

Langerhans Cells at the Interface of Medicine, Science, and Industry

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The 11th International Workshop on Langerhans Cells,* organized by Niki Romani and Georg Stingl, took place in Funchal, Madeira, Portugal, where Paul Langerhans worked and died more than a hundred years ago. Langerhans (1847–1888) is buried at the British Cemetery, a place that was frequently visited by conference attendees (Figure 1). Robert Willan (1757–1812), the founder of modern, systematic dermatology, is also at rest in this cemetery.

Georg Stingl (Vienna, Austria) opened the conference with a historical overview on Langerhans cell (LC) biology. It was intriguing to learn how several observations made many years ago (e.g., finding mitotic LCs) anticipated recent data (e.g., division of LCs within the epidermis), particularly with regard to the ontogeny and homeostasis of LCs. In the two and a half days that followed, the conference concentrated on the ontogeny and functional role of LCs in the skin immune system and on finding ways to ultimately harness these properties for immunotherapy. Highlights are presented here.

Langerhans cell ontogeny and development

Miriam Merad (New York) summarized current knowledge on LC ontogeny and function. She also presented new data on the repopulation of the skin by LCs and the recently discovered dermal langerin⁺ dendritic cells (DCs) (Merad *et al.*, 2008). LC precursors populate murine epidermis during embryonic life, and they proliferate *in situ* to form the typical network of LCs

in the epidermis. In the steady state, the LC network is maintained through local proliferation of LCs themselves. Depending on the kind of inflammation induced in the skin, repopulation occurs by local proliferation of precursor cells, e.g., in the case of contact sensitization or by monocytes recruited from the blood after UV treatment, as shown previously (Merad *et al.*, 2008). CD103⁺ dermal langerin⁺ DCs are repopulated by circulating precursors not only in skin but also in lung, liver, kidney, and gut epithelium (Bogunovic *et al.*, 2009) in a Flt-3 dependent and M-CSF-receptor-independent fashion, as opposed to epidermal LCs, where the inverse occurs.

Frédéric Geissmann and Laurent Chorro (London) demonstrated that in embryonic mice CD115⁺ CX3CR1⁺ LC precursors settle in the epidermis in a single wave of recruitment. They acquire all the typical markers—langerin, major histocompatibility complex (MHC) class II, CD11c—shortly after birth and then fill the epidermal compartment by high (“explosive”) proliferation within the first week after birth. Geissmann and Chorro proposed that it is the LCs themselves that proliferate in the steady state to maintain homeostasis. They demonstrated that increased proliferation of LCs, but not the recruitment of a bone marrow-derived precursor, is induced in some types of skin inflammation, such as atopic dermatitis. In these cases, a still unknown signal released by keratinocytes after stimulation through the vitamin D receptor drives their proliferation.

Florent Ginhoux (Singapore) complemented Geissmann and Chorro’s data, showing that proliferating radioresistant CX3CR1⁺ LC precursors can be found in the epidermis of late mouse embryos. These radioresistant precursors develop into LCs within the first few days after birth; interestingly, they are derived from yolk sac macrophages. Kang Liu (New York) thoroughly dissected the sequence of progenitor stages (myeloid precursor, monocyte/DC precursor, common DC precursor, pre-DC) and the critical branching points for monocytes, plasmacytoid DCs, and conventional DCs in mouse bone marrow. The position of LCs within this developmental tree remains to be defined.

Junda Kel (Rotterdam, The Netherlands) reported that transforming growth factor- β (TGF- β) signaling is important to keep LCs in an immature state in the epidermis. When the TGF- β receptor 1 is deleted under the control of the CD11c-promoter, LCs were present in the epidermis of newborn mice, but their numbers decreased during development when the cells displayed a mature phenotype. This effect was specific for LCs (dermal (langerin⁺) DCs were not affected). As a result of the decreased number of LCs, contact hypersensitivity reactions were impaired. These findings were underscored by Florian Sparber (Innsbruck, Austria), who presented a mouse model in which DCs lacked the p14 adaptor molecule for signal transduction involved in growth factor signaling. LC density in these mice was dramatically decreased, hinting at a malfunction of

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Figure 1. Conference attendees at the gravesite of Paul Langerhans.

the TGF- β receptor pathway. Likewise, Martin Zenke (Aachen, Germany) demonstrated that the transcription factor Id2, involved in TGF- β signaling, is important for population of the skin with both LCs and dermal langerin⁺ DCs in steady state, but that Id2 is not required for LC repopulation in inflammation.

