DISSERTATION

Transcriptional Regulation of Human Langerhans Cell Development

submitted by
Dipl.-Ing. BSc
Corinna KRUMP

for the Academic Degree of
Doctor of Philosophy
(PhD)

at the
Medical University of Graz
Institute of Pathophysiology and Immunology

under the supervision of
Prof. Dr. Herbert STROBL

2017

DECLARATION

I hereby declare that this dissertation is my own original work and that I have fully acknowledged by name all of those individuals and organizations that have contributed to the research for this dissertation. Due acknowledgement has been made in the text to all other material used. Throughout this dissertation and in all related publications I followed the guidelines of “Good Scientific Practice”.

Graz, 14.06.2017

Parts of this thesis have been published in:

Jurkin J.*, **Krump C**.*, Köffel R., Fieber C., Schuster C., Brunner P.M., Borek I., Eisenwort G., Lim C., Mages J., Lang R., Bauer W., Mechtcheriakova D., Meshcheryakova A., Elbe-Bürger A., Stingl G. and Strobl H. "Human skin dendritic cell fate is differentially regulated by the monocyte identity factor Kruppel-like factor 4 during steady state and inflammation"; The Journal of Allergy and Clinical Immunoloy, Volume 139, Issue 6, pp. 1873-1884
OPEN ACCESS, DOI: http://dx.doi.org/10.1016/j.jaci.2016.09.018.
*shared first-authorship (1)

Supported by research grants of the Austrian Sciene Fund (FWF; P19474-B13 to A.E.-B., P22441-B13 and P23228 to D.M., and P22058, P19245, P25720 and SFB-2304 to H.S.) and the PhD programs W1212 "Inflammation and Immunityy", W1248-B13 "Molecular, Cellular and Clinical Allergology" and W1241 "Molecular Fundamentals of Inflammation".

Jennifer Jurkin worked as a Pos-Doc in the lab of Herbert Strobl still at the Medical University of Vienna before she left for IMBA (Institute of Molecular Biotechnology GmbH, Vienna, Austria) in 2011. She performed the microarray screen for the identification of transcription factors this thesis is based on. Initial experiments regarding KLF4 expression by different subsets of the monocytic lineage have been performed by Christina Fieber during her diploma thesis in 2009 under the supervision of Thomas Decker and Jennifer Jurkin. Rene Köffel, now affiliated with the University of Bern, Switzerland, provided data from ChiP experiments. Samples and stainings of prenatal epidermis were provided by Christopher Schuster and Adelheid Elbe-Bürger from the Division of Immunology, Allergy and Infectious Diseases, Department of Dermatology at the Medical University of Vienna. Bioinformatic analysis and heatmap generation using Spotfire was performed by Diana Mechtcheriakova and Anastasia Meshcheryakova from the Departments of Pathophysiology and Allergy Research at Medical University of Vienna.

ACKNOWLEDGEMENTS

After intense months, the day of writing this note of gratitude as finishing touch of my PhD thesis has come. I would like to thank the people who supported and helped me so much during this period of intense learning on both, scientific and personal levels.

Firstly, I would like to express my sincere gratitude to my advisor Prof. Herbert Strobl for the continuous support of my PhD study and related research, for his motivation and the inspiring discussions.

Besides my advisor, I would like to thank the rest of my thesis committee Prof. Gregor Gorkiewicz and Prof. Johannes Stöckl for their encouragement and their time. My sincere thanks also goes to Prof. Adelheid Elbe-Bürger, René Köffel and Jennifer Jurkin for working with me on the publication.
I gratefully acknowledge the funding I received from the doctoral programme "Molecular Fundamentals of Inflammation" (DK-MOLIN) and the FWF- Austrian Science Fund.

I would like to thank my fellow doctoral students for their feedback, cooperation and of course for all the fun we had in the last four years. Special thanks to Christina, Carmen, Elke, Martina and Nicole for not only being great colleagues, but friends. In addition, I would like to express my gratitude to all members of the Institute of Pathophysiology and Immunology at the Medical University of Graz for help whenever needed.

I would like to wholeheartedly thank my family for always believing in me and encouraging me. They helped me in whatever way they could during this sometimes very challenging period of time. With all my heart I am grateful for being able to make my grandparents proud, wherever they now watch from.
I am indebted to all my friends for their constant support, their encouragement and distraction during hard times and their motivation during the final phase of writing this thesis.

TABLE OF CONTENT

ABBREVIATIONS

APC	antigen presenting cell
aN1	active intracellular NOTCH1
BATF3	basic leucine zipper ATF-like transcription factor 3
BMP7	bone morphogenetic protein 7
BLIMP-1	B lymphocyte-induced maturation protein-1
cDC	classical/ myeloid dendritic cell
CDP	common dendritic cell progenitor
cMoP	common monocytic progenitor cell
DC	dendritic cell
FITC	fluorescein isothiocyanate
FLT3L	fms-like tyrosine kinase 3 ligand
GFP	green fluorescent protein
GM-CSF	granulocyte/macrophage-colony stimulating factor
G-CSF	granulocyte-colony stimulating factor
HPC	hematopoietic progenitor cell
ID2	inhibitor of DNA binding 2
IDEC	inflammatory dendritic epidermal cell
IRES	internal ribosomal entry site
KLF4	Kruppel-like factor 4
LC	Langerhans Cell
M-CSF	macrophage-colony stimulating factor
Mac	macrophage
MafB	V-maf musculoaponeurotic fibrosarcoma oncogene homolog B
MDP	monocyte/dendritic cell progenitor
moDC	monocyte-derived dendritic cell
moLC	monocyte-derived Langerhans Cell
MoP	monocyte progenitor
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
pDC	plasmacytoid dendritic cell
p-LC	$CD34^+$ progenitor-derived Langerhans Cell
p-moDC	$CD34^+$ progenitor-derived monocyte-derived dendritic cell

PAMP	pathogen-associated molecular pattern
PPAR	peroxisome proliferator-activated receptor
PRR	pattern recognition receptor
RUNX3	Runt-related transcription factor 3
SCF	stem cell factor
TGF-β1	transforming growth factor beta 1
TLR	Toll-like receptor
TNFα	tumor necrosis factor alpha
VDR	vitamin D receptor
Zbtb46	zinc finger and BTB domain containing 46

KURZFASSUNG

Langerhans Zellen (LZ) stellen einen speziellen Typ von dendritischen Zellen (DZ) dar. Sie bilden Netzwerke in stratifiziertem epithelialem Gewebe und spielen daher eine bedeutende Rolle in der Regulation von Immuntoleranz bzw. –abwehr an Körperoberflächen. LZ entwickeln sich bereits vor der Geburt aus hematopoietischen Stammzellen des Knochenmarks, unterliegen während des Erwachsenenlebens unter Einfluss des Zytokins TGF-β1 jedoch einer Selbstregulation. *In vitro* haben nicht nur Stammzellen die Fähigkeit LZ zu bilden, sondern auch $CD14^+$ Monozyten oder $CD1c^+$ klassische dendritische Zellen zeigen die Fähigkeit zu funktionellen LZ zu differenzieren. Die Differenzierung von Monozyten zu LZ konnte im Maussystem bereits *in vivo* gezeigt werden. Die beteiligten Mechanismen auf Transkriptionsebene, die zur Transdifferenzierung von Monozyten zu LZ führen sind jedoch noch nicht völlig untersucht.

Mithilfe eines Microarrays konnten wir verschiedene Transkriptionsfaktoren identifizieren, die unter Einfluss von TGF-β1 während der Entwicklung von LZ bzw. Monozyten aus $CD34^+$ Stammzellen differenziell reguliert werden. RUNX3 (Runt-related transcription factor 3), der bereits als LZ-instruktiver Faktor beschrieben wurde, zeigte sich als durch TGF-β1 induziert. KLF4 (Kruppel-like factor 4), bestimmend für die Identität von Monozyten, wurde während der Differenzierung von LZ reprimiert. Wir konnten beobachten, dass KLF4 auch während der Differenzierung von Monozyten zu LZ negativ reguliert wird. Interessanterweise bleibt die Expression während der Entwicklung von anderen Subtypen von DZ, wie dermalen DZ, erhalten. Wir konnten weiters demonstrieren, dass der Notch Signalweg für die Inhibition der KLF4-Expression in den Monozyten, die zu LZ differenzieren, verantwortlich ist. Die Notch-abhängige Repression von KLF4 in Monozyten hebt die Inhibition von RUNX3 durch KLF4 auf und erlaubt somit die durch TGF-β1 induzierte Differenzierung zu LZ. Interessanterweise sind hematopoietische Stammzellen und $CD1c^+KLF4^-$ Blut-DZ, anders als Monozyten, in ihrer Differenzierung zu LZ unabhängig von einer exogenen Aktivierung des Notch-Signalwegs. Wir konnten außerdem beobachten dass der Notch-Signalweg, d.h. die Expression von Notch-Rezeptor und -Ligand, in $CD34^+$ hematopoietischen Vorläuferzellen durch TGF-β1 angeregt wird. Mittels Immunfluoreszenz konnten wir eine starke Aktivierung dieses Signalwegs in LZ zeigen, die *in vitro* generiert worden waren. Auch LZ in der gesunden Epidermis zeigten eine Aktivierung des Notch-Signalwegs *in situ*, während KLF4 nicht detektiert werden

konnte. Ebenso beobachteten wir fehlende Expression von KLF4 in HLA-DR$^+$ LZ-Vorläufern in der pränatalen Epidermis, wobei dermale DZ hohe Levels an KLF4 aufwiesen.

Eine Überexpression von KLF4 mittels retroviralen Vektoren in CD34$^+$ Vorläuferzellen führte zu einer Inhibition deren Differenzierung zu LZ, bei gleichzeitig verstärkter Entwicklung von Monozyten; eine Überexpression von RUNX3 hingegen hatte den gegenläufigen Effekt.

Im Blut zirkulierende CD1c$^+$ klassische DZ exprimieren kein KLF4 und differenzieren daher unabhängig einer Aktivierung des Notch Signalwegs zu LZ. Im Vergleich zu Monozten und CD34$^+$ Vorläufern zeigen CD1c$^+$ DZ sogar ein höheres Potential zu LZ zu differenzieren. Dies lässt auf eine Prädisposition von CD1c$^+$ DZ zu LZ zu differenzieren schließen. Andererseits zeigten CD1c$^+$ DZ auch die Fähigkeit, abhängig von verschiedenden Zytokin-Stimuli, sich zu KLF4$^+$ dermalen DZ oder Makrophagen zu entwickeln; diese Beobachtung ist vermutlich auf die bewiesene Heterogenität dieser Zellpopulation zurückzuführen. Dies weist darauf hin, dass CD1c$^+$ Blut-DZ als Vorläufer von LZ als auch von monozytären DZ fungieren können.

ABSTRACT

Langerhans cells (LC) are a special type of dendritic cells (DCs) forming networks in stratified epithelia and play a key role in immunity and tolerance at body surfaces. LCs are established prenatally from hematopoietic progenitor cells (HPCs) and self-maintain in the adult epidermis dependent on TGF-β1. *In vitro*, not only HPCs, but also blood $CD14^+$ monocytes or $CD1c^+$ classical dendritic cells (cDCs) can differentiate into functional LCs. Murine monocytes give rise to LCs under conditions of inflammation *in vivo*. However, the transcriptional mechanisms underlying the transdifferentiation of monocytes into LCs still remained to be elucidated.

We performed microarray studies identifying several transcription factors that are differentially regulated during monocyte vs. TGF-β1-mediated LC differentiation from $CD34^+$ progenitors. Runt related transcription factor 3 (RUNX3), already described as key LC-instructive factor, was induced via TGF-β1. The monocyte identity transcription factor Kruppel-like factor 4 (KLF4) appeared to be oppositely regulated, i.e. being repressed during LC, but induced during monocyte differentiation. We identified KLF4 to be inhibited during LC development from human blood monocytes. Conversely, KLF4 is maintained or induced during dermal DC and monocyte-derived DC (moDC) differentiation. We identified epithelial Notch signaling to repress KLF4 in monocytes undergoing LC commitment. Loss of KLF4 in monocytes transcriptionally de-represses RUNX3 in response to TGF-β1, thereby allowing LC differentiation. Interestingly, unlike monocytes, HPCs and $CD1c^+KLF4^-$ cDCs differentiate into LCs independently of exogenous Notch activation. We could show a TGF-β1-dependent induction of the Notch signaling pathway (e.g. Notch receptor Notch-1, Notch ligand Jagged-2) in $CD34^+$ human hematopoietic progenitors undergoing LC differentiation. Immunofluorescence staining revealed a strong activation of this pathway in $CD34^+$-derived LCs *in vitro* as well as in epidermal LCs *in situ* in healthy adult skin; these cells appeared to be $KLF4^-$. Also $HLA\text{-}DR^+$ LC precursors in the prenatal epidermis lacked KLF4 expression, whereas dermal DCs strongly expressed KLF4. Retroviral gene transduction showed an inhibitory effect of KLF4 on *in vitro* LC differentiation in favor of monocyte development; however ectopic expression of LC-instructive RUNX3 reversed this effect.

$CD1c^+$ blood circulating cDCs do not require exogenous activation of the Notch signaling pathway due to the lack of KLF4 expression. Side-by side comparison revealed a higher

potential of $CD1c^+$ cDCs to differentiate to LCs than monocytes or $CD34^+$ progenitors indicating a pre-commitment towards the LC lineage. However, $CD1c^+$ cDCs showed potential to also give rise to $KLF4^+$ moDCs and macrophages probably due to the described heterogeneity of this cell population. This indicates that $CD1c^+$ cDCs might represent precursors of the LC as well as the monocytic DC lineage.

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

1.1. INNATE VS. ADAPTIVE IMMUNITY

The innate (non-specific) immune system represents the first defense line against invading pathogens and infections (2) thereby triggering a pro-inflammatory response (3). On the other hand, the adaptive (acquired) immune system is responsible for the elimination of pathogens and the development of immunological memory (4). The primary cells involved in innate immune responses are antigen-presenting cells (APCs)/ mononuclear phagocytes, such as macrophages and DCs, which are primarily located at body surfaces, i.e. the epidermis, the ciliated respiratory epithelium or mucosal surfaces. They build the interface between innate and adaptive immunity via antigen uptake and subsequent migration to lymph nodes for antigen presentation to T cells and induction of an antigen-specific T cell responses (5–7). Macrophages can also function as effector cells as they eliminate pathogens via direct uptake in tissues. Apart from instructing T cells, DCs can directly induce an immunological response by the secretion of pro-inflammatory cytokines or interferons as a result of viral infection or shock.

1.2. DENDRITIC CELLS

Dendritic cells (DCs) comprise a heterogeneous family of APCs that are pivotal in the orchestration of adaptive immune responses (8), i.e. they critically determine whether tolerance or specific immune reactions towards certain antigens are initiated (9). DCs mediate the polarization of different T helper cell subsets via different cytokines: IL-12 plays an essential role in the induction of Th1 responses, mostly via IFN-γ. IL-23 and IL-1 in humans and TGF-β and IL-6 in mice contribute to Th-17 responses marked by secretion of mainly IL-17, IL-21 or IL-22 (10–12). Th2-responses consisting of IL-15, IL-4 and IL-13 are strongly dependent on IL-4 (13) (
Figure 1, right panel). Maintenance of tolerance (
Figure 1, left panel) (9) is achieved by negative selection of autoreactive thymocytes (14) and the induction of regulatory T cells (Treg).

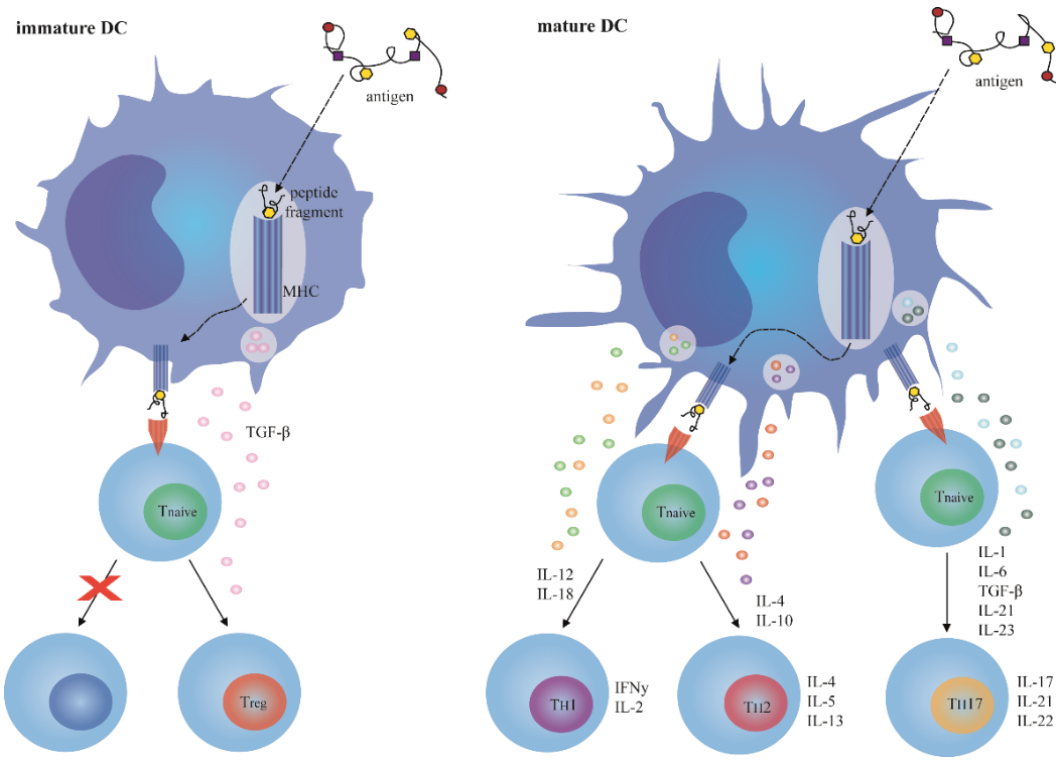

Figure 1: Mature DCs induce different types of T cell response.
DCs present processed antigen to naive T cells in the thymus. In the absence of infection, DCs prime a tolerogenic T cell phenotype (Treg). Upon infection/ inflammatory stimuli, DCs undergo maturation marked by the upregulation of co-stimulatory molecules, enhanced antigen processing/ presentation and the production of pro-inflammatory cytokines. Mature DCs induce different types of $CD4^+$ T cells, such as Th1, Th2 or Th17 depending of the type of the secreted cytokine.

DC subsets in mouse and human have been characterized by their differential location (circulating blood DCs, lymph-node resident DCs, epidermal and dermal DCs), their origin (myeloid or plasmacytoid), by the physiological state they appear in (steady-state or inflammation) or by the expression of surface markers (15).

1.1.1. HUMAN DENDRITIC CELL SUBSETS

Circulating DC subsets

Most of what is known about subtypes of dendritic cells is derived from the murine system, but recent research has also brought detailed insights into the human system. Human blood contains several phenotypically and functionally subsets of plasmacytoid DCs, conventional/myeloid DCs and tissue-resident DCs.

Table 1: Phenotype of human blood circulating DC subsets

	origin		
	myeloid (CD1c$^+$ cDCs)	myeloid (CD141$^+$ cDCs)	lymphoid/ plasmacytoid (pDCs)
phenotype	CD1c$^+$	CD1c$^-$	CD1c$^-$
	CD4$^{+/-}$	CD4$^{+/-}$	CD4$^+$
	CD11c$^+$	CD11c$^{+/-}$	CD11c$^-$
	CD33$^+$	CD33$^{+/-}$	CD33$^-$
	CD45RA$^-$	CD45RA$^-$	CD45RA$^+$
	CD123$^-$	CD123$^-$	CD123$^+$
	BDCA2$^-$	BDCA2$^-$	BDCA2$^+$
	BDCA4$^-$	BDCA4$^-$	BDCA4$^+$
	BDCA3$^-$	BDCA3$^+$	BDCA3$^-$

(*Table adapted from (16)

Plasmacytoid DCs

Plasmacytoid DCs (pDCs) can be identified by the expression of CD123 (IL-3R), CD303 (CLEC4A, BDCA-2) and CD304 (neutropilin, BDCA-4) and the lack of myeloid markers such as CD11b, CD11c, CD13 and CD33 (17) as well as CD45RA (B220). They are not related to plasma cells, though possessing some lymphoid characteristics (18,19). Though pDCs are not present in high numbers in tissues, they comprise about 20 % of MHC-II$^+$ cells in the lymph nodes (45-50 % of blood DCs) and are rapidly recruited to sites of inflammation (20). pDCs were shown to express high levels of TLR7 and TLR9 (21,22) and induce a rapid type I interferon response to viral infections (23–25). INF-α secreted by pDCs is suggested to play an important role in the establishment of CD8$^+$ T cell memory responses. When freshly isolated, pDCs do not induce efficient T cell responses and upon activation they seem to be less „mature“ compared to myeloid DCs (23,24,26).

Conventional/ classical myeloid DCs

Conventional or classical dendritic cells (cDCs) exhibit a unique phenotype, such as expression of high levels of MHC-II and long dendrite extensions. Human cDCs exist in both lymphoid and peripheral tissues. Two subsets have been characterized, both being Lin^- ($CD3^-CD14^-CD19^-CD20^-CD56^-$) and either $CD1c^+$ ($BDCA\text{-}1^+$) or $CD141^+$ ($BDCA\text{-}3/Thrombomodulin^+$). $CD1c^+$ cDCs are the main cDC subset found in blood (45-50 % of blood DCs); $CD141^+$ cDCs are rather rare (5-10 % of blood DCs). $CD1c^+$ cDCs are thought to be equivalents of mouse lymphoid tissue-resident $CD11b^+$ cDCs due to similar gene expression profiles and their preferential priming of $CD4^+$ T cells, whereas $CD141^+$ cDCs act as equivalents of mouse $CD8\alpha^+$ cDCs as they also express XCR1 and CLEC9A and preferably cross-present antigen to $CD8^+$ T cells and promote Th1 differentiation and activate cytotoxic T cells.

$CD1c^+$/$BDCA\text{-}1^+$ cDCs

$CD1c^+$ DCs are the major population of DCs in human blood, tissues and lymphoid organs. Originally this subset was recognized in the blood as $HLA\text{-}DR^+Lin^-$ cells expressing the myeloid markers CD11b, CD11c, CD13, CD33, CD172 (Sirpα) and CD45RO (27). $CD1c^+$ cDCs in tissue seem to have a higher activation status (higher expression of CD80, CD83, CD86 and CD40) than blood $CD1c^+$ cDCs and express higher levels of the homing receptor CCR7 (28,29) They are also contained in spleen and lymph node (30,31), likely originating directly from the blood (32,33), residing interdigitating in T cell areas. $CD1c^+$ cDCs express a wide range of TLRs and other PRRs for antigen uptake, are potent inducers of naive T cell proliferation and possess the ability to cross-present antigens to $CD8^+$ T cells (31–35). They were shown to secrete pro-inflammatory cytokines such as TNFα, IL-8 and IL-10 as well as IL-12 after TLR7/8 stimulation (30).

$CD141^+$/ $BDCA\text{-}3^+$ cDCs

$CD141^+$ cDCs have been detected among resident cDCs in the lymph node, tonsil, spleen and bone marrow (30,31,33,36) as well as in non-lymphoid tissues such as skin, lung and liver (32). CD141 (thrombomodulin) is also found on migratory $CD14^+$ DCs and $CD1c^+$ DCs upon stimulation with vitamin D (37). Compared to $CD1c^+$ cDCs, $CD141^+$ cDCs express lower levels of CD11b and CD11c (38), but high levels of CLEC9A, which mediates the uptake of apoptotic/ necrotic cells (34,39) and TLR3/8 for sensing viral nucleic acids

and cross-presentation to $CD8^+$ T cells (31–34). $CD141^+$ cDCs produce high levels of TNFα and IFN-γ but low levels of IL-12p70 (30,32,40,41).

At steady state, DC-committed precursor cells migrate from the bone marrow via the blood to peripheral and lymphoid tissues, where they undergo final differentiation to different DC subsets. An exception are Langerhans cells (LCs), which self-maintain in the epidermis originating from a local precursor rather than circulating progenitors (18).

Tissue-resident DC subsets

DC subsets in the skin

In the steady-state skin three different DC subpopulations have been identified (epidermal LCs, resident dermal myeloid DCs and plasmacytoid DCs), whereas under inflammatory conditions a fourth population has been identified (myeloid dermal „inflammatory" DCs).

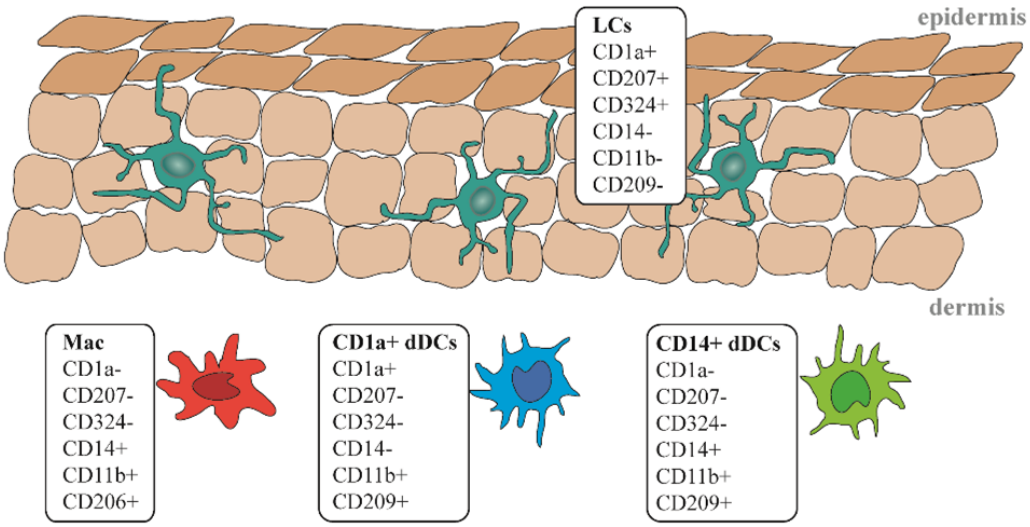

Figure 2: Dendritic cell subsetss residing in human skin.
Langerhans cells form a dense network within the epidermis and uniquely express CD207 (Langerin). In the dermis, DC subsets are identified by the differential expression of CD1a or CD14. Macrophages function in clearance of apoptotic cells and can be distinguished from DCs by surface marker expression.

Healthy human skin contains epidermal LCs and dermal $CD1a^+$ or $CD14^+$ DCs as well as macrophages (Figure 2). Only under inflammatory conditions, an additional large population of myeloid „inflammatory DCs" ($CD1c^+CD11c^+$HLA-$DR^+TNF\alpha^+iNOS^+$) can be detected.

In mouse skin an additional $CD207^+$ DC population is observed in the dermis, whereas CD207 expression in the human is restricted to epidermal LCs (Table 2).

Table 2: Surface characteristics of human and murine skin DC subsets.

	localization	cell type	surface markers
human	epidermis	LC	CD45, MHC-II, CD1a, CD207 (Langerin), E-Cadherin, EpCAM
	dermis	$CD14^-$dDC	CD1c, $CD1a^{+/-}$, CD34, CD11b, CD11c, MHC-II
		$CD14^+$dDC	CD1c, CD45, CD11b, CD11c, CD14, MHC-II, CD209 (DC-SIGN)
mouse	epidermis	LC	CD45, CD11b, CD11c, MHC-II, CD205, CD207, E-Cadherin, EpCAM
	dermis	$CD11b^+$ dDC	CD45, $CD11b^{hi}$, CD11c, MHC-II, CD205
		$CD207^+$ dDC	CD45, $CD11b^{dim}$, CD11c, CD103, MHC-II, CD207

Langerhans cells

Langerhans cells (LCs) have first been described in 1868 by Paul Langerhans as „nerve cells of the skin“. Only more than 100 years later it was recognized that LCs indeed belong to the family of dendritic cells. LCs form dense networks in stratified epithelia such as epidermis and oral and genital mucosae (42). In the epidermis they reside in the supra-basal layers of keratinocytes. LCs are characterized by the expression of high levels of Langerin (CD207) and CD1a. Langerin is an endocytic C-type lectin receptor, which is associated with the unique, characteristic, rocket-shaped Birbeck granule (BG) (43), an endosomal compartment that has specifically been described only in this cell type. In addition to the induction of BG formation, langerin functions as an endocytic receptor involved in the transport of exogeneous mannosylated ligands from the surface of the cell to intracellular BG compartments (44). Langerin-deficient mice were demonstrated to develop normal numbers of $MHC\text{-}II^+$ epidermal LCs; however, these LCs lacked BG (45). LCs are anchored in the epidermis to keratinocytes via E-Cadherin (46), maintaining them in an inactivated state. The Mellman lab (47) discovered that *in vitro* the maturation of LCs can be induced by disrupting E-cadherin bindings. E-Cadherin is induced during TGF-β1-dependent LC differentiation from $CD34^+$ HPCs (48,49) and monocytes (50). Consistently, *in vitro* generated LCs undergo homophilic adhesion and form large E-Cadherin-mediated cell

clusters, which represents a morphologic hallmark of human *in vitro* LC generation cultures (51,52).

Three key functions have been assigned to LCs: (i) Immature LCs in the epidermis serve as sentinels being highly specialized in taking up and processing antigens. They constantly probe the environment for the presence of pathogens by extension and retraction of their dendrites (53). (ii) Upon encountering pathogens and inflammatory cytokine stimulation, LCs undergo phenotypic changes, such as upregulation of surface MHC-II and co-stimulatory molecules (54) as well as downregulation of E-Cadherin (46). E-Cadherin downregulation induces detachment from surrounding keratinocytes and along with upregulation of CCR7 (55), allows migration of LCs to T-cell areas of skin-draining lymph nodes. Upon stimulation of maturation, LCs downregulate their ability to phagocytose and process antigens (54,56,57). In the lymph nodes, LCs appear as fully functional APCs, stimulating T cell proliferation and T cell polarization via the secretion of cytokines. In the steady-state, LCs also undergo the same maturation process, but in the lymph node produce IL-10 to induce regulatory T cells, thereby ensuring tolerance (58).

Resident dermal DCs

Dermal DCs (dDCs) are suggested to be analogous to „interstitial-type“ DCs found in the connective tissue and stroma of other organs (59–61). These cells also possess the ability to take up/ process antigens, migrate to lymph nodes and present processed antigens to T cells and B cells (62,63). The main population of resident DCs in human dermis can be identified by their expression of CD1c. CD1a is expressed on immunostimulatory dermal DCs in healthy (59,64) and psoriatic skin (60), though to a lower extent than on epidermal LCs. Zaba et al proposed that $CD1a^+$ cells might represent a subset of skin resident myeloid DCs, whereas CD1c might be a more useful marker for their identification due to co-localization of this marker with nearly all $CD11c^+$ cells (15). An additional population of dermal APCs/ DCs is characterized by the expression of CD14 (64,65). Interestingly, these cells lack typical markers of myeloid DCs such as CD1c or CD141, co-stimulatory molecules or CCR7 (66–68). These cells have been identified as migratory skin population of $CD14^+$ cells (59); they were previously referred to as „interstitial-type“ or „dermal-type“ DCs. $CD14^+$ DCs express markers found on monocyte-derived DCs (moDCs) or macrophages, such as CD209 (DC-SIGN), FXIIIA or CD163 (66).

$CD14^+$ DCs isolated from explant skin cultures appeared more macrophage-like (28),

though having the ability to differentiate into LC-like cells or more mature DCs upon cytokine stimulation (65,69). Due to low expression levels of CCR7, it is questionable if $CD14^+$ DC do migrate to lymph nodes (28,66), though a certain functional role regarding follicular helper T cell formation or B-cell help has been assigned to $CD14^+$ DCs (68,70). Recently, it was demonstrated that skin $CD14^+$ dermal cells are transcriptionally similar to tissue-resident macrophages and derived from $CD14^+$ blood monocytes (71). Albeit $CD14^+$ DCs share their gene-expression pattern with monocytes, they display phenotypic and functional differences. However, it was suggested, that $CD14^+$ DCs in the dermis represent monocyte-derived macrophages rather than being related to the DC lineage (71)

Inflammatory dendritic cells

Inflammatory DCs are comprised by DC subsets that develop under conditions of inflammation, but are not detectable during steady state (72). Psoriatic inflammation is characterized by a marked increase (up to 30-fold) of $CD11c^+$ dermal DCs (73). Preliminary studies suggest that these „inflammatory" myeloid DCs lack expression of CD1c but can be identified by their uniform expression of CD11c. This indicates that they potentially originate from monocytes (74), circulating „pre-DCs" (75–77) or other steady-state DCs recruited to sites of inflammation via stimulation with pro-inflammatory cytokines. As dermal DCs in psoriasis were shown to produce TNFα and iNOS, they have been termed „Tip-DCs" (78); it is likely that Tip-DCs are contained within the population of „inflammatory" dermal DCs emerging during psoriatic conditions (15).

6-sulfo LacNAc DCs (SlanDCs) have been identified as inflammatory DCs in active lesions of psoriasis (79,80). These cells share expression of CD16 with non-classical monocytes, though possess significant functional and transcriptional differences (81). Phenotypically, slanDCs ($CD16^+CD14^-CD1c^-CD11c^+$) are distinct from $CD1c^+$ cDCs, $CD141^+$ cDCs or pDCs and have been shown to produce high levels of TNFα, IL-12 and IL-23 (82). They function as inflammatory dermal DCs in psoriasis via induction of Th1 and Th17 T cells (79,80).

Also in skin lesions of atopic dermatitis patients, a distinct population of inflammatory DCs was described. These inflammatory dendritic epidermal cells (IDECs) (83) are defined as $HLA\text{-}DR^+Lin^-CD11c^+CD1a^+$ cells co-expressing CD206 (macrophage mannose receptor), CD36, FcεRI, immunoglobulin E, CD1d/c, CD11b and CD209 (DC-SIGN) (84)

In vitro, highly functional „inflammatory-type“ DCs can be generated from classical $CD14^+$ blood circulating monocytes via stimulation with GM-CSF and IL-4 (85), which have been shown to serve as potent stimulators of $CD4^+$ naive T cells and to produce pro-inflammatory cytokines such as IL-1, IL-6, TNFα, IL-12 and IL-23 (40).

1.1.2. MOUSE DC SUBSETS

Plasmacytoid DCs

In the mouse, $CD11c^+B220^+Gr1^+$ DCs present in the thymus represent the counterpart to IFNα-producing human pDCs (86). Similar to the human system, murine pDCs are dependent on stimulation with FLT3L *in vitro* (87,88) and *in vivo* (89). Interestingly, there exists a mouse-strain specific difference in frequency of pDCs and IFNα response *in vitro* and *in vivo* (90).

Non-lymphoid tissue cDCs

In non-lymphoid tissue, mouse cDCs are represented by two major subsets: $CD103^+CD11b^-$ cDCs and $CD11b^+$ cDCs.

The $CD103^+CD11b^-$ cDC subset has a common origin with $CD8^+$ cDCs residing in lymphoid tissues (91,92) and are found in most connective tissues. $CD103^+$ cDCs do not express macrophage-associated markers such as CD11b, CD115, CD172a, F4/80 or CX_3CR1; however, $CD103^+$ cDCs in the intestinal *lamina propria* have been found to express CD11b (93,94). Compared to $CD11b^+$ cDCs, $CD103^+$ cell express higher levels of FLT3L; mice devoid of FLT3L show a marked reduction of $CD103^+$ cDCs (93).

The $CD11b^+CD103^-$ cDC subset is suggested to be comprised by mixed populations of tissue cDCs and macrophages; in the *lamina propria* they differentiate from cDC-restricted precursor cells or monocytes (95).

