HYPOTHESIS
Insights & Perspectives

Tolerogenic and immunogenic states of Langerhans cells are orchestrated by epidermal signals acting on a core maturation gene module

Marta E. Polak1,2 | Harinder Singh3

1 Clinical and Experimental Sciences, Sir Henry Wellcome Laboratories, Faculty of Medicine, University of Southampton, Southampton, UK
2 Institute for Life Sciences, University of Southampton, Southampton, UK
3 Center for Systems Immunology, Departments of Immunology and Computational and Systems Biology, The University of Pittsburgh, Pittsburgh, Pennsylvania, USA

Correspondence
Dr. Marta E. Polak, Systems Immunology Group, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, SO16 6YD, UK.
Email: m.e.polak@soton.ac.uk
Dr. Harinder Singh, Center for Systems Immunology, Departments of Immunology and Computational and Systems Biology, 200 Lothrop Street, Pittsburgh, PA 15213, USA.
Email: harinder@pitt.edu

Funding Information
Wellcome Trust, Grant/Award Number: 109377/Z/15/Z

Abstract
Langerhans cells (LCs), residing in the epidermis, are able to induce potent immunogenic responses and also to mediate immune tolerance. We propose that tolerogenic and immunogenic responses of LCs are directed by signaling from the epidermis and involve counter-acting gene circuits that are coupled to a core maturation gene module. We base our analysis on recent genetic and genomic findings facilitating the understanding of the molecular mechanisms controlling these divergent immune functions. Comparing gene regulatory network (GRN) analyses of various types of dendritic cells (DCs) including LCs we integrate signaling-dependent (TGFβ, EpCAM, β-Catenin) and transcription factor (IRF4, IRF1, NFκB) regulated gene circuits that appear to orchestrate the distinctive LC functional states. Our model proposes, that while epidermal signaling in the steady-state promotes LC tolerogenic function, the disruption of cell-cell contacts coupled with inflammatory signaling induces LC immunogenic programming. The conceptual framework emphasizes the sensing of discrete epidermal and inflammatory cues by resident LCs in dictating their genomic programming and cell state dynamics.

Keywords
epithelial signaling, gene regulatory networks, immune functions, interferon regulatory factors, Langerhans cells

LANGERHANS CELLS INDUCE TOLEROGIC AS WELL AS IMMUNOGENIC RESPONSES

LCs have intrigued scientists since their discovery by Paul Langerhans in 1868. Despite being the longest studied antigen presenting cell (APC) population, and therefore the stereotypical “dendritic cells” (DCs), their functional importance has been continuously evolving.1 Their major immunologic function as stimulators of antigen-specific T-cell proliferation in an MHC II-dependent manner, was demonstrated in late 1970.2,3 LCs were shown to express 10-fold higher levels of MHC II than DCs and recognized as highly efficient APCs.4 These seminal experiments initiated an era of intense investigation of roles of LCs in epidermal, cutaneous, and systemic immunity. Multiple reports confirmed the functions of LCs in promoting immune responses in the context of contact hypersensitivity5 and allograft immunity6 and linked the release of pro-inflammatory cytokines, such as TNF by keratinocytes, with LC activation.7–10

Murine LCs have been shown to generate potent T cell responses to cutaneous pathogens, including herpes simplex virus (HSV) and Staphylococcus aureus.11–13 Langerin (CD207), the LC hallmark

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BioEssays. 2021;43:2000182.
https://doi.org/10.1002/bies.202000182
molecule, has been implicated in both human and murine LCs to recognize opportunistic and pathogenic fungi, including *Candida species* and *Malassezia furfur*.[14,15] We and others have demonstrated that human LCs can efficiently prime CD4 T cell responses and also induce naive CD8 T cell activation via antigen presentation as well as cross-presentation, respectively.[16–19] Human epidermal LCs have been shown to deliver a co-stimulatory signal through CD70 and in conjunction with IL-15 released directly into the immune synapse, to prime naive CD8(+) T cells into potent cytotoxic immune effectors.[16,18–21] TNF potentiates human LC transcriptional programs encoding antigen processing and presentation genes, enhancing their ability to cross-present antigens and induce T cell immunity.[18,22] Finally, LCs from human cutaneous squamous cell carcinoma have been shown to induce potent allogeneic CD8 and CD4 type 1 immune responses.[23]