Adelheid Elbe-Bürger (Vienna) reported studies in human prenatal skin. She described in detail the development of the LC network in the epidermis. Similar to the mouse system, there is a high proliferation rate of CD45⁺ leukocytes in the fetal/embryonic skin; the rate of proliferation decreases after birth. During the first to second trimester, markers on epidermal leukocytes are expressed in a stepwise order: first

HLA-DR, then CD1c, followed by langerin and CD1a. Interestingly, this correlated with TGF- β expression in the epidermis. High expression of IL-10 in embryonic skin seems to keep the LCs in a functional blockade (Schuster *et al.*, 2009). Christopher Schuster (Vienna) supplemented these data by demonstrating that precursors of dermal DCs are detectable at 9 weeks of embryonic life and express markers such as CD14, HLA-DR, and CD36.

In summary, the findings presented in the ontogeny session demonstrated that during embryonic life highly proliferative precursors settle in the epidermis and, upon birth, the dense network of LCs starts to form in both mouse and human skin. The proliferation rate is reduced after birth and during adult life. LCs

themselves repopulate the epidermis in steady state and in some inflammatory settings. TGF- β and IL-10 seem to be important factors for keeping LCs in an immature stage in the epidermis in the steady state. The exact mechanisms that control LC homeostasis in adult skin remain to be established.

DC migration is tightly coupled with antigen uptake

Gwen Randolph (New York) described how DCs in the adipose tissue around lymph nodes acquire soluble antigens that drain from the skin lymphatics for later presentation in the skin-draining lymph node. Ana-Maria Lennon-Duménil (Paris) demonstrated that the ability of DCs to sample antigen is inversely correlated with their ability to migrate. Using microchannel chambers, she showed that shortly after activation, bone marrow-derived DCs coexpress myosin II and the invariant chain in the front portion of the cell, and they move slowly to be able to incorporate and process antigen. Upon further maturation, they translocate myosin and the invariant chain to the rear of the cell and begin rapid migration to lymph nodes, where they meet T cells (Faure-Andre *et al.*, 2008). It would be interesting to validate this mechanism in LCs. Along these lines, Ira Mellman (South San Francisco, CA) stressed that “phenotypically mature” DCs need not necessarily be “functionally mature” DCs. He pinpointed yet another specialization of DCs for efficient presentation: unlike macropinocytosis, receptor-mediated endocytosis via clathrin-coated pits and vesicles does not stop upon maturation. This allows DCs to perform multiple rounds of processing and presentation.

A novel form of antigen uptake by LCs was presented by Akiharu Kubo (Tokyo). Upon activation achieved by mild tape stripping, the investigators observed that LCs extend dendrites into the stratum corneum and form tight junctions with surrounding keratinocytes. This could allow LCs to incorporate antigens or pathogens from the surface of the skin without compromising epidermal barrier function. Nancy Luckashenak (Munich) examined the importance of Cdc42 Rho GTPase in

DC migration. Upon depletion under the CD11c-promoter, LCs stop migrating to the lymph node and cannot exit the epidermis. As a consequence, transport of antigen such as FITC to the lymphatic tissue is greatly reduced. Together these findings indicate that migration and antigen incorporation are tightly coupled, ensuring that LCs/DCs present antigens efficiently in lymphoid tissues.