LCs

In mouse, LCs comprise 3-5 % of all cells in the epidermis. They are characterized by the expression of CD207 (Langerin), CD11c and MHC-II and the presence of cytoplasmic Birbeck granules. In contrast to human LCs, murine LCs also express monocyte/ macrophage markers such as CD11b or F4/80 (96) Unlike other DC subsets, LC development is not dependent on FLT3 or FLT3L *in vivo* (97), though they share dependency for M-CSF-R with macrophages.

Migratory DCs

Peripheral lymph nodes contain an additional DC population termed „tissue-migratory DCs“, which relate to non-lymphoid tissue-resident cDCs as they migrate through the

lymphatics to the respective draining lymph nodes (76) in a CCR7-dependent manner (55,98). Induction of DC migration is initiated by cell maturation accompanied by upregulation of MHC-II and other co-stimulatory molecules in response to certain cytokine stimuli at steady-state and during inflammation (99).

Lymphoid-resident cDCs

Lymphoid-organ resident cDCs are comprised by $CD8\alpha^+$ and $CD11b^+$ cDCs (100). The $CD8\alpha^+$ cDC subset makes up 20-40 % of spleen and lymph node cDCs and specifically lack expression of CD11b or other macrophage-associated markers. The development of $CD8\alpha^+$ cDCs (and their non-lymphoid tissue equivalent $CD103^+CD11b^-$ cDCs) is mediated by the transcription factors ID2, IRF8 and BATF3. The deletion of these factors causes severe reductions in both cell types (97,101–103), while $CD11b^+$ cDCs are not affected. $CD8\alpha^+$ cDCs are marked by high levels of CD205, CLEC9A as well as FLT3; FLT3L stimulation induces their proliferation. Mice lacking FLT3L show a significant reduction in $CD8\alpha^+$ cDCs numbers (104,105). Also $CD11b^+$ lymphoid-resident cDCs proliferate upon FLT3L-stimulation and their development is amongst others regulated by the transcription factors RelB (106), NOTCH2 (107), IRF2 (108) and IRF4 (109). IRF4 in particular also mediates functional features of $CD11b^+$ cDCs (110). $CD11b^+$ cells can be further segregated into subsets based on the differential expression of endothelial cell-specific adhesion molecule (ESAM). $ESAM^{hi}$ cells express higher levels of CD11c and FLT3 but lower levels of M-CSF-R and CCR2 compared to $ESAM^{lo}$ cells. $ESAM^{hi}CD11b^+$ cells are suggested to originate from DC-committed precursors, whereas $ESAM^{lo}CD11b^+$ cDCs seem to develop from circulating monocytes (107).

1.3. TRANSCRIPTIONAL CONTROL OF DC DEVELOPMENT

Hematopoietic stem cells (HSCs) in the bone marrow pass through several commitment steps thereby successively acquiring more restriction towards a specific mature blood cell type. Stem cells/multipotent progenitor cells show a promiscuous expression pattern of genes of different hematopoietic lineages though at low levels (111). As the various hematopoietic lineages develop, the gene expression spectrum gets narrowed and precursor cells turn more restricted. These cell fate decisions are influenced by various signaling pathways and cytokines that modulate the expression of lineage-determining transcription factors (111,112). Up to now several transcription factors have been assigned to DC development and DC subset specification (113).

1.1.3. PU.1

The master transcription factor PU.1 (encoded by the *Sfpi1* gene) is a member of the Ets-family of DNA-binding proteins (114). It is highly expressed in early hematopoietic progenitors, including the circulating monocytic precursors of myeloid DCs (115) and precursors of both thymic T cells and lymphoid DCs (116), but repressed in megakaryocyte-erythrocyte precursor cells (117,118). PU.1 deficiency was shown to result in perturbed hematopoiesis but enhanced granulopoiesis (119–121). PU.1 functions as a critical regulator of DC lineage development; it is expressed in all cDCs and pDCs (117,118). Mice reconstituted with PU.1$^{-/-}$ hematopoietic progenitors show a marked reduction in CD8α^{+} and CD8α^{-} lymphoid DCs (122). *In vitro,* PU.1-deficient murine precursors fail to differentiate into DCs upon stimulation with GM-CSF (114). Overexpression of PU.1 in human multipotent myeloid cells was shown to be instructive for DC development, i.e. ectopic PU.1 in HL60 monocytes induced the expression of the DC marker CD1a whereas CD11b, also present on monocytes, was not altered (123).

LCs strongly express PU.1; ectopic overexpression of PU.1 in human hematopoietic precursors promotes LC development in coordance with TGF-β1 *in vitro* (124). However, in absence of TGF-β1, overexpression of PU.1 in human progenitors was not sufficient to induce LC development (124).

Gene knock-down and gain-of-function studies have shown that PU.1 also participates in cell fate decisions at later stages during myeloid differentiation (122,125–128). This is

mediated by cooperative or competitive actions of other transcription factors with PU.1 in a cell-type specific fashion, e.g. with C/EBPα. The granulocyte-specific transcription factor C/EBPα counteracts PU.1 during LC differentiation (128), whereas on the re-direction of B cells towards the macrophage lineage requires activity of both transcription factors (129). As observed for other myeloid transcription factors, PU.1 seems to control cell fate decisions in a concentration-dependent manner, i.e. the relative levels of PU.1 within progenitor cells determine lineage decisions as determined for the development of macrophages vs. DCs (123).

1.1.4. ID2

Inhibitor of DNA binding 2 (ID2), a helix-loop-helix transcription factor, negatively regulates basic helix-loop-helix (bHLH) transcription factors via formation of heterodimers, thereby inhibiting their transcriptional activity (130). Knockout of ID2 in the murine system significantly influences development of DC subsets, i.e. mice deficient for ID2 have been shown to have a marked increase of pDCs, whereas numbers of $CD8\alpha^+$ and $CD11b^+CD11c^+$ cDCs were significantly reduced and epidermal LCs were completely absent (130,131).
$ID2^{-/-}$ mice showed a phenotype comparable to TGF-β1-deficient animals, which are also characterized by a complete lack of LCs (132).
Hacker et al identified ID2 as a TGF-β1 downstream target, therefore a deletion of ID2 might render LC progenitors insensitive to TGF-β1 signaling causing inhibition of efficient LC differentiation at steady-state (130). Interestingly, under inflammatory conditions, $Gr\text{-}1^+$ monocytes were demonstrated to be recruited to the epidermis and to be able to differentiate into LCs in absence of ID2 (133,134). In fact, it was suggested that LC development during steady-state and inflammatory conditions takes place in an ID2-dependent and ID2-independent pathway (133,134), respectively. Furthermore, LC development from human precursors was demonstrated to be induced by ID2 via PU.1 (124).

1.1.5. RUNX3

Runt-related transcription factor 3 (RUNX3) belongs to the Runt domain family of transcription factors, which are implicated as key regulators of lineage-specific gene expression in various differentiation pathways. It has been shown to be highly expressed during development of myeloid DCs as part of the TGF-β1 signaling cascade (135). Loss of

RUNX3 results in loss of Langerhans cells (135); deletion of RUNX3 in leukocytes was associated with the spontaneous development of inflammatory bowel disease. The deficiency of RUNX3 has led to an increase in DC maturation contributing to enhanced inflammation (135). Fainaru et al determined the complete absence of Langerhans cells in the epidermis of Runx3$^{-/-}$ mice, similar to the observations made for TGF-β1$^{-/-}$ mice (132). This indicates that, upon TGF-β1 stimulation, RUNX3 mediates downstream responses that are essential for LC differentiation (135).

1.1.6. IRFs

Originally, interferon-regulatory factor (IRF) proteins (IRF1-9) were identified as regulators of the type I interferon system. Amongst all members of the superfamily, expression of IRF4 and IRF8 is restricted to the hematopoietic lineage, whereas the other members are expressed ubiquitously. Within the single DC subsets, the expression of IRF8 and IRF4 is differentially regulated (109,136,137). In mouse, IRF8 is essential for the development of CD8α^{+} cDCs, while IRF4 mediates differentiation of CD4^{+} cDCs; both, though IRF4 to a lesser degree, contribute to pDC generation. It was described, that FLT3L-dependent DC development *in vitro* is mainly dependent on IRF8, whereas GM-CSF-induced DC differentiation is mainly mediated by IRF4 (109,136). Epidermal LCs and dermal DCs have been shown to depend on IRF8 for their differentiation and function (138). In particular, it was demonstrated that deletion of ICSBP (interferon consensus sequence-binding protein) in mouse led to a reduced frequency of LCs and a reduced migration of DCs from skin to the lymph node under steady-state conditions.

IRF4

IRF4 was shown to be a key regulator of lymphoid cell, myeloid cell and DC differentiation. The expression of IRF4 is restricted to immune cells (B cells, T cells, macrophages and DCs) and is suggested to selectively control MyD88-dependent gene regulation in a cell-type specific manner (139). Mice devoid of IRF4 display decreased numbers of CD8α^{-} cDCs and pDCs, whereas other DC subsets seemed to be not affected (109,136). IRF4 expression has also been found in macrophages (140,141).

IRF8

IRF8 plays a critical role in the regulation of lineage commitment in myeloid cell maturation (142). $IRF8^{-/-}$ mice show a reduction in frequencies of LCs, pDCs and resident $CD8\alpha^+$ cDCs, but increased $CD8\alpha^-$ cDCs (143). IRF8 interacts with PU.1, which is known as master regulatory factor of macrophage and B cell development. Interaction with chromatin PU.1 allows binding of IRF8 to DNA thereby regulating target gene expression (143). In DCs, IRF8 has been shown to contribute to the TLR9-MyD88-dependent signaling pathway. DCs devoid of IRF8 fail to produce pro-inflammatory cytokines such as TNFα or IL6 when stimulated (144).

1.1.7. C/EBPα

CCAAT/enhancer-binding proteins (C/EBP) account for a family of transcription factors that are characterized by a basic region-leucine zipper (bZIP), which allows homo- or hetero-dimerization with each other and binding to cognate consensus sequences (125). C/EBP proteins control the transcription of genes involved in development and function of cells of the hematopoietic system (125), especially of granulocytic cells. C/EBPα deficient mice show impaired differentiation and/or function of granulocytes leading to severe infections (145).

Expression of a dominant-negative form of C/EBPα in $CD34^+$ human hematopoietic precursors completely abolished granulocyte and monocyte/ macrophage differentiation but favored LC development (125), whereas overexpression of C/EBPα causes enhanced development of $CD15^+$ granulocytes. As development of DCs is dependent on PU.1 activity, C/EBPα might function to a certain degree as negative regulator of DCs.

1.1.8. BATF3

BATF3 (Basic Leucine Zipper ATF-like transcription factor 3/ p21SNFT) is specifically expressed in DCs and functions as transcriptional repressor upon hetero-dimerization with JUN. Deletion of BATF3 in mice results in ablation of $CD8\alpha^+$ cDCs, while not affecting other cell types of the hematopoietic system (103). Also numbers and function of other DC subsets such as $CD8\alpha^-$ cDCs were not influenced.

1.1.9. MafB

The bZip factor V-maf musculoaponeurotic fibrosarcoma oncogene homolog (MafB) was shown to be selectively expressed in monocytes and macrophages, but not in other cells of the myeloid compartment (146,147). MafB acts as transcriptional activator or repressor and plays a pivotal role in the regulation of lineage-specific hematopoiesis by repressing erythroid-specific genes though direct protein interactions with Ets-1 in the myeloid cell compartment (146). It has been shown to be required for differentiation/survival/maturation of monocytes, macrophages and osteoclasts. MafB-directed monocyte/macrophage differentiation or PU.1-mediated DC differentiation seem to be alternative but exclusive fate options (123). DC differentiation from human HL60 monocytes via overexpression of PU.1 was demonstrated to be accompanied by a repression of MafB mRNA levels. This is due to a direct inhibitory effect of PU.1 on Mafb for directing DC vs. monocyte/ macrophage cell fate (123).

1.1.10. Zbtb46

In mouse, Zbtb46 is induced at the pre-cDC stage and remains present on lymphoid $CD8\alpha^+$ and $CD11b^+$ cDCs and non-lymphoid $CD103^+$ cDCs but is not expressed on monocytes and macrophages (148,149), though moDCs acquire Zbtb46 upon their differentiation from monocytes (149).

1.1.11. NF-κB

Transcription factors of the NFκB/ Rel family (Rel-A, c-Rel, RelB, NF-kB1 (p105/50), NF-κB2 (p100/52)) play an important role in regulating inflammatory responses (150) via influencing the development and function of DCs. Upon activation of the NF-κB signaling pathway, IκB inhibitory proteins get phosphorylated and rapidly degraded through the ubiquitin-proteasome pathway thereby releasing NF-κB to enter the nucleus to regulate target gene expression (151,152). In mouse, lack of the non-classical NFκB-signaling pathway member RelB was shown to inhibit development of $CD8\alpha^-$ DCs, though numbers of $CD8\alpha^+$ cells and LCs remained unaltered (153).

RelB has been shown to positively regulate the generation of monocytic $CD14^+CD11b^+$ precursors of interstitial-type DCs from human hematopoietic precursors (154). It has been

associated with mature DCs (155–157) as it is upregulated and translocated to the nucleus upon stimulation of DC maturation (158,159).

1.1.12. KLF4

Kruppel-like factor 4 (KLF4, GKLF) is a member of the KLF4 family (KLF1- KLF17) of zinc finger transcription factors involved in the regulation of cell proliferation and differentiation (160). Initially, KLF4 was identified in the epithelial lining of the gut (161). KLF4 was demonstrated to be strongly expressed in the human monocyte/ macrophage lineage (162) and to be essential for the development of monocytes (163). $KLF4^{-/-}$ mice showed defects in CMP and HPCs downstream differentiation reflected by a decrease in monocytes but elevated levels of granulocytic cells (162). Downstream of PU.1, KLF4 functions as transcriptional regulator of the monocyte-specific marker CD14 by binding to its promoter (162). KLF4 is a downstream target of PU.1 as demonstrated by murine $PU.1^{-/-}$ fetal liver cells lacking KLF4, whereas ectopic overexpression of PU.1 causes an increase in KLF4 expression (162). Upon induction via PU.1, KLF4 serves as lineage determinant in guiding myeloid cell differentiation towards the monocyte cell fate. Expression of KLF4 in human macrophages functions as regulator of pro-inflammatory signaling (160) and is induced in response to inflammatory cytokines such as IFNγ or TNFα. Mouse and human embryonic stem (ES) cells show high levels of KLF4; recently expression of KLF4, together with three other transcription factors (Oct2/3, Sox2 and c-Myc) was found to allow efficient developmental reprogramming of murine fibroblasts to gain characteristics of pluripotent ES cells (164–166).

KLF4 was demonstrated to be a direct target of IRF8, and IRF8 is essential for the development for both monocyte subsets, especially for $Ly6C^{+}$ monocytes (167). The deficiencies of monocyte development observed in $IRF8^{-/-}$ mice were similar to those seen in $KLF4^{-/-}$ chimeric mice. KLF4 expression was shown to be lost in IRF8-deficient MDPs; KLF4 overexpression slightly restored monocyte development in $IRF8^{-/-}$ deficient mice (167).

1.1.13. NOTCH SIGNALING PATHWAY

Firstly identified in *Drosophila* and being highly conserved among most multicellular organisms, the Notch signaling pathway is involved in regulation of growth, differentiation

and maintenance of a wide range of organs (168). In the mammalian system, four different Notch receptors (Notch 1-4) and five ligands (Delta-like (1-4) or Jagged1/Jagged-2) have been identified (168,169). Notch signaling is activated by cell-cell contact, i.e. the ligand on one cell interacts with the respective receptor on the other cell. Upon ligand binding, the receptor is trimmed in consecutive proteolytic cleavage events resulting in the release of the Notch intracellular domain (NICD) and its translocation to the nucleus. The first cleaveage is mediated by metalloproteases of the ADAM family; the second cleavage step is mediated by complex of proteolytic enzymes (γ-secretase complex). The NICD mediates transcriptional repression through interaction with repressor CSL proteins converting them into transcriptional activators (170) and recruitment of co-activator proteins, such as mastermind proteins (MAML1-3). Major targets of CSL activaton are basic helix-loop-helix proteins encoded by the HES1 gene (see Figure 3). This pathway is involved at various stages of cell-fate decisions of many different lineages, the spectrum of possible Notch target genes being greatly enhanced by crosstalk with other signaling pathways (e.g. NF-κB, TGF-β1) (171,172). The Notch and TGF-β1 signaling pathways share similarities in their mode of action. In both pathways the signal is transduced by intracellular mediators translocating to the nucleus upon ligand binding to the respective receptor, i.e. the Notch intracellular domain (NICD) and TGF-β1- induced Smad proteins. Also the TGF-β1-dependent differentiation of endothelial progenitor cells into endothelial cells was shown to be partially mediated via the concomitant activation of the Notch signaling pathway (173). There is also evidence that the „strength“ of the exerted Notch signal contributes to the variety of possible effects, i.e. different cellular responses can be induced depending on the levels of Notch signaling (174).

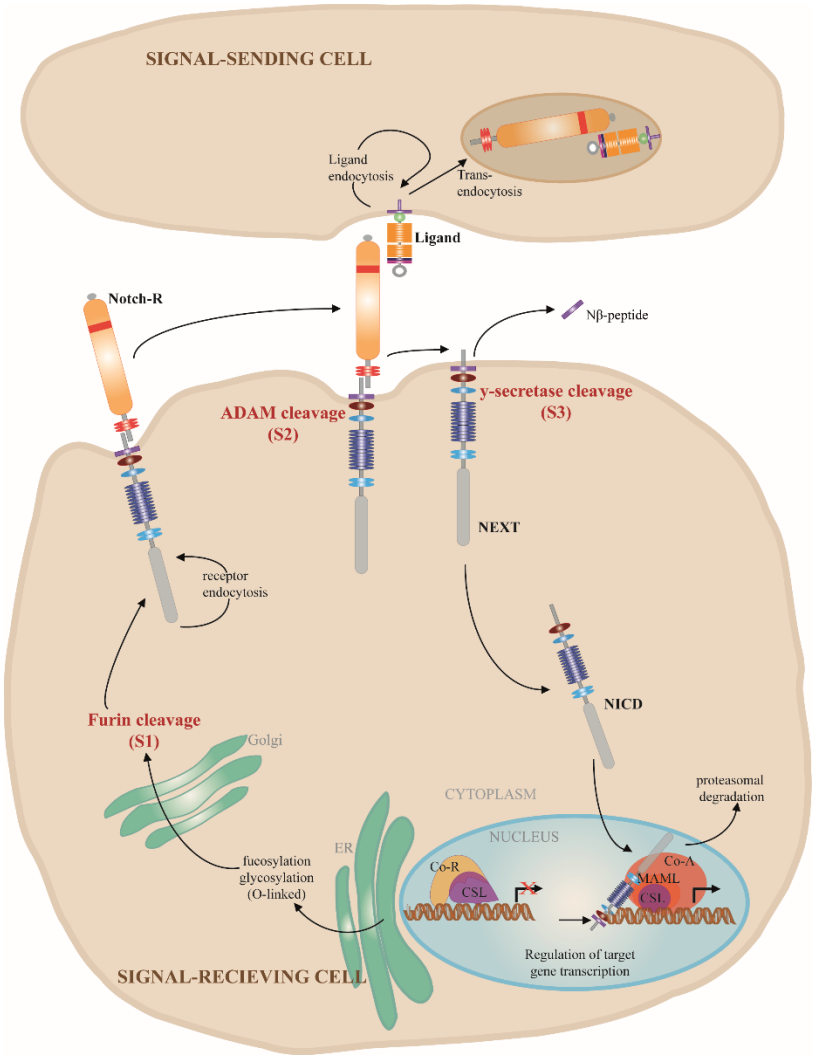

Figure 3: The core Notch signaling pathway is mediated by regulated proteolysis.
Upon translation, the Notch protein is glycosylated, which is essential for full function of the receptor. The fully mature receptor is established by proteolytic cleavage by Furin at site S1 followed by translocation to the cell surface in its heterodimeric form. Receptor activation is mediated by binding to its ligand presented on the surface of the neighboring cell leading to a conformational change of the receptor that allows cleavage at site S2 by ADAM metalloproteases. This cleavage step generates a membrane-anchored receptor fragment (NEXT, Notch extracellular truncation), which is further trimmed by the γ-secretase complex at site S3 generating the NICD (Notch intracellular domain); the NICD translocates to the nucleus and regulates Notch target gene expression. In the absence of the NICD, the DNA-binding protein CSL is associated with receptor proteins (Co-R) repressing the transcription of target genes. Binding of NICD to CSL replaces Co-R proteins with transcriptional co-activators (Co-A) such as MAML (Mastermind-like) proteins, thereby allowing transcription of target genes. (figure adapted from (175))

The absence or presence of Notch signaling plays a critical role in hematopoiesis in guiding progenitors into the correct lineage direction (176). It was suggested that Notch signaling is especially important for definite, but dispensable for primitive hematopoiesis as germline mutant embryos deficient for Notch-1 failed to give rise to intra-embryonic HPCs while yolk sac hematopoiesis of these mice was not affected (177,178). Cultivation of human $CD34^+$ hematopoietic cord blood progenitors on immobilized Notch ligand Delta-1 was shown to allow long-term maintenance and *ex vivo* expansion of HPCs (179,180). As suggested for

differentiation processes, also the efficiency of expansion and/or maintenance of HPCs via Notch activation is dose-dependent (180). Notch-1 receptor and Jagged-2 ligand have been shown to be expressed by hematopoietic stroma cells (181,182). Overexpression of NICD or HES-1 in bone marrow-derived progenitors led to increased self-renewal and/or proliferation of HPCs (183,184).

1.4. CYTOKINE CONTROL OF THE DC LINEAGE

Various hematopoietic cytokines control differentiation and maintenance of subsets of the DC lineage. From *in vitro* studies specific roles could be assigned to specific cytokines.

1.1.14. GM-CSF

GM-CSF (granulocyte/macrophage colony-stimulating factor) is a key cytokine for the development of DCs and LCs from human and mouse hematopoietic precursors and monocytes *in vitro* (185,186), whereas it is not required for the generation of pDCs (187). Although GM-CSF is essential for *in vitro* DC generation, mice devoid of GM-CSF or its receptor show normal numbers of DCs. As PU.1 levels were shown to be increased by GM- CSF, one could assign the positive effect of GM-CSF stimulation on DC development to the concomitant induction of PU.1 (123). The same effect could be exerted by TNFα, as PU.1 can replace TNFα requirements in LC differentiation from human $CD34^+$ hematopoietic precursors (128). GM-CSF levels rise during inflammatory conditions and there is a marked increase in dermal DC numbers in inflammation (15). Therefore, GM-CSF might be essential for inflammatory DC development *in vivo* although it is dispensable under steady-state conditions (18).

1.1.15. FLT3L

Mice deficient for Fms-related tyrosine kinase 3 8FLT3; CD135) or its ligand (FLT3L) show reduced DC numbers (104,105), whereas treatment with FLT3L induces an increase in DC numbers (188), i.e. pDCs and all lymphoid-resident and migratory DC subsets (188,189). *In vitro*, the development of all human DC subsets (intDCs, LCs and pDCs) from $CD34^+$ hematopoietic precursors is promoted by FLT3L (72,190–192), where it functions as key regulator of subsets of the DC lineage (18,193).

CD135 is expressed on HPCs (194), common lymphoid progenitors (CLPs) and on a subset of common myeloid progenitors (CMPs) (195). It is maintained on DC precursors such as monocyte/dendritic cell progenitors (MDPs) (105,196), common dendritic cell progenitors (CDPs) (193,197) and pre-cDCs (198) with the expression levels decreasing as commitment to a specific DC subset increases; expression is lost as soon as cells get (pre-)commited to the non-DC lineage (195).

1.1.16. M-CSF (Csf-1)

Macrophage- colony stimulating factor (M-CSF, Csf-1) regulates survival, proliferation and differentiation of macrophages (199) with its receptor (CD115) being expressed on GMPs, MDPs, monocytes and macrophages. CD115 is also expressed on CDPs, but gets lost as commitment towards the DC lineage increases. However, Csf-1 is maintained on a subset of murine $CD11b^+$ cDCs, suggesting a monocytic origin of these cells (93). Epidermal LCs are competely absent in M-CSF-R knockout mice (200,201) but are not dependent on M-CSF ligand, suggesting an alternative ligand for CD115 being involved in LC development. IL-34, a high affinity Csf-1 receptor ligand, is strongly expressed by epidermal keratinocytes (202) and was determined to play a key role in the development of LCs.

1.1.17. TGF Superfamily

Transforming growth factor β 1 (TGF-β1) and bone morphogenetic protein 7 (BMP7) are two key members of the TGF-superfamily and were determined to play an important role in the differentiation of various cell types.

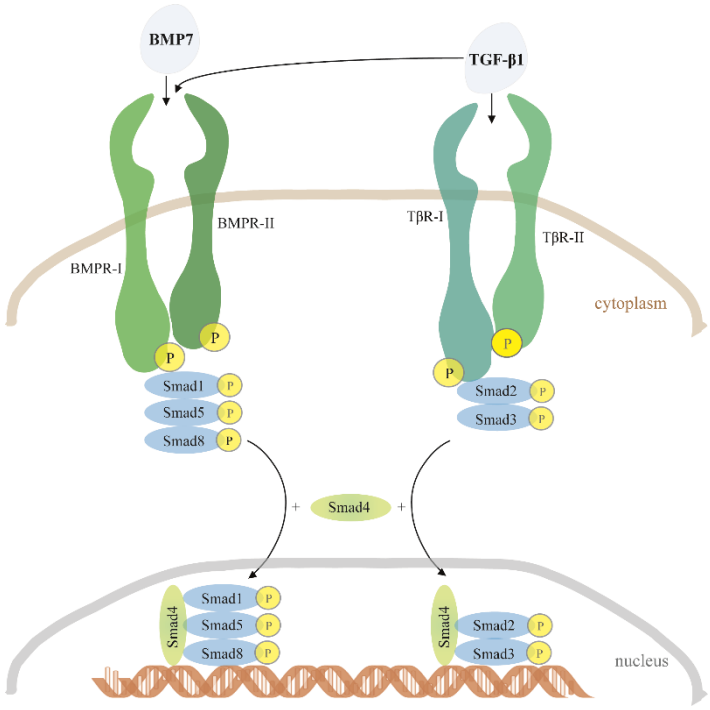

Figure 4: TGF-β1 and BMP7 signal via different ALK receptors.
TGF-β1 binds TGF-β-receptor type II (TβR-II), which then recruits and phosphorylates TGF-β-receptor type I (TβR-I, ALK5). ALK4 phosphorylates Smad2 and Smad3. In some cell types, TGF-β1 was also shown to be able to also activate the ALK3-induced Smad1/5/8 pathway, which was originally considered as the BMP7-induced pathway (203,204). The phosphorylated Smad2/3 or Smad1/5/8 complexes associate with Smad4 and translocate to the nucleus to regulate transcription of target genes. Additionally, inhibitory Smads get activated, of which Smad6 mainly inhibits BMP7, whereas Smad7 has inhibitory effects mainly on TGF-β1 signals (figure adapted from: (205)).

TGF-β1

In vivo, TGF-β1 is produced by leukocytes and non-hematopoietic cells, including keratinocytes, and exerts pleiotropic effects on cells of the immune system (Li et al, 2006, Annu Rev Immunol 24: 99-146). Of the three existing isoforms of TGF-β, TGF-β1 is the dominant form; it binds to TGF-β-receptor type II (TGF-βRII/ALK5) leading to the activation of Smad2/3 and 4 (see
Figure 4).

LC development is strongly dependent on TGF-β1. *In vitro*, bone marrow hematopoietic precusors give rise to LC in presence of GM-CSF and TGF-β1 (51) Adding TGF-β1 to GM-CSF/TNFα-supplemented cultures of $CD34^+$ progenitors enables the generation of $CD1a^+$/Birbeck granule$^+$ cells under serum-free conditions (51). *In vivo*, LCs are completely absent in TGF-$\beta1^{-/-}$ mice (132) and these animals spontaneously develop lethal multifunctional autoimmune inflammation (206,207). Kaplan et al aimed to investigate whether TGF-β1 acts directly on LC precursors to induce maturation or whether it influences accessory cells which in turn can affect LC precursors (208). Therefore, they

generated different mouse models: Langerin-Cre-TGF-βRII mice, in which LCs were unable to respond to TGF-β1 stimulation or Langerin-Cre-TGF-β1 mice, where LCs were not able to produce TGF-β1. Both animal models displayed significant reductions in the numbers of epidermal LCs, suggesting that TGF-β1 derived from LCs acts directly on LCs via an autocrine/paracrine feedback loop and that presence of TGF-β1 is essential for LC development and/or survival (208). The notion, that TGF-β1 is a key instructive factors for LC generation from HPCs was confirmed by the demonstration that neutralizing anti-TGF-β1 antibodies abrogate the differentiation of $CD1a^+$ cells in serum-containing GM-CSF/TNFα-dependent LC cultures (209).

TGF-β1 is secreted in a biologically non-active form bound to latency-associated peptide (LAP) (210). Integrins $a_v\beta_6$ and $a_v\beta_8$ have been demonstrated to bind to a specific region within LAP leading to its dissociation consequently resulting in the activation of TGF-β1. Recently, it was demonstrated that both integrins are expressed by epidermal keratinocytes and contribute to maintenance of LCs within the epidermis; inhibition of $a_v\beta_6$ and $a_v\beta_8$ caused lower levels of active TGF-β1 within the epidermis and the consequent emigration of LCs (211).

BMP7

Even though the signaling through the BMP7 pathway functions similar to TGF-β1, different regulatory Smad proteins are involved (see

Figure 4). BMP7 has been demonstrated to play an essential role in the differentiation of LCs (212). LCs do express high amounts of BMP7 and mice devoid of BMP7 show reduced numbers of LCs (213). In mouse, BMP7 was detected in prenatal epidermis at timepoints where LC precursors seed the embryonic skin (214–217). Also in human, basal/suprabasal keratinocyte layers have been demonstrated to express high levels of BMP7 (212), whereas TGF-β1 coud not be detected; i.e. TGF-β1 and BMP7 showed an inverse expression pattern with TGF-β1 being expressed suprabasally and BMP7 being expressed only in the basal layers. This suggested that LC precursors, which reside in the basal keratinocyte layers are exposed to a $BMP7^+$ environment (212).

In vitro, stimulation of $CD34^+$ cord blood progenitors with BMP7 induced differentiation of LCs exhibiting a higher proliferative capacity when compared to TGF-β1–dependent LCs (212). BMP7-induced LCs were also shown to be more potent in allogeneic T cell stimulation as well as in the production of pro-inflammatory cytokines and when compared to their TGF-β1-dependent counterparts (212). However, addition of TGF-β1 to BMP7-

supplemented LC cultures assimilated cells to the TGF-β1-dependent phenotype. Martínez et al (218) demonstrated that the canonical BMP signaling pathway is also involved in the maturation of monocyte-derived dendritic cells, i.e. BMP4 induced upregulation of co-stimulatory molecules on immature moDCs. BMP4 has also been described to enhance human LC development (212).

1.5. DENDRITIC CELL ONTOGENY

DCs, monocytes and macrophages comprise the system of mononuclear phagocytes. Current models propose that monocytes, macrophages and DCs originate from common hematopoietic stem cell progenitors with a differentiation potential restricted towards the myeloid lineage (219). Successive commitment steps in the bone marrow include common myeloid progenitors (CMPs), granulocyte-macrophage precursors (GMPs) and macrophage/DC progeniors (MDPs). MDPs are proliferating cells in the bone marrow which already show myeloid characteristics possessing the potential to give rise to macrophages and subsets of DCs, but not to granulocytes. Within the bone marrow, these MDPs would differentiate into monocytes and the common DC precursors (CDPs). CDPs differentiate into pDCs and pre-cDCs, but not into monocytes in the bone marrow (193,198,220). Pre-cDCs in turn give rise to mature cDCs upon exiting the bone marrow into blood and lymph nodes (221,222).

Macrophages and DC subpopulations are renewed from the bone marrow, but epidermal LCs seem to represent their own precursors, i.e. renewal of the LC network under inflammatory conditions is not dependent on bone marrow progenitors (18,223). LCs were shown to develop from an embryonic precursor, which colonizes the epidermis already before birth, differentiates *in situ* and proliferates during the first week of life, thereby establishing the epidermal LC network (217). In contrast to LCs, the renewal of other DC types depends on hematopoietic precursors which re-circulate between the periphery and the bone marrow.

There are differences between the DC networks in the human and the mouse. The classical model of DC development was set up based on the findings in the mouse (Figure 5), where a bi-potent progenitor derived from stem cells in the bone marrow (CMP) gives rise to MDPs, which in the end further develop into either to DCs or monocytes (105,196,224).

Early progenitors include the CLPs and CMPs, which have been identified in both, mouse and human. The commitment to the lineage of mononuclear phagocytic cells is suggested to occur at the MDP stage which in mouse are phenotypically described as Lin^{-}c-$kit^{+}CX_3CR1^{+}$ (196) and $Lin^{-}CD34^{+}CD117^{+}CD115^{+}CD135^{+}CD45RA^{+}$ in human (225). MDPs develop from CMPs or GMPs (granulocyte macrophage progenitors) (198) and differentiate into spleen macrophages, monocytes, lymphoid-resident cDCs (196), non-lymphoid-resident cDCs (93,97,226) and pDCs (224) but not into granulocytes (196).

Recently a DC-specific progenitor has been identified (193,224,227): the CDP (common DC precursor) is phenotypically similar to MDPs but gives rise exclusively to cDCs and pDCs (193,220). $Lin^{-}c\text{-}kit^{+}Flt3^{+}Csf\text{-}1R^{+}$ CDPs are immediately downstream from MDPs and appear to be the first dedicated DC precursor cells giving rise to the lymphoid and non-lymphoid cDC precursors as well as pDCs. $CD8^{+}$ and $CD11b^{+}$ cDCs originate from $CD11c^{+}MHC\text{-}II^{+}$ pre-cDCs; these do not give rise to pDCs, monocytes or macrophages (197,228). Pre-cDCS emigrate from the bone marrow to differentiate locally in lymphoid and non-lymphoid tissues (93,198).

Also for monocytes and macrophages a more restricted precursor was described: the cMoP (common monocyte progenitor), which differentiates into monocyte subsets and macrophages but lacks differentiation potential towards the DC lineage.

