Commentary

Is Maturation Required for Langerhans Cell Migration?

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Key papers in scientific literature sometimes bring a definitive resolution to a long-awaited issue. Others may be far less decisive but importantly implore us to question the fitness of prevailing models. So it is that a paper in this issue by F. Geissmann and colleagues (1), albeit subject to some variety of interpretation, nevertheless compels us to reexamine the widely accepted idea that migration of Langerhans cells in response to inflammatory stimuli is necessarily coupled to and follows from their maturation. It is well established that in the steady-state Langerhans cells turn over very slowly, but they can be mobilized en masse by inflammatory or antigenic stimuli. In vitro and in vivo, these inflammatory mediators have also been observed to promote maturation, and Langerhans cells that migrate from skin explants in culture usually display a mature phenotype. In further agreement with the idea that migration is linked with maturation is evidence that molecules associated with migration, particularly CCR7 (2), are induced during Langerhans cell maturation (3).

Dendritic cell (DC) "maturation" is defined functionally as the acquisition of potent immunogenic capacity. Among DC biologists, this term signifies a particular program of gene expression that typically includes the upregulation of CD40, CD83, CD86, MHC products, and other molecules associated with antigen presentation, along with simultaneous induction of CCR7 (4). At the same time, the ability to acquire and process antigen, a feature that characterizes immature DCs, is downregulated. Understanding the extent to which maturation and migration are coupled lies at the heart of understanding how DCs regulate T cell differentiation. If immature DCs promote naive T cells to develop a tolerogenic phenotype (5), then one is left wondering whether immature DCs traffic efficiently to lymph nodes from the periphery. If they do, then what of the belief that CCR7 is needed for DC migration to lymph nodes and that CCR7 is expressed only by mature DCs? Clearly, the fit of some pieces of the puzzle have to be reevaluated.

Evidence that Immature Langerhans Cells May Accumulate in Lymph Nodes. The paper by Geissmann et al. contains two provocative observations that build on work the group had previously published (6). First, the authors present immunophenotypic analysis of human lymph node sections taken from patients that suffer from dermatopathic lymphadenopathy, a pathological disorder marked by excessive accumulation of nonproliferative Langerhans cells in lymph nodes that drain a chronically inflamed skin site (7). These lymph nodes contain, not surprisingly, abundant DCs that express low levels of E-cadherin and what has been suggested to be a Langerhans cell–restricted marker, Langerin. The striking observation is that none of the Langerin$^+$ E-cadherin$^+$ CD1a$^+$ cells (CD1a$^+$ data from reference 6) show expression of mature DC markers, including CD86 and CD83. In contrast, neighboring DCs that lack Langerin express CD86 and CD83. These data suggest that Langerhans cells migrated to lymph nodes as immature DCs, although several cautions to this interpretation are applicable, as follows. (a) Although widely expressed by Langerhans cells, Langerin is not restricted in expression entirely to these epidermal DCs. For instance, Langerin is expressed by dermal Langerhans cell precursors (8) and can also be found in some other tissues (9). However, in contrast to Langerhans cells and the lymph node cells characterized by Geissmann and colleagues, dermal Langerhans cell precursors do not express E-cadherin when freshly isolated from skin (8). (b) Some of the results could be specific to this particular inflammatory disease and not broadly applicable to inflamed lymph nodes. (c) We cannot be certain that the Langerin$^+$ cells did not access lymph nodes from the blood, given evidence that potential DC precursors like plasmacytoid cells (10) and monocytes (11) can enter inflamed lymph nodes by traversing high endothelial venules (HEVs), rather than gaining access through afferent lymph. However, that the disease occurs within the inflamed skin and many of the Langerin$^+$ CD83$^-$ DCs were found in or near afferent sinuses strongly suggests that the cells recently arrived to the lymph node via afferent lymphatic vessels rather than via HEVs. Furthermore, although a small fraction of Langerin$^+$ DCs have been spotted in unlikely places for Langerhans cells, such as in spleen, questioning the specificity of Langerin for LCs, one would have to argue that none of the Langerin$^+$ cells observed by Geissmann et al. were derived from Langerhans cells. This extreme view seems unlikely, and because none of the Langerin$^+$ cells showed signs of maturation, it is reasonable to conclude that Langerhans cells and/or

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their dermal precursors accumulate within lymph nodes in an immature state during this chronic inflammatory disease. Even if this observation does not extend to all inflammatory settings that involve Langerhans cell migration, the data nevertheless imply that, at a basic level, Langerhans cell maturation and migration may be quite separable events and that maturation need not be strictly upstream of migration.