Conversely, a key role of LCs in immune tolerance, promoting the maintenance of cutaneous homeostasis, has been elaborated in both murine models and in human cell culture systems.[18,21,24–27] LC migration from the epidermis is a continuous process during steady-state conditions and the transport of self-antigens to skin draining lymph nodes does not result in inflammatory disease.[1,28,29] Antigen processing and presentation of the keratinocyte protein desmoglein (Dsg3) by murine LCs, enabled by Langerin-mediated capture, has been shown to induce regulatory T cells (Tregs).[27] Consequently, ablation of LCs leads to autoimmune dermatitis in the murine model. Correspondingly, we and others have demonstrated that during steady-state (non-pathogenic) conditions, human LCs cocultured in vitro with skin resident and naïve CD4 T lymphocytes that during steady-state (non-pathogenic) conditions, human LCs co-cultured in vitro with skin resident and naïve CD4 T lymphocytes that during steady-state (non-pathogenic) conditions, human LCs co-cultured in vitro with skin resident and naïve CD4 T lymphocytes selectively induce the activation and proliferation of Foxp3 expressing CD4+CD125+CD127−, IL-10 producing regulatory T cells.[21,24,25] Interestingly, expansion of Tregs correlated with poor re-stimulation of antibacterial memory CD4(+) T cells and inefficient induction of *S. aureus*-specific effector CD4(+) T cells from naive T cells by bacteria-pulsed LCs.[27]

While LC-induced tolerance plays an important role in cutaneous immune homeostasis, the nature of signaling and transcriptional programs that regulate the functioning of steady-state, migratory LCs in priming regulatory T cell responses to innocuous antigens, remain to be elucidated. Furthermore, it is not known how such homeostatic transcriptional programing of LCs is counteracted by pathogen-triggered signals. These pathogen-associated inflammatory signals enable LCs to prime effector T cell responses as well as to activate skin-resident effector memory T cells. Herein, based on recent genomic and epigenomic analyses of human LCs and drawing upon findings with other types of DCs, we put forward a hypothesis that tolerogenic and immunogenic states of LCs are directed by counter-acting gene regulatory networks that are both coupled to a shared core maturation gene module. The latter is induced by the disruption of cell-cell contacts and programs efficient antigen presentation during LC migration to draining lymph nodes.

**LCs—PROGRAMED FOR TOLERANCE BY THE EPIDERMIS**

The cellular interactions between LCs and keratinocytes have been shown to be important for their development and residence in the epidermis as well as their tolerogenic function.[30–33] LC progenitors are generated during development of the fetus and colonize skin shortly before birth. Importantly, the cross-talk of differentiating LC progenitors with keratinocytes is dependent on two cooperating signaling pathways, namely TGF-β and E-cadherin:β-catenin.[32,34] TGF-β, is a soluble mediator whose activity is contextually regulated by its cleavage and release from the extracellular matrix.[35] TGF-β has long been recognized as a key factor for LC development both in vivo and in vitro.[32,36,37] The pivotal role of TGF-β in LC development has been established using TGF-β -deficient mice, which are unable to generate LCs.[30] Importantly, TGF-β is also required to maintain the epidermal residence of LCs and this in turn is dependent on the proteolytic activation of its latent precursor that is facilitated by integrins αvβ6 and αvβ8 that are expressed by keratinocytes.[38,39] Given the major function of TGF-β signaling in retention of LCs in the epidermis, it is attractive to consider its potential role in programing of their tolerogenic state. In this regard, a key role for TGF-β-mediated tolerance of DCs has been documented in multiple tissues,[40,41,42] including the uterus[43] and the gut.[35,44] While it has been proposed that the impact of TGF-β signaling depends on the stage of maturity of DCs, its roles have been demonstrated both in induction of self-tolerance, for example, prevention of EAE,[45] in tissue homeostasis[35,40,44] and in resolution of inflammation.[45]

