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Editorial

Editorial: **Faux amis: Langerin-expressing DC in humans and mice**

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LC are a phenotypically and spatially defined population of myeloid cells that form a contiguous layer across the epidermis of the skin. In 2000, Valladeau and colleagues [1] reported the cloning of a novel human C-type lectin receptor that was thought to be unique to LC and named it Langerin (CD207). Two years later, the murine ortholog was cloned and showed considerable homology to the human gene [2, 3]. Expression of Langerin subsequently became synonymous with identification of LC in both humans and mice, although early RNA expression data suggested that murine Langerin was expressed more broadly in other tissues and DC populations [3]. Langerin binds to glycoconjugates on viruses, fungi, and mycobacteria and plays a key role in the internalization and degradation of HIV-1 by human LC, thus preventing transfer of the virus to T cells [4]. The receptor is internalized into the endosomal recycling compartment, where it accumulates in subdomains known as BGs. However, the function of these unique compartments is unclear and, although Langerin is required for BG biogenesis [2, 5, 6], LC from Langerin-deficient mice are phenotypically and functionally indistinguishable from wild-type cells. In particular, Langerin-deficient mice are not susceptible to cutaneous infections, including *Mycobacterium* [6]. A single nucleotide polymorphism that enhances the ability of Langerin to bind certain glycoconjugates has been identified recently in humans [7], but it is not known whether this mutation provides an advantage in the recognition and uptake of pathogens.

The cloning of the *Langerin* gene led to the generation of a collection of murine models, in which LC were both fluorescently labeled and could be depleted in vivo [8]: two groups used the endogenous murine *Langerin* locus to control expression of a high-affinity DTR-GFP DNA cassette (Langerin-DTR mice), whereas in an alternative approach, the DTA was inserted into bacterial artificial chromosome DNA containing the human *Langerin* gene and its surrounding regulatory sequences to generate the human Langerin-DTA transgenic mice. Characterization of Langerin-expressing populations and cell depletion in these mice led to 3 unexpected observations. The 1st was that contrary to accepted dogma at the time, epidermal LC were not required for the activation of many skin immune responses; the 2nd was that in addition to CD8α^+ DC in lymphoid organs, the murine Langerin promoter was expressed by a novel population of CD103$^+$ tissue DCs, which were specialized for cross-presentation of antigens to CD8$^+$ T cells and were developmentally distinct from LC. These cells have subsequently been shown to fulfill many of the roles traditionally ascribed to LC [9]. The 3rd finding was that transgenes controlled by the murine or human Langerin promoters were differently expressed, and genes under the human promoter were only switched on in epidermal LC. This difference between species was subsequently confirmed with the characterization of human cross-presenting DCs, which do not express Langerin but can be identified by the expression of thrombomodulin (CD141; blood DC antigen 3) [10]. A comparison of Langerin expression by murine and human LC and DC populations is illustrated in **Fig. 1**. The level of Langerin expression on murine CD103$^+$ DC is not sufficient to induce formation of BG, and it is not know whether Langerin plays a functional role in these cells. Indeed, antigens conjugated to anti-Langerin antibodies are processed efficiently and presented to T cells by Langerin$^+$ CD103$^+$ dermal DCs, demonstrating that Langerin-bound molecules can access intracellular processing pathways in the absence of BG [11]. In addition, the question remained as to whether Langerin was unique to LC in humans.

In this issue of *JLB*, Bigley et al. [12] have addressed this question and investigated the presence of Langerin-expressing immune cells in human tissues. They clearly demonstrate the presence of a small subset of myeloid DCs that express low levels of Langerin in the dermis of the skin and also find similar DCs in other tissues. Further characterization of the phenotype of these cells demonstrates that they more closely resemble other dermal CD1a$^+$ cells than CD141^{high} cross-presenting DCs. In elegant experiments, the authors exploited access to samples from patients that have received HSCTs or that carry mutations in the *Gata2* gene to demonstrate unequivocally that CD1a$^+$ Langerin-expressing

Abbreviations: BG = Birbeck granule, DC = dendritic cell, DT = diphtheria toxin, DTA = diphtheria toxin A subunit, HSCT = hematopoietic stem cell transplant, LC = Langerhans cell(s)