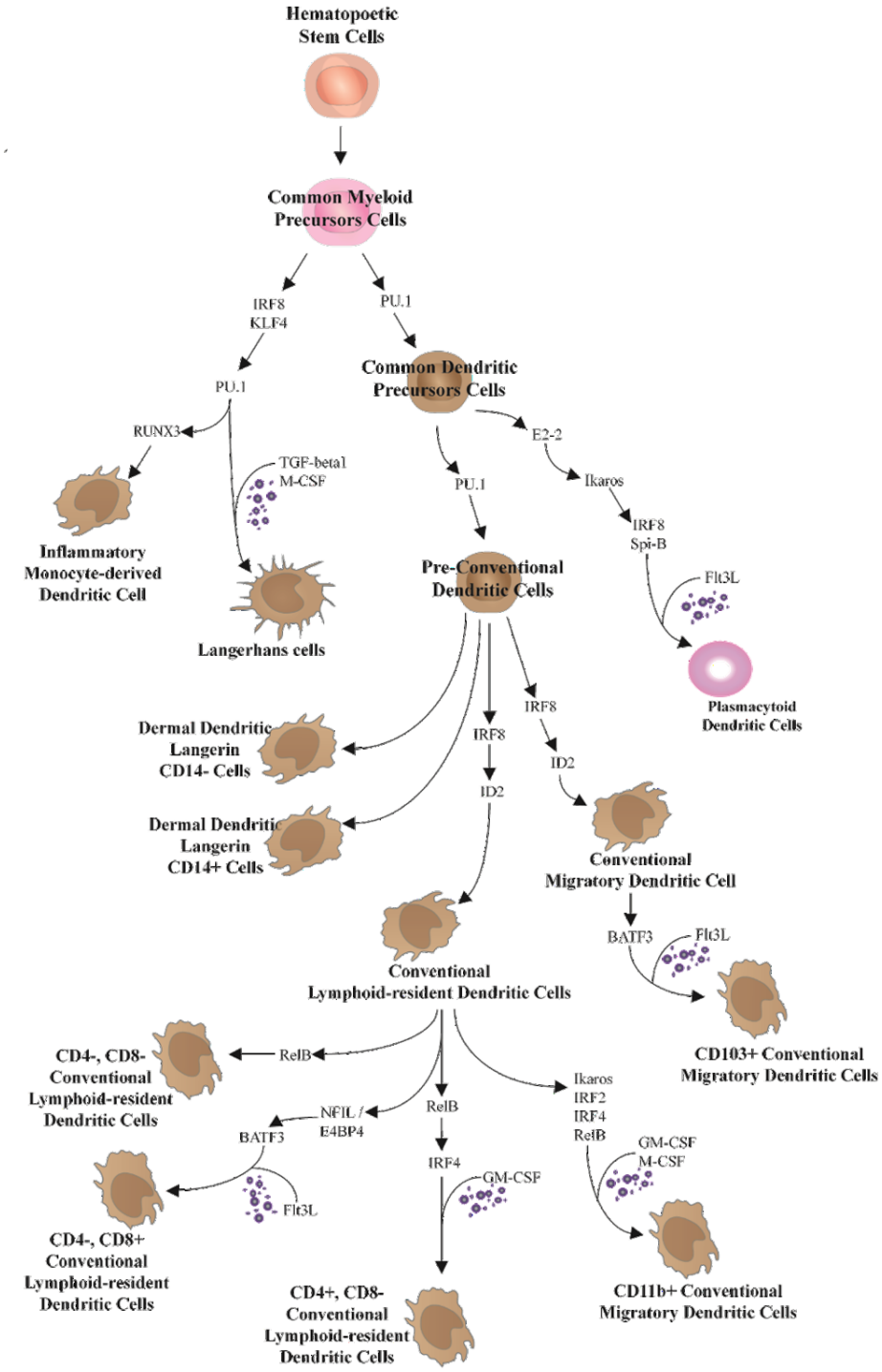

Figure 5: Current Model of mouse DC development.
The earliest precursors from HSCs are CMPs (229,230). CMPs in turn give rise to cells pre-commited for the monocyte- or DC-lineage (CDPs), which give rise to monocyte-derived DCs, pre-cDCs and pDCs, respectively. Pre-cDCs differentiate into all known lymphoid-resident and non-lymphoid-resident DC subsets (figure adapted from https://www.rndsystems.com/pathways/dendritic-cells-developmental-lineage-pathway).

Development of DCs in the human (Figure 6) is far less well understood compared to mouse; the definition of the human DC lineage is complicated by the limited access to human tissues, the low number of blood circulating DCs as well as difficulties to effectively

distinguish them from monocytes. *In vitro* cultures of human monocytes in presence of specific cytokines allow differentiation into potent APCs with certain phenotypic characteristics of DCs (185). However, these cells more closely resemble activated monocytes rather than DCs (35,197,231). Only recently, deeper insights into human DC lineage development was gained using an *in vitro* differentiation system from $CD34^+$ hematopoietic progenitor cells (221). Lee et al revealed a sequential origin of DCs from restricted progenitor cells: a human granulocyte-monocyte-DC progenitor (hGMDP) gives rise to a human monocyte-dendritic progenitor (hMDP) that in turn develops into monocytes and a human CDP (hCDP) giving rise to the three main DC subsets (221) (see Figure 6). These precursors were defined based on the expression of surface markers (225) and were found in the bone marrow, but were absent in blood or tissues and showed some similarities with granulocyte-macrophage progenitors (GMPs).

Only recently, the immediate precursor of human cDCs could be identified (222,232). Originating from committed bone marrow DC precursors, this migratory hpre-cDC is present in bone marrow, cord blood, peripheral blood and peripheral lymphoid organs and exclusively give rise to $CD1c^+$ cDCs and $CD141^+$ cDCs, but not tipDCs or monocytes.

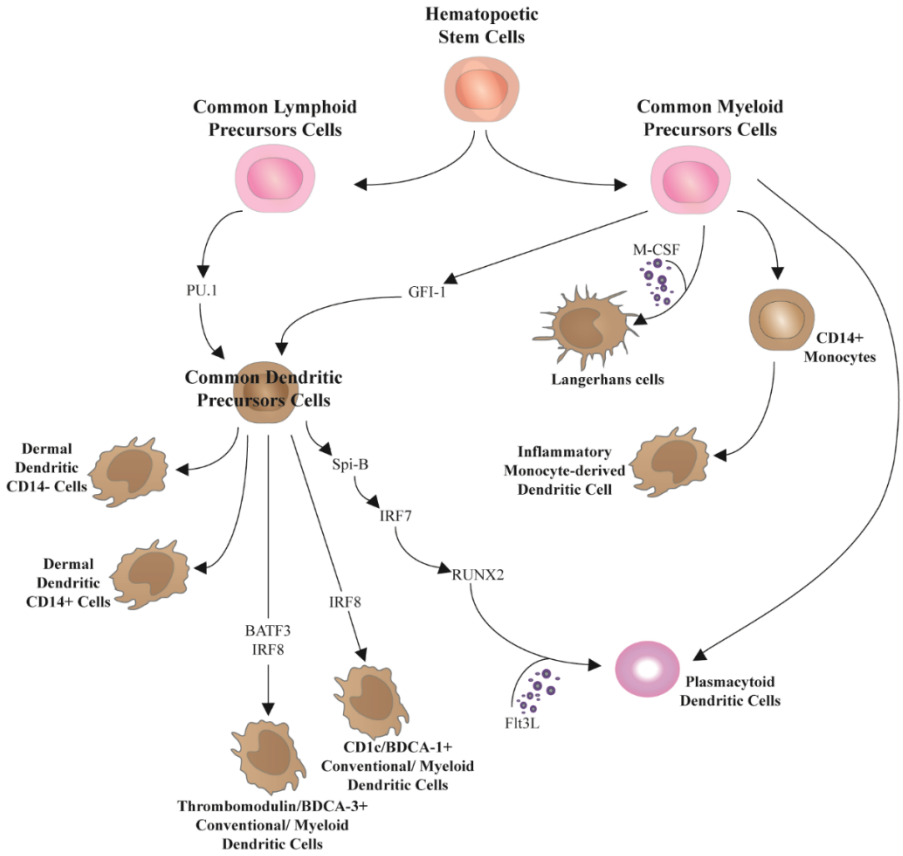

Figure 6: Current Model of human DC development.
Bone marrow precursors give rise to common dendritic cell precursors (CDPs) which give rise to all known subsets of tissue-resident and blood-circulating DCs but not pDCs. CDPs also give rise to common myeloid progenitors which differentiate into monocytes, inflammatory DCs, pDCs and LCs (figure adapted from https://rndsystems.com/pathways/dendritic-cell-developmental-lineage-pathway).

1.6. MONOCYTE PLASTICITY

Monocytes develop from HPCs in the bone marrow and spleen via several intermediate myeloid-committed progenitors. The earliest precursors from HPCs are $CD34^{+}Sca\text{-}1^{-}$ common myeloid precursors (CMPs) (229,230). CMPs in turn give rise to multipotent $CD16^{+}CD32^{+}$ granulocyte/macrophage precursors (GMPs); CD115 (CSF-1R/M-CSFR)$^{+}$ $CX_3CR1^{+}CD135$ (FLT3)$^{+}$ macrophage/ DC precursors (MDPs) are included within GMPs. MDPs lack granulocytic potential but give rise to monocytes and macrophage subsets; monocytes themselves give rise to some DC subsets including inflammatory DCs (94,224,227). The cMoP, being restricted towards the monocytic lineage exclusively gives rise to $Ly6C^{+}CX3CR1^{int}CCR2^{+}$ and $Ly6C^{-}CX_3CR1^{hi}CCR2^{-}$ monocyte subsets and macrophages (Figure 7).

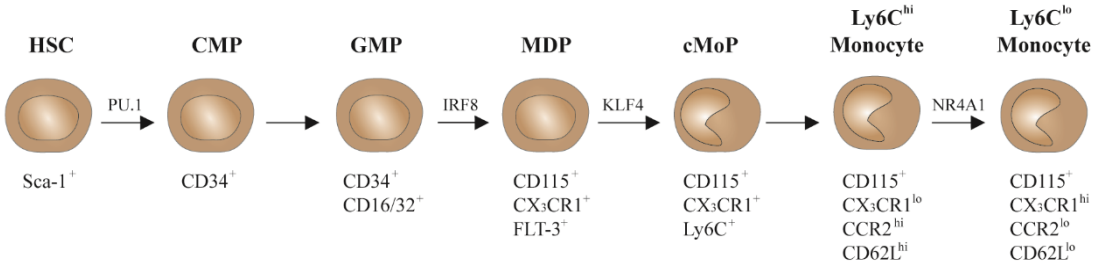

Figure 7: Developmental pathway of monocytes in the mouse.
Monocytes are derived from HPCs in the bone marrow via progenitors restricted to the myeloid lineage. $Sca\text{-}1^{+}$ HSCs give rise to $CD34^{+}$ CMPs, which in turn differentiate into $CD34^{+}CD16/32^{+}$ GMPs. MDPs ($CD115^{+}$ $CX_3CR1^{+}FLT3^{+}$) lose expression of FLT3 and start expressing Ly6C as they differentiate to cMoPs. cMoPs ($CD115^{+}CX_3CR1^{+}Ly6C^{+}$) cells differentiate into classical and inflammatory $Ly6c^{hi}$ and $Ly6c^{lo}$ monocytes subsets, respectively (figure adapted from (233)).

Monopoeisis is tightly controlled by the activity of different transcription factors expressed at defined timepoints of cellular development (233). As for DCs, PU.1 also plays an essential role in monocyte differentiation by antagonizing pro-granulocyte transcription factors, such as GATA-1, GATA-2 and C/EBPα, and inducing myeloid-specific factors, such as IRF8 and KLF4 (162,234). IRF8 is expressed at low levels in HSCs, but gets up-regulated during the successive differentiation to CMPs and GMPs (235). Together with PU.1, IRF8 promotes the differentiation of GMPs into monocytes; mice devoid in IRF8 are characterized by a strong expansion of granulocytes but an impaired development of monocytes (142,236–238). The interaction of IRF8 with PU.1 activates KLF4; KLF4 is a major downstream target of IRF8 and PU.1 and is critical for the development of the

monocytic lineage as murine KLF4-deficient HPCs fail to differentiate into Ly6C^{hi} monocytes (163).

The most important cytokine in monocyte development in the steady-state is macrophage colony stimulating factor (M-CSF); monocytes express the M-CSF receptor (239,240). Additionally, IL-34 is suggested to be involved in monocyte differentiation as it was shown to induce human monocyte proliferation and survival *in vitro* (241). Also GM-CSF has been demonstrated to impact monocyte differentiation at least under inflammatory conditions (242,243). GM-CSF is not detectable in the serum at steady state-conditions (244,245); however it is induced during inflammation (246–250), and therefore, in contrast to M-CSF, might be important for monopoiesis during inflammation. Consistent with this, stimulation with M-CSF induces a homeostatic steady-state phenotype in monocytes, whereas GM-CSF imprints an inflammatory phenotype (251).

The heterogeneity of monocytes has also been described in the human system (252). Based on phenotype and function, human analogs to the described mouse inflammatory and steady-state subsets have been defined (81,240,253–255). As shown in mice, human monocytes also express CD115 (CSF-R1) and CX_3CR1 (240,253) but can be characterized via CD14 and CD16 instead of the mouse-specific Ly6C marker (256). Classical monocytes in human are CD14^{hi}CD16^{-}, whereas the second subset is characterized by a CD14^{+}CD16^{hi} phenotype (253,254). In humans, an additional third population of "transitional/ intermediate" monocytes (CD14^{hi}CD16^{+}) has been recognized (257). The CD14^{+}CD16^{-} classical monocytes comprise about 90 % and the CD16^{+} nonclassical, inflammatory subset comprises about 10 % of all monocytes under steady-state conditions (258). However, numbers of CD16^{+} cells can increase under conditions of excessive stress exercise or inflammation (259). These cells have also been shown to producer higher levels of TNFα and IL-12 (260,261), but show no responsiveness to CCL2 in contrast to CD14^{+} classical monocytes (262,263).

Mouse and human monocytes have been demonstrated to be able to undergo markable phenotypic and functional changes upon various cytokine stimuli. MurineLy6C^{hi} classical/inflammatory monocytes exhibit a marked potential to change their phenotype; especially under inflammatory conditions, Ly6C^{hi} monocytes show high plasticity. In response to specific pro-inflammatory stimuli monocytes can transdifferentiate into DCs (61,219,264,265) or macrophages (266,267) (see *Figure 8*).

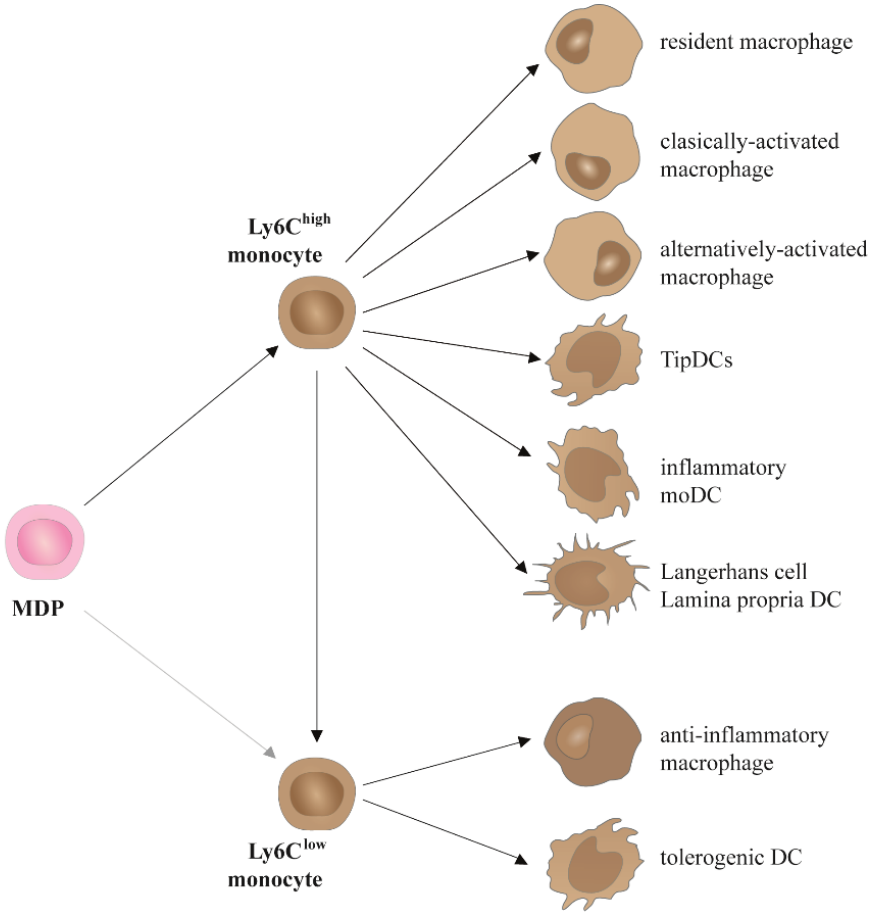

Figure 8: Developmental pathways and differentiation potential of mouse Ly6Chi and Ly6Clo monocytes.
During inflammatory reactions (e.g. infection, allergy, injury, tumor growth), Ly6C^{high} monocytes are recruited to affected tissues and differentiate into different types of macrophage and DCs depending on the cytokines they are exposed to. Also Ly6C^{low} monocyte have been determined to give rise to macrophages and DCs upon inflammation (figure adapted from (268)).

1.1.18. Monocytes give rise to LCs

Lauvau (269) described that the migration of Ly6C^{hi} inflammatory monocytes to sites of inflammation is dependent on the expression of the chemokine receptor CCR2 (74) and that these cells can transdifferentiate into effector cells such as tissue-macrophages and monocyte-derived dendritic cells. This is in line with previous findings, demonstrating that Ly6C^{hi}Gr-1^{hi}CCR2^{+} monocytes actively migrate into the epidermis upon UV irradiation in CCR2$^{-/-}$ mice and there undergo transdifferentiation into LCs (133,200). It was observed that the re-establishment of the epidermal LC network occurs in two waves: the first wave being comprised by blood- circulating monocytes that differentiate into short-lived LCs followed by a second wave of bone-marrow precursors that differentiate into the final long-term resident LCs (see Figure 9). The short-term LCs were shown to phenotypically differ by

means of Langerin and MHC-II expression and their developmental dependency on the transcription factor ID2 (133). Short-term LCs exhibited lower expression levels of Langerin and a more uniform distribution of MHC-II expression over the cell body and developed more dendrites than long-term LCs. Gene expression analysis revealed more similarities of short-term LCs with Gr-1^{hi}/Ly6C^{hi} inflammatory monocytes rather than with steady-state ID2-dependent LCs (133).

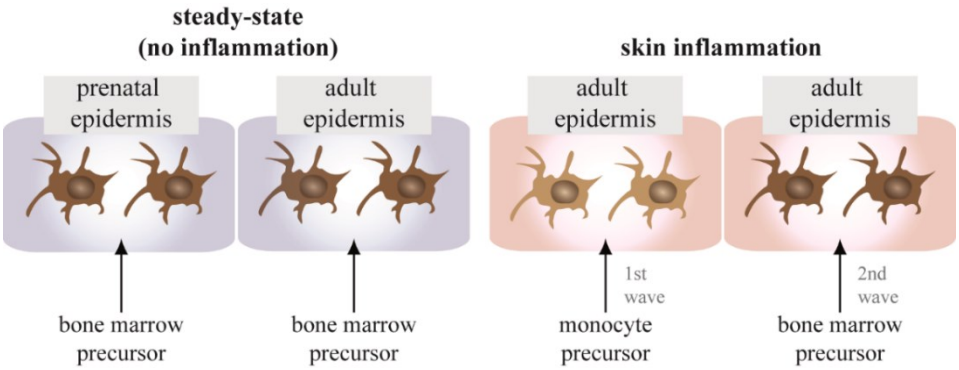

Figure 9: Langerhans cells populate the epidermis in two waves.
In the mouse, a wave of bone-marrow progenitors migrates to the epidermis and undergoes proliferation and LC differentiation in situ. During steady-state, LC precursors continuously immigrate into the epidermis thereby maintaining the network. Upon inflammation-induced emigration of LCs from the epidermis, the network is re-established by LC precursors in two waves: the first wave is comprised by Gr1^{+}Ly6C^{hi} blood circulating monocytes that transiently replenish the LC network, followed by a second wave presumably of bone-marrow derived precursors that then set-up the long-term steady-state LC network (figure adapted from Romani, Tripp, et al. 2012).

2. AIM OF THE STUDY

DCs are essential regulators of the immune system either maintaining the steady-state or inducing an immune response upon danger signals. In mouse and human, different subsets of DCs have been described based on their phenotype and their localization within different tissues. Due to their localization of residence in stratified epithelia, LCs are in contact with and mediate tolerance/immune reactions to epithelial and environmental antigens. The network of LCs is established before birth from bone marrow-derived hematopoietic precursors which differentiate *in situ* and self-maintain in the epidermis during steady-state. Upon activation via contact with environmental antigens, LCs emigrate from the epidermis to the skin-draining lymph nodes in order to induce immune reaction or tolerance. Local precursors in the skin replenish these emigrating cells ensuring maintenance of the network. However, under severe inflammatory conditions, it was observed in the mouse system, that blood-circulating monocytes are recruited to the epidermis and function as locally differentiating LC precursors. Also in the human system, *in vitro* studies gave hints for blood-circulating cells possessing capability to transdifferentiate into LCs in response to stimulation with certain cytokines.

The different cells of the mononuclear phagocyte system such as DCs, monocytes and macrophages have been shown to develop from a common monocytic precursor in a transcriptionally tighty controlled fashion. PU.1 has been demonstrated to act a master regulator of cell fate decisions of the hematopoietic system; depending on the interaction partner and the level of PU.1 expression, hematopoietic common precursor cells differentiate into different subsets of the mononuclear phagocyte system. Additionally, the nature and levels of different cytokines present in specific tissues contribute to the expression of transcription factors finally regulating the differentiation/ maintenance of DC subsets. Being highly expressed by epidermal keratinocytes, the immune-suppressive cytokine TGF-β1 has been shown to function as key cytokine inducing the development of LCs from monocytic precursors *in vitro* and *in vivo*. The differentiation of blood-circulating monocytes has been clearly described in the murine system; however, the transcriptional mechanisms underyling monocyte-to-LC conversion remained to be elucidated. *In vitro*, both monocytes and LCs can be generated from common precursor cells dependent on the absence or presence of TGF-β1. In this study we aimed to identify transcription factors which are regulated by stimulation of TGF-β1 and to describe the role the identified transcription factors play in LC cell fate decisions. As it was described in the mouse that blood-borne cells can replenish the

epidermal LC network, we also aimed to identify potential blood-circulating LC precursors in the human system.

The identification of potential LC precursors might add on a better understanding of LC function and might allow improvement of vaccination strategies directed towards antigen-presenting cells in the skin and other stratified epithelia.

3. MATERIALS AND METHODS

3.1. Cytokines and Reagents

Stem cell factor (SCF), thrombopoetin (TPO), tumor necrosis factor α (TNFα), granulocyte/macrophage-colony stimulating factor (GM-CSF), Fms-related tyrosine kinase 3 ligand (FLT3L), interleukin-6 (IL-6), interferon-γ (IFNγ), interleukin-4 (IL-4) and macrophage colony-stimulating factor (M-CSF) were purchased from Peprotech. Transforming growth factor β1 (TGF-β1) was from R&D Systems. Pam_3CSK_4 was purchased from InvivoGen. The recombinant extracellular domain of Notch ligand Delta-1 (Delta-$1^{ext\text{-}IgG}$) was kindly provided by I. Bernstein (Seattle, WA, USA) (1).

3.2. Sources of cells and skin tissues

$CD34^+$ cells were obtained from cord blood samples from healthy donors. Blood samples were collected during healthy full-term deliveries and prepared as previously described (190). $CD34^+$ hematopoietic progenitors were isolated by means of immunomagnetic positive selection (EasySep; STEMCELL Technologies).

For the microarray screen, $CD34^+CD19^-$ cells were subsorted into $CD45RA^{low/-}$, $CD45RA^{int}$, and $CD45RA^{hi}$ subsets by means of flow cytometry (FACS) and analyzed separately. For all differentiation experiments, the total $CD34^+$ cell fraction was used. $CD14^+$ monocytes were isolated from PBMCs obtained from blood of healthy volunteers in cooperation with the Department of Pharmacology of the Medical University of Graz by positive selection with magnetic beads (Miltenyi Biotec). For microarray experiments, $CD14^+$ monocytes were FACS sorted, as described below. $CD1c^+$ blood cDCs along with $CD14^+$ or $CD16^+$ monocyte subsets were purified from buffy coats that underwent thrombopheresis from blood samples of healthy donors provided by the Department of Transfusion Medicine of the University Hospital Graz via magnetic bead isolation (Miltenyi Biotec).

Skin was obtained from healthy adults undergoing elective surgery (breast reduction or abdominoplasty) (271). For prenatal skin stainings, specimen of human embryonic trunk skin (range 9-10 weeks of estimated gestational age (EGA)) were studied after legal termination of pregnancy. Approval was obtained from the Vienna Medical University Institutional Review Board. Informed consent was provided in accordance with the Declaration of Helsinki (1).

3.3. Isolation of immune cells for microarray studies

Epidermal LCs and keratinocytes, as well as dermal cell populations, were isolated from healthy human skin. Briefly, after separation of dermal and epidermal sheets by incubation with Dispase I (3 U/mL, Roche), single cells were released from epidermal sheets by using 0.25 % trypsin/EDTA (Invitrogen) for 30 min at 37 °C. $CD11b^{-}CD1a^{+}$ LCs (n=4) and $CD11b^{-}CD1a^{-}$ keratinocytes (n=1) were sorted from epidermal cell suspensions on a FACSAria (BD BioSciences). For preparation of dermal cell suspensions, dermal sheets were dissociated with 0.5 U/mL collagenase IV (Worthington Biochemical; Lakewood, NJ, USA) for 90 min at 37 °C. Dermal cells were sorted into $CD1a^{+}$ dDCs ($CD1a^{+}CD11b^{+}CD14^{-}$, n=3), and $CD14^{+}$ dDCs ($CD14^{+}CD11b^{+}CD1a^{-}$, n=3).

Monocytes were sorted from buffy coats obtained from the Austrian Red Cross. Briefly, after Ficoll Hypaque (Pharmacia) density gradient centrifugation. Briefly, PBMCs were depleted of Lin^{+} cells (CD3, CD16, CD34, CD56, and glycophorin A) and $CD14^{+}CD11b^{+}CD19^{-}CD1c^{-}$ monocytes were sorted by using a FACSAria (BD BioSciences). The purity of all cell populations used was at least 98 %. Sorted cells were pelleted and lysed in TRI Reagent (Sigma-Aldrich), and RNA was isolated according to the manufacturer´s recommendations (1).

3.4. *In vitro* culture of $CD34^{+}$ cord blood cells

For $CD34^{+}$ progenitor cell-derived LCs (p-LCs), $CD34^{+}$ progenitor cell-derived monocyte-derived dendritic cell (p-moDC) or monocyte (p-Mo) generation, a previously described two-step culture model was used with slight modifications (272,273). In short, sorted $CD34^{+}$ cells were cultured in CellGro DC medium (CellGenix) supplemented with 10% FCS, 100 ng/mL GM-CSF, 20 ng/mL SCF, 50 ng/mL FLT3L, and 2.5 ng/mL TNFα for 5 days before sub-culturing in RPMI-1640 medium (Sigma-Aldrich) + 10% FCS under lineage-specific cytokine conditions (100 ng/mL M-CSF, 50 ng/mL FLT3L, 20 ng/mL SCF, 2.5 ng/mL TNFα and 2 ng/mL IL-6 for monocytes; 100 ng/mL GM-CSF, 2.5 ng/mL TNFα and 25 ng/mL IL-4 for p-moDCs; and 100 ng/mL GM-CSF, 2.5 ng/mL TNFα and 1 ng/mL TGF-β1 for p-LCs). All cultures were supplemented with Glutamax (2.5 mmol/L; Gibco/Invitrogen) and penicillin/streptomycin (125 U/mL each; PAA). p-LC clusters were purified by means of 1 g sedimentation, as previously described (1).

3.5. *In vitro* culture of CD14$^+$ peripheral blood monocytes

For generating moDCs, purified CD14$^+$ blood monocytes were cultures in RPMI-1640 supplemented with 100 ng/mL GM-CSF and 25 ng/mL IL-4 in the presence of 10% FCS for 7 days. moLC differentiation was performed by culturing CD14$^+$ monocytes in presence of 100 ng/mL GM-CSF, 10 ng/mL TGF-β1 and 10% FCS in 24-well plates coated with Delta-1, as previously described (274). To immobilize Delta-1 ligand, plates were first coated with 10 µg/mL goat polyclonal anti-human IgG (Affinipure F(ab')$_2$ Fragment (goat Anti-Human IgG, Fcy fragment specific; Jackson ImmunoResearch) for 60 min, washed, blocked (60 min, RPMI-1640 supplemented with 20% FBS) and then coated with 1 µg/mL Delta-1$^{ext\text{-}IgG}$ for 3 h. All cultures were supplemented with Glutamax (2.5 mmol/L; Gibco/Invitrogen, Grand Island, NY) and penicillin/streptomycin (125 U/m Leach; PAA) (1).

3.6. *In vitro* cultivation of CD1c$^+$ blood cDCs

Circulating BDCA-1$^+$/CD1c$^+$ blood cDCs were obtained from buffy coats from the local transfusion medicine department via magnetically labelled beads according to manufacturer´s instructions (Miltenyi). Briefly, CD1c$^+$CD19$^+$ B cells were depleted prior to positive selection of CD1c$^+$ cDCs. Differentiation into LC-like DCs was performed by cultivation of freshly isolated cells in presence of 100 ng/mL GM-CSF and 10 ng/mL TGF-β1 or 200 ng/mL BMP7 in RPMI-1640 supplemented with 10% FCS in presence or absence of immobilized Notch-ligand Delta-1.

In vitro CD1c$^+$ blood cDC equivalents were generated as described by Lee et al (221) with slight modifications. In brief, purified CD34$^+$ cord blood progenitors were cultivated in presence of 100 ng/mL FLT3L, 20 ng/mL SCF and 10 ng/mL GM-CSF on plate-bound Delta-1 under serum-free conditions (X-VIVO-15 medium, 1% penicillin/streptomycin, 1% Glutamax) for 7 days. For further differentiation into LCs, *in vitro* generated CD1c$^+$ cDCs were isolated via magnetic beads isolation as described (Miltenyi) and stimulated under moDC- (100ng/mL GM-CSF), LC- (100 ng/mL GM-CSF, 10 ng/mL TGF-β1) or macrophage- (100 ng/mL M-CSF, 2 ng/mL IL-6) promoting cytokine conditions.

3.7. RNA isolation and quantitative PCR

Cells were harvested and total RNA was isolated with the RNeasy Micro Kit (Qiagen, Hilden, Germany). Purified RNA was reverse transcribed with oligo-dT primers (Eurofins MWG GmbH, Ebersberg, Germany) and reverse transcriptase (M-MLV-RT-H-; Fermentas, Waltham, Mass), according to the manufacturer´s instructions. Quantitative PCR was performed in a Roche LightCycler (Roche) with Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen). Obtained expression values were normalized to hypoxanthine phosphoribosyltransferase (HPRT) (1). Primers are listed in Table 3.

Table 3: Primers used for quantitative PCR

Primer name	Orientation	Sequence (5´→ 3)
RFX2	Forward	ATA GAT GTC TCC CAC TGC TTC
	Reverse	TCT CGA TGT AGT GGA ACT GGA G
TIEG	Forward	CCA GGA TGT GGC AAG ACA TAC
	Reverse	TTC ACA ACC TTT CCA GCT ACA G
BLIMP-1	Forward	CGG CAA GAT CAA GTA CGA ATG
	Reverse	GAG CTG AGT AAA GCC CTT GTT G
ETS-2	Forward	TTG TGG GTG ACA TTC TCT GG
	Reverse	ATG AGG AAC GGA GGT GAG G
DEC2	Forward	CCT ACC GTC CCA CAG ATT G
	Reverse	CCT TGG TGT CGT CTC GTT TC
DEC1	Forward	TGA CCG GAT TAA CGA GTG C
	Reverse	GAG CAG AAC ATC TCT TGA CCT G
KLF4	Forward	GCC GCT CCA TTA CCA AGA G
	Reverse	GTG CCT TGA GAT GGG AAC TC
PPARδ	Forward	TCA CAC AGT GGC TTC TGC TC
	Reverse	TCT ACA GGG TGG TTC CCA TC
RXRα	Forward	CGA CCC TGT CAC CAA CAT TTG C
	Reverse	GAG CAG CTC ATT CCA GCC TGC C
VDR	Forward	AGA TGA CCC TTC TGT GAC CC
	Reverse	AGC TTG TTC AGT CCC ACC TG
HPRT	Forward	GAC CAG TCA ACA GGG GAC AT
	Reverse	AAC ACT TCG TGG GGT CCT TTT C

3.8. mRNA microarray and data analysis

Cells were collected at indicated time points (0, 6 and 24 h after addition of TGF-β1). Total RNA from 6 independent donors was isolated using the RNeasy Micro Kit (Qiagen). RNA samples were then combined into two separate pools (each containing RNA from 3 independent donors), labeled, and hybridized onto U133 Plus 2.0 Affymetrix GeneChips (Affymetrix, Santa Clara, California). Hundred nanograms of total RNA per sample were processed using the Ambion´s Message Amp II Biotin Enhanced Labeling Kit (Ambion,

Thermo Fisher, Waltham, Massachuesetts). Gene chips were stained, washed and scanned according to Affymetrix standard procedures. The probe level data (CEL files) were processed for local normalization, and expression values were generated by using the robust multiarray average algorithm of the „affy package“ in the R software environment (http://www.R-project.org). The microarray data have been deposited in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/goe/) and can be accessed as GSE31318. Form RNA profiling of immune cells isolated from skin, total RNA was subjected to 2 rounds of linear amplification, as previously described (275,276). Biotin-labeled ribonucleotides were incorporated by using the ENZO Bio-Array High-Yield RNA Transcript Labeling Kit (Affymetrix) during the second round of in vitro transcription. Fragmented cDNA (10 µg) was hybridized to Human Genome U133 Plus 2.0 Array (Affymetrix). Microarray data were normalized by using the robust multiarray analysis, as implemented in Bioconductor (277,278). All analyses were performed with log2-transformed data. The Ingenuity Pathway Analysis (http://www.ingenuity.com) tool was used to assign microarray data sets to common biological pathways and to define gene sets attributed to Notch signaling. Analysis was performed for genes showing regulated expression under TGF-β1-supplemented vs. non-supplemented culture conditions after 6 and 24 h (cut-off: fold change, 1.3). Differential expression of Notch was determined by using a heat map with Spotfire software (http://www.spotfire.tibco.com) (1).

3.9. Flow cytometry

Flow cytometric staining and analyses were performed, as previously described (279). Briefly, 0.5-1x10^6 cells were blocked with human serum for 10 min at 4 °C, followed by antibody staining for 30 min at 4 °C in FACS wash buffer (PBS+BSA+azide). Cells were washed twice with buffer, resuspended and analyzed. Flow cytometric analysis was performed with an LSRII or LSR Fortessa Instrument (BD BioSciences) and FlowJo (TreeStar) or Diva (BD BioSciences) software. For FACS sorting, the BD FACSAria flow cytometer (BD Biosciences) was used. Used antibodies are listed in Table 4 (1).

Table 4: Antibodies used for flow cytometry

Primary antibodies

Antigen	Conjugate	Distributor
CD34	FITC	BD Biosciences
CD14	FITC	BD Biosciences
HLA-DR	FITC	BD Biosciences
CD1a	FITC	BD Biosciences
CD1c	FITC	Miltenyi Biotec GmbH
CD11b	FITC	Immunotech
CD207	FITC	Miltenyi Biotec GmbH
CD207	PE	Immunotech
CD203	PE	Immunotech
CD1a	PE	BD Biosciences
CD14	PE	BD Biosciences
HLA-DR	PE	BD Biosciences
CD45RA	PE	BD Biosciences
CD11c	PE	BD Biosciences
Lactoferrin	PE	Caltag/ An der Grub
CD14	PE	ImmunoTools
Jagged-2	PE	BioLegend
CD19	PerCP	BD Biosciences
NGFP	PerCP-Cy5.5	BD Biosciences
CD45	ECD	Immunotech
CD117	CyChrome	BD Biosciences
CD1a	APC	BD Biosciences
CD14	APC	BD Biosciences
E-Cadherin	APC	BioLegend
Notch-1	APC	BioLegend
E-Cadherin	AF647	BD Biosciences
CD11b	PE-Cy7	BioLegend
CD11b	APC-Cy7	BD Biosciences
CD80	Biotinylated	BD Biosciences
CD86	Biotinylated	BD Biosciences
CD209	Biotinylated	BD Biosciences
CD11b	Biotinylated	BD Biosciences
CD1a	BV421	BD Biosciences
CD1a	Pacific Blue	BioLegend

The second-step reagent for biotinylated antibodies was streptavidin-PerCP (BD Biosciences).
APC, Allophycocyanin; ECD, Phycoerythrin-Texas Red; PE, phycoerytrhin; PerCP, peridinin-chlorophyll-protein complex

3.10. Western Blot analysis

For Western Blot analysis, lysates of 1-2x10^6 cells were loaded per lane, and resolved proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore). Membranes were probed with antibodies against KLF4 (Santa Cruz Biotechnology), RUNX3 (a kind gift of S. Sakaguchi, Vienna, Austria) or β-actin (Sigma-Aldrich), followed by horseradish peroxidase-conjugated goat anti-rabbit IgG antibodies

(Pierce Biotechnology). Antibody binding was visualized with the chemiluminescent substrates, SuperSignal West Pico or West Dura (Pierce Biotechnology) (1).

