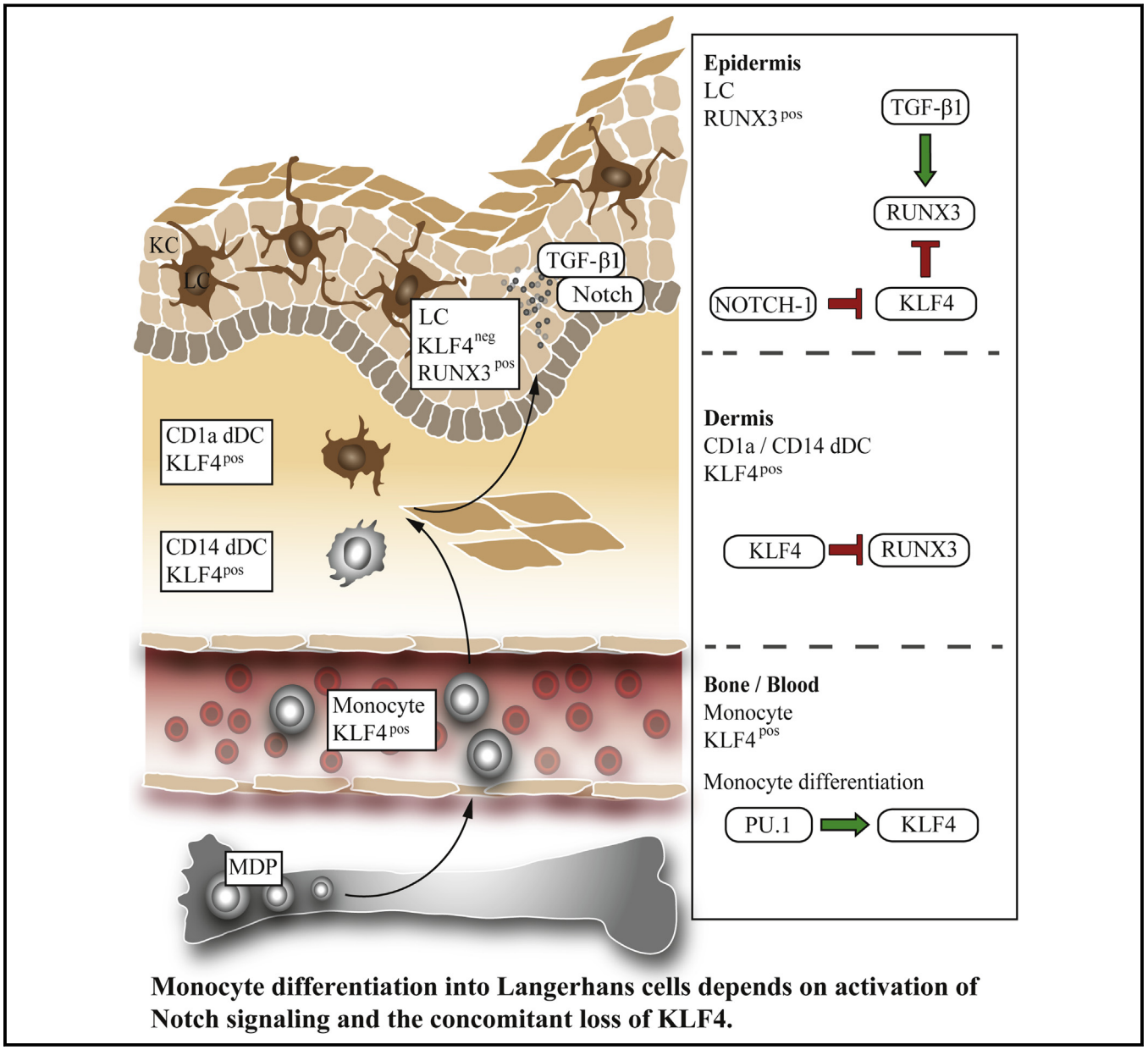
Human skin dendritic cell fate is differentially regulated by the monocyte identity factor Kruppel-like factor 4 during steady state and inflammation

Jennifer Jurkin, PhD,[b]*‡ Corinna Krump, MSc,[a]* René Köffel, PhD,[b]§ Christina Fieber, MSc,[b] Christopher Schuster, MD,[c] Patrick M. Brunner, MD,[c] Izabela Borek, MSc,[a] Gregor Eisenwort, MD,[b] Clarice Lim, BSc(Hon),[a,b] Jörg Mages, PhD,[d] Roland Lang, MD,[d] Wolfgang Bauer, MD,[c] Diana Mechtcheriakova, PhD,[e] Anastasia Meshcheryakova, MSc,[e] Adelheid Elbe-Bürger, PhD,[c] Georg Stingl, MD,[c] and Herbert Strobl, MD[a,b] *Graz and Vienna, Austria, and Erlangen, Germany*

GRAPHICAL ABSTRACT



Monocyte differentiation into Langerhans cells depends on activation of Notch signaling and the concomitant loss of KLF4.

Background: Langerhans cell (LC) networks play key roles in immunity and tolerance at body surfaces. LCs are established prenatally and can be replenished from blood monocytes. Unlike skin-resident dermal DCs (dDCs)/interstitial-type DCs and inflammatory dendritic epidermal cells appearing in dermatitis/eczema lesions, LCs lack key monocyte-affiliated markers. Inversely, LCs express various epithelial genes critical for their long-term peripheral tissue residency.