Informative animal models with regard to the study of the phenotype of migratory lymph DCs are those in which afferent lymph is sampled from cannulated thoracic ducts of rats or sheep, after the animals have undergone surgical resection of intervening lymph nodes. These models demonstrate the existence of at least two major phenotypes of DCs within lymph, one of which is less mature, as exhibited, for example, by less robust activity in stimulating proliferation of allogeneic T cells (12). The flux of DC emigration via lymph increases 8–15-fold in this model after systemic administration of LPS (13), a well known DC maturation stimulus. Much overlooked has been the finding that the relative proportion of the less mature DC population does not change after LPS treatment (13), suggesting that the migration-enhancing effects of LPS may not entirely stem from its maturation-inducing properties.

One, however, might argue that data on the behavior of intestinal DCs may not be pertinent to Langerhans cells. Indeed, even more so than for other potential sources of DCs, Langerhans cells have been thought to be especially dependent on maturation for migratory exit from the skin. This study, however, suggests that in analogy to the diversity of phenotypes observed in lymph-borne intestinal DCs, there appears also to be diverse phenotypes or states of maturation among migratory Langerhans cells in vivo. Thus, a more accurate conclusion may be that, like intestinal DCs, Langerhans cell migration to lymph nodes includes a balance between immature and mature cells. Whereas all Langerin$^+$ cells in the lymph nodes studied by Geissmann et al. appear immature, their own work (see Fig. 5 C, page 424) and that of others (8, 9) indicate that fully mature Langerhans cells may completely downregulate Langerin. Balancing the apparent presence of the immature Langerin$^+$ Langerhans cells in the lymph nodes examined are the neighboring Langerin$^-$ mature DCs, some of which are likely to have at least partly derived from Langerhans cells.

CCR7 and Migration of Immature DCs. As mentioned earlier, we have learned through experimentation that mobilization of Langerhans cells to lymph nodes is triggered by inflammatory mediators like the "maturation factors" TNF-α and IL-1β. Geissmann and colleagues, in a second series of experiments, show us that TNF-α, at least under some circumstances, can induce CCR7 without otherwise causing maturation. The authors indicate that it is impossible to determine whether immature Langerin$^+$ DCs expressed CCR7 in inflamed human lymph nodes, due to the ubiquitous expression of CCR7 by neighboring lymph node T cells. Therefore, they turn to an in vitro culture system to generate cells resembling Langerhans cells. Here they utilize monocytes cocultured in IL-4, GM-CSF, and TGF-β1. The rationale for using TGF-β1 is reasonable, given that Langerhans cells depend on this cytokine for development (14) and the extent to which it promotes a Langerhans cell phenotype, including expression of E-cadherin, Langerin, and the formation of Birbeck granules in vitro. Geissmann et al. show that TGF-β1–generated Langerhans cells do not become CD83$^+$, nor express high levels of CD86, when they are exposed to inflammatory mediators like TNF-α or even live *Escherichia coli*. However, these triggers were sufficient to induce expression of CCR7 and migratory responses to CCR7 chemotactic ligands, suggesting that these Langerhans cells were rendered able to migrate upon triggering by inflammatory stimuli, but that they were incapable of maturing fully unless they were engaged by CD40 ligand (CD154).

The model that emerges provides a means by which immune responses could be properly held in check, as it diminishes the idea that a simple inflammatory trigger sends numerous Langerhans cells to the lymph node in a mature form that could promote immune responses to undesirable targets such as self-antigens. In this model, full maturation would be at least somewhat restricted to the situation in which a DC productively engages a T cell such that CD154 is induced on the T cell and positively feeds back to drive activation of the antigen-presenting cell. The widely reported immune-enhancing power of anti-CD40 mAb mimetics of CD154 speak to the potency of such engagement.

However, it is difficult to accept the in vitro model and interpretation in whole, because ex vivo–derived Langerhans cells mature much more easily than do these in vitro–derived, TGF-β1–treated Langerhans cells. Furthermore, it is important to note that withdrawal of TGF-β1 during the late phases of the in vitro culture period permits substantial Langerhans cell maturation in response to inflammatory mediators (6), in contrast to the effects observed when this cytokine is continuously present. Nonetheless, an important observation arises from these experiments: namely, that DCs can exist in a state/environment in which "maturation" factors like TNF-α are able to trigger the onset of a migratory phenotype, including CCR7 expression, without promoting full antigen presentation capacity. Thus, we are left with a model in which immature DCs express CCR7, suggesting that they may very well migrate via afferent lymphatic vessels, a conclusion bolstered by the pathologic evidence from inflamed lymph nodes.

An assumption that remains outstanding, however, is the very fact that CCR7 is necessary for all DCs, regardless of state of maturation, to migrate to afferent lymphatic vessels. The relative scarcity of DCs in lymph nodes of CCR7-deficient mice supports this notion but may be misleading. It is quite possible that the lack of T cells in the lymph nodes of CCR7$^{-/-}$ mice secondarily depresses the migration of Langerhans cells because DC mobilization from skin is impaired in recombination activation gene (RAG)2$^{-/-}$ mice (15). Experiments are needed to probe more thoroughly the role of CCR7 in DC migration, including the repetition of "FITC painting" contact

hypersensitivity experiments using skin transplants (16) in which CCR7-deficient mice serve as skin transplant donors and recipients are $CCR7^{+/+}$ with normally developed lymph nodes. Other adoptive transfer approaches in which $CCR7^{-/-}$ circulating DC precursors (17) are transferred to normal hosts would also be extremely valuable for assessing the conditions under which CCR7 may and may not be essential for DC migration.

