tissue chemokine (SLC; CCL21) is constitutively expressed by stromal cells in T-cell zones of lymph nodes and by lymphatic endothelial cells in peripheral tissue, such as the dermis (Fig 3).⁴⁷ On the basis of these observations, it was proposed that immature DCs, such as epidermal LCs, exposed to danger-maturation signals in the periphery begin to upregulate CCR7 and thus become chemotactically attracted to constitutively expressed SLC released by lymphatic vessels. Indeed, Saeki et al⁴⁶ recently demonstrated that migration of skin-derived DCs to the draining lymph node could be inhibited when the activity of SLC was blocked by neutralizing antibodies. Similarly Gunn et al,⁴⁸ who analyzed the spontaneous mouse mutant line *plt*, which lacked measurable levels of SLC in lymphoid tissues, reported that in comparison with control mice, the application of contact allergen resulted in a reduced accumulation of skin-derived DCs in the regional lymph nodes. What at first did not fit the above model was the observation that the entry of LCs into lymphatic vessels in *plt* mice was found to be normal. The discovery that the mouse genome contains 2 genes that encode SLC-like proteins provided an explanation. Although both genes encode very similar proteins with equal biologic activities, they greatly differ in their expression pattern. One gene is expressed at high levels in lym-

phoid organs, and the other is predominantly found in lymphatic endothelium of nonlymphoid tissues.⁴⁹ In *plt* mice only the former gene was deleted, whereas the latter one seemed to be expressed at normal levels,⁴⁹ explaining why the migration of LCs from the epidermis into the afferent lymphatics was not altered in *plt* mice. The only other known ligand for CCR7 is the EBV-induced molecule I ligand (ELC; CCL19), which is produced by DCs and stromal cells in T-cell areas of lymphoid tissue.⁵⁰ ELC production by resident DCs in draining lymph nodes may facilitate the correct positioning of newly arriving migratory DCs that have been recruited from peripheral tissue. The importance of CCR7 and its ligands SLC and ELC in directing the migration of LCs to the draining lymph node has now been corroborated by the most recent finding of an almost complete block of this process in CCR7-deficient animals.⁵¹

Thus constitutive expression of SLC by lymphatic endothelium seems to provide the first chemotactic gradient for activated CCR7-positive LCs, leading to a selective recruitment of LCs from the epidermis to the afferent lymphatics (Fig 4). Once they enter the lymphatics, they are likely to be transported passively with the lymph to the subcapsular region, where they then encounter an additional chemotactic gradient of ELC (and SLC) that directs their migration into the paracortical T-cell zone of the lymph node.

RECRUITMENT OF IMMATURE DC/LC PRECURSORS INTO THE SKIN

The enigmatic LC precursor

Although we are beginning to understand the mechanisms that regulate the migration of LCs from the epidermis to regional lymph nodes, far less is known about the recruitment of LCs into the epidermis. Bone marrow transplant studies introduced the concept that epidermal LCs are derived from a mobile pool of bone marrow-derived precursors.⁵ Although LCs are clearly myeloid cells, immediate *in vivo* precursors of LCs have not been identified or characterized. There may be a small population of LCs (<1% of all LCs present) that can proliferate *in situ*, but there is general consensus that the bulk of LCs are postmitotic.⁵² Thus intraepidermal LCs must derive from blood-borne committed precursors or from less-committed precursors that assume the characteristics of LCs locally. *In vitro* studies have provided evidence that the commitment to LC differentiation is already established at the level of circulating DC precursors early during ontogeny.⁵³ However, LC-like DCs have also been generated from peripheral blood monocytes,¹⁴ suggesting that LC differentiation may be determined by the microenvironment encountered by precursor cells once they enter the interstitial tissue of the skin.

Rolling home: mechanisms of homing to skin

Regardless of the nature of the LCs' precursor, they must exit the blood stream to eventually localize to the epidermis. In 1990, Cruz et al⁵⁴ reported that intra-