Given the interplay of TGF-β and E-cadherin:β-catenin signaling in the context of LC development,[34] similar cooperation between the two signaling systems is plausible in programing of the LC tolerogenic state. The role of E-cadherin mediated signaling in programing of the tolerogenic state of DCs has been invoked in several studies of E-cadherin-mediated DC-DC adhesion has been shown to induce upregulation of MHC class II and costimulatory molecules as well as chemokine receptors.[46] Such DC maturation was shown to be partly dependent on the activation of the beta-catenin pathway. Importantly, E-cadherin-stimulated DCs induced regulatory T cells and tolerance in vivo. Consistent with these findings, Wnt-β-catenin signaling in intestinal DCs has been shown to be required for expression of anti-inflammatory mediators including retinoic acid-metabolizing enzymes and interleukin-10 as well as Treg induction.[47] In spite of these promising findings with murine DCs, deletion of E-cadherin in their LC counterparts has been recently shown to be dispensable for their retention in the skin as well as homeostatic migration.[48] Notably loss of E-cadherin does not exacerbate imiquimod-instigated psoriatic skin inflammation and T cell inflammatory responses. In spite of these findings, it remains possible that E-cadherin functions in a redundant manner in LCs to negatively regulate Wnt-β-catenin signaling. The role of the latter pathway in the tolerogenic programing of LCs remains to be tested using lineage-specific deletion strategies.
In addition to the above signaling systems the epithelial cell adhesion molecule, EpCAM, has a major role regulating LC interactions with keratinocytes and therefore could function in programming their tolerogenic state. EpCAM is a membrane glycoprotein that is expressed on the surfaces of LCs, as well as on epithelial cells.[49–51] Analysis of EpCAM-deficient LCs in mice demonstrated the importance of this cell adhesion molecule in controlling of LC mobility and migration in the absence of inflammation.[52] Notably, EpCAM expression on LCs prevented an excessive inflammatory response to OVA allergen challenge.[52] Intriguingly, EpCAM may function as an outside-in signaling molecule that regulates gene expression and cross-talk with E-cadherin and β-catenin.[53] Furthermore, EpCAM can be up-regulated by accumulation of β-catenin thereby establishing a positive auto regulatory loop.[54] Finally, recent studies suggest a close relationship of EpCAM with other signaling molecules in the epidermis including E-cadherin, claudins, and TGF-β.[55–57] thus highlighting EpCAM an important hub in the network of signaling interactions between keratinocytes and LCs.

THE TRANSCRIPTION FACTOR IRF4 PROGRAMS LC TOLERANCE

Analyses of transcription factors regulating murine and human LC development and function have highlighted RUNX3, PU.1, Interferon Regulatory Factors (IRFs)[18,58–61] and the NF-κB system[62,63] as key regulators. RUNX3 and PU.1 are crucial for LC development as well as restraining LC maturation.[60,61] Our recent transcriptomic and epigenetic analyses of primary human LCs has revealed the likely dependence of LC migration and tolerogenic function on the transcription factor IRF4.[18,59]

Amongst its diverse functions in regulating the development and activation of various types of immune cells, IRF4 has long been implicated in promoting tolerance, driving regulatory T cell expansion and IL-10 expression.[64–68] While analyzing transcriptional determinants of DC function in a bone marrow-derived DC (BMDC) culture system we have shown that in the absence of microbial products, IRF4 promotes regulatory T cell (Treg) generation by enhancing expression of genes required for antigen presentation together with those for T cell tolerance.[69] In keeping with these findings, IRF4-deficient DCs were impaired for Treg generation in vivo, resulting in decreased FoxP3+ Tregs in numerous organs, most evident in peripheral tissues such as skin and small intestine.[69] Intriguingly, IRF4 has been suggested to cooperate with the aryl hydrocarbon receptor (Ahr) to form a complex that binds to the promoter of the IL-10 gene and regulate its expression.[70] Conversely, IRF4 appears to be important for dampening inflammatory cytokine genes as the expression of IL-12 and TNF is essential for the transport of self-antigens to skin draining lymph nodes and expansion of regulatory T cells preventing autoimmunity.[21,27,29,74] we and others have demonstrated that human LCs activated by TLR2 signaling or pro-inflammatory cytokines, such as TNF, can very potently activate cytotoxic T lymphocytes, as well as prime Th2 and Th17 adaptive immune responses.[16,18,19,75] Similarly, external antigen uptake by LC, as well as trans-epidermal exposures to contact sensitizers, or danger signals, drive strong adaptive immune responses.[52,75–77]