3.11. γ-secretase inhibitor treatments

$CD14^+$ peripheral blood monocytes were cultivated under moLC differentiation conditions (100 ng/mL GM-CSF, 10 ng/mL TGF-β1, immobillized Delta-1). Treatment with γ-secretase inhibitors DAPT (N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; Sigma Aldrich) or L-685,458 (5S-(tert-Butoxycarbonylamino)-6-phenyl-(4R)-hydroxy-(2R)-benzylhexanoyl)-L-leucy-L-phenylalaninamide; Tocris) was performed either in a continuous fashion (addition of inhibitor on day 0 and day 4 of cultivation) or during the initial differentiation phase (day 0- day 4) of cultivation. DMSO-treated and untreated cells served as controls.

3.12. Retroviral vectors

RV-GFP and RV-KLF4-GFP vectors were kindly provided by M.W. Feinberg (162). KLF4-coding DNA was inserted into the BglII/XhoI sites of the MSCV-IRES-GFP vector. Cutting of RV-KLF4 with BglII and XhoI and insertion into the MCSV-IHRES-NGFR vector generated MIN-KLF4. MIG-RUNX3 was kindly provided by S. Sakaguchi (Vienna, Austria). HR-KLF4-IGFP was generated by cutting RV-KLF4-GFP with BglII and XhoI and inserting it into the BamHI/XhoI site of the pHR-IGFP vector (kindly provided by F.Rossi, Vancouver, British Columbia, Canada) (1).

3.13. Transfection of packaging cell lines and gene transduction

Gene transduction was performed as previously described (280,281). In short, the packaging cell line Phoenix-Gag-Pol (Ph-GP) was used for generating GALV envelope-containing retroviral particles. Target cells were plated on RetroNectin (Takara Bio)-coated non-tissue culture plates coated with virus (3-5 h, 37 °C) in specific growth medium. Infections were repeated 2 to 3 times at intervals of 12 to 24 h. $CD34^+$ cells were infected in expansion mix (50 ng/mL SCF, 50 ng/mL FLT3L, 50 ng/mL thrombopoietin/ TPO). The retroviral tetracycline-inducible system (tet-on system) was described previously (280).

Briefly, the first vector encoded the Tet activator pTA-mCD8α. The second vector encoded human KLF4-IRES-GFP under the control of a Tet-responsive element. $CD34^+$ cells were first infected with TA-mCD8α, followed by infection with the Tet-responsive vector. Expression of the KLF4 transgene was induced by addition of 1 μg/mL doxycycline (DOX). Fresh DOX was added every 2 to 3 days of LC differentiation cultures to sustain KLF4 expression (100 ng/mL GM-CSF, 2.5 ng/mL TNFα, 1 ng/mL TGF-β1 secondary cultures) (1).

3.14. Confocal microscopy

Multicolor immunofluorescence staining procedures were performed on frozen sections, as previously described (282). In short, specimen were embedded in optimum tissue compound (Tissue-Tek, Sakura) and snap-frozen in liquid nitrogen. Sections of 6 μm were air-dried, fixed in acetone (10 min) and washed with PBS. Samples were then stained with primary antibody overnight at 4 °C; secondary antibodies were applied for 60 min at room temperature. Cytospins were prepared by centrifugation of cells (1-2x10^5 cells/ sample) using FlexiPERM chambers (Greiner BioOne) mounted in SuperFrost microscropy slides (Menzel, Thermo Scientific). Cells were washed twice with PBS, fixed (10 min, 4% PFA), washed, air-dried and stored at -20 °C. For immunofluorescence stainings, slides where thawed at room temperature for 20 min and washed with 1x TBS-T. Permeabilization for nuclear stainings was performed using 0.1% Triton-X (10 min, RT).

Negative controls were obtained in all staining experiments by substituting primary antibody with the isotype-matched IgG. Slides were mounted in Permafluor (Themo Fisher Scientific), VECTASHIELD (Vector Laboratories) or Fluoroshield (Sigma-Aldrich). Immunofluorescently labeled sections were analyzed with a Zeiss LSM 520 confocal microscope (x40/1.3NA; Zeiss,), and images were captured with Zen 2008 Software (Zeiss). The following primary antibodies were used: polyclonal rabbit anti-KLF4 (Sigma-Aldrich); mouse anti-CD207 (Immunotec, Vaudreuil-Dorion, Quebec, Canada), fluorescein isothiocyanate (FITC)-conjugated mouse anti-CD11b (Immunotec), FITC-labeled mouse anti-HLA-DR (BD Biosciences); polyclonal rabbit anti-activated Notch-1 (Abcam); monoclonal mouse anti-CD1a (Novus Biologicals), polyclonal goat anti-CD14 (Novus Biological) and FITC-labeled mouse anti-CD14 (BioLegend, San Diego, California). Alexa Fluor 488-conjugated anti-Laminin 5 (Millipore, Temecula, California) was used to visualize the epidermal-dermal junction on skin sample stainings. Anti-FITC polyclonal goat

IgG (Invitrogen) and anti-rabbit polyclonal F(ab')2 fragment (JacksonImmunoResearch) or goat anti-rabbit Alexa Fluor 647 (Invitrogen), donkey anti-rabbit Rhodamine Red-X (JacksonImmunoResearch), donkey anti-mouse Alexa Fluor 488 (JacksonImmunoResearch), and donkey anti-goat Alexa Fluor 647 (JacksonImmunoResearch) served as secondary reagents. All secondary antibodies have been cross-absorbed to avoid cross-reactivity with IgG of other species (1).

3.15. Immunohistochemical staining

Double-labeled immunohistochemical staining was performed on paraffin-embedded sections or cytospin preparations by using the LabVision Polymer Detection System (anti-mouse AP, anti-rabbit horseradish peroxidase), according to the commercial protocol (Thermo Fisher Scientific). The following primary antibodies were used: monoclonal mouse anti-CD1a (Novus Biologicals), polyclonal rabbit anti-activated Notch-1 (Abcam), and polyclonal rabbit anti-KLF4 (Sigma-Aldrich) (1).

3.16. Chromatin immunoprecipitation assay

$CD14^+$ peripheral blood monocytes were induced to differentiate with GM-CSF (100 ng/mL) and IL-4 (25 ng/mL) into moDCs. KLF4 chromatin immunoprecipitation was performed with the KLF4 ExactaChIP Kit (R&D Systems), according to the manufacturer´s protocol. Briefly, moDCs ($6x10^6$) were treated for 15 min at 37 °C with 1 % (v/v) formaldehyde, followed by addition of glycine (final concentration, 125 mmol/L). Cells were pelleted, re-suspended in lysis buffer, lysed on ice, and sonicated to obtain chromatin fragments of 0.5 to 1 kb in length. Equal amounts (5 µg) of goat anti-human KLF4 antibody or of normal goat IgG were used per reaction. The following primers were used for detection of the RUNX3 promoter region: 5'-GCAGCCCCAGAACAAATC-3' and 5'-GCTACGACCCGAGAGAGG-3'. The abundance of distinct DNA fragments was quantified by means of semi-quantitative PCR. The PCR products were resolved by using 2 % agarose gel electrophoresis (1).

3.17. Cytokine measurements

Cells were seeded ($1x10^4$ to $2x10^4$ per 200 µL) in 96 well plates, and supernatants were collected 48hours later, as previously described (280). Cytokine (IL-6, IL-10, IL-8, TNFα and IL-12p40) levels were quantified with the Luminex system (Luminex) (1).

3.18. Mixed leukocyte reaction (MLR)

Allogeneic mixed leukocyte reactions (MLRs) were performed as described previously (212,283). In brief, purified T cells (100.000 cells/well) were co-cultivated with graded numbers of purified stimulator APCs (moLCs, $CD1c^+$ DC-derived LCs, $CD34^+$-derived p-LCs; 100- 27.000 cells/well) in 96-well U-bottom cell culture plates (Sarstedt) in RPMI-1640 medium supplemented with 10% FBS. Expansion of T cells was determined after cultivation for 5 days by the addition of thymidine [methyl-3H] TdR (Perkin-Elmer). Cells were harvested (TOMTEC Harvester 96) onto a filter mat (glass fibre filter), sealed together with scintillation paper (Metilex; sealed by Wallac Microsealer 1495-021) followed by measuring the incorporation of thymidine after 18 h using a β-counter (Wallac Microbeta 1450 Plus). Assays were performed in triplicates.

3.19. Statistical analysis

Statistical analysis was performed with the paired, 2-tailed Student t test or ANOVA. P values of less than 0.05 were considered significant.

4. RESULTS

4.1. KLF4 is inversely regulated during LC vs. monocyte differentiation of myeloid progenitor cells

TGF-β1 induces the generation of $CD1a^+CD207^+$ progenitor cell- derived (p-) LCs at the expense of monocytes/ macrophages when added to serum-free, cytokine supplemented cultures (GM-CSF, SCF, FLT3L and TNFα) of $CD34^+$ cord blood hematopoietic precursor cells (190).

We screened for TGF-β1- regulated transcription factors during p-LC commitment. To obtain cell fractions highly enriched in consecutive differentiation stages of early myeloid cells, $CD34^+$ cells devoid of initial B cell committed cells ($CD34^+CD19^-$) were sub-fractionated into $CD45RA^{low/neg}$, $CD45RA^{int}$ and $CD45RA^{hi}$ subsets (Figure 10) (284–287). These subsets were pre-stimulated with GM-CSF, SCF, FLT3L and TNFα for 48 h to bias their differentiation to monocytes. Thereafter, TGF-β1 was added to induce p-LC differentiation; parallel cultures were maintained without TGF-β1 addition to further promote monocyte development (Figure 10) (190). Under these conditions, only $CD45RA^{hi}$ cells, representing the most differentiated myeloid progenitor subset, exhibited potent capacity to differentiate into $CD1a^+CD207^+$ p-LCs or $CD14^+CD11b^+$ p-monocytes (Figure 10). To enrich for functional LC precursors, we therefore used these $CD45RA^{hi}CD34^+$ cells for microarray profiling (Figure 11, A). Conversely, all the subsequently described cell culture experiments were done with total $CD34^+$ cells (1).

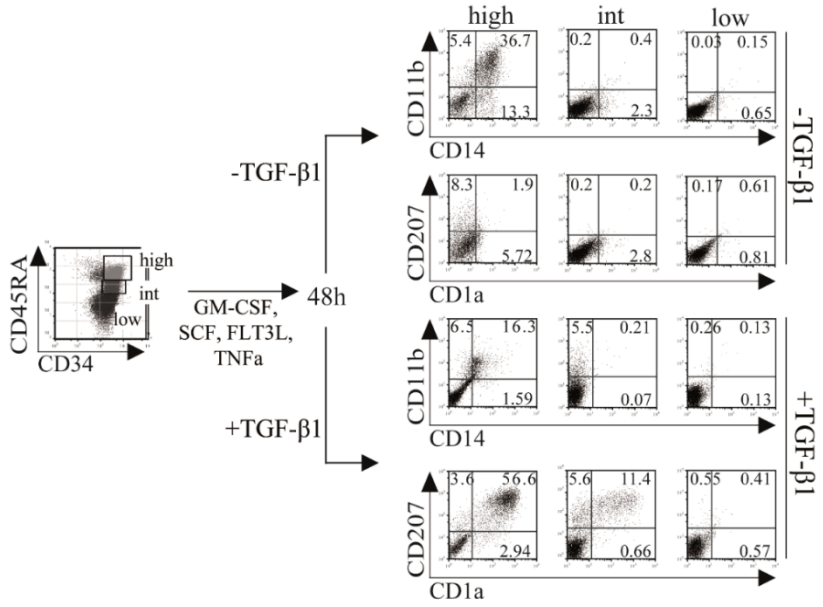

Figure 10: CD34^{+}CD45RA^{hi} progenitors give rise to monocytes and LCs.
Sorted CD34^{+}CD45RA^{lo}, CD34^{+}CD45RA^{int} and CD34^{+}CD45RA^{high} progenitor subsets were cultured under monocyte-promoting cytokine conditions (GM-CSF, FLT3L, SCF, TNFα) for 48 h before further cultivation for 7 days under monocyte (-TGF-β1) or LC- (+TGF- β1) promoting conditions and analyzed for surface marker expression (figure published in (1)).

From mRNA analysis (Figure 11, B) we identified several "proof of concept" molecules being induced in TGF-β1-supplemented cultures such as Claudin-1, E-Cadherin, CD207 or CCR6 (Table 5); vice versa, monocyte/moDC-associated markers (CD36, CLEC10, MRC1 or FXIIIA) were repressed under LC conditions (Table 6). Three groups of differentially regulated transcription factors were identified: (i) factors rapidly induced in TGF-β1-containing cultures including DEC1, VDR, RUNX3, RXRα, RFX2; (ii) factors induced late at 24 h in presence of TGF-β1 (DEC2, PPARγ) or slightly induced in absence of TGF-β1 (BLIMP-1); (iii) factors rapidly repressed in TGF-β1-containing cultures including ETS2 and KLF4. Among all these transcription factors, KLF4 was the only factor significantly inversely regulated under p-LC vs. monocyte differentiation conditions; i.e. repressed during p-LC (+TGF-β1) but induced during monocyte (-TGF-β1) differentiation (Figure 11, C).
KLF4 was shown to induce monocyte differentiation (162,163); therefore it might be involved in regulating alternative lineage fate options of shared monocyte/ LC progenitor cells.
KLF4 is a member of the family of Kruppel-like factors, carrying conserved DNA binding zinc finger domains, but lacking homology outside of the DNA binding regions (288). Apart from KLF10 (TIEG), an immediate early TGF-β1-inducible factor in various cell types (289), and KLF6, none of the other KLF family members underwent significant regulation in the presence or absence of TGF-β1 in our screen (Table 7) (1).

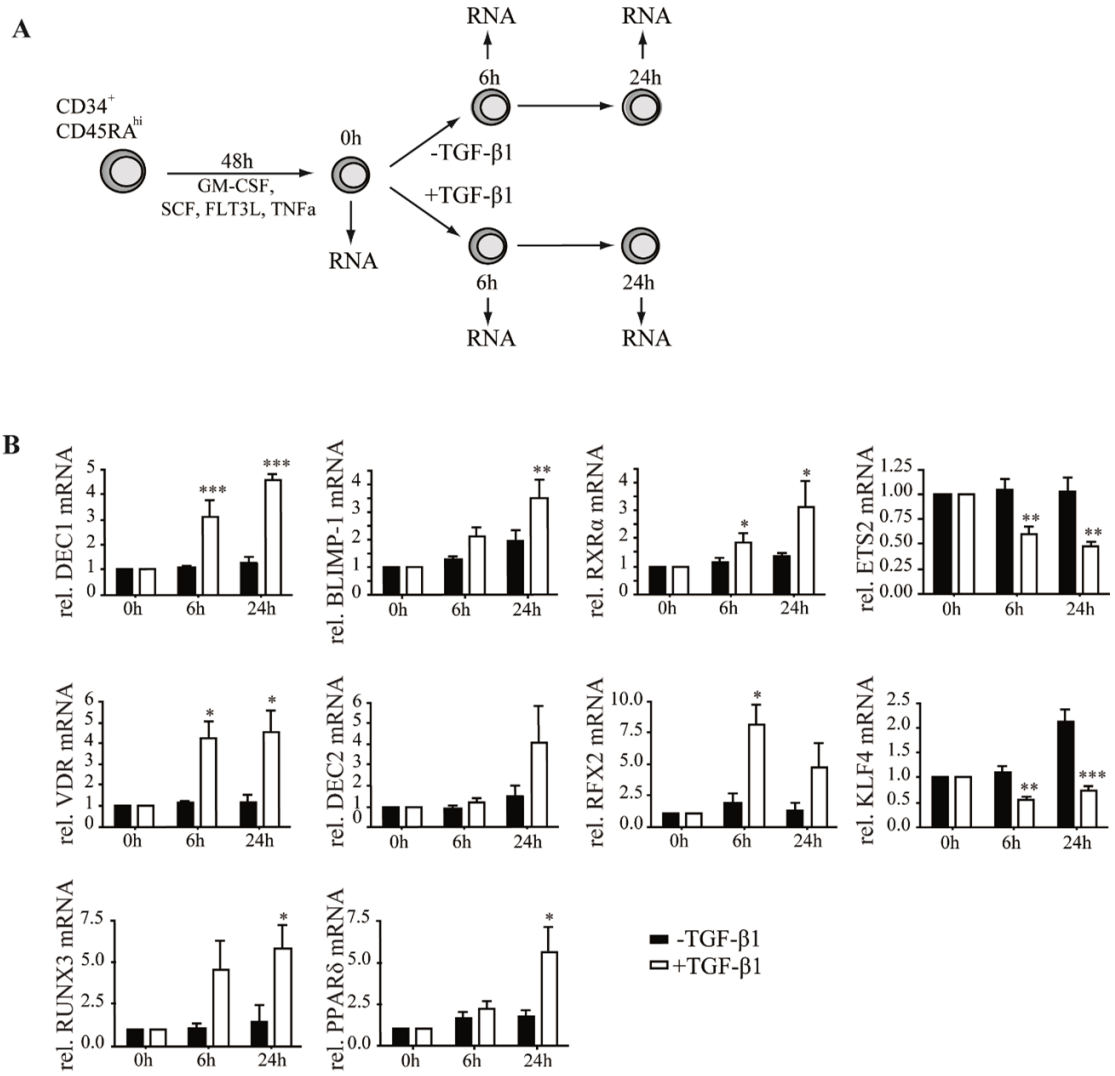

Figure 11: Identification of transcription factors regulated during LC commitment.
(A) Schematic representation of the set-up of microarray analysis of LC vs. monocyte differentiation of $CD34^+$ progenitors. (B) qPCR validation of selected transcription factors (n=4-8, +/- SEM, *t-test $p<0.05$, **t-test $p<0.01$, ***t-test $p<0.001$) (figure published in (1)).

Table 5: Microarray data: mRNAs induces in TGF-β1- stimulated vs. non stimulated cultures

probe set	gene symbol	0 h	- TGF-β1		+ TGF-β1		sum of calls	F.p. value
			6 h	24 h	6 h	24 h		
201131_s_at	CDH1	13.3§	14.9	26.1	69.8	470.0	8	<0.0001
222549_at	CLDN1	8.8	11.2	12.8	34.6	253.8	6	<0.0001
220428_at	CD207	7.2	6.8	8.2	7.7	197.9	2	<0.0001
206337_at	CCR7	19.0	16.3	25.4	51.9	77.9	6	0.0015

§microarray expression values generated using the robust multi-array average algorithm (RMA) and processed for global normalization

Table 6: Microarray data: mRNAs repressed in TGF-β1-stimulated vs. non-stimulated cultures

probe set	gene symbol	0 h	- TGF-β1		+ TGF-β1		sum of calls	F.p. value
			6 h	24 h	6 h	24 h		
209555_s_at	CD36	312.3[§]	389.9	730.6	165.1	343.5	10	0.00007
206682_at	CLEC10A	217.2	295.3	517.1	214.7	350.1	10	0.00310
204438_at	MRC1	661.0	775.3	1771.1	372.7	93.6	10	0.00001
203305_at	FXIIIA	75.7	73.8	73.8	64.4	42.1	7	0.00435

[§]microarray expression values generated using the robust multi-array average algorithm (RMA) and processed for global normalization

Table 7: Microarray data: KLF4 family member mRNA regulation in TGF-β1-stimulated vs. non-stimulated cultures

Probe set	gene symbol	0 h	- TGF-β1		+ TGF-β1		sum of calls	F.p. value
			6 h	24 h	6 h	24 h		
210504_at	KLF1	35.9[§]	34.7	38.9	55.6	41.8	10	0.827
219371_s_at	KLF2	18.6	16.8	19.2	15.6	17.4	1	0.531
222913_at	KLF3	65.8	63.0	67.0	63.1	67.2	10	0.953
221841_s_at	KLF4	111.4	137.6	205.7	52.2	65.0	10	0.057
209212_s_at	KLF5	31.5	38.4	31.7	26.6	32.9	7	0.444
1555832_s_at	KLF6	442.8	411.8	605.6	352.4	555.3	10	0.010
1555420_s_at	KLF7	28.8	32.0	29.9	30.3	29.5	10	0.976
219930_at	KLF8	5.0	4.6	5.4	4.5	5.2	10	0.295
203543_s_at	KLF9	4.8	5.0	6.2	4.7	7.5	5	0.056
202393_s_at	KLF10	116.9	94.9	110.3	221.3	181.6	10	0.014
218486_at	KLF11	52.0	47.1	55.7	47.2	63.4	10	0.211
227261_at	KLF12	89.1	77.4	76.8	88.2	104.2	10	0.062
225390_s_at	KLF13	490.0	458.2	578.2	586.3	635.8	10	0.129
1552814_s_at	KLF14	9.9	8.9	9.7	8.4	9.4	0	0.747
221302_at	KLF15	25.7	23.4	27.6	28.2	26.0	0	0.668
226328_at	KLF16	51.8	50.6	60.8	46.5	58.4	2	0.976
1553891_at	KLF17	6.7	7.4	7.3	5.9	5.9	0	0.628

[§]microarray expression values generated using the robust multi-array average algorithm (RMA) and processed for global normalization

4.2. KLF4 protein is inversely regulated during moLC or moDC generation from CD14$^+$ peripheral blood monocytes

Murine inflammatory monocytes are KLF4$^+$ and require KLF4 for differentiation (163). These cells can give rise to LCs under inflammatory conditions (200). As our microarray data suggests that LCs lack KLF4, we investigated the regulation of KLF4 protein expression during LC development from monocytes.

Congruent with previous observations (Hoshino et al, 2005, J Leukoc Biol 78: 921-929), Notch ligand Delta-1 cooperates with TGF-β1 to induce LC differentiation from CD14$^+$ monocytes (moLCs; CD1a$^+$CD207$^+$ cells). Under these conditions, KLF4 was repressed to virtually undetectable levels (Figure 12, A). Conversely, addition of TGF-β1 alone (i.e. without Delta-1) to GM-CSF-supplemented cultures resulted in the preferential generation of macrophages (CD14$^+$CD11b$^+$CD1a$^-$) exhibiting marked up-regulation of KLF4 protein (Figure 12, B). In fact, KLF4 expression levels were elevated in GM-CSF plus TGF-β1 supplemented cultures as compared to cells stimulated with GM-CSF only. Stimulation of monocytes with GM-CSF in the presence of Delta-1 caused repression of KLF4 (Figure 12, B). KLF4 was also abundantly expressed in CD1a$^+$ moDCs generated in the presence of GM-CSF+IL-4 (Figure 12, B) (1).

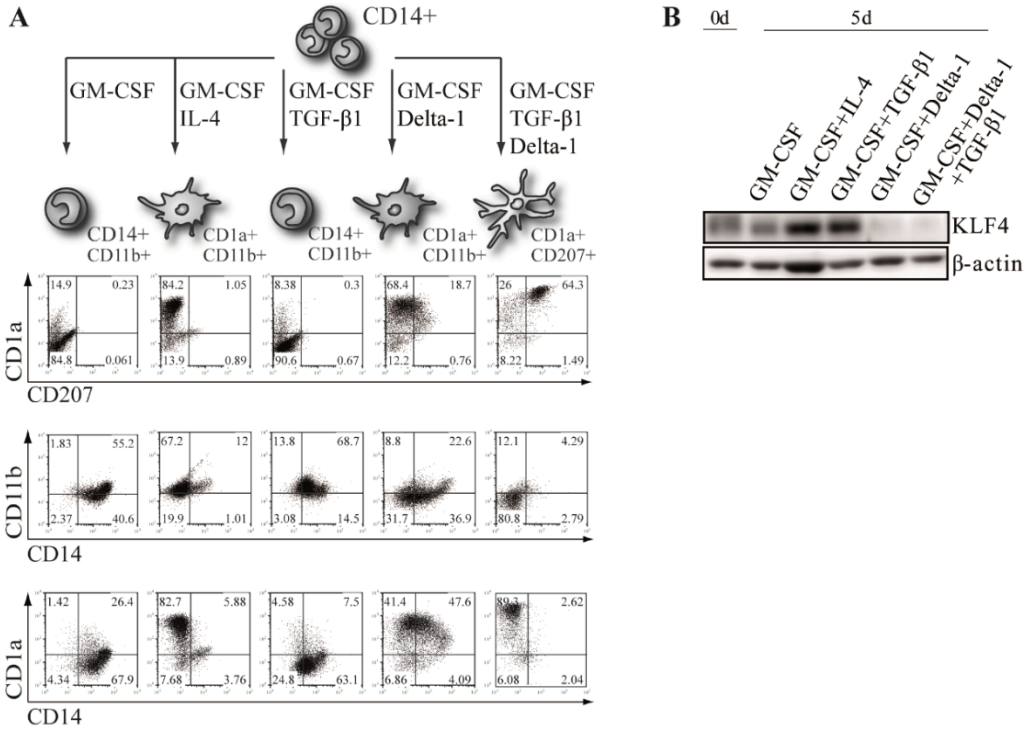

Figure 12: Delta-1 represses KLF4 during LC differentiation from peripheral blood monocytes:
$CD14^+$ blood monocytes were cultured for 5 days with indicated cytokines and analyzed (A) for expression of lineage markers by FACS and (B) KLF4 protein expression by Western Blot (figure published in (1)).

We subsequently analyzed the regulation of KLF4 during p-macrophage, p-moDC and p-LC generation from $CD34^+$ hematopoietic precursors. Time kinetic analyses revealed weak KLF4 expression by monocyte/ LC precursors generated from $CD34^+$ cells in the presence of GM-CSF, SCF, FLT3L and TNFα plus 10% FCS as described previously (186) (day 0, Figure 13, A). These precursors can be induced to differentiate into $CD1a^+CD207^+CD209^-CD11b^-$ p-LCs, $CD1a^+CD207^-CD209^+CD11b^+$ p-moDCs (Figure 13, A and B) or $CD14^+CD11b^+$ p-macrophages (Figure 13, A). p-LCs clearly lacked detectable KLF4 (Figure 13, C), thus the presence of low levels of KLF4 in p-LCs (Figure 13, A) might be attributed to contaminating monocytes. Conversely, p-moDC differentiation (IL-4, Figure 13, C) was marked by rapid and persistent KLF4 induction (day 2 to day 7). M-CSF-induced macrophage differentiation (Figure 13, A) was accompanied by a transient KLF4-upregulation. Omission of TGF-β1 from the LC-inducing cytokine mix abrogated p-LC differentiation but favored generation of $CD14^+CD11b^+$ monocytes from Mo/LC precursors, similarly to what has been observed for pre-stimulated $CD34^+$ cells (compare Figure 13, D and Figure 10, A). KLF4 was expressed by Mo/LC precursors at day 0 and increased during monocyte (-TGF-β1) but declined during LC differentiation (+TGF-β1) (Figure 13, D). IFNγ

was previously shown to enhance KLF4 expression in monocytes (160). Consequently, we analyzed whether KLF4 is induced in $CD34^+$-derived p-LCs or p-moDCs in response to IFNγ stimulation for 2 days. Whereas IFNγ failed to induce KLF4 in p-LCs, p- moDCs showed marked upregulation of KLF4 upon IFNγ treatment (Figure 13, E) (1).

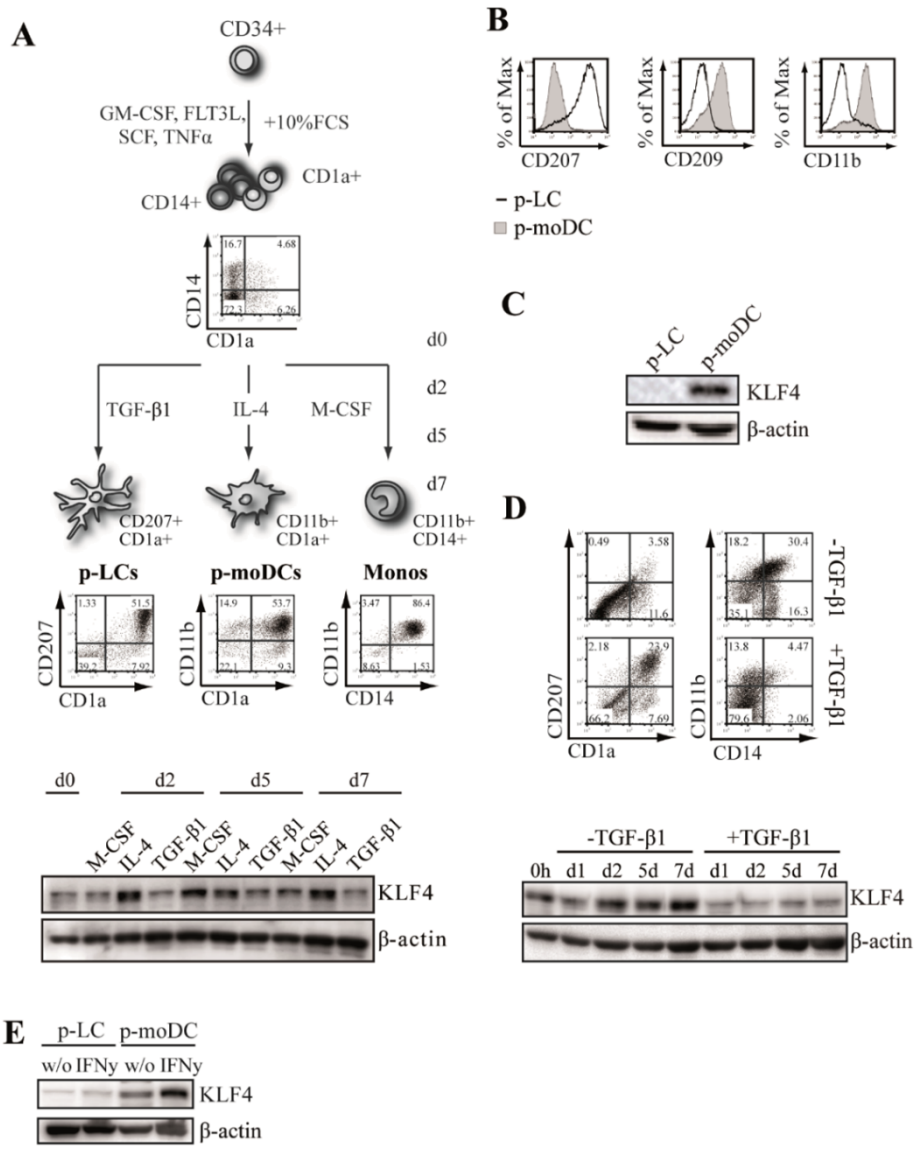

Figure 13: KLF4 is inversely regulated during moDC and LC differentiation from common progenitors.
(A) $CD34^+$ cells were cultured in presence of GM-CSF, FLT3L, SCF and TNFα for 5 days (= day 0) and subsequently plated under p-moDC, p-LC or p-monocyte differentiation conditions for 7 days. Surface marker expression was analyzed by FACS (upper panel); KLF4 expression was determined by Western Blot (lower panel). (B) Lineage marker profile and (C) KLF4 expression of $CD1a^+$ cells of day 7 p-moDC- (grey filled histograms) or p-LC (black lines) cultures. (D) FACS and KLF4 expression analysis of day 5 progenitors cultured in presence or absence of TGF-β1. (E) KLF4 expression of FACS-purified p-LCs or p-moDCs cultured for 2 days +/-IFNγ (figure published in (1)).

Guironnet et al previously (290) described an antagonistic effect of TGF-β1 and IL-4 on the differentiation of moLCs when cultivated in absence of immobilized Notch ligand. The presence of Delta-1 in GM-CSF/TGF-β1-supplemented cultures significantly enhanced expression of CD207 and CD1a (Figure 14, A). Addition of IL-4 to these differentiation conditions resulted in downregulation of CD207, but enhancement of CD11b expression (Figure 14, A). In line with previous observations (290), we observed induction of E-Cadherin expression upon addition of IL-4 to our moLC differentiation cultures (data not shown). moLCs cultured in presence of Delta-1 developed the classical LC clusters, whereas supplementation of IL-4 resulted in a decrease in dendrite formation though similar formation of homotypic clusters was observable (Figure 14, B).

In order to determine the requirement of a constitutive activation of the Notch signaling pathway, moLC cultures were re-plated from immobilized Delta-1 ligand on consecutive days of differentiation. We observed that the presence of Delta-1 is essential in the initial phase of differentiation, whereas re-plating at later timepoints did not affect $CD1a^{+}CD207^{+}$ moLC development. Continuous abrogation of plate-bound Delta-1 ligand did not allow moLC development (Figure 14, C). Treatment of moLCs with γ-secretase inhibitor L-685,458 showed a concentration-dependent reduction of $CD1a^{+}CD207^{+}$ cells (Figure 14, D) compared to control conditions. Viability was not affected (data not shown). Treatment of moLCs with DAPT during the initial differentiation phase or throughout the course of differentiation resulted in a complete abrogation of moLC development. Interestingly, at lower concentrations (5 μM) CD1a expression was still induced comparable to GM-CSF/TGF-β1-supplemented culture conditions in absence of Delta-1 (Figure 14, D). As seen for DAPT, the inhibitory effect of L-685,458 was concentration-dependent and did not affect cell viability (data not shown).