Objective: Dendritic cells (DCs) are functionally involved in inflammatory diseases; however, the mechanisms remained poorly understood.

Methods: *In vitro* differentiation models of human DCs, gene profiling, gene transduction, and immunohistology were used to identify molecules involved in DC subset specification.

Results: Here we identified the monocyte/macrophage lineage identity transcription factor Kruppel-like factor 4 (KLF4) to be inhibited during LC differentiation from human blood monocytes. Conversely, KLF4 is maintained or induced during dermal DC and monocyte-derived dendritic cell/inflammatory dendritic epidermal cell differentiation. We showed that in monocytic cells KLF4 has to be repressed to allow their differentiation into LCs. Moreover, respective KLF4 levels in DC subsets positively correlate with proinflammatory characteristics. We identified epithelial Notch signaling to repress KLF4 in monocytes undergoing LC commitment. Loss of KLF4 in monocytes transcriptionally derepresses Runt-related transcription factor 3 in response to TGF-β1, thereby allowing LC differentiation marked by a low cytokine expression profile.

Conclusion: Monocyte differentiation into LCs depends on activation of Notch signaling and the concomitant loss of KLF4. (J Allergy Clin Immunol 2016;■■■:■■■-■■■.)

Key words: *Kruppel-like factor 4, Runt-related transcription factor 3, TGF-β1 signaling, Notch, lineage decision, monocyte differentiation*

Abbreviation used

aN1:	Active intracellular NOTCH1
DC:	Dendritic cell
FITC:	Fluorescein isothiocyanate
FLT3L:	FMS-like tyrosine kinase 3 ligand
GFP:	Green fluorescent protein
IDEC:	Inflammatory dendritic epidermal cell
IRES:	Internal ribosomal entry site
KLF4:	Kruppel-like factor 4
LC:	Langerhans cell
moDC:	Monocyte-derived dendritic cell
moLC:	Peripheral blood $CD14^+$ monocyte-derived Langerhans cell
p-LC:	$CD34^+$ progenitor cell–derived Langerhans cell
p-moDC:	$CD34^+$ progenitor cell–derived monocyte-derived dendritic cell
PPAR:	Peroxisome proliferator-activated receptor
RUNX3:	Runt-related transcription factor 3
SCF:	Stem cell factor

Langerhans cells (LCs) are abundantly occurring dendritic cells (DCs) in environment-exposed stratified epithelia.[1] They represent a well-defined DC subset marked by unique immunophenotypic ultrastructural and functional characteristics.[2] Among various known human DC family members, LCs are identified as $CD1a^{hi}CD207^+$ cells that express several epithelial molecules (eg, E-cadherin, tumor-associated calcium signal transducer 2 [TACSTD2]/TROP2,[3] epithelial cell adhesion molecule [EpCAM]/TROP1,[4] and claudin-1[5]), allowing them to undergo long-lasting adhesion to keratinocytes. LCs efficiently stimulate regulatory T cells and are involved in mediating tolerance to epithelial and environmental antigens.[6] Consistently, even in the absence of microbial or danger signals, LCs continuously migrate to T-cell areas of skin-draining lymph nodes.[1]

LCs lack expression of monocyte/macrophage lineage-affiliated markers, including CD11b, CD36, lysozyme, CD209/DC-specific intercellular adhesion molecule–grabbing nonintegrin, and c-fms/monocyte colony-stimulating factor,[7] as well as the early myeloid lineage marker myeloperoxidase.[8] However, LC development is dependent on macrophage colony-stimulating factor receptor (CD115, c-fms), and blood monocytes can differentiate into LCs.[9-12]

Human monocytes can also differentiate into monocyte-derived dendritic cells (moDCs; induced by GM-CSF and IL-4), resembling inflammatory DCs *in vivo* (eg, inflammatory dendritic epidermal cells [IDECs]), or into macrophages. Unlike what is observed for LCs, monocyte lineage markers are maintained in these (sub)lineages.[13] Moreover, moDCs possess a much higher capacity to synthesize proinflammatory cytokines.[14,15] The transcriptional mechanism underlying monocyte/macrophage/moDC versus peripheral blood $CD14^+$ monocyte-derived Langerhans cell (moLC) differentiation remains elusive.

LC differentiation from human hematopoietic progenitor cells is marked by upregulation of transcription factors.[16-18] LCs express high levels of PU.1, and ectopic PU.1 promotes LC differentiation from myeloid progenitors.[19] PU.1 transactivates Runt-related transcription factor 3 (RUNX3), which is essential

From [a]the Institute of Pathophysiology and Immunology, Medical University of Graz; [b]the Institute of Immunology, [c]the Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology, and [e]the Departments of Pathophysiology and Allergy Research, Medical University of Vienna; and [d]the Institute of Clinical Microbiology, Immunology and Hygiene, University Hospital Erlangen, Friedrich-Alexander-University Erlangen-Nürnberg, Erlangen.

*These authors contributed equally to the work.

‡Dr Jurkin is currently affiliate with the IMBA-Institute of Molecular Biotechnology GmbH, Vienna, Austria.

§Dr Köffel is currently affiliated with the Institute of Anatomy, University of Bern, Bern, Switzerland.

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Corresponding author: Herbert Strobl, MD, Institute of Pathophysiology and Immunology, Medical University of Graz, Heinrichstrasse 31A, 8010 Graz, Austria. E-mail: herbert.strobl@medunigraz.at.

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