LC IMMUNOGENICITY IS PROMOTED BY THE TRANSCRIPTION FACTORS IRF1 AND NFκB

While LC migration from the epidermis during the steady-state is essential for the transport of self-antigens to skin draining lymph nodes and expansion of regulatory T cells preventing autoimmunity,[21,27,29,74] we and others have demonstrated that human LCs activated by TLR2 signaling or pro-inflammatory cytokines, such as TNF, can very potently activate cytotoxic T lymphocytes, as well as prime Th2 and Th17 adaptive immune responses.[16,18,19,75] Similarly, external antigen uptake by LC, as well as trans-epidermal exposures to contact sensitizers, or danger signals, drive strong adaptive immune responses.[52,75–77]

As noted above, LC activation can lead to induction of efficient CD4 and CD8 T cell responses.[16,19,20] The transcription factor IRF8, an IRF4 paralog, and IRF1 have been shown to be critical for programming immunogenic responses in murine APCs.[78–82] IRF8-regulated genomic responses in such APCs have been shown to protect against numerous infections including cytomegalovirus and gram-negative bacteria.[79,83,84] The importance of IRF8-mediated protective immune responses in humans is exemplified by a patient with...
an IRF8 mutation.\textsuperscript{85} Loss of IRF8 expression resulted in depletion of several DC populations, and severe disseminated mycobacterial and mucocutaneous fungal infections. Finally, IRF8 and IRF1 regulated gene modules have been implicated in chronic pathological inflammation in mouse models of neuroinflammation, pulmonary tuberculosis, and cerebral malaria.\textsuperscript{79,83}

Surprisingly human LCs lack IRF8 expression even when stimulated with TNF and under conditions in which they prime immunogenic CD8 responses.\textsuperscript{18} This is consistent with inactive chromatin at the IRF8 locus, thereby highlighting an epigenetic distinction of LCs from both DCs and macrophages. The apparent lack of dependence of LCs on IRF8 could represent a mechanism for their distinctive genomic programming, enabling them to be adapted to their environmental niche and the safeguarding of epidermal homeostasis. IRF8 has been shown to regulate production of pro-inflammatory cytokines in DCs and macrophages.\textsuperscript{83} In contrast, numerous reports point to the restricted repertoire of cytokines produced by LCs, at steady state or upon activation, and their superior ability to activate antigen-specific adaptive immune responses, in place of broad-spectrum inflammation. We propose that by utilizing IRF4 rather than IRF8, LCs uncouple efficient antigen presentation and co-stimulation of T cells from production of pro-inflammatory mediators, and thus prevent excessive inflammatory responses and promote epidermal homeostasis.