LCs in infection

The role of LCs in skin infection is still largely unknown. However, several reports highlighted the fact that skin DCs can incorporate various pathogens and are involved in the clearance of pathogens. Teunis Geijtenbeek and colleagues (Amsterdam) observed that LCs derived from human epidermis are able to prevent transmission of HIV (Cunningham *et al.*, 2008), herpes simplex virus (HSV), hepatitis C virus, and measles virus after binding to langerin. This would endow LCs with a special (innate) function in neutralizing viruses, in contrast to other DCs (such as dermal DCs) that *transmit* HIV to T cells. Geijtenbeek further reported that in the case of inflammation or when doses of virus are too high, LCs are no longer able to eliminate them (de Jong *et al.*, 2008). The picture is slightly different in the model of vaginal epithelium as presented by Florian Hladik (Seattle, WA). Here, the main receptors for HIV uptake are CD4 and CCR5. In vaginal LCs these receptors do not support productive fusion, but rather nonproductive endocytosis followed by transmission of infectious virions to T cells through infectious synapses. In vaginal mucosa langerin expression is low on LCs facing the lumen, indicating that this could explain why vaginal LCs might not be able to eliminate HIV through the langerin pathway.

Yonatan Ganor (Paris) observed that inner foreskin was highly permissive of HIV infection and that in this tissue virus was transmitted by LCs to T cells, whereas the outer foreskin is thicker and less permissive of HIV infection. Vassili Soumelis (Paris) showed that human papillomavirus (HPV)-infected skin lesions are devoid of LCs. He presented evidence supporting a role for

thymic stromal lymphopoietin, which was expressed by HPV-infected keratinocytes, in inducing emigration of LCs. M. de Jong (Amsterdam) reported that human LCs can transfer UV-inactivated measles virus to cocultured monocyte-derived DCs, which in turn are readily able to cross-present viral antigen to CD8⁺ T cells. On the basis of these observations, she speculated that antigen transfer from LCs to other antigen-presenting cells may occur in the dermis.

Consistent with this, Bill Heath (Melbourne, Australia) noted that during the second wave of HSV infection, which corresponds to a recrudescence phase involving the entire innervated dermatome, cutaneous CD103⁺ DCs (but no other migratory DCs from skin) and CD8⁺ lymph node-resident DCs present HSV antigen to CD8⁺ T cells. LCs play a minor direct role in antigen presentation, possibly because they are infected and then killed by this cytolytic virus. However, they may contribute to antigen transfer. In the primary wave of infection, only CD8⁺ DCs are capable of performing cross-presentation in the node, again indicating antigen transfer from infected skin DCs to lymph node resident DCs (Bedoui *et al.*, 2009). Miriam Merad (New York) has observed that lung CD103⁺ DCs express high levels of TLR3. In a mouse model of influenza infection, lung DCs that coexpress langerin were shown to be mandatory for the induction of CD8⁺ T cell responses because depletion of langerin⁺ cells prevented virus clearance, as presented by Hamida Hammad (Ghent, Belgium) (Geurtsvankessel *et al.*, 2008). In this model, however, it was impossible to distinguish between CD103⁺ and CD8⁺ DCs because langerin is expressed on most lung CD103⁺ DCs and on a proportion of lung-draining lymph node-resident DC populations.

LCs might also be important in regulating immune responses to nonviral pathogens. In a Leishmania infection model, the depletion of LCs enhanced T helper type 1 responses, resulting in smaller lesions after intradermal infection with physiological doses of parasites. Concomitantly, numbers of regulatory T cells were decreased, as demonstrated by Kordula Kautz-Neu

(Mainz, Germany), suggesting a suppressive role for LCs during Leishmania infection. In contrast, LCs might be less important in antibacterial immunity—Marcel Teunissen (Amsterdam) showed that, in addition to a selective lack of Toll-like receptors essential for bacterial recognition, human LCs take up, process, and present bacterial antigens poorly compared with dermal DCs. Dermal DCs also express more Fcγ receptors and more molecules associated with the MHC class II processing/presentation pathway than do LCs. LCs' poor responsiveness to bacteria might be an essential feature to maintain tolerance to the commensal bacterial flora on the skin.

In summary, LCs may play different roles depending on the agent and site of infection.

Depletion of LCs and what we learn from the new mouse models

Four years ago, three groups independently developed mouse models in which LCs can be depleted either constitutively or conditionally. For this purpose, either the diphtheria toxin subunit A or the receptor for diphtheria toxin was expressed under the langerin promoter (Kaplan *et al.*, 2008). These models were presented for the first time at the 9th International Workshop on Langerhans Cells in Madeira in 2005, and they revealed initially surprising differences in contact hypersensitivity responses. In the meantime, these mice were freely distributed, and many labs have used them to investigate the role of LCs in the induction of immunity and tolerance. Therefore, expectations were high for the presentations at the 11th workshop.