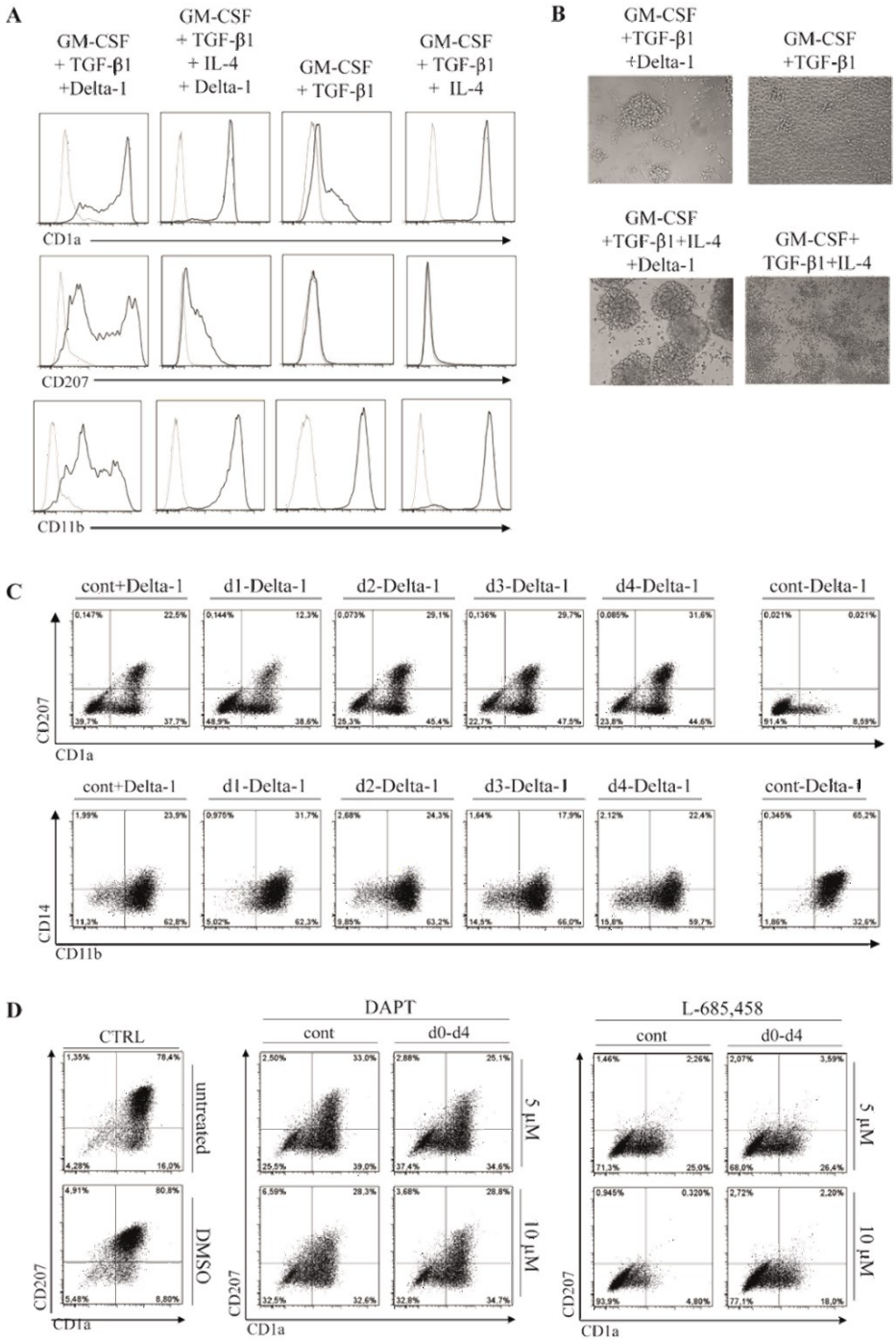

Figure 14: Activation of Notch signaling is essential during early phase of moLC differentiation: Freshly isolated $CD14^+$ blood monocytes were incubated with the indicated cytokines and analyzed (A) by FACS) and (B) by light micrscopy to assess morphology. (C) Cells were removed from immobilized Notch ligand Delta-1 on consecutive days during moLC differentiation and analyzed by FACS). (D) The influence of inhibition of Notch activation by two γ-secretase inhibitors (DAPT; L-685,458) at different concentrations on moLC differentiation was evaluated by FACS analysis.

4.3. LCs and their precursors lack detectable KLF4, whereas dermal DCs are KLF4$^+$

LCs in adult human skin (HLA-DR$^+$CD207$^+$ epidermal cells) lacked detectable KLF4 (Figure 15, A, arrowS), whereas all keratinocyte layers exhibited nuclear KLF4 expression pattern. Moreover, KLF4 was readily detectable in HLA-DR$^+$ dermal cells (Figure 15, arrow heads). Since LCs develop prenatally from epidermal resident precursor cells, we also analyzed prenatal human skin. HLA-DR$^+$ cells in embryonic epidermis skin at 9 weeks estimated gestational age (EGA) lacked KLF4 expression (Figure 15, left panel, arrows), whereas HLA-DR$^+$KLF4$^+$ cells could be identified in the dermis (Figure 15,left panel, arrows and arrowheads).

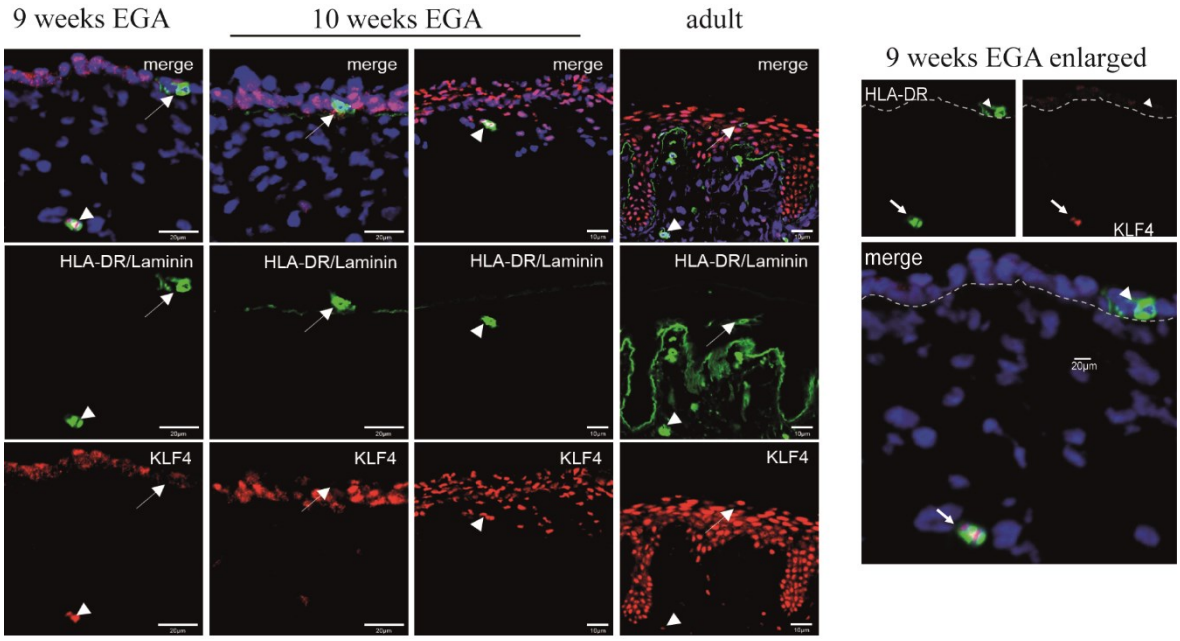

Figure 15: Prenatal LC precursors in the epidermis lack KLF4 expression.
Frozen sections of healthy human skin (fetal skin at 9 and 10 weeks EGA, adult skin) were stained for the indicated markers. Nuclei were stained with DAPI and are displayed in blue. LCs and prenatal HLA-DR$^+$ LCs are indicated by arrows, dermal HL-DR$^+$ cells are indicated by arrowheads (n=4 per developmental stage); Bars equal 20 µm (figure published in (1)).

Two major subsets of dDCs exist in adult skin, marked by CD1a and CD14 expression, respectively (Figure 16, A). KLF4 could be detected at varying levels in most adult CD1a$^+$ and CD14$^+$ dermal DCs, whereas epidermal CD1a$^+$ cells lacked KLF4 (Figure 16, A; B). Grading was performed according to reference levels of KLF4 in keratinocyte layers (outer layers, high; suprabasal, int; basal, low). Gene profiling of FACS-sorted cell subsets

confirmed that both dDC subsets, monocytes and keratinocytes express KLF4 mRNA at high levels, while it is virtually undetectable in LCs (Figure 16, C).

It was proposed that migratory $CD14^+$ dDCs can differentiate into LCs when stimulated with TGF-β1 (69). Skin explant cultures revealed two subsets of $CD14^+$ dDCs, one of which displayed higher migratory potential and readily differentiated into LCs in presence of TGF-β1. One might speculate that these cells contribute to the replenishment of the epidermal LC network upon resolution of inflammation. In immunofluorescence stainings we observed that lesional psoriatic skin is characterized by an increase in numbers of dermal $CD1c^+KLF4^+$ or $CD14^+KLF4^+$ cells (Figure 16, D). This is in line with previous findings (291) showing that $CD1a^+CD14^{dim}$ moLCs in the epidermis and HLA-$DR^+CD14^{dim/bright}$ moDCs in the dermis can be detected in lesions of skin of psoriasis patients (1).

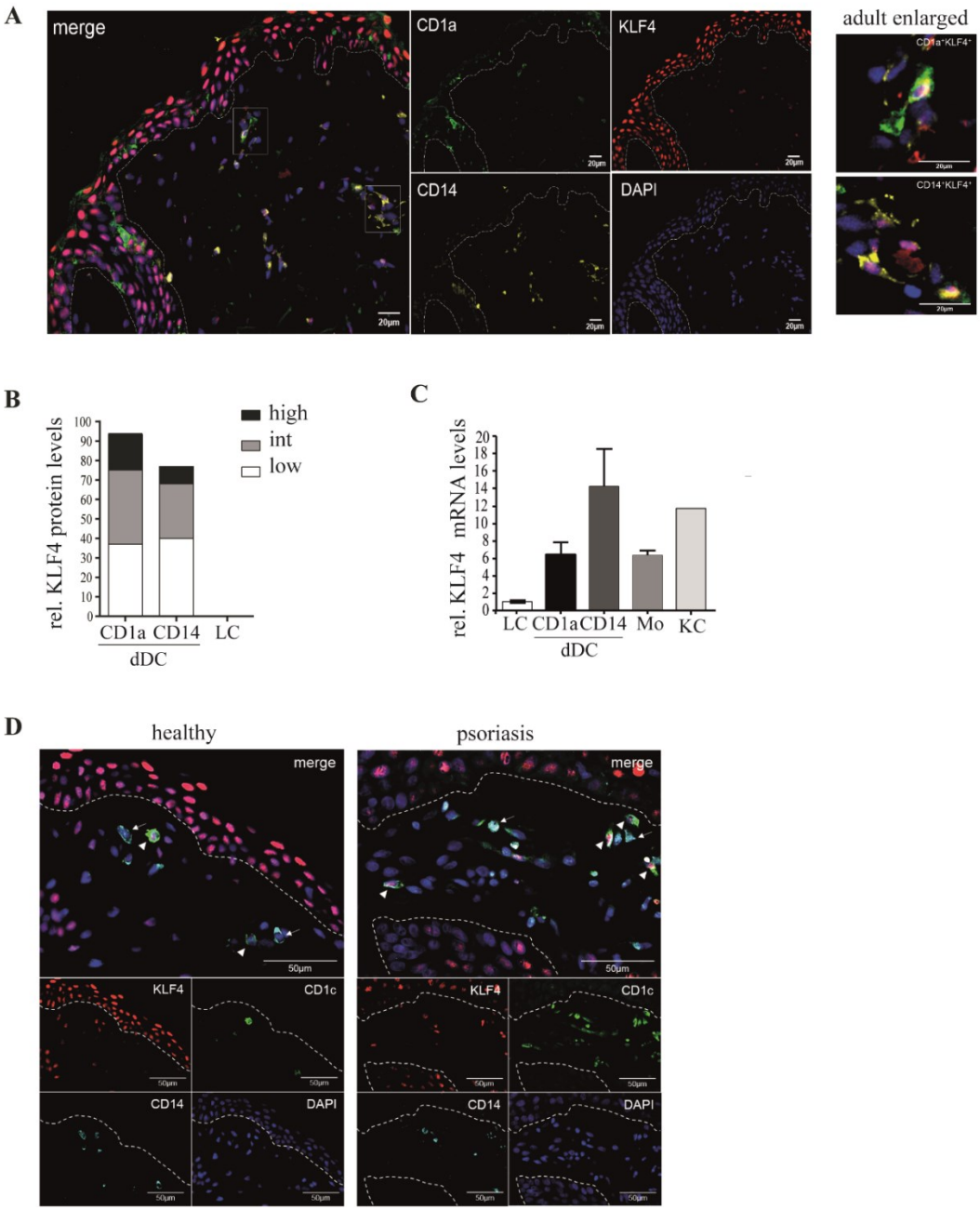

Figure 16: Dermal CD1a$^+$ or CD14$^+$ DCs express varying levels of KLF4:
Sections of paraffin-fixed adult skin were stained for the indicated markers. Nuclei were stained with DAPI and are displayed in blue. (A) Dermal CD1a$^+$ and CD14$^+$ DCs were evaluated for KLF4 expression (Bars equal 20 µm) and (B) graded for KLF4 levels (n=6), (C) KLF4 mRNA levels of LCs, blood monocytes, CD1a$^+$ dDCs or CD14$^+$ dDCs and keratinocytes isolated from healthy adult human skin (n=3). (D) Healthy and psoriatic adult skin samples were compared. Bars 50 µm (parts of figure published in (1)).

4.4. Notch signaling is activated in LCs

Notch activation in response to Delta-1 represses KLF4 as well as monocyte lineage markers CD14 and CD11b in blood monocytes (Figure 12, A). Moreover, Delta-1 is required for TGF-β1- induced moLC differentiation (Figure 12, A). $CD1a^+$ epidermal LCs stain strongly positive for an antibody specific for active intracellular NOTCH1 (aN1) (292,293); conversely they lack KLF4 (Figure 17, A).

Consistently, $CD207^+$ cells generated from monocytes or $CD34^+$ cells are $aN1^+$ but lack KLF4 (Figure 17, B). Inversely, moDCs lack aN1 expression, but are strongly $KLF4^+$. Positive aN1 reactivity of LCs was confirmed using a chromogen-based immunohistochemistry method (Figure 18, A and B) (1).

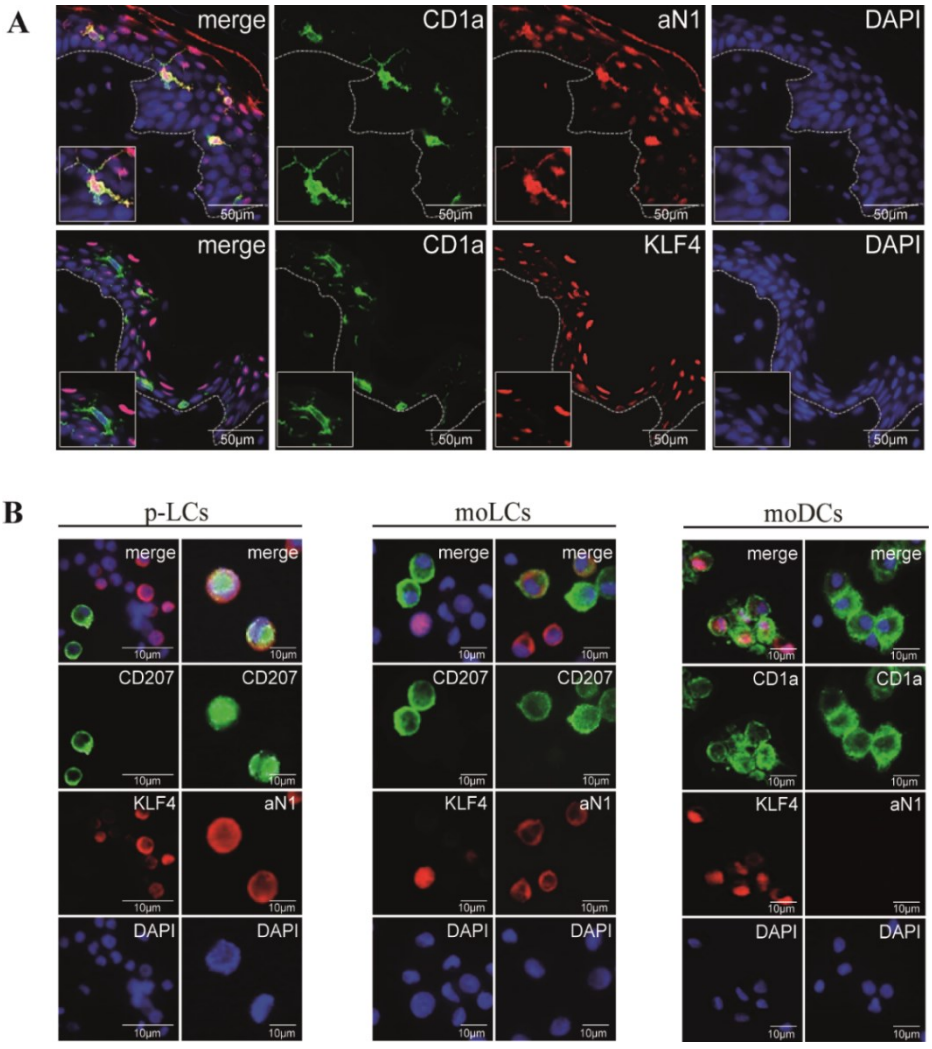

Figure 17: LCs are strongly positive for activated Notch signaling.
(A) Sections of healthy human skin and (B) cytospins of in vitro generated p-LCs, moLCs and moDCs were stained for the indicated markers. Nuclei were stained with DAPI and are displayed in blue. Bars equal 50 µm and 10 µm, respectively (figure published in (1)).

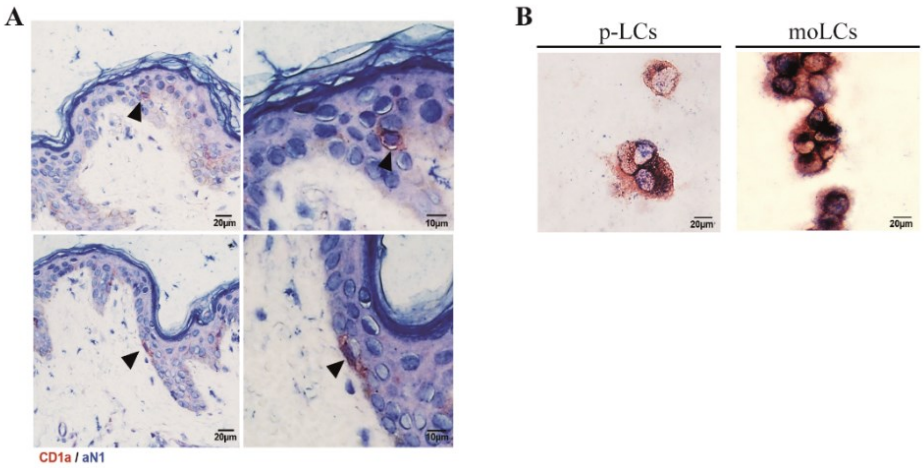

Figure 18: LCs in vivo and in vitro show activation of the Notch signaling pathway.
Immunohistochemical analysis of healthy human skin and in vitro-generated LCs. (A) Paraffin-embedded sections of healthy human skin were stained for CD1a (red) and aN1 (blue); Bars equal 20 µm. (B) p-LCs and moLCs were sorted using anti-CD207 mAb using magnetic beads. Cells were spun on slides, fixed, permeabilized and stained for CD1a and aN1; Bars equal 20 µm (figure published in (1)).

Since $CD34^+$ cell-derived p-LCs develop in absence of exogeneous Notch ligand in vitro, we assumed an endogenous activation of this pathway in these cells. Ingenuity pathway analysis of above- described microarray screen (Figure 11, A) revealed induction of Notch signaling pathway members by TGF-β1 within 6 h. Notch signature gene HES1 was induced along with NOTCH1 and JAG2 ligand, as well as components of the Notch activating γ-secretase complex (PSEN1, PSEN2) (Figure 19, A; Table 8). Consistently, NOTCH1 was induced in response to LC-instructive TGF-β1 signaling in hematopoietic progenitors within 24 h to 72 h (Figure 19, B, upper panel). Moreover, a portion of day 5 p-LC precursors expressed JAG2, which was further up-regulated in day 7 generated $CD1a^+CD207^+$ p-LCs (Figure 19, B, lower panel). Notch signaling occurs via a cell contact-dependent mechanism (294). Consistently, p-LC generation cultures exhibited typical cell clustering involving E-Cadherin adhesion (48,49) and aN1 positivity (Figure 19, C). These characteristics were not observed in GM-CSF/IL-4-dependent p-moDC differentiation cultures irrespective of the presence or absence of TGF-β1 (1).

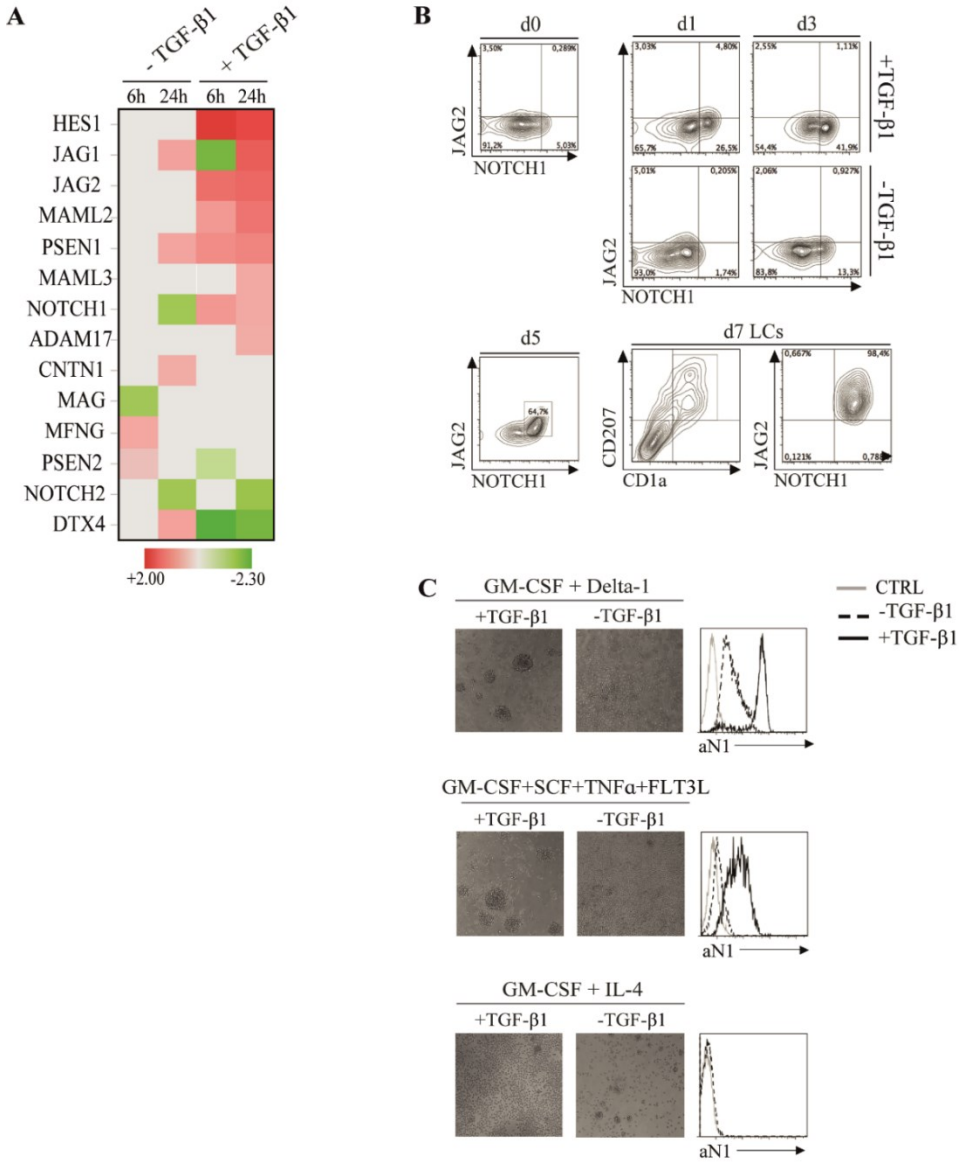

Figure 19: TGF-β1 induces expression of Notch signaling genes.
Microarray data of $CD34^+$ progenitors cultivated +/-TGF-β1 were subjected to Ingenuity Pathway Analysis. (A) Heat map shows TGF-β1-dependent up- or down-regulation of Notch target genes after 6 h and 24 h (n=3). (B) $CD34^+$ progenitors were stimulated with SCF, FLT3L and TPO for 3 d (d 0) and subsequently plated under LC- or monocyte-differentiation conditions (GM-CSF+SCF+TNFα+FLT3L+/-TGF-β1). D 1, d 3, d 5 and d 7 generated cells were analyzed for NOTCH1 and JAG2 surface expression by FACS (n=4). (C) FACS analysis of intracellular active NOTCH1 (aN1) of day 5 moLCs (GM+CSF+Delta+/-TGF-β1), day 7 p-LCs (GM-CSF+SCF+TNFα+FLT3L+/-TGF-β1) and d 5 moDCs (GM-CSF+IL4+/-TGF-β1) (n=4); (figure published in (1)):

Table 8: Ingenuity pathway analysis: Notch pathway genes regulation in TGF-β1-stimulated vs. non-stimulated cultures

Gene symbol	- TGF-β1		+ TGF-β1	
	0 h vs 6 h	0 h vs 24 h	0 h vs 6 h	0 h vs 24 h
HES1	1	1	1.974	1.926
JAG1	1	1.365	-1.975	1.857
JAG2	1	1	1.764	1.814
MAML2	1	1	1.419	1.707
PSEN1	1	1.354	1.522	1.573
MAML3	1	1	1	1.319
NOTCH1	1	-1.341	1.446	1.316
ADAM17	1	1	1	1.3
CNTN1	1	1.304	1	1
PSEN2[§]	1	1	-1.356	1
MAG	-1.305	1	1	1
MFNG	1.342	1	1	1
PSEN2	1.375	1	1	1
NOTCH2	1	-1.367	1	-1.545
DTX4	1	1.374	-2.293	1.994

[§]PSEN2 expression was detected by two Affymetrix probe sets: 204261_s_at for "0 h vs 6 h -" and 211373_s_st for "0 h vs 6 h +"

4.5. Ectopic KLF4 represses LC differentiation from Mo/LC precursors

To investigate whether KLF4 interferes with LC development, $CD34^+$ progenitors were transduced with KLF4-IRES-GFP or empty control vector and subsequently induced to differentiate for 5 days into Mo/LC precursors as schematically shown in (Figure 13, A) (GM-CSF/SCF/FLT3L/TNFα + 10% FCS) (295). Ectopic KLF4 reduced the percentages of $CD1a^+$ cells in favor of $CD14^+CD1a^-$ cells (Figure 20, A), while a KLF4 mutant (KLF4ΔZNF-IRES-GFP), lacking DNA binding activity, but still retaining the trans-activation/ repression domain (160,296), showed the opposite effect (Figure 20, A). Consistently, KLF4 overexpression strongly inhibited, whereas KLF4ΔZNF promoted p-LC generation from Mo/LC precursors (Figure 20, B) (1).

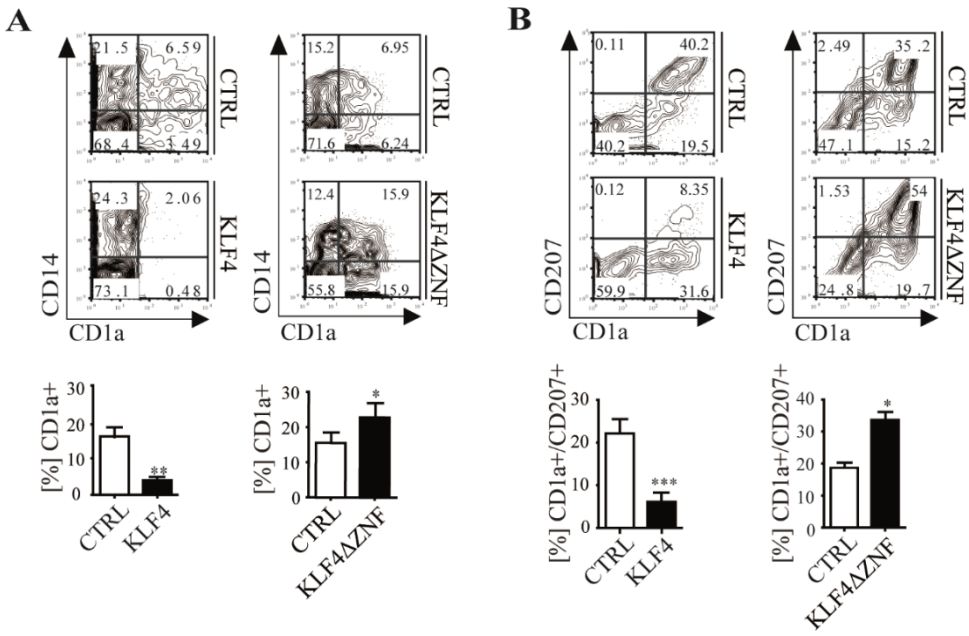

Figure 20: Overexpression of KLF4 interferes with LC differentiation.
$CD34^+$ cord blood progenitors were transduced with KLF4-IRES-GFP, KLF4ΔZNF-IRES-GFP or empty vector CTRL. (A) GFP^+ day 5 progenitors and (B) GFP^+ day 5 progenitors were further cultivated with TGF-β1 and analyzed by FACS for surface marker expression (n=6, *t-test $p<0.05$, **t-test $p<0.01$, ***t-test $p<0.005$); (figure published in (1)).

We next generated day 5 Mo/LC precusors (according toFigure 13, A; FACS plots day 0) and analyzed the consequences of KLF4 overexpression on p-LC differentiation using an inducible retroviral system (Figure 21, A) (280). Expanded progenitor cells were transduced with two retroviral vectors (inducible "tet-on" system (280)) followed by the generation of

DC precursors in primary cultures. Thereafter, cells were sub-cultured under secondary LC generation conditions in presence of doxycycline (DOX) to induce KLF4-IRES-GFP or empty control-GFP (Figure 21, B). FACS analysis of GFP^+ cells was then performed at day 7 in p-LC generation cultures. The induction of KLF4 at the stage of DC precursors was sufficient to inhibit p-LC generation (Figure 21, C). Conversely, ectopic KLF4 expression in $CD34^+$ cells failed to inhibit but rather promoted generation of p-moDCs ($CD207^-CD209^+CD11b^+CD1a^+$ cells, Figure 21, D) (1).

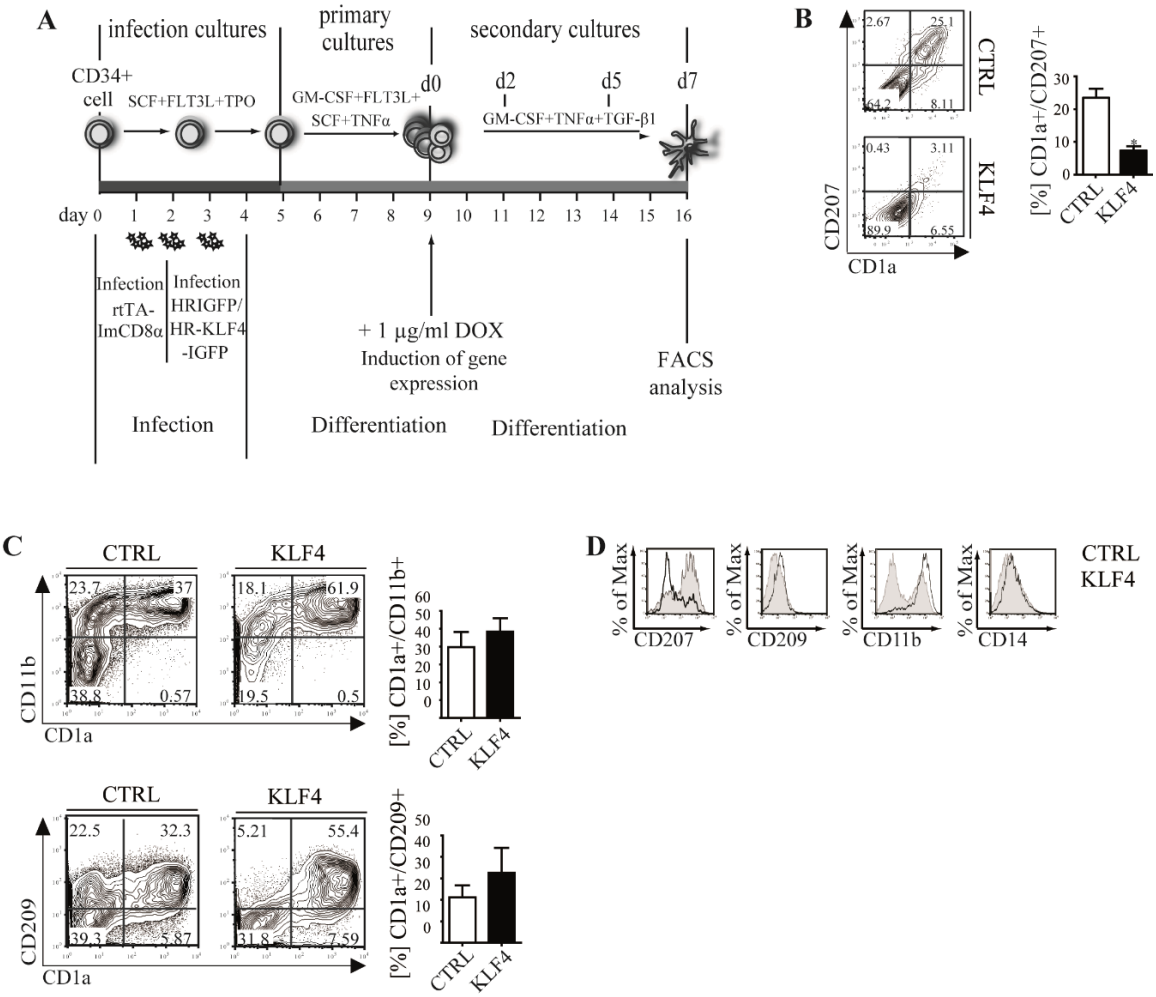

Figure 21: Induced expression of KLF4 promotes moDC differentiation.
(A) Schematic representation of experimental set-up for KLF4 induction. (B, C) Day5 progenitors cultivated according to (A) were sub-cultured under LC-promoting conditions (GM-CSF+TNFα+TGF-β1). GFP^+ cells were analyzed by FACS on day 7 (n=6, *t-test) for CD1a/CD207 or moDC-specific surface markers (C). FACS of day 5 progenitors sub-cultured under p-moDC conditions (n=6). (D) KLF4-IRES-GFP- (grey filled histograms) or CTRL-tranduced (black lines) cells were cultivated +TGF-β1 and analyzed for the indicated surface makers (figure published in (1)).

Apart from phenotypic characteristics, LCs and moDCs also differ in functional properties. p-LCs expressed substantially lower levels of pro-inflammatory cytokines than p-moDCs (Figure 22, A and B), confirming previous observations (68,272,297). KLF4-transduced p-moDCs showed slightly higher mean production levels of pro-inflammatory cytokines relative to controls (Figure 22, B). However, these data did not reach statistical significance in paried t-test statistics. Consistent with the observed repression of KLF4 during LC differentiation, KLF4ΔZNF-transduced p-LCs equaled control transduced cells in low levels of cytokine production (1).

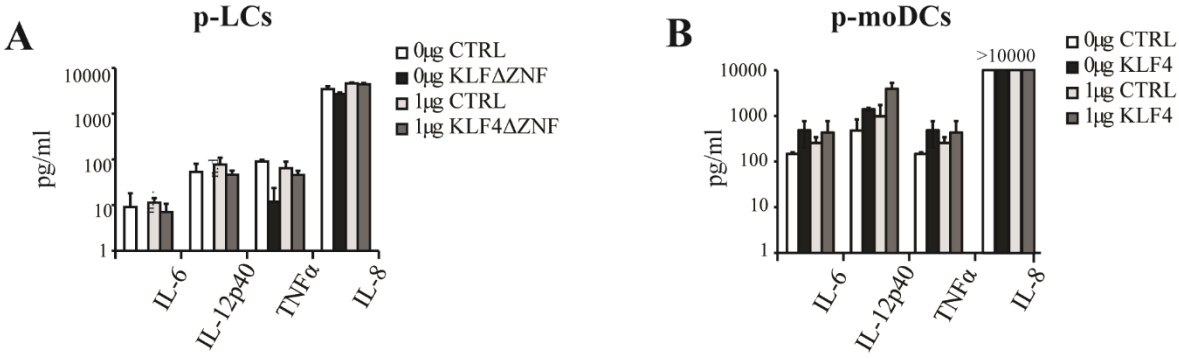

Figure 22: p-LCs exhibit a less pro-inflammatory phenotype.
(A) GFP$^+$CD1a$^+$CD207$^+$ p-LCs or (B) GFP$^+$CD1a$^+$CD11b$^+$ p-moDCs were sorted at d 7 and stimulated for 2 days in presence of 1 µg Pam_3CSK_4 or left untreated (0 µg). Cells were analyzed for the secretion of the indicated cytokines. (n=3, +/-SEM); (figure published in (1)).