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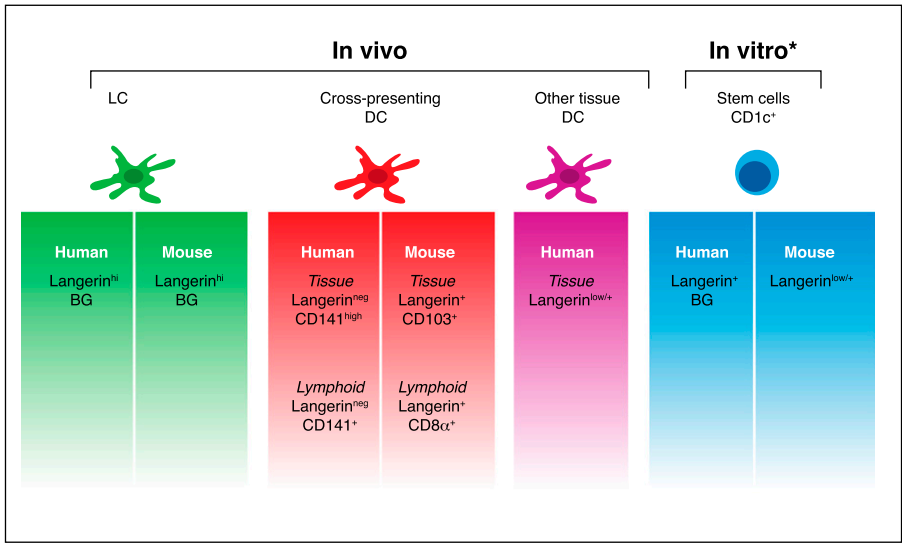

Figure 1. Langerin expression on LC and other DC populations in mice and humans. A summary of the different LC and DC populations in humans and mice that express Langerin and contain BGs. *Mouse: bone marrow cells cultured with GM-CSF and TGF-β. Langerin expression is low and heterogeneous in these cultures. Human: CD34$^+$ progenitors or blood CD1c$^+$ DCs cultured with GM-CSF/TNF-α/TGF-β/thymic stromal lymphopoietin.

DCs are not a subset of epidermal LC: host LC persist following HSCT, whereas CD1a$^+$ Langerin$^+$ DCs are replaced by cells derived from the donor; and patients with a global deficiency in DCs but not LC also lack CD1a$^+$ Langerin$^+$ DCs. Interestingly, immunohistochemistry on sorted cells demonstrates that the intracellular distribution of Langerin is different between LC and Langerin$^+$ dermal DCs. The significance of this difference is unclear but implies that Langerin does not accumulate in endosomal recycling compartments in CD1a$^+$ Langerin$^+$ DCs and, therefore, is unlikely to trigger formation of BGs. The costaining with other endosomal markers is needed to test this hypothesis.

Whereas the authors convincingly demonstrate that human and mouse dermal Langerin$^+$ DCs are nonorthologous "faux amis," a lack of functional experiments performed with CD1a$^+$ Langerin$^+$ DCs makes it difficult to determine whether they represent a functionally distinct DC population or merely heterogeneity within CD1a$^+$ DC. These experiments are challenging, given the rarity of these cells in human skin; however, the authors do demonstrate that incubation of blood DC with TGF-β can induce expression of Langerin by some cells. It would be interesting to know whether TGF-β can also drive up-regulation of Langerin on the dermal CD1a$^+$ population.

This study highlights the ongoing difficulties in defining a unified model of DC populations across species. Global gene expression analyses have provided powerful tools in recent years to compare the expression of human and mouse othologs, and such analyses have been used recently to demonstrate that key transcriptional profiles and regulation of differentiation are largely conserved between humans and mice across immune cell populations, including DCs [13]. However, key differences do exist, and regulation of expression of the *Langerin* gene provides a prime example. These differences likely reflect the strong evolutionary pressures on mammalian immune systems by the different pathogens they encounter.

The potency of DCs in controlling the activation of adaptive immunity has led to the development of novel immunotherapy strategies that are focused on delivering antigens, such as cancer-specific proteins, to defined DC subsets. One popular approach has been the direct conjugation of antigens to antibodies specific for C-type lectin receptors, such as Langerin, thus promoting uptake and intracellular processing of peptides for presentation to T cells. However, studies, such as the one presented by Bigley et al. [12] in this issue, are vital for our understanding of which cells we may be targeting if these approaches are to be translated successfully into the clinic.