Bernard Malissen (Marseille, France) started the discussion by subdividing skin DCs into five subsets: epidermal LCs, LCs in transit through the dermis, dermal langerin⁺ DCs, and two types of dermal langerin-negative DCs. He noted that CD103, which has been used as a marker to distinguish LCs and dermal langerin⁺ DCs, is not expressed on all of the latter cells. Therefore, caution is warranted when using it as a marker for separating skin DC subsets in the lymph node. Björn Clausen (Rotterdam) presented data on a mouse

model in which β -catenin is stabilized specifically in DCs. As a consequence, the number of regulatory T cells was increased and contact hypersensitivity reactions were downregulated, providing *in vivo* evidence that this pathway promotes a tolerogenic DC function. In a mouse model lacking the IL-10 receptor in DCs, contact hypersensitivity reactions were increased, indicating that IL-10 signaling in skin DCs is important to contain and regulate effector T-cell responses. In langerin-DTR mice, high doses of contact sensitizer are presented by both LCs and dermal DCs, but low amounts require LCs for the efficient induction of an immune response. Dermal langerin⁺ DCs play a minor role in this system.

This is in contrast to the langerin-DTA mice studied by Dan Kaplan (Minneapolis, MN) that exhibit exaggerated contact hypersensitivity reactions with both high and low doses of contact allergen in the absence of epidermal LCs. Kaplan replicated these results in a newly developed ablation model in which only epidermal LCs (but not dermal langerin⁺ DCs) are depleted upon administration of diphtheria toxin. This suggests that it is not the lifelong absence of LCs in the langerin-DTA mice that is responsible for their augmented contact hypersensitivity. In the langerin-DTA mice, more antigen-specific effector T cells are present in skin and lymph nodes, and LC expression of MHC class II and IL-10 is important for the enhanced responses. Also, in *Candida* infection experiments, both LC-depletion models revealed increased responses to topical infection. Tetsuya Honda (Kyoto, Japan) devised a clever chimera model in which langerin-DTR mice could be depleted only of dermal langerin⁺ DCs and not of LCs. These mice exhibited no difference in contact hypersensitivity, although the degree of dermal DC chimerism has not been clearly established in these mice. This model will be helpful for further investigation of the relative roles of LCs and dermal langerin⁺ DCs.

Discrepancies between the inducible and the constitutive ablation models were also evident in tumor models. Patrizia Stoitzner (Innsbruck) reported increased

numbers of chemically induced tumors in depleted langerin-DTR mice in comparison with wild-type mice. This would indicate a role for langerin⁺ cells in tumor immunosurveillance. Interestingly, langerin-DTA mice were completely protected from tumors in the same tumor setting (Strid *et al.*, 2008). Using a murine atopic dermatitis model, Sandrine Dubrac (Innsbruck) found that LCs were required for the development of disease. After depletion of LCs, Th2 responses and, as a consequence, disease severity, were markedly reduced.

The role of LCs in tolerance induction was directly addressed by Elena Shklovskaya (Sydney, Australia). In an elegant chimeric mouse model, in which MHC class II presentation was restricted to either LCs or dermal DCs, LCs induced tolerance and dermal DCs stimulated immunity after subcutaneous and topical immunization (Shklovskaya *et al.*, 2008).

So, are we any further along? Yes. Some questions were indeed resolved; but discrepancies and seemingly contradictory observations among the various models remain. One important lesson can be learned from these models: the role of LCs in the skin immune system is manifold, depending on the circumstances in the skin (steady state vs. inflammation), the pathogens, and the doses of antigens, among others. The notion (and the evidence) that LCs *in vivo* can act in a tolerogenic/downregulatory fashion has certainly been reinforced by the data presented in Funchal.

Skin DCs in pathology and potential new vaccination strategies

The closing session of the conference dealt mostly with human skin DCs in various pathologies and highlighted potential novel vaccination strategies.