4.6. RUNX3 and KLF4 are inversely regulated during LC and moDC differentiation

We next aimed to identify the mechanism underlying KLF4-mediated repression of LC differentiation. Since RUNX3$^{-/-}$ mice lack LCs (135), and RUNX3 is strongly induced concomitant with LC differentiation (Figure 23, A), we addressed whether RUNX3 is involved in KLF4-mediated repression of LC development.

Mo/LC precursors were stimulated under monocyte or LC differentiation conditions (i.e. +/- TGF-β1, Figure 23, A) and monitored in parallel for KLF4 and RUNX3 expression. RUNX3 was induced during during LC differentiation (+TGF-β1), but remained low/ undetectable under monocyte differentiation conditions (-TGF-β1), while KLF4 showed an inverse expression pattern (Figure 23, A). Furthermore, RUNX3 was expressed by moLCs but neither moDCs nor macrophages generated from peripheral blood monocytes (Figure 23, B) (1).

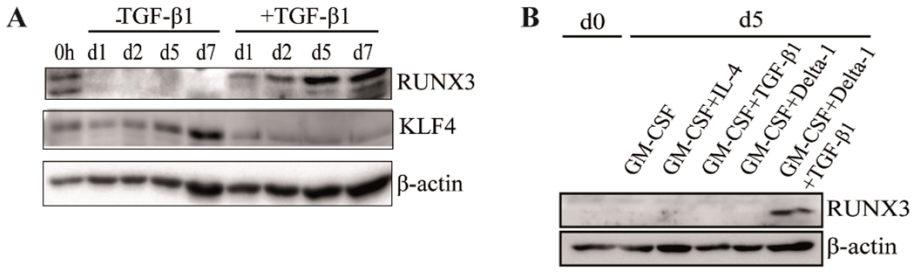

Figure 23: RUNX3 and KLF4 show an inverse expression pattern during moLC/moDC differentiation. KLF4 and RUNX3 protein expression of (A) day 5 p-LC precursors further cultivated +/-TGF-β1 for 7 days and (B) CD14^{+} monocytes stimulated with the indicated cytokines for 5 days (figure published in (1)).

Ectopic expression of RUNX3 in CD34^{+} cells promoted the generation of day 5 p-LC precursors (CD1a^{+} cells) at the expense of monocyte precursors (CD1a^{-}CD14^{+} cells) (Figure 24, A). Accordingly, RUNX3 promoted the generation of CD1a^{+}CD207^{+} p-LCs in secondary cultures in absence or presence of TGF-β1 (Figure 24, B). Furthermore, RUNX3 reduced percentages of CD11b^{+}CD209^{+}CD1a^{+} moDCs generated in the presence of GM-CSF/IL-4 in favour of CD11b^{-}CD209^{-}CD1a^{+}CD207^{+} p-LCs (Figure 24, C and D) (1).

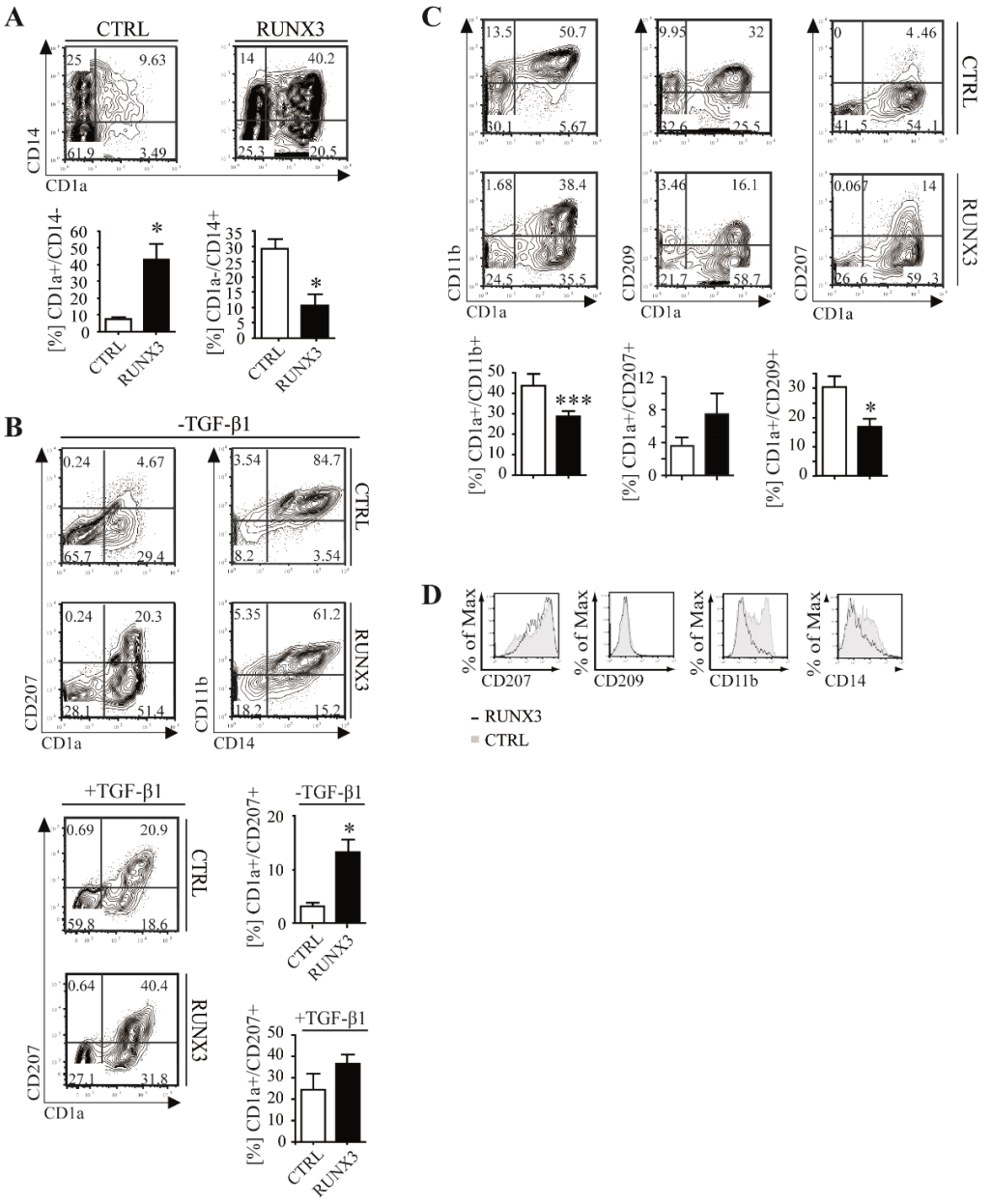

Figure 24: RUNX3 and KLF4 are inversely regulated during LC and moDC differentiation.
CD34$^+$ cells were transduced with RUNX3-IRES-NGFR or empty CTRL vector and induced to differentiate into d 5 precursor cells (A) Representative FACS plots of gated GFP$^+$ cells. Day 5 precursors were stimulated +/-TGF-β1 and analyzed for (B) p-LC or (C) p-moDC phenotype (n=6, *t-test $p<0.05$, ***t-test $p<0.005$). (D) RUNX3-IRES-NGFR- or CTRL-transduced day 5 precursors were cultured +TGF-β1. GFP$^+$CD1a$^+$ cells were analyzed for expression of CD207, CD209 (DC-SIGN), CD11b and CD14 (figure published in (1)).

4.7. Ectopic KLF4 in progenitor cells inhibits TGF-β1-mediated RUNX3 induction and RUNX3 overexpression restores LC differentiation in KLF4-transduced progenitors

We next analyzed whether KLF4 inhibits LC differentiation via repression of RUNX3. Thus, $CD34^+$ cells were transduced with KLF4-IRES-GFP or empty control vector; subsequently, cells were induced to differentiate into Mo/LC precursors in the presence of GM-CSF/SCF/FLT3L and TNFα +10% FCS as schematically shown in Figure 21, A. GFP^+ cells were sorted and then stimulated in p-LC (+TGF-β1) or p-monocyte (-TGF-β1) differentiation conditions. In control transduced cells, RUNX3 protein and mRNA was strongly induced under LC conditions, as expected (Figure 25, A and B). Conversely, TGF-β1-dependent RUNX3 protein and mRNA induction was substantially impaired in KLF4 transduced cells (Figure 25, A and Figure 25, B). In comparison, other TGF-β1- induced genes from the screen, such as TIEG1 and SMAD7, were not repressed by KLF4 (Figure 25, C). Id2 has been described to be essential during LC development (102). Consistently, we observed a slight downregulation of Id2 mRNA by KLF4 overexpression (Figure 25, D). Thus ectopic KLF4 in progenitor cells inhibits TGF-β1-mediated RUNX3 induction under LC-promoting differentiation conditions, but does not generally interfere with TGF-β1 signaling. In line with this, KLF4 binds to the RUNX3 promoter in moDCs, suggesting an inhibitory role on RUNX3 expression (Figure 25, E) (1).

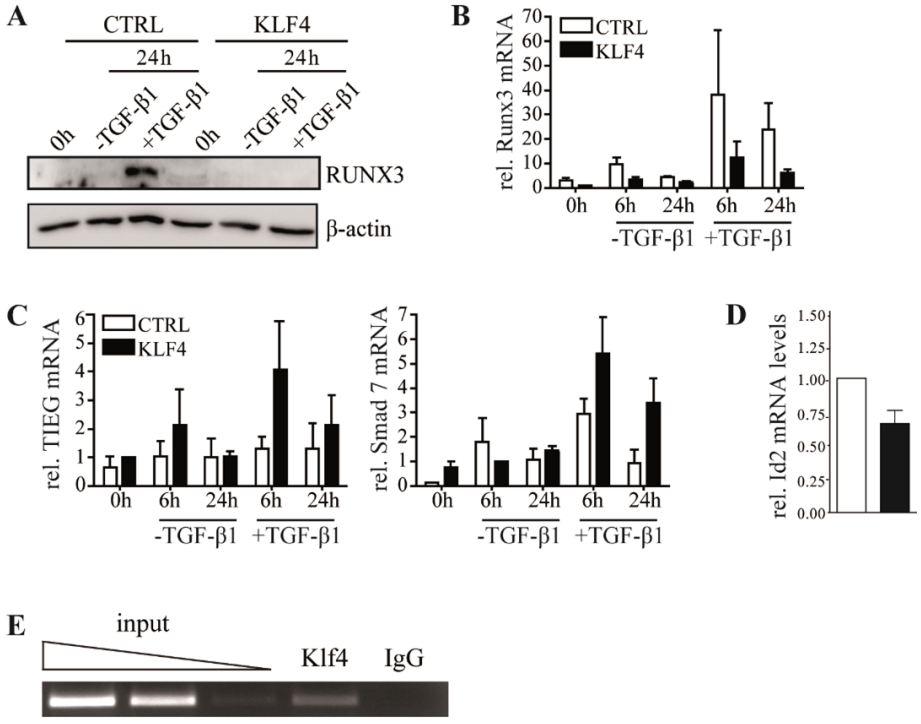

Figure 25: KLF4 represses RUNX3 promoter activity.
(A) RUNX3 protein and (B) RUNX3, (C) TIEG and SMAD7 mRNA expression of GFP$^+$ day 5 precursors cultivated +/- TGF-β1. (D) KLF4-IRES-GFP- or CTRL-transduced cells were cultured + TGF-β1 and sorted day 1 GFP$^+$ cells were analyzed for ID2 mRNA expression by qPCR (n=3, +/- SEM). (E) Semi-quantitative PCR analysis of KLF4 chromatin immunoprecipitation from CD1a$^+$ moDCs (n=3); (figure published in (1)).

We further evaluated whether ectopic overexpression of RUNX3 in progenitor cells could rescue LC differentiation in KLF4-transduced progenitors. Thus, CD34$^+$ progenitor cells were simultaneously transduced with KLF4-IRES-GFP and RUNX3-IRES-NGFR or respective empty control vectors and day 5 generated GFP$^+$NGFR$^+$ Mo/LC precursors were analyzed. Expectedly, ectopic KLF4 inhibited the generation of CD1a$^+$ cells in favor of CD14$^+$CD1a$^-$ cells, while RUNX3 showed an inverse effect (Figure 26, A, FACS and bar diagrams). Notably, ectopic RUNX3 was able to overcome the inhibitory effect of KLF4 on the generation of CD1a$^+$ cells (Figure 26, A, FACS and bar diagrams). In line with this, RUNX3 was furthermore able to re-establish the generation of CD1a$^+$CD207$^+$ p-LCs from KLF4 transduced progenitor cells (Figure 26, B, FACS and bar diagrams),(1).

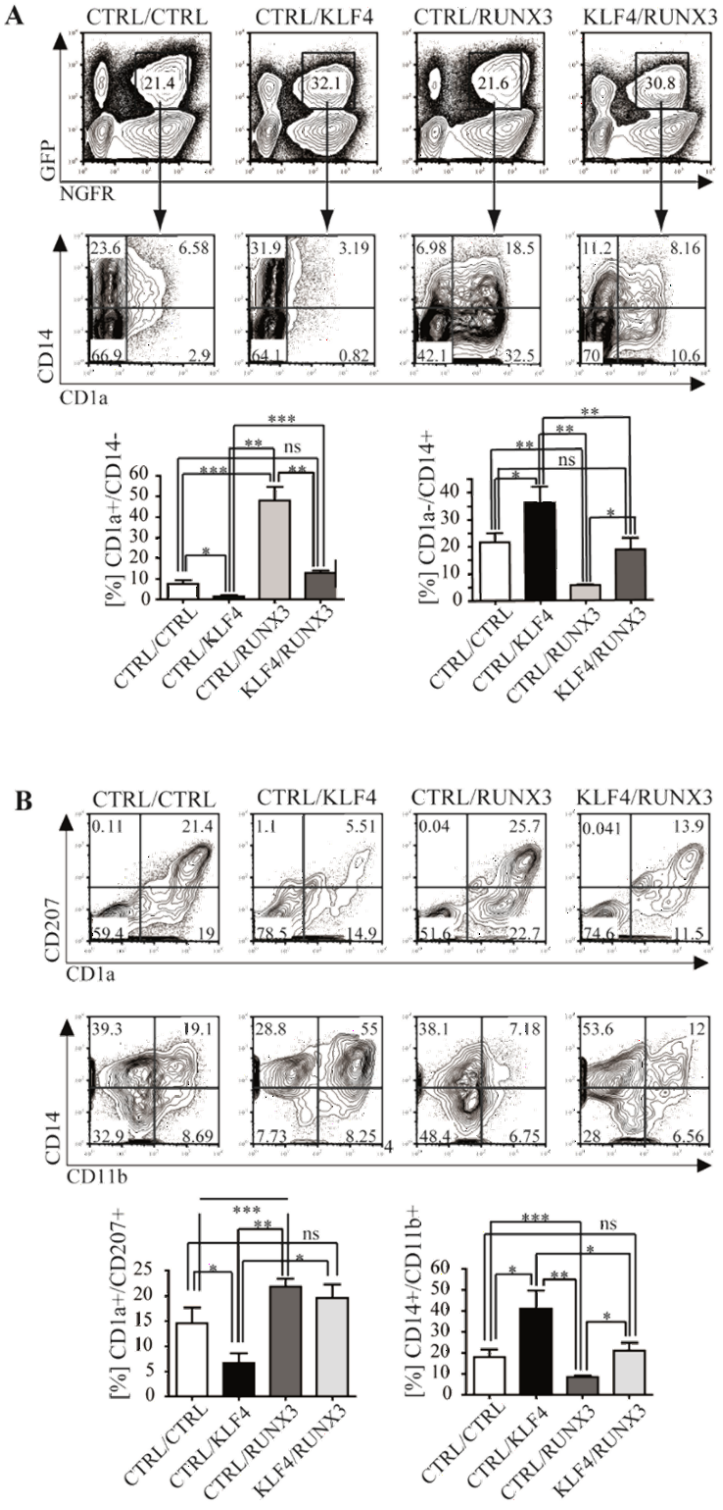

Figure 26: RUNX3 restores LC differentiation from KLF4-transduced progenitors.
$CD34^+$ cells were co-transduced with KLF4-IRES-GFP plus RUNX3-IRES-NGFR vectors or respective empty CTRL vectors. (A) FACS analysis of co-transduced GFP^+NGFR^+-gated day 5 precursors and (B) p-LCs (+TGF-β1) (n=6, *t-test $p<0.05$, **t-test $p<0.01$, ***t-test $p<0.001$); (figure published in (1)).

4.8. Lack of KLF4 in CD1c$^+$ cDCs is indicative for their LC differentiation potential

Human CD1c$^+$/BDCA-1$^+$ cDCs comprise 0.02-0.06 % of cell in the peripheral blood (0.3-0.9 % of leukocytes). Due to this low frequency we isolated cDCs from apheresis products rather than peripheral blood samples of healthy donors along with CD14$^+$ monocytes and CD4$^+$ T cells using magnetic beads (Figure 27, A). Initial removal of CD14$^+$ monocytes followed by CD1c$^+$CD19$^+$ B cell depletion resulted in high purities of CD1c$^+$ blood cDCs (CD1c$^+$CD11c$^+$HLA-DR$^+$CD19$^-$CD14$^-$CD4$^-$) as analyzed by FACS (Figure 27, B).

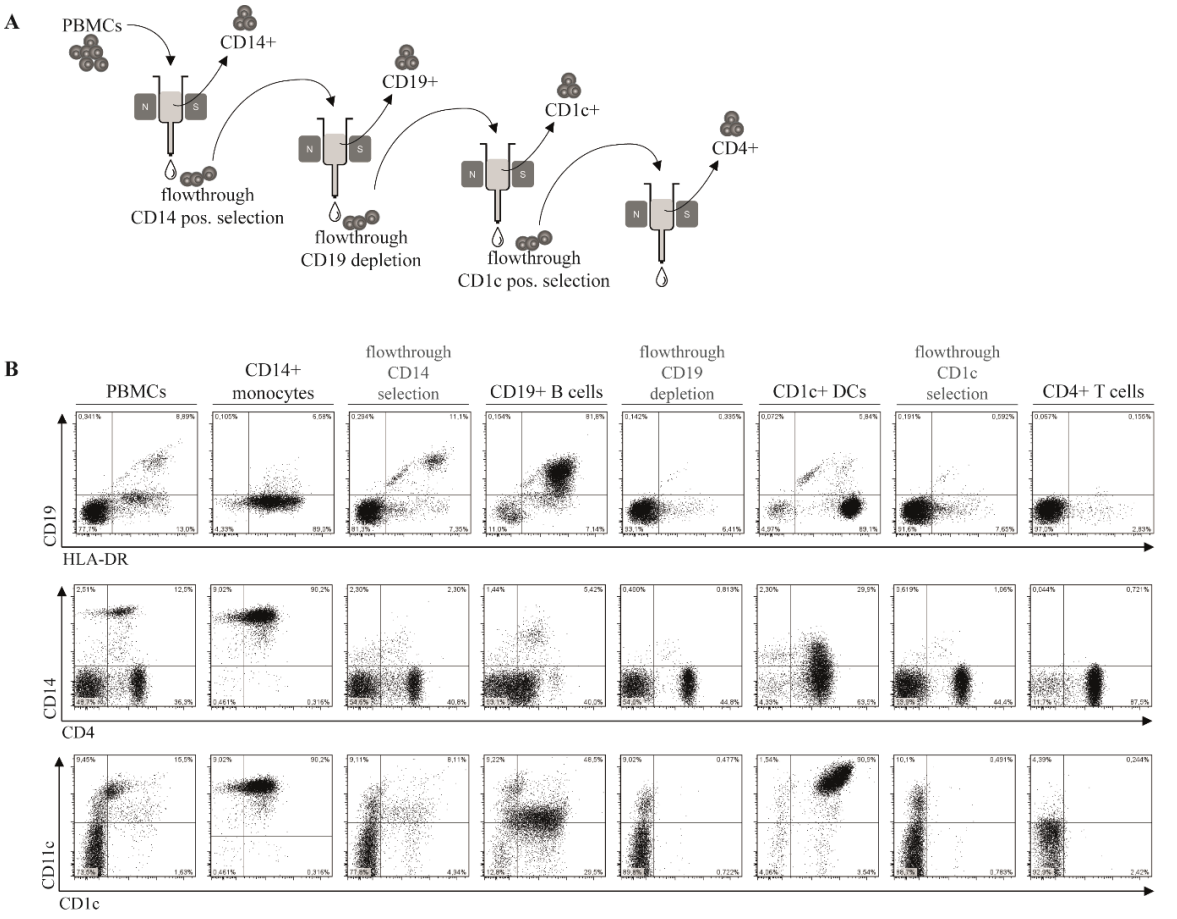

Figure 27: Isolation of CD1c$^+$ cDCs and CD14$^+$ monocytes from buffy coat apheresis products.
(A) Schematic representation of isolation protocol. CD14$^+$ monocytes, CD1c$^+$ cDCs and CD4$^+$ T cells were isolated using magnetic beads. (B) The purity of the individual blood cell type was analyzed by FACS; plots are representative for 5 independent donors.

Unlike monocytes, CD1c$^+$ blood circulating cDCs have been shown to differentiate into LCs in presence of TGF-β1 or BMP7 independent of exogenous Notch ligands (298). As monocytic KLF4 had to be repressed in CD14$^+$ monocytes via Notch activation to allow

for moLC differentiation, we investigated the level of KLF4 expression of $CD1c^+$ cDCs. Immunofluorescence staining revealed absence of KLF4 protein within nuclei of freshly isolated $CD1c^+$ cDCs (Figure 28, A), suggesting these cells to be pre-committed towards the LC linage but not the monocytic lineage. $CD1c^+$ cDCs stimulated with GM-CSF+ TGF-β1/BMP7 differentiated into substantial percentages of $CD1a^+CD207^+$ LCs; immobilized Delta-1 further enhanced the differentiation of LCs by nearly 30 % (Figure 28, B, upper panel). Interestingly, stimulation with only GM-CSF in presence of Delta-1 induced LC-differentiation to higher levels when compared to GM-CSF/BMP7 conditions only. This suggests an intrinsic LC-instructive function of the activated Notch signaling pathway in $CD1c^+$ cDCs, similar to what we observed for $CD14^+$ monocytes (Figure 28, B, lower panel). As mentioned, abrogation of immobilized Delta-1 from moLC cultures did not result in $CD1a^+CD207^+$ cell differentiation (Figure 28, B, lower panel). Presence of Delta-1 in $CD1c^+$ cDC cultures stimulated with TGF-β1 repressed CD11b, whereas in all other conditions CD11b was expressed at substantial levels. (Figure 28, B, upper panel). As we demonstrated that LCs differentiated from monocytes or hematopoietic precursors are KLF4 negative, we also stained $CD1c^+$-derived LCs for KLF4. Indeed, we could verify their LC lineage identity, as no nuclear KLF4 expression could be detected (Figure 28, C).

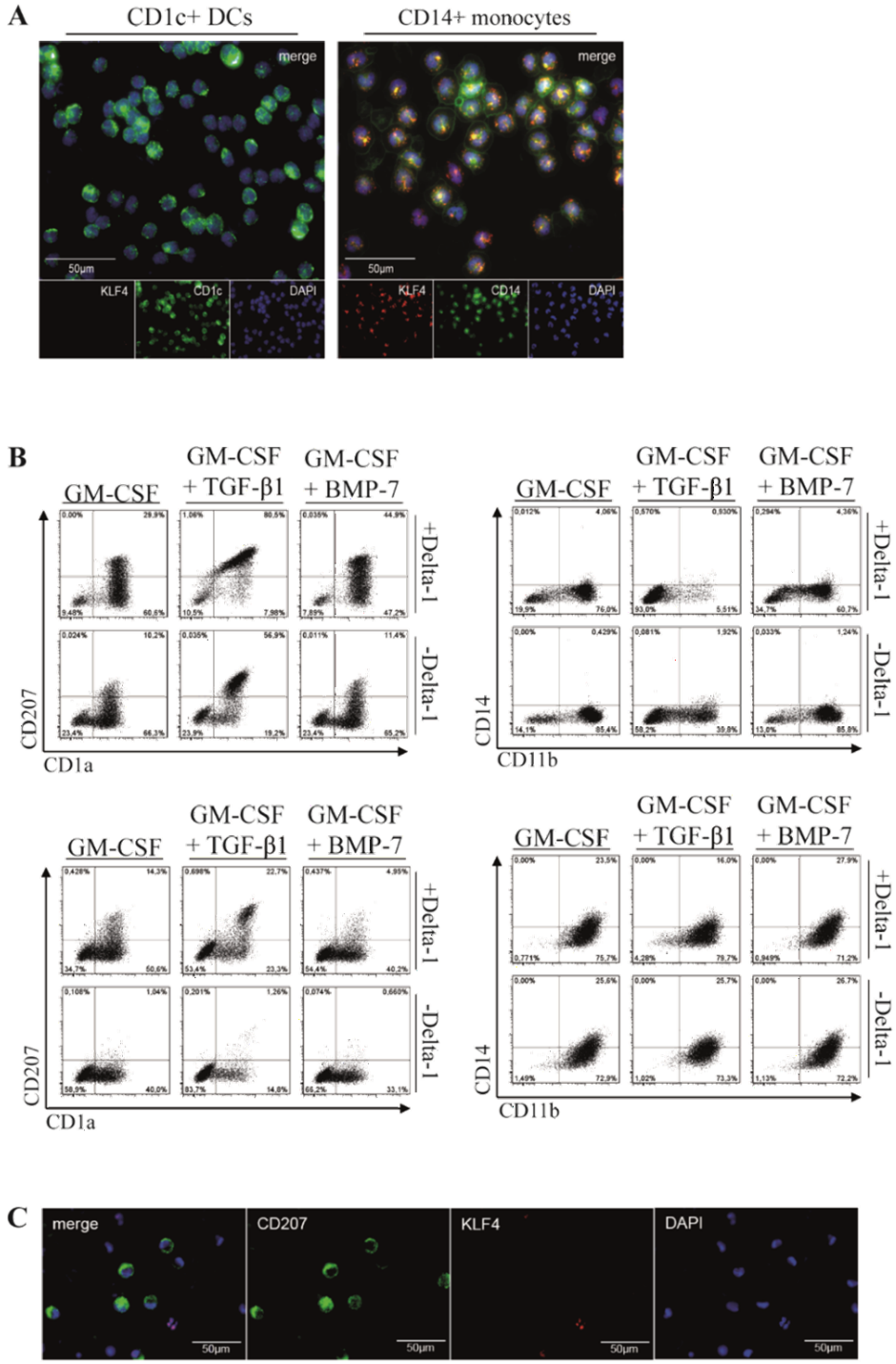

Figure 28: CD1c+ blood DCs are KLF4- and do not depend on activation of Notch signaling.
(A) Cytospins of CD1c$^+$ cDCs and CD14$^+$ monocytes were stained for the indicated markers; Bars equal 50 µm (n=3). (B) Freshly isolated CD1c$^+$ blood cDCs and CD14$^+$ blood monocytes were cultured for 5 days under LC promoting conditions (100 ng/mL GM-CSF, 10 ng/ml TGF-β1) in presence or absence of immobilized Notch ligand Delta-1 and analyzed by FACS for surface marker expression. Plots are representative for 4 independent experiments. C) LCs generated from CD1c+ blood DCs were stained for indicated markers. Bars equal 50 µm. (n=3)

Additionally, CD1c$^+$ cDCs differentiate into LCs in presence of lower concentrations of TGF-β1 when compared to CD14$^+$ monocytes (Figure 29, A). Phenotypically, CD1c$^+$ cDC-derived LCs, moLCs and CD34$^+$-derived p-LCs slightly differed by the appearance of longer

dendrites in case of CD1c$^+$-derived and moLCs, indicative for a higher activation status of these cells (Figure 29, B); in all cultures we observed the LC-characteristic formation of homotypic clusters mediated by E-Cadherin adhesion. Stronger activation would be in line with a potential role of these cells in inflammatory conditions.

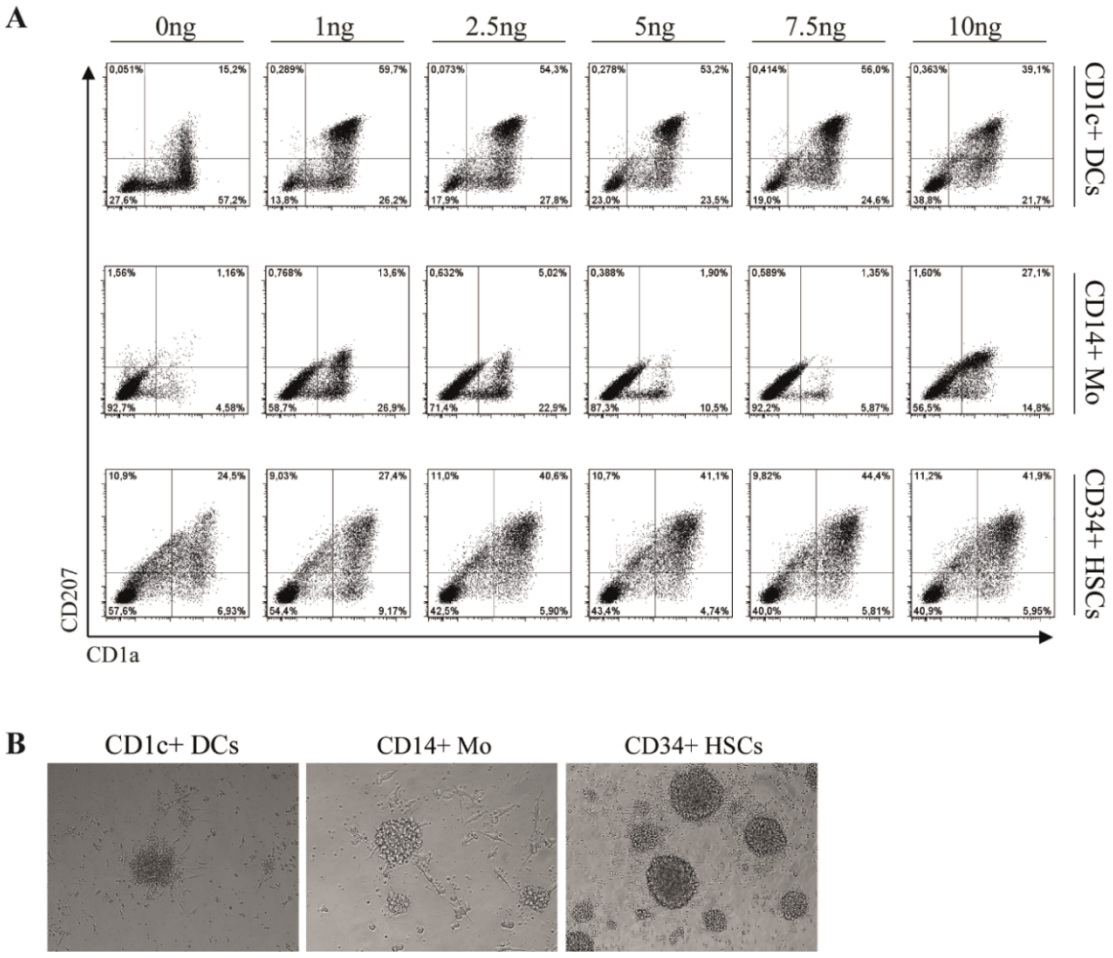

Figure 29: CD1c$^+$ DCs and CD34$^+$ HPCs show similar capactiy to differentiate into LCs.
(A) CD1c$^+$ cDCs, CD14$^+$ monocytes and CD34$^+$ hematopoietic progenitors were induced by different concentrations of TGF-β1 (n=3) (D) LCs generated from CD1c$^+$ cDCs, CD14$^+$ monocytes and CD34$^+$ HPCs were analyzed by light microscopy.

4.9. CD1c$^+$ cDCs can acquire features of moDCs and macrophages

Hypothesizing that CD1c$^+$ cDCs are pre-committed to give rise to LCs, we investigated their potential to differentiate into other cells of the mononuclear phagocyte system. Surprisingly, CD1c$^+$ cDCs also showed potential to develop into interstitial-type DCs/moDCs and macrophages in presence of GM-CSF/IL-4 (Figure 30, A) or M-CSF/IL-6 (Figure 30, B),

respectively. Indeed, $CD1c^+$ cDCs were more potent in differentiating into $CD1a^+CD11b^+CD209^+$ moDCs compared to $CD14^+$ monocytes (Figure 30, B and C). $CD14^+$ derived moDCs expressed higher levels of CD11b but less CD1a compared to $CD1c^+$ cDC-derived moDCs (Figure 30, D). CD209 (DC-SIGN) expression of $CD14^+$-derived moDCs exceeded CD209 expression of $CD1c^+$-derived cells (Figure 30, D). We identified macrophages by surface expression of CD14, CD11b and mannose receptor (CD206). As expected, $CD14^+$ monocyte gave rise to high numbers of macrophages (Figure 30, C). Though $CD1c^+$ cDC-derived macrophage cultures developed into $CD14^+CD11b^+$ cells, expression of CD206 was substantially lower compared to monocytes. Compared to $CD34^+$ hematopoietic progenitors, $CD1c^+$ cDCs displayed higher capacity to give rise to $CD1a^+CD209^+$ moDCs (Figure 30, E). This suggests $CD1c^+$ cDCs being pre-committed towards the DC lineage, but not to the monocyte/ macrophage lineage, though some surface characteristics of macrophages can be induced on $CD1c^+$ cDCs in in vitro cultures. The development of monocytes and macrophages as well as $CD14^+$-derived moDCs was demonstrated to be dependent on KLF4 (162,163). We therefore performed immunofluorescence stainings of moDC and macrophage differentiation cultures (Figure 30, F). $CD14^+CD11b^+CD206^+$ macrophages and $CD1a^+CD11b^+$ moDCs derived from $CD1c^+$ cDCs exhibited nuclear KLF4 expression comparable to $CD14^+$-derived cells. In $CD1c^+$ cDC-derived macrophage cultures not all cells expressing CD14 were stained similarly positive for KLF4. This is in line with the described heterogeneity of $CD1c^+$ blood cDC population (299).