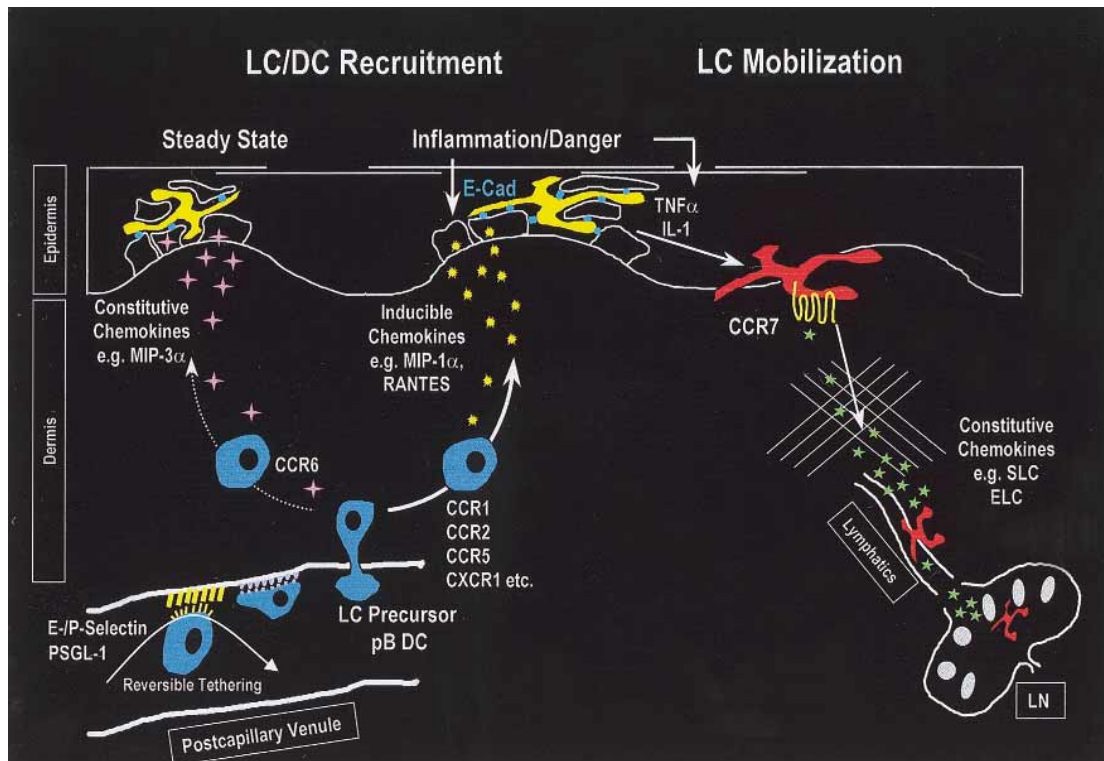


FIG 4. Multistep navigation of LCs/DCs in and out of the skin. The recruitment of LC/DC precursors into skin under steady-state or inflammatory conditions and the mobilization of resident LCs from epidermis to regional lymph nodes are regulated by a spatially and temporally coordinated sequence of events involving various sets of adhesion/homing molecules and distinct pairs of chemokines and their receptors. For details, see text.

venously injected LCs localized in skin. At about the same time, cutaneous lymphocyte-associated antigen (CLA) was identified as a skin-homing molecule for a subset of memory T cells.⁵⁵ CLA interacts with selectins expressed on endothelial cells and mediates tethering of leukocytes to the endothelium as the first step in a series of distinct events that leads to transendothelial migration. The subsequent demonstration of CLA on epidermal LCs⁵⁶ and on putative LC precursors expanded from peripheral blood⁵³ led to the suggestion that CLA participates in the homing of LC precursors to skin. Recently Robert et al⁵⁷ demonstrated that circulating DCs express and use P-selectin glycoprotein ligand 1 (PSGL-1; an isoform of PSGL-1 is known as CLA) to tether and roll on E- and P-selectin-expressing capillary endothelial cells in vitro and in vivo. Using intravital video microscopy, they could demonstrate that under normal conditions, approximately 50% of circulating DC precursors were rolling on endothelium of dermal postcapillary venules. This process was strikingly dependent on the presence of E- and P-selectin because no rolling interaction was observed in E- and P-selectin double-deficient mice. Both selectins are expressed on endothelial cells at low levels and upregulated by inflammatory stimuli. Consistent with that, Robert et al⁵⁷ observed a preferential extravasation of DCs at sites of cutaneous inflamma-

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FIG 5. Recruitment of immature DCs from the circulation into inflamed skin in vivo. Ex vivo calcein-labeled murine DCs (green) injected into the tail vein extravasate in vivo from CD31-positive blood vessels (red) into the dermis of inflamed skin of the ear. For details, see text. Courtesy of C. Robert and T. Kupper, Harvard Skin Disease Research Center, BWH, Harvard Institutes of Medicine, Boston, MA. Reproduced from *J Exp Med*. 1999;189:627-36 by copyright permission of The Rockefeller University Press.

tion (Fig 5). On the basis of these findings, it was suggested that DC precursors constantly monitor skin endothelium ready to extravasate on encounter of additional inflammatory activation signals.

LC/DC recruitment into skin: constitutive, on demand, or both?