Michelle Lowes (New York) further characterized the complex populations of skin DCs in diseased skin. Intriguingly, phenotypically mature-appearing DCs in squamous cell carcinoma were functionally impaired, made tumor necrosis factor- α and inducible nitric oxide synthase, and may contribute to the ability of the tumor to evade immunosurveillance

(Bluth *et al.*, 2009). For the past 2 years, we have been searching for a human counterpart of mouse dermal langerin⁺ DCs. At this meeting, Matthew Collin (Newcastle, UK) reported a very likely candidate, namely, a langerin⁺ EpCAM⁻ DC in human dermis. CD103 seems to be an unreliable marker for these cells. The dermal langerin⁺ DC equivalent was found even in human bone marrow transplant patients (Haniffa *et al.*, 2009), emphasizing the value of patient-oriented research by excellent physician-scientists. As in the mouse, this cell type is of donor origin.

Hideki Ueno (Dallas, TX) presented data on a functional dichotomy between human LCs and dermal DCs. LCs are superior in the induction of cytotoxic T cells but can also stimulate strong Th2 responses. Dermal DCs produce IL-12, which induces follicular T helper cells that in turn regulate antibody secretion by B cells, making them perfect stimulators of humoral responses (Banchereau *et al.*, 2009). Carl Allen (Houston, TX) discussed the origin of the LC histiocytosis (LCH), which consists of an accumulation of langerin⁺ cells in tissue and occurs mostly in children. It is still not certain whether LCH is a neoplastic or an inflammatory disease. Expression-profile analysis using microarray suggested that LCH cells are more closely related to bone marrow myeloid cells than to LCs or dermal langerin⁺ DCs that are present in normal skin. Therefore, Allen proposed LCH as a "myeloid proliferative disorder."

The novel therapeutic approach of targeting antigen to DCs via lectin receptors was discussed in several talks. Irina Caminschi (Melbourne) presented Clec9A and Clec12A as potential candidates that specifically target CD8⁺ DCs and plasmacytoid DCs. The requirement for adjuvants differs between the two delivery antibodies; however, both are efficient in the stimulation of CD4⁺ and CD8⁺ T-cell responses. Juliana Idoyaga (New York) discovered that all CD8⁺ DCs in the spleen of BALB/c mice (i.e., spleen-resident DCs) coexpress langerin, making them potential targets for langerin antibody-conjugated antigens. Indeed, intravenously injected anti-langerin-antigen conjugates were able to stimulate potent CD4⁺ and CD8⁺

T-cell responses *in vivo*. Regarding CD205-conjugated antigens, the observation by Ira Mellman (San Francisco, CA) that even mature DCs can take up and present such conjugates (because receptor-mediated endocytosis is operative in this case) may be of great value for developing immunotherapies. Vincent Flacher (Innsbruck) demonstrated that topical imiquimod (Aldara cream) can induce migration of skin DCs and increase the number of CD205-targeted cells in the lymph nodes after intradermal immunization. Interestingly, most targeted cells were langerin⁺ DCs. Anti-CD205-antigen conjugates induced potent cytotoxic responses, emphasizing their future therapeutic potential.

Summary

The presence of 140 scientists from all over the world, working on various aspects of LC and DC biology, made this meeting exciting and productive. The functions of LCs in the skin immune system *in vivo* seem to be manifold. Clearly, LCs are involved in immune responses against pathogens and skin tumors and perform a regulatory role by downmodulating immune responses—or even promoting disease progression. These characteristics make them attractive targets for therapeutic interventions through the skin. Nevertheless, many questions must be answered before LCs and other skin DCs can be harnessed for immunotherapy on a large scale. With new mouse models, new sorting strategies for the various skin DC

subsets, and—most importantly—more studies with human cells, human diseases, and patients, we will be able to illuminate these remaining mysteries. This will ultimately be of benefit for medicine and, as a consequence, for industry.

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* *The 11th International Workshop on Langerhans Cells was held at the CS Madeira Atlantic Resort and Sea Spa in Funchal, Portugal, 3–6 September 2009.*

The 12th International Workshop on Langerhans Cells will take place in the fall of 2011 in Bethesda, Maryland, USA (<http://www.lc2011.org>).

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