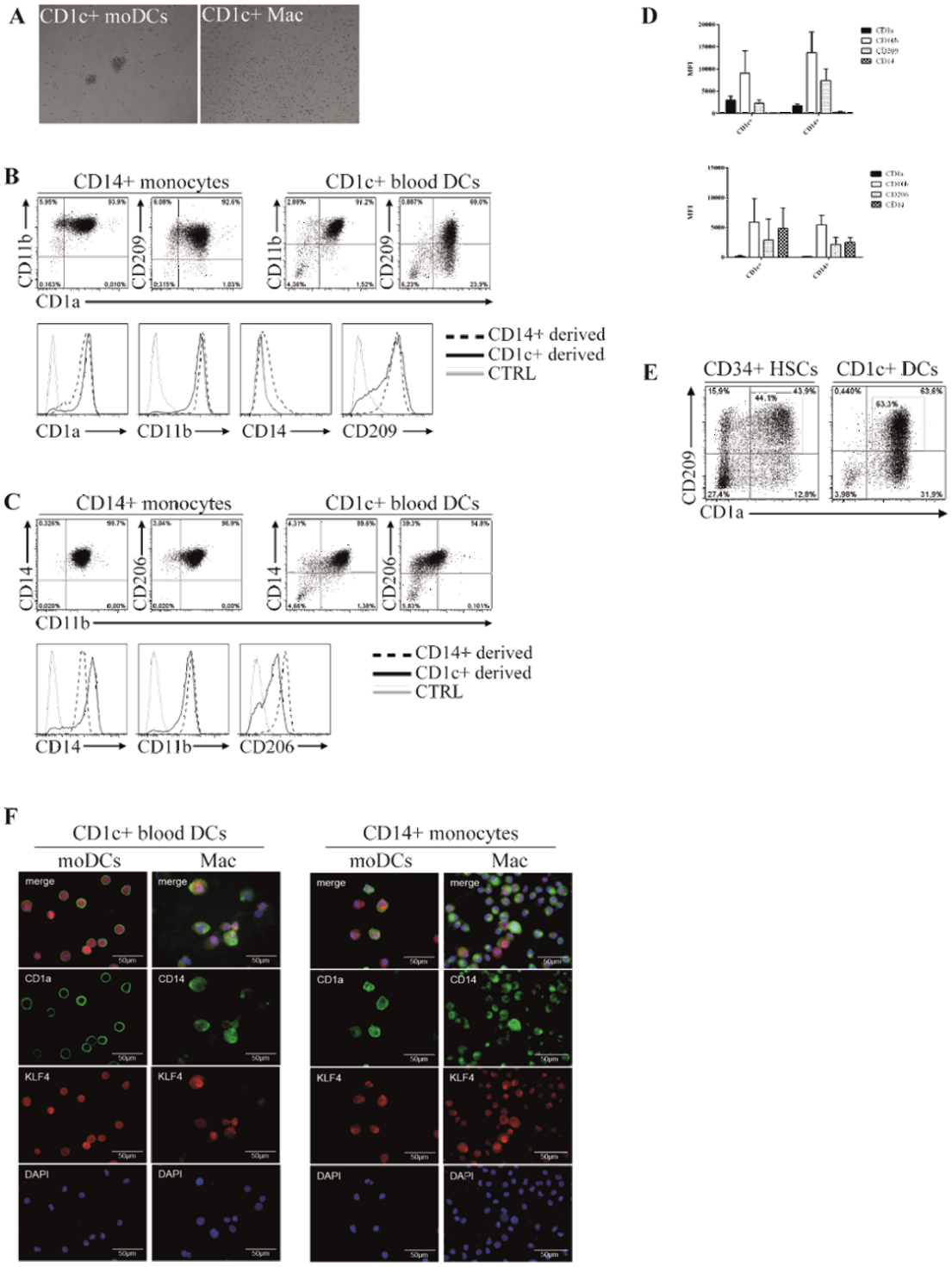

Figure 30: CD1c$^+$ cDCs can differentiate into moDCs and macrophages.
Freshly isolated CD14$^+$ monocytes and CD1c$^+$ cDCs were cultured under (A, B) moDC (GM-CSF/IL-4) or (A, C) macrophage (M-CSF/IL-6) differentiation conditions and (D) analyzed by FACS for surface marker expression (n=3). (E) CD34$^+$ progenitors and CD1c$^+$cDCs were compared for their moDC differentiation potential when cultivated in presence of GM-CSF and IL-4. (F) Cytospins of CD1c$^+$ cDC-derived and CD14$^+$ monocyte-derived moDCs and macrophages (Mac) were stained for the indicated markers, Bars equal 50 µm.

4.10. CD1c$^+$ blood cDCs are a heterogeneous population of CD5^{high} and CD5^{low} subsets differentially expressing CD14

From our differentiation experiments we observed that not all cells within the population of purified CD1c$^+$ cDCs display the same potential to give rise to either LCs, moDCs or macrophages. Previously, CD1c$^+$ cDCs have been described to be comprised of sub-populations of CD5^{hi} and CD5^{lo} cells (299), (Figure 31, B) being enriched for DC-related or monocyte-associated genes, respectively. Interestingly, we observed low CD14 expression by a subset of freshly isolated CD1c$^+$ cDCs (Figure 31, A; purity ~90 %). Based on these findings we investigated whether the CD14$^+$ cells within purified CD1c$^+$ cDCs (Figure 31, C) can be assigned to one of the beforehand mentioned CD5-expressing subsets. Indeed, CD1c$^+$CD14$^+$ cells showed lower expression of CD5 when compared to CD1c$^+$CD14$^-$ DCs (Figure 31, C and D). CD1c$^+$CD5^{hi} cells (~35 % of all CD1c$^+$ cells) displayed a 12-fold higher expression of CD5 compared to CD1c$^+$CD5^{low} cells (~63 % of all CD1c$^+$ cells); CD14 expression was higher on the CD5^{low} subset (3-fold), but negative on the CD1c$^+$CD5^{high} subset (Figure 31, C and D). We did not observe differential expression of CD11b of any subset (data not shown). One might speculate that the subset of CD1c$^+$CD5^{lo} DCs expressing high levels of CD14 preferentially differentiate into moDCs or macrophages, whereas higher CD1c$^+$CD5^{hi}CD14$^-$ cells might be pre-commited towards the LC lineage.

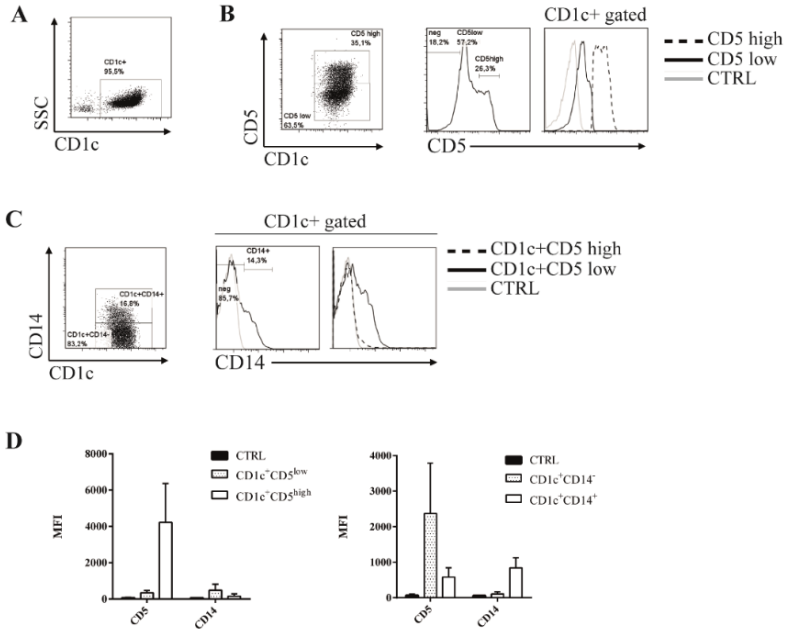

Figure 31: CD1c$^+$ cDCs segregate into CD14$^{+/-}$ and CD5$^{high/low}$ subsets.
(A) CD1c$^+$ cDCs were isolated from buffy coats to high purity and analyzed for (B) CD5 and (C) CD14 expression (n=3) (D) MFIs of CD5 or CD14 expressing CD1c$^+$ DC subsets (+/- SD).

4.11. Immobilized Notch ligand Delta-1 allows *in vitro* generation of CD1c$^+$ cDCs from CD34$^+$ progenitors

As seen for monocytes, the application of genetic manipulation of freshly isolated primary cells is hampered by means of cell viability and susceptibility for viral transfection. Recently it was shown, that CD1c$^+$ cDCs can be differentiated from CD34$^+$ hematopoietic progenitors *in vitro* (221) by use of MS5 stromal feeder cells. As the bone marrow stroma was described to express high levels of Notch ligands being involved in dendritic cell differentiation (300), we used this approach and replaced the described MS5 feeder cells with immobilized Delta-1 for the differentiation of CD1c$^+$ cDCs from CD34$^+$ HSCs. Stimulation of CD34$^+$ cells with GM-CSF/FLT3L/SCF and plate-bound Delta-1 under serum-free conditions resulted in the differentiation of a small population of CD1c$^+$ cDCs (Figure 32, A). As differentiation of LCs in vitro was shown to be enhanced if the initial population of CD34$^+$ hematopoietic progenitors is enriched with CD1a$^+$CD14$^-$ monocytopoeitic precursors (186), we tested if the same strategy could be applied also for the generation of CD1c$^+$ cDCs.

CD34$^+$ cord blood progenitor cells were induced to differentiate into the CD1a$^+$CD14$^-$ and CD1a$^-$CD14$^+$ precursor subsets in presence of GM-CSF/TNFα/FLT3L/SCF and 10 % serum for 5 days and then sorted for CD1a$^+$CD14$^-$ and CD1a$^-$CD14$^+$ cells using magnetic beads targeted either against CD1a or CD14 (Figure 32, B). Both progenitor sub-populations were then incubated under CD1c$^+$ cDC- inducing culture conditions (GM-CSF/FLT3L/SCF and Delta-1, serum-free. FACS analysis revealed that only the CD1a$^+$CD14$^-$ population is potent in differentiating into CD1c$^+$DCs, whereas CD1a$^-$CD14$^+$ cells did not give rise to CD1c$^+$ cells (Figure 32, B).

FACS analysis of *in vitro* generated and freshly isolated CD1c$^+$ cDCs revealed similar expression levels of characteristic markers such as CD11c, HLA-DR and CD80. Expression of CD1a on *in vitro* generated cells was due to GM-CSF supplementation to the culture medium (221) (Figure 32, C). Both populations showed similar capacity in differentiating into CD1a$^+$CD207$^+$LCs (Figure 32, D) when stimulated with GM-CSF and TGF-β1. Interestingly, expression of E-Cadherin (CD324) was higher on LCs differentiated from in vitro-generated CD1c$^+$ cDCs (Figure 32, D) compared to freshly isolated cells.
The *in vitro* generation of CD1c$^+$ cDCs from CD34$^+$ progenitors would allow the application of gene transduction experiments to investigate of the role of different transcription factors involved in their initial differentiation or during the transdifferentiation into LCs/ DCs upon cytokine stimulation.

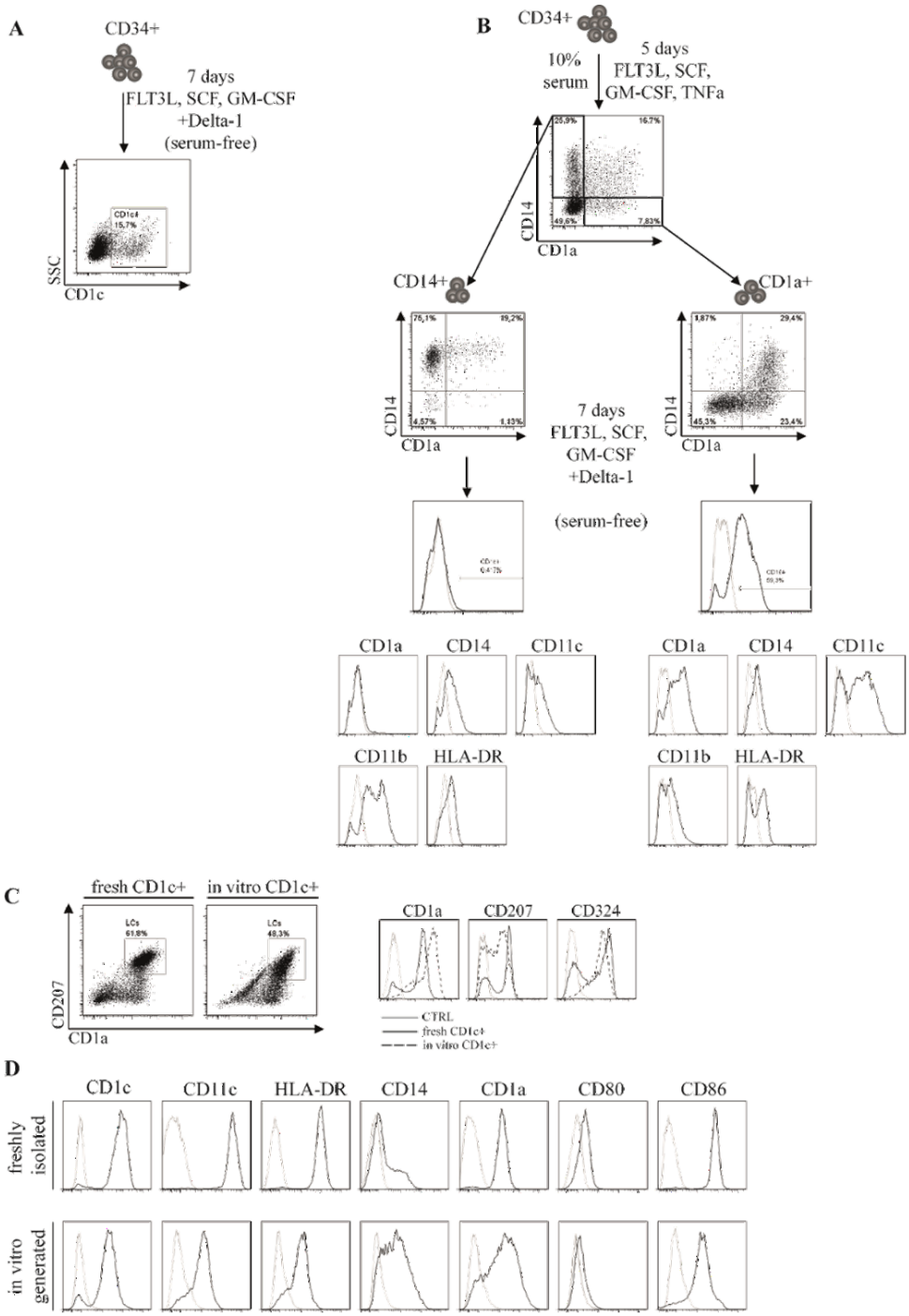

Figure 32: CD1c$^+$ DCs can be generated from CD34$^+$ hematopoietic progenitors:
(A) CD34$^+$ cells were plated for 7 days in presence of 100 ng/mL FLT3L, 20 ng/mL SCF and 10 ng/mL GM-CSF and immobilized Delta-1 under serum-free conditions and analyzed for CD1c expression by FACS. (B) CD34$^+$ cells were differentiated into common monocytic progenitors (100 ng/mL GM-CSF, 50 ng/mL FLT3L, 20 ng/mL SCF,2.5 ng/mL TNFα, 10 % serum) prior sorting of CD1a$^+$CD14$^-$ or CD1a$^-$ CD14$^+$ cells. Isolated CD1a$^+$CD14$^-$ or CD1a$^-$CD14$^+$ cells were further differentiated in presence of 100 ng/mL FLT3L, 20 ng/mL SCF and 10 ng/mL GM-CSF and immobilized Delta-1 under serum-free conditions and analyzed by FACS for expression of CD1c. (C) FACS analysis of freshly isolated CD1c$^+$ cDCs compared to magnetically sorted in vitro generated CD1c$^+$ cDCs. (D) Freshly isolated CD1c$^+$ cDCs from buffy coats and in vitro generated CD1c$^+$ cDCs were differentiated into LCs (+100 ng/mL GM-CSF, 10 ng/mL TGF-β1) and analyzed by FACS. (n=3)

4.12. CD1c$^+$ cDCs are potent in inducing proliferation of T cells

LCs are known to be involved in T cell regulation (58). Upon phenotypic analysis of CD1c$^+$ DC-derived and monocyte-derived LCs we next aimed to determine their functionality compared to CD34$^+$ progenitor derived p-LCs and performed mixed leukocyte reactions (MLR). LCs were generated as described and purified by use of magnetic beads targeted against CD207 prior co-cultivation with naïve CD4$^+$ T cells. Proliferation of T cells was measured by thymidine incorporation. CD1c$^+$-derived cells seemed to be less potent than p-LCs (TGF-β1-LCs) in induction of T cell proliferation though exceeding moLCs (Figure 33) at conditions were higher numbers of LCs were plated. At conditions where lower numbers of LCs were used (i.e. 300-3000 LCs), CD1c$^+$-derived cells appeared to be much stronger inducers of T cell proliferation compared to p-LCs (TGF-β1-LCs) and moLCs. Whether co-cultivation with either LC-type induces Th1 or Th2 cell polarization remains to be elucidated.

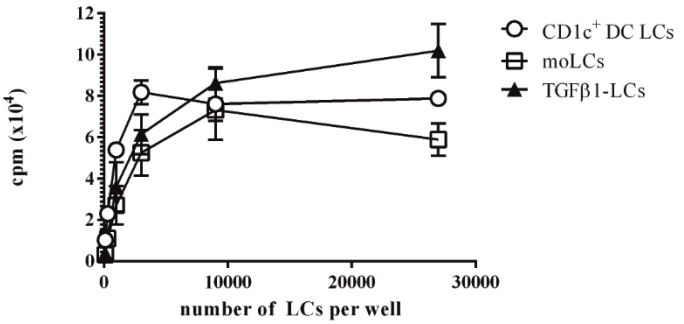

Figure 33: CD1c$^+$-derived LCs are potent inducers of T cell proliferation.
CD1c$^+$ DCs (circles), monocytes (boxes) or CD34$^+$ cord blood progenitors (triangles) were differentiated into LCs as described and co-cultivated with CD4 naïve T cells. Proliferation of T cells was measured via incorporation of ^{13}thymidine (n=3).

5. DISCUSSION

Among all members of the DC family, LCs are unique as they form a tight three-dimensional network in the basal and suprabasal keratinocytes layers in the epidermis, which is mediated by the expression of epithelial type adhesion molecules. Also DCs and LCs have been recognized as being involved in inflammatory skin diseases and are known to function as potent inducers of innate and adaptive immune responses (301). However, the epithelial micro-environmental mechanisms underlying the differentiation of human DCs/LCs in the steady-state and under inflammatory conditions are poorly defined.

Here we demonstrated that Notch signaling-dependent repression of KLF4 is critical for LC commitment from monocytes. Loss of KLF4 enables LC commitment at least partially through de-repression of RUNX3 thereby inhibiting the induction of the LC differentiation program in monocytes. Moreover, we identified KLF4 as a key switch factor regulating differentiation of monocytes into LCs vs. moDCs/dDCs. Unlike LCs, moDCs and dDCs express KLF4 (Figure 34). Our study highlights the role of signals within the epidermal/epithelial microenvironment for instructing LC commitment of monocytes and sheds light on the known pleiotropic effect of TGF-β1 on differentiation and activation of the monocyte/macrophage and DC system (1).

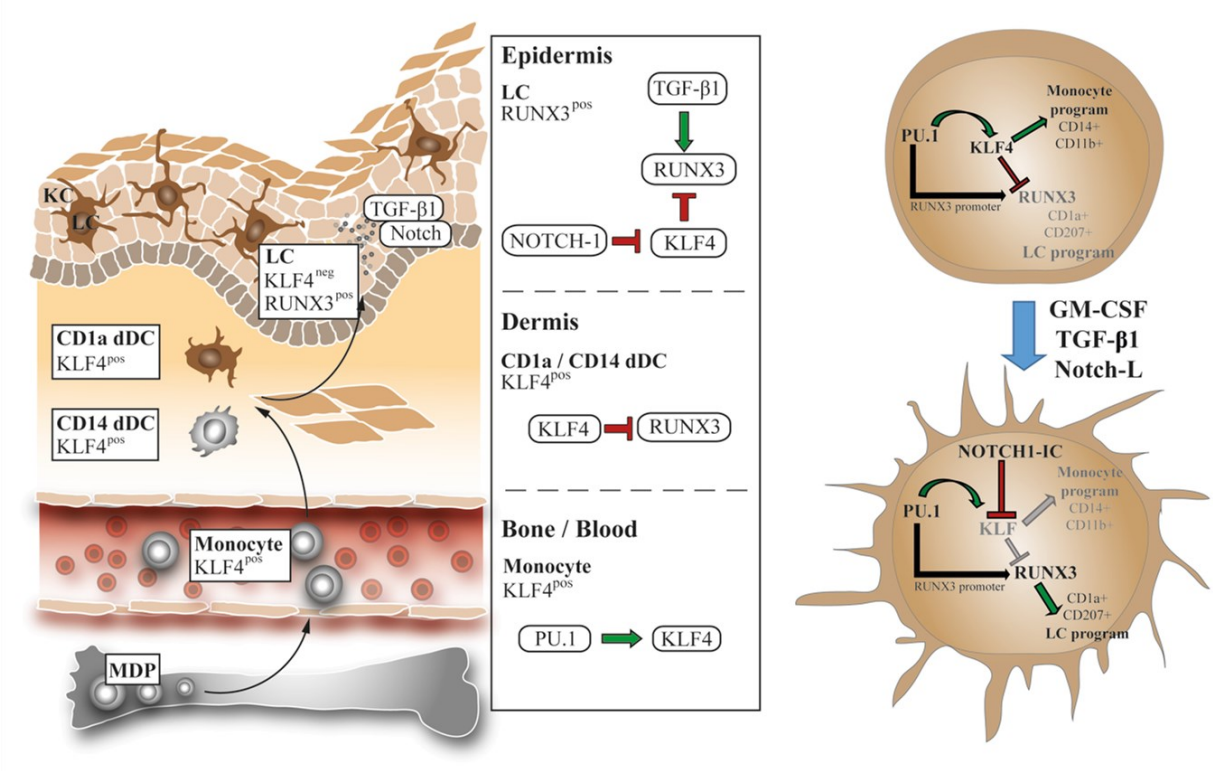

Figure 34: KLF4 acts as switch factors regulating monocyte-to-LC differentiation.
Monocytes differentiate in the bone marrow from common monocyte/ DC precursor cells (MDP). Upon recruitment to the skin, KLF4^{+} monocytes can give rise to KLF4^{+} dermal DC subsets (CD1a^{+} dDCs or CD14^{+} dDCs) retaining KLF4 expression. In the epidermis, keratinocytes provide LC-instructive TGF-β1 and express Notch-ligands which bind to Notch receptors expressed on monocytic cells. The synergistic action of the TGF-β1 and Notch signaling pathways induces transdifferentiation of blood-borne monocytes into LCs (left panel). On the transcriptional level, PU.1 functions as master regulator. In monocytes PU.1 induces KLF4, which in turn inhibits LC-instructive RUNX3 expression, thereby promoting expression of CD14 and CD11b. However, the activation of the Notch pathway causes repression of KLF4. Notch activation in concert with TGF-β1 -mediated induction of RUNX3 via PU.1 turns the differentiation pathway towards the LC lineage marked by the induction of CD1a and CD207 expression (right panel); (parts of figure published in (1)).

KLF4 is a monocyte lineage identity factor known to promote monocyte commitment of myeloid progenitors (162). As such, KLF4 induces a set of monocyte lineage-associated molecules. Among these, CD11b is well-known as a critical marker for classifying DC subsets. Epidermal LCs *in vivo* lack CD11b, whereas it is strongly expressed by *in vitro*-generated moDCs. Also, high levels of CD11b are a hallmark of IDECs, representing putative monocyte-derived dendritic cells in atopic dermatitis/ eczema lesions (302). Additionally, CD11b is expressed by the two major subsets of dermal DCs (CD1a^{+} dDCs, CD14^{+} dDCs). We detected KLF4 in dDCs and in *ex vivo*-generated moDCs, which were previously shown to be phenotypically similar to IDECs (302).

The previous observation, that ectopic wild-type KLF4 induces CD11b in human myeloid progenitor cells (162) is therefore supportive to the here observed positive correlation between KLF4 and CD11b among skin DC subsets. Consistently, stage-dependent loss of LC but not moDC differentiation potential during successive steps of

monocyte/macrophage differentiation marked by CD11b up- and CD14 down-regulation was previously noted in cultures of $CD34^+$ precursors (303). Our observation that KLF4 promotes $CD11b^+$ moDC/dDC differentiation is reminiscent oF previously observed functions of RelB (154). RelB promotes $CD11b^+$ moDC/dDC differentiation from human $CD34^+$ progenitors by inducing the generation of monocytic $CD11b^+$ intermediates. Inhibition of RelB by the use of a truncated version of RelB interaction partner p100 caused arrest of $CD34^+$ HSC differentiation in a $CD11b^-$ myeloid cell stage retaining LC differentiation capacity (154). Interestingly, $CD11b^+$ classical DCs dependent on KLF4 or RelB have also been described in the murine system (304,305).

Here we describe epithelial Notch activation-dependent KLF4 repression, which might allow LC differentiation from monocyte-committed cells under inflammatory conditions (Figure 34) (1). Human $CD14^+$ dDCs express KLF4; these cells can reconstitute LCs *in vivo* (69). Similarly, murine $Gr1^{hi}$ monocytes are dependent on KLF4 (163) and can reconstitute LCs *in vivo* (200). We also detected KLF4 in fetal dermal HLA-DR^+ cells, opening the possibility that such cells might give rise to LCs in prenatal epidermis. Reminiscent of similar observations in epithelial cells (306), here we demonstrate that TGF-β1 induces Notch signaling pathway members in monocytopoietic cells undergoing LC commitment. Moreover, cleaved NOTCH-1 was previously shown to bind to a specific site within the KLF4 promoter (307) and antagonism between KLF4 and Notch signaling has been documented in non-hematopoietic cells (307). Additionally, KLF4 has been demonstrated to inhibit cell proliferation in the intestinal epithelium (308). Notch signaling has previously been shown to be up-regulated in intestinal tumors (309,310). The expression of KLF4 seems to be increased by inhibition of the Notch signaling pathway in murine goblet cells, counteracting proliferation and thereby acting as a potential mediator for the effect of Notch inhibitors (308).

It has previously been shown, that KLF4 can inhibit the negative effect of TGF-β1 on pro-inflammatory cytokine expression (160,311). Since it has been demonstrated that LCs can exert a tolerogenic function by the induction of regulatory T cells (312–316), lack of KLF4 by LCs might indicate their involvement in tolerogenic functions. We demonstrated here, that TGF-β1-dependent LC differentiation is marked by the repression of KLF4 (1). Nevertheless, KLF4 levels further increased upon TGF-β1 addition to GM-CSF dependent macrophage cultures. These data are reminiscent of previously observed positive effects of TGF-β1 in KLF4 expression in non-hematopoietic cells (311,317). Our study confirms previous observations claiming that the Notch ligand Delta-1 facilitates DC differentiation

from monocytes by inhibiting default macrophage differentiation. Delta-1 alone repressed KLF4 in monocytes along with the induction of certain DC characteristics, such as CD1a expression. A portion of cells generated under these conditions still expressed low levels of CD14. It is interesting to speculate that these cells represent intermediate stages of LC differentiation from monocytes. Downregulation of KLF4 through Delta-1 in these cells might allow TGF-β1-induced terminal differentiation of LCs within the epidermis. KLF4 down-regulation is not generally required for skin DC development because, unlike LCs, dDCs and moDCs express KLF4. Congruent with this, keratinocytes in the TGF-β1-rich environment of the epidermis exhibit a strong nuclear KLF4 expression pattern (1). Thus the effect of TGF-β1 on KLF4 expression seems to be largely influenced by the cellular context. Cultivation of monocytes in presence of immobilized Notch-ligand Delta-1 and GM-CSF caused inhibition of their differentiation into macrophages (318); addition of TNFα to these cultivation conditions allowed enhanced monocyte-to-DC differentiation (318). Combination of Delta-1 with M-CSF was determined to induce apoptosis of monocytes, whereas Delta-1/Notch signaling together with GM-CSF appeared to protect from cell death (318). This suggests that the type of cytokine present at the time of Notch activation also strongly influences cell fate decisions. Decisions of cell fate such as for macrophages or DCs are generally made in specific microenvironments assigning a major regulatory role to cyokines present in the respective niche of each particular cell type. In line with this, keratinocytes in human epidermis have been observed to express different levels of Notch ligands depending on the layer the reside in, with the highest level of ligand expression being concentrated at those regions where stem cells reside (319); via immunofluorescence stainings we detected higher numbers of LCs in those regions site enriched of LCs/LC precursors.

RUNX3-deficient mice lack LCs and similarly, LCs are missing in mice in which RUNX3 has been conditionally deleted in $CD11c^+$ cells (134). The here presented data support the concept that RUNX3 is a critical positive regulator of LC differentiation (1). In addition to RUNX3, LC development *in vivo* requires ID2 (130). Similar to RUNX3, ID2 is induced concomitant with LC differentiation downstream of TGF-β1. However, unlike observed for RUNX3, ectopic ID2 failed to promoted LC differentiation (124). Instead, ID2 repressed monocyte development, opening the possibility that ID2 is involved in narrowing lineage options of shared monocyte/LC precursors in response to TGF-β1 signaling. Analysis of *in vitro* differentiation of murine BM precursors revealed the existence of subsets with differential capacity to give rise to LCs (134). These subsets were identified

based on the expression of CD205 (DEC205) and EpCAM upon 3 d stimulation with LC-instructive TGF-β1. $DEC205^{+}EpCAM^{+}$ and $DEC205^{-}EpCAM^{+}$, the latter further differentiating into $DEC205^{+}EpCAM^{+}$ cells, showed highest expression of genes associated with LCs (ID2, RUNX3, IRF4), but lowest expression of genes associated with monocytes (e.g. KLF4) (134). This correlates with the described existence of LC-restricted and monocyte-restricted precursors in *in vitro* cultures of human $CD34^{+}$ hematopoietic progenitors (186,273,320). Conditional deletion of ID2 resulted in an almost complete absence of epidermal $CD11c^{+}$ LCs, whereas neither subset of $Ly6C^{hi}$ or $Ly6C^{lo}$ monocytes was affected mice (134). This is in line with the demonstration that a short-lived subset of inflammatory LCs is developing from monocytes independently of ID2 (133). However, the development of both, steady-state LCs and inflammatory moLCs, is strictly dependent on PU.1 (133,134). This suggests that depending on the progenitor and the physiological conditions, different transcriptional networks regulate the development of LCs.

Our screen identified additional factors induced during LC differentiation which might also be involved in a transcription factor network regulating LC differentiation, e.g. VDR (321), as well as DEC1, DEC2, Blimp-1 and PPARδ (1).

Blimp1, encoded by the PRDM1 gene, plays an important role in the development/proliferation of antibody-secreting plasma cells and is implicated as a possible factor in autoimmune diseases, such as systemic lupus erythematosous (SLE) (322). Recently, it was shown that KLF4, being strongly expressed in moDCs but absent in B cells, is involved in regulating Blimp1 expression in patients assigned to SLE risk factor polymorphisms (323). They could show that the level of KLF4 expression in SLE-associated moDCs inversely correlates with the level of Blimp-1 expression. SLE is characterized by defective clearance of apoptotic cells, which is normally mediated by the TAM (Tyro3, Axl, Mertk) receptor tyrosine kinase family. TAM family members inhibit innate inflammatory responses of DCs thereby aiding in the prevention of lupus-like autoimmunity (324). One of the TAM receptors, Axl, has been identified to be induced in response to TGF-β1 during LC differentiation (325) from human hematopoietic precursors, but being absent from GM-CSF/IL-4-dependent moDCs. Axl is suggested to exhibit an anti-inflammatory role, as TAM-deficient mice show a marked impairment of the epidermal LC network along with severe spontaneous inflammation of the skin. This suggests TGF-β1 mediated Axl expression on LCs to be involved in maintaining a tolerogenic environment. (325).

It was demonstrated that TGF-β1 mediated LC differentiation from $CD34^+$ progenitors *in vitro* is accompanied by the induction of β-catenin; ectopic overexpression in these cultures even enhanced $CD1a^+CD207^+$ LC development (326). KLF4 has been shown to interact with β-catenin and to repress β-catenin-mediated gene expression (327) via inhibition of binding of β-catenin to its co-activator p300/CBP (328,329). Additionally, β-catenin specifically interacts with VDR during LC differentiation (321), whereas moDC differentiation is associated with down-regulation of VDR (330). Interestingly VDR was demonstrated to be a direct downstream target of KLF4 (331). Considering the strong expression of VDR ligand (1,25VD3), Notch ligands, β-catenin and TGF-β1 of epidermal keratinocytes, the concerted interplay of these signaling pathways seems to be responsible for the induction of the LC differentiation programme from monocytes. Immigration of monocytes into the epidermis induces activation of the Notch pathway upon interacting with Notch ligands, which leads to KLF4 repression. Repression of KLF4 de-represses β-catenin, allowing its interaction with VDR which is further enhanced by TGF-β1 finally allowing LC-instructive gene transcription.

As seen from our microarray screen (1), E-Cadherin is strongly induced in response to TGF-β1 stimulation during *in vitro* LC differentiation(52). Expression of E-Cadherin is responsible for the formation of the characteristic homotypic clusters formed in *in vitro* LC cultures (52). We observed activation of the Notch signaling pathway upon 4 days of *in vitro* cultivation, representing the time-point where E-Cadherin mediated cluster formation is observed. This is in line with the suggestion that $CD34^+$ progenitors endogenously activate Notch signaling via cell-cell contact, whereas monocytes require spatial interaction with keratinocytes. We could not detect aN1 in *in vitro* generated moDCs or dermal $CD1a^+$ or $CD14^+$ dermal DCs *in situ*; this is in line with the absence of E-Cadherin expression of dermal/interstitial DCs developing independently of TGF-β1 (154).

We demonstrated that $CD14^+$ monocytes can efficiently be differentiated into $CD1a^+CD207^+$ moLCs in presence of immobilized Notch ligand Delta-1 and TGF-β1 (1). However, replacement of TGF-β1 by BMP7, another TGF superfamily member, did not allow moLC generation independent of exogenous Notch activation. An interactive function of the Notch and TGF-β1 signaling pathways was recently suggested from murine myoblasts. It was shown that, the NICD interacts with TGF-β1 downstream effector molecules Smad3 and Smad4 thereby enhancing Notch downstream target HES-1; i.e. TGF-β1 signaling potentiates Notch signaling (332). This is in line with the finding, that Notch signaling is dosage dependent and might lead to different outcomes of cell fate decisions

depending on the strength of the signal and the cell type (174); i.e. potentiation of the Notch pathway by LC-instructive TGF-β1 turns on the LC differentiation machinery but does not permit monocyte commitment. As mentioned, BMP7 in combination with Notch activation does not allow monocyte-to-LC conversion. This might be attributed to the induction of different effector molecules in the BMP7 signaling pathway; i.e. Smad1/5/8 do not interact with the NICD and the LC differentiation programme cannot get induced under these conditions.

$CD34^+$ progenitors have been shown to effectively differentiate into LCs in presence of BMP7 (212). Canonical TGF-β1 signaling occurs via ALK5; however it was demonstrated that TGF-β1 can also induce its signaling via the alternative canonical BMP7 receptor, ALK3 (212). Additionally, the superfamily of ALK receptors is subject to alternative splicing (333); i.e. different isoforms can be expressed on different cell types rendering the target cells more or less sensitive to ligand stimulation

TGF-β1 and BMP7 show an opposite expression pattern in adult healthy skin: TGF-β1 is mainly detectable suprabasally, whereas BMP7 is highly expressed in the basal layers of epidermal keratinocytes (212). Additionally, other BMPs, such as BMP2, BMP4 and BMP6, have been shown to be expressed in the epidermis (334). Indeed, BMP4 was shown to replace BMP7 in *in vitro* LC differentiation cultures from $CD34^+$ precursors; BMP2 or BMP6 did not allow LC development (212). Notch signaling was described to interact with BMP4 in murine muscle cells (335); i.e. BMP4 induced the expression of Notch downstream targets HEY-1 and HES-1. Interestingly, human $CD1c^+$ blood DCs differentiate into LCs without exogeneous Notch ligand (i.e. GM-CSF plus either TGF-β1 or BMP7 (298)). One might suggest a higher expression of ALK3 on $CD1c^+$ cDCs compared to monocytes, explaining their responsiveness to stimulation with BMP7; however, the expression levels of ALK3 or ALK5 on blood circulating monocytes or $CD1c^+$ cDCs remain to be determined.