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skin · Birbeck granules · diphtheria toxin receptor (DTR)

Editorial: **Crossing the divide: a novel *Cd8* enhancer with activity in CTLs and CD8$\alpha\alpha^+$ dendritic cells**

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Transcription of the genes encoding the CD8 coreceptor (*Cd8a* and *CD8b1*) is subject to extensive regulatory control, allowing expression to be responsive to environmental cues. For example, the CTL lineage is identified, in part, by expression of the CD8$\alpha\beta$ heterodimer, which is essential for the development and subsequent effector function of this cell lineage. CTL progenitors first express CD8$\alpha\beta$ in the DP stage during thymic development, where CD8$\alpha\beta$ expression, in concert with CD4, allows the developing thymocyte to determine if its newly rearranged TCR is capable of signaling, following interaction with MHC molecules (positive selection). If the TCR is capable of signaling, then the CD8$\alpha\beta$ coreceptor is down-regulated in a process thought to allow the DP thymocyte to determine whether its TCR signals in response to MHC I or to MHC II [1].

Abbreviations: Bcl11b = B cell lymphoma 11b, CRE = *cis*-regulatory element, DC = dendritic cell, DP = CD4$^+$CD8$\alpha\beta^+$ double-positive, ECR = evolutionary-conserved region, IEL = intraepithelial lymphocyte, Runx = runt-related transcription factor, SP = CD4$^+$ or CD8$\alpha\beta^+$ single-positive, T_{CM} = CD44$^+$CD62 ligand$^+$ central memory T lymphocyte

Cd8a and *Cd8b1* are closely linked genetically, and only the CD8$\alpha\beta$ heterodimer is a functional coreceptor [2]. However, mature CTLs also selectively up-regulate CD8α during antigen priming to generate a pool of memory cells [3]. The selective expression of CD8α is also a feature of non-CTL lineages, including $\alpha\beta$TCR IELs, $\gamma\delta$TCR IELs, and a subset of DCs [2]. Both the fine-tuned developmental control of CD8$\alpha\beta$ expression and the numerous differentiated lineages that express genes from the *Cd8* locus point to a complex and modular set of signals that regulate CD8 expression. This idea is supported by the array of CREs found within the *Cd8* locus that are important determinants of this complex patterning of CD8 expression.

Early study of the *Cd8* locus used a transgenic approach to show that an 80-kb region spanning the *Cd8a* and *Cd8b1* genes contained a sufficient complement of CRE to reconstitute a normal pattern of CD8 expression [4]. The authors characterized this region further by identifying 4 clusters of DNAse I hypersensitivity sites in chromatin isolated from thymocytes. Subsequent research groups characterized the capacity of these DNAse hypersensitivity clusters to promote expression of a transgenic reporter in CD8$^+$ lymphocytes from mice. This transgenic reporter approach led to the identification of the 4 enhancers, labeled $E8_I$, $E8_{II}$, $E8_{III}$, and $E8_{IV}$ (**Fig. 1A**). The initial enhancer identified, $E8_I$, is located midway between the *Cd8b1* and *Cd8a* coding sequences. $E8_I$ transgenic mice showed reporter expression in mature SP thymocytes, peripheral CTLs, and both $\alpha\beta$TCR and $\gamma\delta$TCR CD8$\alpha\alpha^+$ IELs [6]. Reporter expression in $\gamma\delta$TCR IELs, which do not express CD8β, provided the first indication that $E8_I$ may play a role in selective expression of CD8α. Surprisingly, DP thymocytes from $E8_I$ transgenic mice lacked reporter expression. The 3 remaining enhancers ($E8_{II}$, $E8_{III}$, and $E8_{IV}$) are closely linked, with $E8_{III}$ and $E8_{IV}$ overlapping the 3′ end of *Cd8b1*. In contrast to the $E8_I$ enhancer, the *Cd8b1*-proximal enhancers all facilitate reporter expression in DP thymocytes, and extrathymic activity for these enhancers is only seen for $E8_{II}$ in peripheral CTLs [7]. Thus, the bias of this array of enhancers to thymic development underscores the complex regulatory choreography

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