How do LCs coordinate protective immune responses in the absence of IRF8? Our recent studies document that TNF signaling induces expression of IRF1 in migratory LCs.\textsuperscript{18,22} Similarly to IRF8, IRF1 has been implicated in mediating pro-inflammatory responses.\textsuperscript{86–88} Interestingly, IRF1 controls anti-viral transcriptional programs,\textsuperscript{87,88} and coordinates the expression of MHC I antigen processing and presentation genes, activating transcription by binding to ISRE genomic elements.\textsuperscript{89} We propose that the transcription factor IRF1 in conjunction with NF-xB could perform this vital function in the genomic programming of Immunogenic LCs. In many cell types pathogen-derived signals induce expression of IRF1 along with activation of the NF-xB system; the two transcription factors in turn physically cooperate to induce MHC class I gene expression. Induced by pathogens, IRF1 coordinates IFN responses in both immune and non-immune cell types.\textsuperscript{90–92} IRF1 binding to interferon stimulated response elements (ISREs) induces HLA-A and B2M expression\textsuperscript{89,93} and activates inflammasome gene expression.\textsuperscript{92,94} Interestingly, IRF1 expression is potently induced in human migratory LCs by TNF signaling, correlating with their enhanced ability to cross-present antigens to CD8 T cells.\textsuperscript{16,18,22} NF-xB is classically regarded as central to the activation of immune responses. Signaling via the TNF superfamily member Receptor Activator of NF-xB (RANK) and its ligand, RANKL, has been shown to prevent LC apoptosis through NF-xB activation, resulting in the enhanced migration of LCs to regional lymph nodes. This in turn is associated with an improved induction of MHC class I–restricted HSV-1–specific antiviral immunity, dependent on TLR3 signaling.\textsuperscript{112} NF-xB also appears to be important for human LC activation through TLR2 signaling, resulting in up-regulation of CD86, CD83, CCR7, TNF and IL-6, and the priming of CD4 T cell responses.\textsuperscript{95}

Thus, it is likely, that while EpCAM – β-catenin – IRF4 regulatory circuit controls tolerogenic LC function, proinflammatory signaling from keratinocytes or danger signals from pathogen derived components and PRR activation, program the immunogenic state of LCs via IRF1 and NF-xB.

### COUNTERACTING GENE REGULATORY NETWORKS UNDERPIN TOLEROGENIC AND IMMUNOGENIC PROGRAMING OF LCS

GRNs along with their signaling inputs have been used to describe and analyze the regulation of transcriptional programs during immune cell development, activation and function.\textsuperscript{96,97} Within a GRN, transcription factors act in a combinatorial manner to coordinate expression of functionally important sets of target genes that comprise transcriptional modules. Some of these transcription factors are responsive to extrinsic signals either being activated via a post-translational mechanism or via transcriptional induction of the gene encoding them. GRNs generate causal regulatory frameworks for decision-making circuits.\textsuperscript{59,96–98} Herein we elaborate such a conceptual framework to assemble a GRN comprising counter-acting modules that appears to control LC tolerance versus immunogenic states (Figure 1). Given, the accumulating evidence that points to the importance of LC maturation in underpinning their immunogenic and tolerogenic functions, our GRN model couples the divergent transcriptional programing of tolerogenic and immunogenic LCs with the induction of a shared core gene module that enables efficient antigen processing, presentation and co-stimulatory signaling to prime either regulatory or effector T cell immune responses. The counter-acting GRN is proposed to be controlled by direct cell–cell contacts of LCs with keratinocytes and via their secretory signals. We emphasize that while some regulatory components and connections of the GRN discussed below have direct experimental support others remain to be experimentally tested in the context of human LCs as they are based on insights in other APCs or non-immune cell contexts.

Maintaining immune tolerance is a key LC function in the epidermis. We suggest that the stability of this functionally critical transcriptional state is controlled by several feed forward and feedback regulatory loops. TGF-β activation and signaling enabled by integrins expressed on keratinocytes is proposed to retain immature LCs in the epidermis by promoting their cell-cell interactions with keratinocytes. Importantly, LC interactions with keratinocytes, mediated by TGF-β and EpCAM and its signaling functions, prevents their LC migration.\textsuperscript{38,39,52} Thus LC-KC interactions reinforce a stable immature LC state state with tolerogenic potential. Inhibition of TGF-β signaling and/or loss of EpCAM enables LC migration, maturation and their tolerogenic programing. This process is likely dependent on the activation of β-catenin signaling and the upregulation of IRF4. Intriguingly, IRF4 has been shown to be positively regulated post-translationally by the ROCK2 kinase, which manifests reciprocal antagonism with E-cadherin,\textsuperscript{99,100} raising the possibility of similar regulation of ROCK2 by other cell-cell
FIGURE 1 Proposed GRN orchestrating maturation of Langerhans cells into their tolerogenic (blue) or immunogenic states (red). Tolerogenic maturation occurs under homeostatic conditions whereas the immunogenic state is manifested during infection of the epidermis and inflammation. Key transcription factors (TFs) comprising the GRN and their regulatory connections are shown including major signaling inputs that modulate the expression or activities of the TFs. Activating and inhibitory regulatory connections are shown with black and red lines, respectively. Dashed lines indicate plausible regulatory connections based on experimental evidence in other cell contexts. Open double headed arrows indicate physical interactions between TFs. Binding of TFs to their cognate sites within promoters and enhancers (yellow rectangles) of their target genes leads to activation of three distinct transcriptional modules in Langerhans cells (grey ovals) designated core maturation, tolerogenic or immunogenic. The GRN depicts the core maturation module as one that is shared by both the tolerogenic and immunogenic states. Counteracting functions of select TFs for example, IRF4 and IRF1 result in an overlay of the core maturation module with either the tolerogenic or immunogenic modules. Table S1 summarizes the evidence for each interaction edge.