Once DCs have left the blood stream, migration in the dermal tissue seems to be regulated by distinct chemokines and their receptors. Immature DCs express a number of CCRs, such as CCR1, CCR2, CCR5, and CXC chemokine receptor 1, which make them responsive to inducible chemokines, such as MIP-1 α , MCP-3, or RANTES.^{43,44} It is tempting to speculate that the epidermal expression of these chemokines during a local inflammatory response may recruit immature DCs (LC precursors) into the epidermis, where they may replace resident LCs that have been activated during the same inflammatory response to leave the epidermis and migrate to the regional lymph node (see above). However, it is still unresolved whether immature DCs recruited from the circulation into the dermis during inflammation can give rise to resting LCs in the epidermis. Alternatively, they may represent a precursor population that gives rise to a distinct subset of DCs, the inflammatory dendritic epidermal cells that were recently identified in the epidermis of chronic inflammatory skin diseases⁵⁸ and represent a CD1a-positive population that displays many characteristics of LCs but shows a distinct surface phenotype and lacks Birbeck granules, the hallmark of LCs.

It is likely that under steady-state conditions (ie, in the absence of inflammation), the recruitment of LC precursors into the epidermis is regulated by different mechanisms. An interesting model has been proposed by Charbonnier et al,⁵⁹ who analyzed the chemokine receptor repertoire on different sets of *in vitro*-generated DC precursors. In contrast to non-LC precursors that displayed a wide range of receptors for inducible chemokines, LC precursors expressed primarily CCR6 and responded selectively to the CCR6 ligand MIP-3 α (CCL20). The observation that LCs *in situ* express CCR6 and that MIP-3 α is constitutively expressed by basal and suprabasal keratinocytes lends further support to the notion that this receptor-ligand pair may be responsible for the constitutive recruitment of LCs in the absence of inflammation. The recent demonstration by Dieu-Nosjean et al⁶⁰ that inflammatory signals, such as IL-1 or TNF- α , induced a dramatic upregulation of keratinocyte MIP-3 α expression suggested that under inflammatory conditions, increased numbers of LC precursors can be recruited into the epidermis through an MIP-3 α -dependent mechanism. These findings suggest a scenario in which the function and migration of different DC subpopulations is determined at the level of their chemokine receptor repertoire and at the level of factors derived from the local microenvironment. One type, the LC, is, under steady-state conditions, constitutively recruited into the epidermis by MIP-3 α to sustain the homeostasis of the epidermal LC population. Under inflammatory conditions, the MIP-3 α -dependent recruitment of LCs is upregulated to compensate for the increased mobilization of LCs leaving the epidermis. The other type, the inflam-

matory DC, is additionally recruited under inflammatory conditions by a wide array of different inducible chemokines and serves in the amplification and modulation of the ensuing immune response.

SUMMARY AND PERSPECTIVES

Epidermal LCs are highly specialized antigen-presenting cells expressing surface molecules that allow them to home to skin and localize in the epidermis. Perturbants of epidermal homeostasis seem to provide danger signals that, through induction of promigratory mediators, promote the mobilization of LCs from the epidermis to the lymphoid tissue. Simultaneously, signals are generated that recruit DC precursors into the skin to reconstitute the epidermal LC population. LCs seem well adapted for this multistep navigation through the cutaneous tissue, in which cell-adhesion molecules, proteolytic enzymes, and distinct pairs of chemokines and their receptors seem to regulate the migration from the circulation to the epidermis and from there through the afferent lymphatics to the regional lymph nodes.

The understanding of mechanisms that regulate LC trafficking has in the past and will in the future help to improve the development of therapeutic strategies in which LCs/DCs are used to modulate immune responses in human subjects. The availability of technologies that allowed the generation of LCs and other DCs in large numbers *ex vivo* has opened new opportunities to use these cells as therapeutic agents in different clinical settings. Methods for loading these cells with antigen or peptide, for transferring genes into them, or for augmenting their immunostimulatory capacity have been developed and are being used in various clinical protocols.^{61,62} Most progress has been made in the field of tumor vaccination, in which autologous DCs obtained from patients with tumors are expanded and loaded with tumor antigen *ex vivo* and subsequently reinjected into the patient to induce tumor-specific immune responses.^{63,64} Understanding the mechanisms that regulate LC/DC trafficking was the basis on which to test different routes of DC administration to patients and has helped to optimize these protocols. Besides using LCs/DCs as nature's adjuvants to augment immune responses, novel approaches are being developed to generate DCs that selectively induce antigen-specific anergy⁶⁵ or that induce antigen-specific regulatory T cells⁶⁶ that downregulate ongoing immune responses. Anergizing or downmodulatory DCs could have wide clinical use in the specific treatment of autoimmunity or allergy. Although numerous approaches already exist to modulate DC function *in vitro*, similar techniques may become available that allow the modulation of LC/DC function *in vivo*. The epidermal location of LCs makes them a prime target for therapeutic interventions to suppress, stimulate, or deviate cutaneous and systemic immune responses.

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