Nature's Course: Stage of Inflammation Influences the Character of Migratory DCs? Even if we accept that it is likely that the $Langerin^+$ cells in the lymph node are Langerhans cells, the possibility that these Langerhans cells may have migrated to the lymph node in immature form should be considered together with the alternative possibility that the cells migrated in a mature state but downregulated costimulatory molecules once in the lymph node. Considering that dermatopathic lymphadenopathy is a chronic inflammatory disease, it is likely that the cytokine environment within the tissue and that sampled by the draining lymph node includes a number of cytokines, but may be particularly rich in cytokines that act to counterbalance the positive feedback of proinflammatory cytokines. IL-10, for example, has the capacity to downregulate costimulatory and MHC molecules on antigen-presenting cells, even in the presence of proinflammatory mediators IL-1 and TNF-α. IL-10 is clearly a key cytokine in limiting inflammatory responses, since $IL\text{-}10^{-/-}$ mice tend to suffer from more robust inflammatory reactions than wild-type counterparts.

A simple experiment using human skin explants, however, supports the argument and model laid forth by Geissmann and colleagues suggesting that immature Langerhans cells express functional CCR7 as they migrate from skin. When human skin explants are cultured, a large fraction of Langerhans cells exit the explants via dermal lymphatic vessels (18). Most of these migratory cells are mature, since many express, for example, surface CD83. All are $CCR7^+$. Addition of the proinflammatory cytokine IL-1β increases the degree of maturation in this population and somewhat upregulates CCR7. Culturing the skin in IL-10 does not block DC migration but leads to a population of emigres that appear immature, and most lack CD83. These cells, however, still express CCR7 and migrate to its ligand CCL19 (unpublished data).

Another cytokine crucial to the regulatory control of inflammatory reactions is TGF-β1. TGF-β1–deficient mice succumb to massive inflammatory disease if this response is not pharmacologically quelled. Geissmann et al. employ TGF-β1 to generate Langerhans-like cells in vitro, and leave TGF-β1 present in the cell cultures even during steps in which maturation stimuli are added. Although Langerhans cells require TGF-β1 for development, it is not clear whether they are continuously producing or are exposed to active forms of TGF-β1. Thus, it is possible that the continuous use of TGF-β1 by Geissmann and colleagues not only induces a Langerhans cell phenotype but then also mimics later stages of inflammation when TGF-β1 would be present and operate to keep the response in check (19). Thus, the cells that they study may inadvertently, but interestingly, tell us something about the effects of antiinflammatory cytokines not only on Langerhans cells but on DCs in general—that they prevent maturation but do not block migration. If so, then a new version of the model develops in which the cytokine profile of an advanced inflammatory response containing molecules like IL-10 and TGF-β1 promote the transit of immature DCs to the lymph node, which in turn may participate in downregulating immune responses. At earlier stages of an inflammatory response, when antiinflammatory cytokine levels may be very low, Langerhans cells and other DCs may be more likely to be found in mature form. Although dermatopathic lymphadenopathy is an inflammatory response that apparently does not resolve properly, many of the cytokine networks that normally operate in late stages of inflammation may nevertheless be present. Future research using animal models in which the state of maturation of migrating Langerhans cells at different phases of an inflammatory response will be required to distinguish between the intriguing new models raised by this study.

Langerhans Cells and Contact Sensitivity: An Uncertain Role? While considering that Langerhans cells may arrive to the lymph node in an immature state, it seems reasonable to review the evidence that Langerhans cells per se are required for induction of contact sensitivity, a prototypical immune response initiated from the skin surface. After epicutaneous application of FITC, it is clear that skin-derived $FITC^+$ DCs migrate to the lymph node and mediate contact sensitivity (16, 20), as particularly evident from adoptive transfer of lymph node $FITC^+$ DCs as mediators of contact sensitivity. However, it is not certain that all of the transferred cells derive from Langerhans cells, since $FITC^+$ migratory DCs are heterogeneous with regard to a number of markers, including differing levels of CD11c (21). Although Sato and colleagues present evidence that $FITC^+$ cells mainly derive from the epidermis, they show that dermal cells which express a lectin called MMGL are required for the onset of contact hypersensitivity (22). They initially described these cells as macrophages, but they may also be DCs (23, 24). The possibility that these cells, rather than Langerhans cells, are the primary inducers of contact sensitivity and that Langerhans cells play a more regulatory role cannot be eliminated. In conclusion, it is perhaps sobering to reflect on the reality that the most well characterized migratory population of DCs, Langerhans cells, still harbors so many secrets.

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