adhesion molecules such as EpCam. The tolerogenic LC programme consists of a functional gene module that includes the IDO1, LGALS1, and IL4I1 genes. While LGALS1 and IL4I1 are directly regulated by IRF4, IDO1 is likely co-induced by IRF4 and the aryl hydrocarbon receptor (AHR) on migration. IDO1 is known to be regulated by the ligand activated AHR. Notably, the β-catenin pathway has been shown to induce AHR expression in hepatocytes raising the possibility that it also does so in LCs. Finally, AHR has been shown to co-bind with IRF4 to promoters of tolerance associated genes including IDO1. Importantly, kynurenine metabolites, produced by IDO-mediated catabolism of tryptophan, could feedback to augment AHR activity in sustaining IDO expression, creating a feedforward loop previously proposed to operate in other tolerogenic DCs. Thus, we propose that a self-reinforcing control circuit involving loss of EpCAM mediated LC:KC interactions and the induction of IRF4, ROCK2 and β-catenin signaling, and AHR activation could orchestrate a cell migration-coupled maturation program and its overlaying with a tolerance-inducing genomic module in LCs.

Our scRNA-seq analyses of the LC compartment under homeostatic conditions suggests the existence of an immunocompetent sub-population able to prime regulatory T cell responses. The nature of the homeostatic signal inducing such a sub-population of LCs is currently unknown, but it is plausible that immunocompetent cells are generated continuously by transient disruption of cell adherens junctions with keratinocytes and consequently the upregulation of IRF4. We note that for their sentinel functions as sensory cells LCs need to alter their shape and positioning of dendrites. Actin cytoskeleton rearrangements required for re-positioning of dendrites would naturally lead to re-arrangements of adherence junctions and may induce the migratory and maturation program in a subset of LCs. Thus, the generation
of such immunocompetent and tolerogenic LC subpopulation in the steady state could reflect the dynamic nature of LC-keratinocyte interactions.

During infection of the epidermis, TLR, CD207, and pro-inflammatory cytokine signaling override TGF-β signaling. Induction of the immune activated LC state requires breaking away from the intricate network of self-reinforcing mechanisms promoting immunotolerance. Based on the existing evidence, we propose that proinflammatory signaling from keratinocytes or danger signals from pathogen derived molecular determinants induce an immunogenic transcriptional program regulated by IRF1 and NF-κB. Notably, IRF1 and IRF4 have been shown to counter regulate each other’s functions in a variety of cellular contexts. Consistent with this proposition, TNF signaling results in the induction of IRF1-mediated transcriptional programs including an increased ability to activate cytotoxic T lymphocytes. Importantly, TNF signaling dampens IRF4 expression. The effect of TNF signaling is enhanced when delivered during LC migration from the epidermis, suggesting that overriding the GRN controlling the tolerogenic program in LCs is more efficient prior to IRF4 induction.

As discussed above, LCs do not appear to express the IRF4 paralog, IRF8, which is critical for the immunogenic functions of DCs and macrophages. We suggest that the notable lack of IRF8 expression in LCs accounts for their major function in epidermal homeostasis. IRF4 and IRF8 have redundant as well as unique functions in spite of interacting with the same set of transcriptional partners such as PU.1 and binding to identical DNA sequences within promoters and enhancers of their target genes. IRF8 has been shown to regulate production of pro-inflammatory cytokines in DCs and macrophages, the latter contributing to chronic inflammation. Furthermore, IRF8 has a well-established role in promoting the expression of the gene encoding the shared p40 subunit of the immunogenic cytokines, IL-12 and IL-23. Interestingly, β-catenin signaling can drive differentiation and immunogenic function of IRF8-dependent DCs, resulting in elevated IL-12 expression upon in vitro microbial stimulation. Therefore, lack of IRF8 expression in LCs can promote tolerogenic immune responses induced by β-catenin signaling following the disruption of adherence junctions in the epidermis. Thus, we propose that by utilizing IRF4 rather than IRF8, LCs uncouple the activation of antigen-specific adaptive immune responses from production of pro-inflammatory mediators, and thus prevent excessive inflammation instead promoting epidermal homeostasis.

CONCLUSIONS AND EXPERIMENTAL TESTING OF GRN MODEL

In conclusion, in our GRN model for LCs, we hypothesize that tolerogenic and immunogenic states are directed by counter-acting gene modules that are both coupled to a shared core maturation gene module, which is induced by the disruption of cell-cell contacts and cell migration to draining lymph nodes. For LCs, migration out of the epidermis is a critical event for inducing the core maturation module, comprising genes encoding antigen presentation components and co-stimulatory receptors, that is necessary for both the efficient priming of tolerogenic as well as immunogenic responses. Depending on the conditions or signals that lead to disruption of LC-keratinocyte interactions and the induction of counter-acting transcription factors, this core module is overlaid by distinctive regulatory modules that promote either tolerogenic or immunogenic LC states. To formally test the hypothesis, a series of genetic perturbations in murine and human LCs of key regulatory components including IRF4, IRF1, SMADs, β-catenin, ROCK2, and AHR, followed by analyses of their transcriptional programing and priming of tolerogenic versus immunogenic T cell responses is critical. It is now possible to utilize Cas9 mediated genome editing to engineer mutations in these key regulatory components in human LCs and to assess the effects of such perturbations on LC transcriptional programing and the priming of regulatory versus effector T cell responses. While genomic analyses of human LCs are possible using scRNA-seq and scATAC-seq the functional examination of their perturbed states will be challenging, given the small numbers of primary LCs and the limitations of the in vitro cell culture systems. Such experiments will therefore need to be complemented by LC specific deletion of these components in murine models. By generating a mathematical model of the proposed GRN and using biologically plausible parameters, one can compute the outcome of signaling inputs and predict TF regulated gene expression dynamics and in turn the frequency of cells that acquire a tolerogenic versus immunogenic state. Perturbations of the GRN can then be simulated using the model. Both the parameters used to generate the simulations and the consequences of specific perturbations can then be experimented tested using the aforementioned experimental approaches with murine and human LCs. The conceptual framework we propose herein offers an opportunity to delineate the genomic programing and cell state dynamics of LCs. It provides a foundation for investigating genome-based mechanisms underpinning LC functions in skin health and inflammation.

ACKNOWLEDGMENTS

We are grateful to Dr Sofia Sirvent for contributing the figure. The study was funded by a Sir Henry Dale Fellowship from Wellcome Trust, 109377/Z/15/Z.

CONFLICT OF INTERESTS

The authors declare there is no conflict of interests.

DATA AVAILABILITY STATEMENT

A review manuscript, no new data generated or shared.

This article is commented on in the Idea to watch paper by Clare L. Bennett, https://doi.org/10.1002/bies.202100072

ORCID

Marta E. Polak https://orcid.org/0000-0003-2878-476X

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https://doi.org/10.1002/bies.202000182

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How to cite this article: Polak, M. E., & Singh, H. (2021). Tolerogenic and immunogenic states of Langerhans cells are orchestrated by epidermal signals acting on a core maturation gene module. *BioEssays*, 43, e2000182. https://doi.org/10.1002/bies.202000182