Martinez-Cingolani et al (336) demonstrated that simultaneous stimulation of $CD1c^+$ blood DCs with TGF-β1 and thymic stromal lymphopoietin (TSLP) efficiently drives LC differentiation. TSLP is an epithelial cell-derived cytokine playing a critical role in inflammation as it causes activation of blood circulating and tissue-resident DCs (337). TSLP is strongly produced by keratinocytes during atopic dermatitis, whereas DCs express high levels of the receptor (TSLPR); ligand binding induces the NFκB pathway leading to the induction of a Th2 response (338,339). LCs generated from $CD1c^+$ DC in presence of TLSP and TGF-β1 displayed high expression of skin homing receptors; CCR2 is highly

expressed on CD1c$^+$ DCs, whereas CCR6 is induced upon stimulation with TSLP (336). CCR2 has been demonstrated to be responsible for the recruitment of mouse Ly6C^{hi} monocytes to inflamed tissues, which finally differentiate into short-lived moLCs (133). In the human system, CCR6 was shown to mediate trafficking of monocytes into lesions of psoriatic skin (291) giving rise to moDCs and moLCs, eventually.

Martinez-Cingolani et al claim, that CD1c$^+$ DCs were more potent precursors of CD1a$^+$CD207$^+$ LCs when compared to CD34$^+$ hematopoietic progenitors or CD14$^+$ monocytes (336). In line with their observations, our experiments reveal a higher potential for CD1c$^+$ cDCs to develop into LCs than monocytes or HPCs. Additionally, we showed that CD1c$^+$ cDCs more readily differentiate into CD1c$^+$CD11b$^+$ moDCs in response to GM-CSF/IL-4 compared to CD34$^+$ cells. However, CD14$^+$ monocytes exceeded CD1c$^+$ cDCs in giving rise to CD11b$^+$CD206$^+$ macrophages when stimulated with M-CSF/IL-6. These observations strengthen the concept of CD1c$^+$ cDCs resembling precursor cells pre-committed to the DC lineage and monocytes functioning as progenitor for macrophages. Macrophages generated from CD1c$^+$ cDCs displayed a different phenotype compared to CD14$^+$ monocyte derived macrophages; CD1c$^+$ derived macrophages showed lower expression of CD206.

It was shown that CD1c$^+$ blood cDCs can be separated in distinct subsets based on the expression of CD5 (299). CD5^{hi}CD1c$^+$ cells displayed gene expression associated with the DC lineage (e.g. IRF4), whereas CD5^{lo}CD1c$^+$ cells were more associated with monocyte identity (e.g MafB) (299). It is interesting to speculate that the CD5^{hi} subset is enriched in cells pre-committed towards LC differentiation, whereas the CD5^{lo} subset is primed to differentiate into cells still associated with monocytes, i.e. moDCs and macrophages. This is in line with the fact, that we detected *de novo* KLF4 expression in CD1c$^+$-derived moDC and CD1c$^+$-derived macrophage cultures, but not in CD1c$^+$-derived LCs. Yin et al (299) determined a higher expression of genes associated with inflammation/ inflammatory conditions, such as CD163 in CD5^{lo}CD1c$^+$ cDCs. CD5^{hi}CD1c$^+$ cDCs expressed higher levels of the CD207 gene, indicative for their high potential to differentiate into LCs. One might suggest that stimulation of CD5^{hi} cells with TGF-β1 either *in vitro* or *in vivo* upon interaction with epidermal keratinocytes induces CD207 protein expression. Also, expression of AXL mRNA was determined to be higher in the CD5^{hi} subset; AXL is strongly expressed by epidermal LCs *in vivo* (325). The differential mRNA expression of genes associated with LCs of CD5^{hi}CD1c$^+$ cDCs compared to CD5^{lo}CD1c$^+$ cDCs might suggests, that the CD5^{hi} subset is already equipped with the genetic information required for LC development;

stimulation of TGF-β1 might be the key switch in turning on the differentiation program. Additionally, $CD5^{hi}$ cells were determined to be more potent in the induction of IL-10 producing T cells suggesting a regulatory function of these cells (299) similar to what is observed for LCs.

Heterogeneity within cDCs has also been described in the rodent system; $CD11b^+$ cDCs, the $CD1c^+$ cDC murine counterparts where shown to segregate based on the expression of CD4 and ESAM (endothelial cell-specific adhesion molecule) (340). $CD4^-ESAM^{lo}CD11b^+$ cDCs displayed higher expression of genes associated with a monocyte signature, such as CSF1R or CCR2, suggesting that these cells derive from circulating monocytes (107). On the contrary, $CD4^+ESAM^{hi}CD11b^+$ cDCs express higher levels of CD11c and FLT3L marking them as progeny of DC-restricted precursors. Interestingly, these cells are also dependent on Notch (340).

Congruent with previous experiments (336) we determined not all blood-circulating $CD1c^+$ cDCs being able to differentiate into LCs. The addition of Delta-1 to TGF-β1-stimulated $CD1c^+$ cDC cultures slightly increased the percentage of $CD1a^+CD207^+$ LCs. This is supportive to the concept of a heterogeneity of the population of $CD1c^+$ blood cDCs (299). Our results are indicative for the fact that a subset of $CD1c^+$ cDCs does not require exogenous activation of the Notch pathway, whereas the other subset required addition of Notch ligands to the culture system.

$CD1c^+$ and $CD141^+$ cDCs have been shown to differentiate from their individual precursor cells distinguishable by the expression of CD172a/ SIRPα (341). Pre-cDCs can be separated into $CD172a^-$ and $CD172a^+$ subsets, functioning as immediate precursors for $CD141^+$ cDCs and $CD1c^+$ cDCs, respectively (341). An additional $CD172a^{int}$ subsets comprises a mixed population of progenitors giving rise to both cDC subsets. Epidermal LCs are marked by high expression of CD172a (36). However, in the current hematopoiesis tree, LCs are considered to be derived from a common myeloid precursor cells also giving rise to monocytes/macrophages and inflammatory monocyte-derived DCs, but not to classical DCs; i.e. $CD1c^+$ cDCs and LCs do not share a common precursor though $CD1c^+$ cDCs readily convert into LCs *in vitro* (298,336). Additionally, it has recently been demonstrated, that LCs display a dual cell fate, i.e. being detectable via MafB-lineage tracing though expressing the DC-specific transcription factor Zbtb46 (342).

Similar to the murine system, cDCs in the human are comprised by two main subsets: $CD1c^+$/BDCA-1^+ cDCs and $CD141^+$/$CLEC9A^+$ cDCs, both of which being highly responsive to Flt3L (222).

$CD141^+$ cDCs express XCR1, Clec9A, IRF8 and TLR3 (31,32,34,35,343,344). $CD1c^+$ cDCs express IRF4 (35,343,345,346).

Previoulsy, it was reported that in humans a migratory pre-cDC, direclty developing from commited DC precursors in the bone marrow (221), serves as immediate precursor for $CD1c^+$ or $CD141^+$ cDCs, but not for pDCs or monocytes. As seen for mice, the population of pre-cDCs comprises a small percentage of all cells in the peripheral blood (0.001 %) (347). Pre-cDCs reside in circulation only for a short time thereby increasing their flux through the blood stream but showing limited potential to proliferate (198). However, the immediate progeny of $CD1c^+$ cDCs and $CD141^+$ cDCs have the potential to further proliferate and thereby expand the pool of cDCs in the periphery (198,222,348,349). Breton et al speculated that this highly dynamic pool of pre-cDCS and cDCs allows a rapid adaptation to acute antigenic challenges. Additionally, a proportion of $CD1c^+$ cDCs has been demonstrated to express the proliferation marker Ki67, whereas Ki67 expression in lymphoid organ-resident $CD1c^+$ cDCs was low (36). This is in line with recent findings demonstrating a higher expression of cell cycle associated genes of $CD1c^+$ cDCs compared to pDCs or $CD141^+$ cDCs (350). This indicates that these cells might not be fully differentiated but functioning as partially differentiated precursors induced to fully differentiate upon conditions of inflammation. $CD1c^+$ cDCs leave the blood stream and continue their maturation in the lymphoid organs. It would be interesting to speculate that, Notch ligands expressed by cells in the lymphoid organs (351) interacting with Notch receptors expressed on $CD1c^+$ cDCs already partially inducing the LC differentiation program or at least inhibiting the monocyte lineage and turning them into the DC/LC lineage. Once these cells emigrated from lymphoid organs and enter the epidermis they encounter keratinocyte derived TGF-β1 and then are capable to fully differentiate into LCs *in situ*. $CD11b^+$ cDCs are considered as $CD1c^+$ cDC murine analogs and were demonstrated to gather in marginal zones of lymphoid organs (352) supplying them with critical factors regulating growth or differentiation (348).

Recent transcriptional comparison of $CD1c^+$ and $CD141^+$ cDC subsets revealed differential expression of the transcription factors IRF8 and IRF4, respectively (341). IRF8 expression is higher in $CD141^+$ cDCs. Depending on the formation of heterodimers with different interaction partners or target DNA elements, IRF8 functions either as a transcriptional repressor or activator (238,353). IRF8 has been shown to stimulate murine monocyte/macrophage differentiation by induction of KLF4 via interaction with PU.1 (167). In the human system, an autosomal recessive IRF8 deficiency was reported to cause a severe

reduction in blood circulating monocytes and pDCs as well as reduced numbers of dermal $CD1a^+$ and $CD14^+$ DCs; however, epidermal LCs were not affected (354). In mouse the role of IRF8 in LC development is controversial, as it was shown that $IRF8^{-/-}$ mice show reduced numbers of epidermal LCs (138), whereas others state that LC development occurs independent of IRF8 (355). However, IRF8 deficiency did not lead to complete loss of epidermal LCs. This could be explained by the results published recently, demonstrating a dual macrophage/DC role of LCs. It would be interesting to speculate that the portion of epidermal LCs affected by deletion of IRF8 has a monocytic origin, whereas the other subset remaining in the epidermis despite of IRF8 deletion is derived from a dendritic cell progenitor developing independent of IRF8. IRF4 expression was higher in $CD1c^+$ cDCs, but low in epidermal LCs (134). Deletion of IRF4 in mouse $CD11c^+$ cells did not cause a reduction of LCs in the epidermis (134). However, IRF4 was up-regulated upon migration. Absence of IRF4 caused a reduction of LCs migrating to the skin draining lymph nodes, suggesting an important role of IRF4 either in survival or migration of LCs (134).

Apart from cytokines and transcription factors, microRNAs (miRNAs) represent an additional class of key regulators in DC differentiation/function. miRNAs are short non-coding RNA molecules (356) that post-transcriptionally regulated expression of target genes via binding to 3' untranslated regions of respective mRNAs thereby causing its inhibition or degradation (357). A role of miRNAs has been assigned to LCs as deletion of Dicer, a key enzyme in the miRNA biosynthesis pathway, caused increased turnover and apoptotis, leading to a progressive ablation of LCs in the epidermis. Additionally, Dicer-deficient LCs showed lack of Birbeck granules (357). LCs and intDCs/moDCs do not only differ by means of TGF-β1-induced expression of transcription factors, but also by levels of miRNAs expressed (358). Several studies showed that differential expression of miRNAs rather affects DC/LC function rather than actual development. miR-146a was shown to be constitutively expressed in LCs but detectable at low levels in intDCs and monocytes. Induced by PU.1, this miRNA primarily affected LC function by interfering with TLR2 downstream signaling rather than LC development (358). Also lack of miR-150 was demonstrated to affect LC cross-presentation capacity without interfering with normal differentiation (359).

Two miRNAs have been described as to interfere with key Notch and Wnt signaling pathways which cooperate in the regulation of DC differentiation (360). miR-21 and miR-34a were demonstrated to down-regulate WNT1 and JAG1 expression, respectively, thereby leading to reduced moDC differentiation/ maturation (361). In line with the described role

of the Notch pathway in enhancing $CD34^+$ hematopoietic cell survival (180), we observed a marked increase in apoptosis in $CD34^+$ cells upon lentiviral transfection with anti-miR34a (data not shown). However, knockdown of miR-21 in our p-LC differentiation cultures did not affect the development of $CD1a^+CD207^+$ cells. A possible impairment of LC function regarding cytokine production and T cell stimulatory capacity remains to be determined. As miR-21 was shown to be increased in conditions of psoriasis (362,363) it would be interesting to investigate if over-expression or knockdown of this miRNA renders LCs/moDCs pro- or anti-inflammatory, respectively. miR-424 is induced by PU.1 and plays an important role in the development of monocytes via inducing expression of monocyte-differentiation specific genes ((364)). Hypothesizing that KLF4 might also be a target of miR-424 we compared p-moDC and p-LC development from $CD34^+$ cells in which miR-424 was knocked down (data not shown). However, we did not see enhancement of $CD1a^+CD207^+$ p-LC or reduced $CD1a^+CD11b^+CD209^+$ p-moDC development in our experiments.

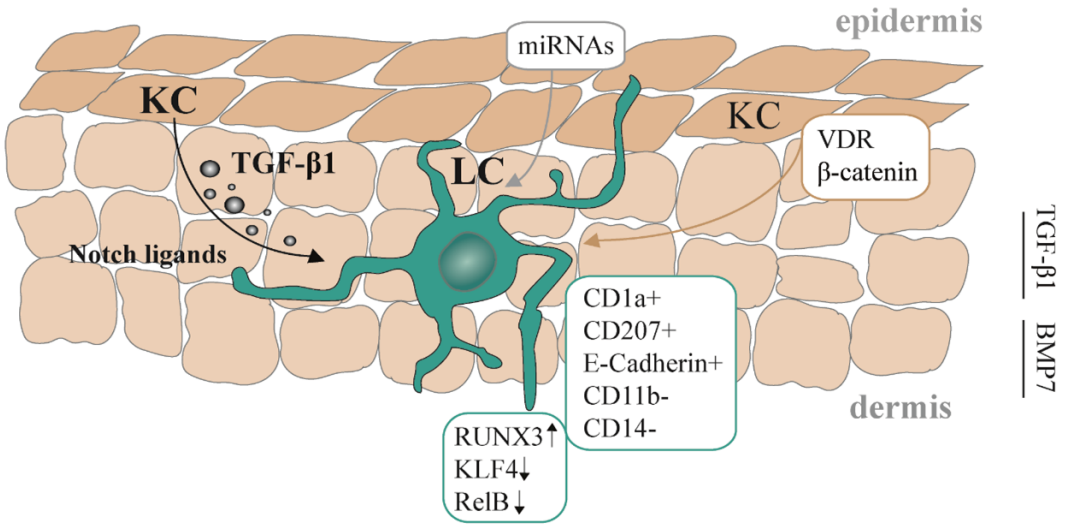

Figure 35: The epidermal microenvironment drives LC differentiation.
The key instructive cytokine TGF-β1 inducing RUNX3 in LCs is secreted by keratinocytes (KC); additionally, KC provide Notch ligands inducing the differntiaiton of LCs from monocytes. Interaction of LC precursors with KC induces β-catenin/ VDR signaling pathways inducing the down-regulation of monocyte-associated genes and surface markers. miRNAs present in the epidermal niche might influence functional aspects of residing LCs.

The development of different subsets of DCs in mouse and men is regulated by a complicated network of interplaying transcription factors and signaling pathways. Depending on the micro-environment the DC/LC precursors home to, different cytokines stimuli induce the expression of a broad variety of downstream targets. Previous work has demonstrated that

keratinocytes in the epidermal niche provides numerous factors promoting the differentiation of LCs either from hematopoietic precursors in the steady state or from blood-circulating progenitors under inflammatory conditions. The physiological state plays an important role on the mode of differentiation, i.e. steady-state differentiation of DCs/LCs seems to occur following different pathways from different progenitor cells when compared to conditions of inflammation. We determined two subsets of blood circulating cells with the capacity to give rise to epidermal LCs and suggest these cells to replenish the epidermal network as short-term cells upon resolution of inflammation followed by the differentiation of local LC precursors re-establishing the long-term network. We describe the interaction of the Notch and the TGF-β1 signaling pathways in the transcriptional regulation of monocyte-to LC differentiation via regulating key transcription factors specific for each cells type. KLF4 was identified as critical regulatory factor identifying monocytic cell identity and required to be repressed to allow LC differentiation.

6. APPENDIX

6.1. CD16$^+$ blood monocytes differentiate into moDCs

Peripheral blood monocytes have been demonstrated to be comprised of a heterogeneous population of cells expressing differential levels of CD14 and CD16 (365–368): CD14^{hi}CD16$^-$ classical, CD14^{hi}CD16$^+$ intermediate and CD14$^+$CD16^{hi} non-classical monocytes. CD16$^-$ classical monocytes comprise about 90 %, whereas CD16$^+$ cells make up 10 % of the total monocyte population under steady state conditions (367). However, the number of CD16$^+$ monocytes can increase under conditions of inflammation/ infection such as sepsis (365,367). Additionally, the CD16$^+$ subset has been demonstrated to produce high amounts of TNFα and IL-1β (260,369), indicating that they might play a role in promoting inflammation. The classical CD14$^+$CD16$^-$ subset was shown numerous times to possess capability to differentiate into moDCs or moLCs, respectively (370,371).

CD16$^+$ monocytes were isolated via magnetically labeled beads (Miltenyi) from total MNCs from peripheral blood of healthy donors. As isolation was performed based on anti-CD16$^+$ magnetically labelled beads, the resulting population included CD16$^+$CD14$^-$ non-classical as well as CD16$^+$CD14$^+$ intermediate monocytes (Figure 36, A). Recently, it was suggested that the non-classical and intermediate monocyte subsets are transcriptionally closely related, suggesting a developmental relationship, i.e. intermediate monocytes give rise to non-classical monocytes by down-regulation of CD14 (257,372). When stimulated under moDC-differentiation conditions (GM-CSF/IL-4), CD16$^+$ monocytes showed similar capacity to give rise to CD1a$^+$CD11b$^+$ moDCs as observed for classical CD14$^+$ monocytes (Figure 36, B), though CD1a expression seemed to be slightly higher for cells derived from the non-classical subset. We next aimed to determine the ability of the CD16$^+$ subset to give rise to CD1a$^+$CD207$^+$ moLCs. Compared to the classical monocyte subset, CD16$^+$ cells gave rise to a lower percentage of moLCs in presence of GM-CSF/TGF-β1 and plate-bound Notch ligand Delta-1 (Figure 36, C). However, CD16$^+$ moLC cultures resulted in a higher percentage of CD1a$^+$CD207$^-$ cells. Abrogation of LC-instructive TGF-β1 did not allow development of LCs from both monocyte subsets, although CD1a$^+$ cells were induced from CD14$^+$ monocytes, which was not observed for CD16$^+$ cells (Figure 36, C). This indicates that CD14$^+$ classical monocytes possess higher capacity to transdifferentiate into LCs that CD16$^+$ monocytes, albeit their capacity of differentiation into moDCs is similar. CD16$^+$ monocytes have been described to be numerically increased under inflammatory conditions

(365,367) and the TGF-superfamily member BMP7 has been associated with pro-inflammatory conditions (212). Therefore, we aimed to determine whether stimulation with this cytokine is effective in inducing moLC differentiation from $CD16^+$ monocytes. Indeed in presence of Delta-1 some cells appeared to acquire CD1a and CD207 expression; however, this was similar to the observations made for $CD14^+$ monocytes and might be assigned to the presence of Delta-1 but not to the stimulation with BMP7. In presence of Delta-1, $CD16^+$ monocytes also gave rise to $CD1a^+CD209^+$ moDCs, which could not be detected under conditions where Delta-1 was abrogated from the system (Figure 36, D).
Recently it was observed that an additional population of blood circulating DCs (slanDCs) shares CD16 expression with the non-classical subset of monocytes (373). As we isolated $CD16^+$ monocytes by magnetic beads targeted to the CD16 antigen, it is possible that slanDCs were contained within our culture system. It is interesting to speculate that the small subset of $CD16^+$ cells giving rise to moLCs in presence of BMP7 is comprised of slanDCs rather than monocytes. SlanDCs have been demonstrated to readily migrate to tissues in response to inflammatory cytokine conditions (374) and might contribute to the pool of inflammatory DCs/LCs.

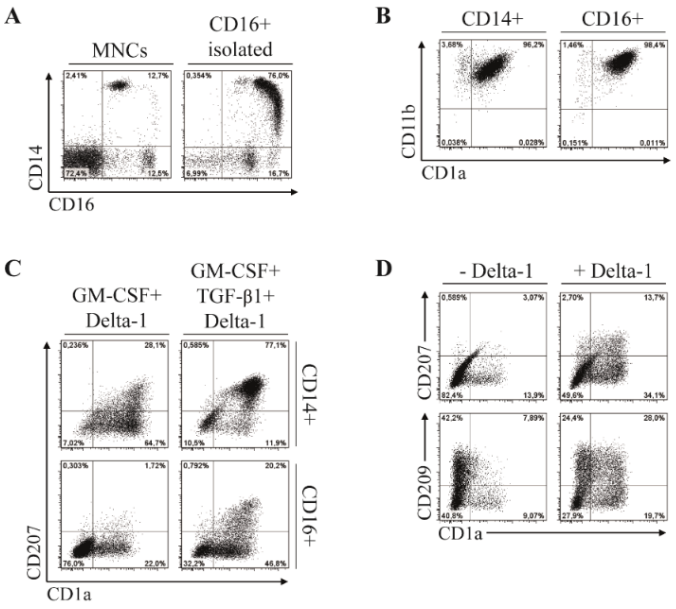

Figure 36: CD16+ monocytes differentiate to moLCs and moDCs.
(A) $CD16^+$ monocytes were isolated from PBMNs of peripheral blood and incubated under (B) moDC (GM-CSF/IL-4) and (C) moLC (GM-CSF/TGF-β1 +/- Delta-1) differentiation conditions and compared to $CD14^+$ monocytes. (D) $CD16^+$ monocytes were stimulated with BMP7 +/-Delta and analyzed for moDC or moLC marker expression. (n=3)

6.2. Band-stage neutrophils differentiate into KLF4$^+$ monocytes

Band-stage neutrophils have been shown to display high plasticity and to acquire features of monocytes or antigen-presenting cells under inflammatory conditions (375–379). These immature form of neutrophils is enriched in blood of G-CSF-treated stem cell donors as well as under inflammatory conditions (e.g. rheumatoid arthritis) or cancer (380–382). Previously it has been shown that a particular subset of human band-stage neutrophils of G-CSF mobilized blood (CD15$^+$CD54$^-$ cells) exhibits potential to differentiate into monocytes/macrophages in presence of pro-inflammatory cytokines (GM-CSF, IL-1β, TNFα) *in vitro* (376). *In vivo*, neutrophils with APC-like features have been observed in experimentally induced inflammatory lesions in mice (383). As monocytes are capable to transdifferentiate into moDCs or moLCs *in vitro*, we investigated whether neutrophil-derived monocytes possess capability to give rise to LCs under the same culture conditions.

We isolated G-CSF mobilized band-stage neutrophils from blood of healthy stem cell donors collected at the Department of Hematology of the University Hospital Graz. Briefly, upon density gradient centrifugation, highly pure CD15$^+$CD16$^+$ granulocytes were obtained following erythrocyte lysis (Figure 37, A). Differentiation into monocytes was performed by stimulation with pro-inflammatory cytokines (100ng/mL GM-CSF, 25ng/mL TNFα, 10ng/mL IL-1β) for 4-5 days. CD14$^+$ neutrophil-derived monocytes were isolated via magnetically labeled beads (Miltenyi) (Figure 37, B) and then further incubated under moDC (100 ng/mL GM-CSF, 20 ng/mL IL-4) differentiation conditions. G-CSF mobilized band-stage neutrophils effectively differentiated into CD14$^+$CD11b$^+$ monocytes upon stimulation with pro-inflammatory cytokines (Figure 37, B). However, these neutrophil-derived monocytes did not give rise to CD1a$^+$CD11b$^+$CD209$^+$ moDCs in presence of GM-CSF/IL-4 (Figure 37, C). Interestingly, we observed induction of CD209 but no CD1a expression after 5 days of culture. CD14 was down-regulated, whereas CD11b expression was maintained. Freshly isolated blood- circulating CD14$^+$ monocytes express high levels of KLF. We also detected KF4 protein expression in nuclei of neutrophil-derived monocytes (Figure 37, D, lower panel); freshly isolated neutrophils were KLF4$^-$ (Figure 37, D, upper panel).

Oehler et al demonstrated the capactiy to acquire DC features of G-CSF mobilized band-stage neutrophils upon stimulation with GM-CSF, IL-4 and TNFα (379). Under these conditions, the expression of classical DC markers such as CD1a, HLA-DR, CD1c and co-

stimulatory molecules (CD40, CD80, CD86) was induced on neutrophils. However, we did not observe CD1a expression in our culture system. This could be due to (i) abrogration of pro-inflammatory TNFα in our culture system, (ii) longer incubation times used by Oehler et al. In our moLC differentiation experiments, we also observed stronger induction of CD1a expression in presence of TNFα (data not shown). In their experiments, neutrophils were stimulated with DC-inducing cytokines for 9 days, whereas in our system FACS analysis of differentiated cells was performed after 5 days of incubation in order to maintain high viability of the cells. According to their experiments, induction of CD1a or CD1c was only determined after the longer cultivation times, they also did not detect expression of CD1 antigens until 6 days of cultivation.

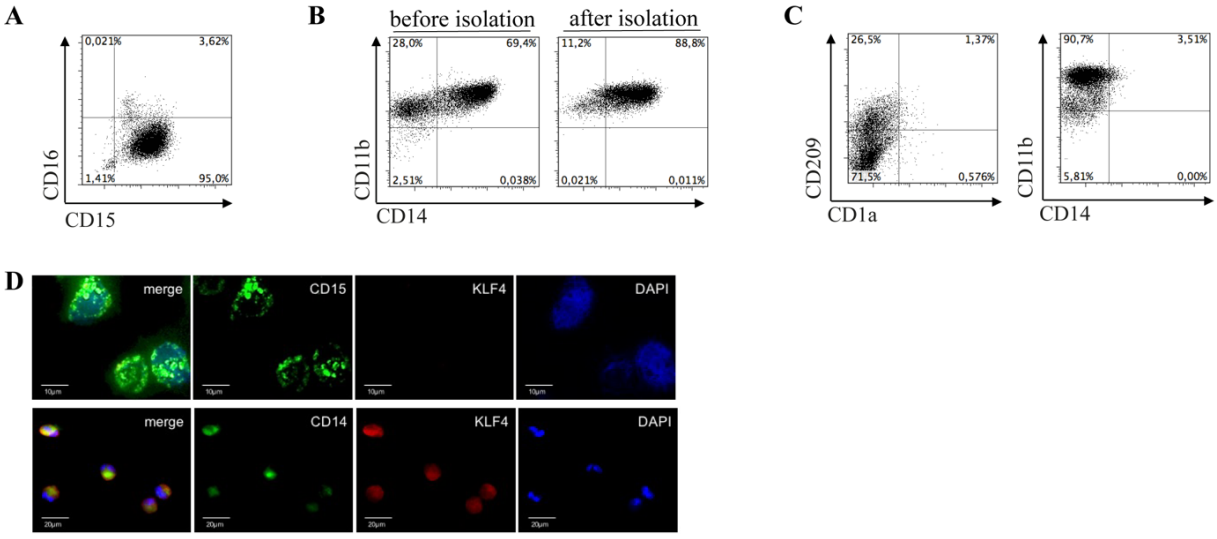

Figure 37: G-CSF mobilized neutrophils differentiate into KLF4+ monocytes.
(A) $CD15^+$ neutrophils were isolated from blood of G-CSF mobilized stem cell donors. (B) Upon stimulation with pro-inflammatory cytokines (100 ng/mL GM-CSF; 10 ng/mL IL-1β, 25 ng/mL TNFα) $CD14^+$ monocytes were purified via magnetic beads and (C) incubated under moDC differentiation conditions (100 ng/mL GM-CSF, 25 ng/mL IL-4). (D) Freshly isolated neutrophils (upper panel) and neutrophil-derived monocytes (lower panel) were stained for the indicated markers (n=3).Bars equal 20 µm.

7. REFERENCES

1. Jurkin J, Krump C, Köffel R, Fieber C, Schuster C, Brunner PM, et al. Human skin dendritic cell fate is differentially regulated by the monocyte identity factor Kruppel-like factor 4 during steady state and inflammation. J Allergy Clin Immunol. 2017;139(6):1873–84.
2. Clark G, Angel N, Kato M, Lopez A, Macdonald K, Vuckovic S, et al. The role of dendritic cells in the innate immune system. Microbes Infect. 2000;2:257–72.
3. Mezhitov R, Janeway C. Innate Immunity. N Engl J Med. 2000;343(5):338–44.
4. Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev. 2009;22(2):240–73.
5. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, et al. A Toll-like receptor recognizes bacterial DNA A Toll-like receptor recognizes bacterial DNA. Nature. 2000;408:740–5.
6. Honda K, Sakaguchi S, Nakajima C, Watanabe A, Yanai H, Matsumoto M, et al. Selective contribution of IFN-alpha/beta signaling to the maturation of dendritic cells induced by double-stranded RNA or viral infection. Proc Natl Acad Sci U S A [Internet]. 2003;100(19):10872–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=196895%7B&%7Dtool=pmcentrez%7B&%7Drendertype=abstract
7. Hoshino K, Kaisho T, Iwabe T, Takeuchi O, Akira S. Differential involvement of IFN-beta in Toll-like receptor-stimulated dendritic cell activation. Int Immunol [Internet]. 2002;14(10):1225–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12356687
8. Clausen BE, Kel JM. Langerhans cells: critical regulators of skin immunity? Immunol Cell Biol. 2010;88(4):351–60.
9. Steinman RM, Hawiger D, Nussenzweig MC. Tolerogenic Dendritic Cells. Annu Rev Immunol [Internet]. 2003;21(1):685–711. Available from: http://www.annualreviews.org/doi/10.1146/annurev.immunol.21.120601.141040
10. Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature. 2006;441(7090):231–4.
11. Muruve DA, Pétrilli V, Zaiss AK, White LR, Clark SA, Ross PJ, et al. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. Nature [Internet]. 2008;452(7183):103–7. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=18288107&retmode=ref&cmd=prlinks%5Cnpapers3://publication/doi/10.1038/nature06664
12. van Beelen AJ, Zelinkova Z, Taanman-Kueter EW, Muller FJ, Hommes DW, Zaat SAJ, et al. Stimulation of the Intracellular Bacterial Sensor NOD2 Programs Dendritic Cells to Promote Interleukin-17 Production in Human Memory T Cells. Immunity. 2007;27(4):660–9.
13. Powrie F, Mason D. Phenotypic and functional heterogeneity of CD4+ T cells. Trends Immunol. 1988;9(9):274–6.
14. Brocker T, Riedinger M, Karjalainen K. Targeted expression of major histocompatibility complex (MHC) class II molecules demonstrates that dendritic cells can induce negative but not positive selection of thymocytes in vivo. J Exp Med [Internet]. 1997;185(3):541–50. Available from: http://www.jem.org/cgi/content/full/185/3/541%5Cnpapers2://publication/uuid/6E21D455-0AFF-4056-9E65-D10512BF61E2
15. Zaba L, Krueger J, Lowes M. Resident and "inflammatory" dendritic cells in human skin. J Invest Dermatol. 2009;129(2):302–8.
16. Grabbe S, Kämpgen E, Schuler G. Dendritic cells: multi-lineal and multi-functional. Immunol Today. 2000;21(9):431–3.
17. Reizis B. Classical dendritic cells as a unique immune cell lineage. J Exp Med [Internet]. 2012;209(6):1053–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3375637&tool=pmcentrez&rendertype=abstract
18. Merad M, Manz MG. Dendritic cell homeostasis. Blood [Internet]. 2009;113(15):3418–27. Available from: http://171.66.121.249/cgi/content/abstract/113/15/3418
19. Colonna M, Trinchieri G, Liu YJ. Plasmacytoid dendritic cells in immunity. NatImmunol [Internet]. 2004;5(1529–2908 (Print)):1219–26. Available from: file:///G:/Article_database/GAC_pdfs/PM15549123.pdf%5Cnhttp://dx.doi.org/10.1038/ni1141
20. Cox K, North M, Burke M, Singhal H, Renton S, Aqel N, et al. Plasmacytoid dendritic cells (PDC) are the major DC subset innately producing cytokines in human lymph nodes. J Leukoc Biol [Internet]. 2005;78(5):1142–52. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16260587
21. Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang Y-H, Homey B, et al. Plasmacytoid

dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 2007;449(7162):564–9.
22. Gilliet M, Cao W, Liu Y-J. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nat Rev Immunol. 2008;8:594–606.
23. Grouard G, Rissoan MC, Filgueira L, Durand I, Banchereau J, Liu YJ. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. J Exp Med [Internet]. 1997;185(6):1101–11. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2196227&tool=pmcentrez&rendertype=abstract
24. Siegal FP, Kadowaki N, Shodell M, Fitzgerald-bocarsly PA, Shah K, Ho S. The Nature of the Principal Type 1 Interferon – Producing Cells in Human Blood. Science (80-). 1999;284(June):1835–8.
25. Perussia B, Fanning V, Trinchieri G. A leukocyte subset bearing HLA-DR antigens is responsible for in vitro alpha interferon production in response to viruses. Nat Immun Cell Growth Regul. 1985;4(3):120–37.
26. O'Doherty U, Peng M, Gezelter S, Swiggard WJ, Betjes M, Bhardwaj N, et al. Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. Immunology [Internet]. 1994;82(3):487–93. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1414873&tool=pmcentrez&rendertype=abstract
27. Cella M, Jarrossay D, Facchetti F, Alebardi O, Nakajima H, Lanzavecchia A, et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. Nat Med. 1999;5(8):919–23.
28. Angel CE, George E, Brooks AES, Ostrovsky LL, Brown TLH, Dunbar PR. Cutting edge: CD1a+ antigen-presenting cells in human dermis respond rapidly to CCR7 ligands. J Immunol [Internet]. 2006;176(10):5730–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16670277
29. McLellan AD, Heiser A, Sorg R V., Fearnley DB, Hart DNJ. Dermal dendritic cells associated with T lymphocytes in normal human skin display an activated phenotype. J Invest Dermatol. 1998;111(5):841–9.
30. Mittag D, Proietto AI, Loudovaris T, Mannering SI, Vremec D, Shortman K, et al. Human dendritic cell subsets from spleen and blood are similar in phenotype and function but modified by donor health status. J Immunol [Internet]. 2011;186(11):6207–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21515786
31. Poulin LF, Salio M, Griessinger E, Anjos-Afonso F, Craciun L, Chen J-L, et al. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. J Exp Med [Internet]. 2010;207(6):1261–71. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2882845&tool=pmcentrez&rendertype=abstract
32. Haniffa M, Shin A, Bigley V, McGovern N, Teo P, See P, et al. Human Tissues Contain CD141 hi Cross-Presenting Dendritic Cells with Functional Homology to Mouse CD103 + Nonlymphoid Dendritic Cells. Immunity. 2012;37(1):60–73.
33. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. J Exp Med [Internet]. 2010;207(6):1247–60. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2882828&tool=pmcentrez&rendertype=abstract
34. Bachem A, Güttler S, Hartung E, Ebstein F, Schaefer M, Tannert A, et al. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. J Exp Med. 2010;207(6):1273–81.
35. Crozat K, Guiton R, Contreras V, Feuillet V, Dutertre C-A, Ventre E, et al. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. J Exp Med [Internet]. 2010;207(6):1283–92. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2882835&tool=pmcentrez&rendertype=abstract
36. Segura E, Valladeau-Guilemond J, Donnadieu M-H, Sastre-Garau X, Soumelis V, Amigorena S. Characterization of resident and migratory dendritic cells in human lymph nodes. J Exp Med [Internet]. 2012;209(4):653–60. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=22430490&retmode=ref&cmd=prlinks%5Cnpapers3://publication/doi/10.1084/jem.20111457
37. Chu C-C, Ali N, Karagiannis P, Di Meglio P, Skowera A, Napolitano L, et al. Resident CD141 (BDCA3)+ dendritic cells in human skin produce IL-10 and induce regulatory T cells that suppress

skin inflammation. J Exp Med [Internet]. 2012;209(5):935–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22547651%5Cnhttp://jem.rupress.org/content/209/5/935.full.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/22547651%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3348099

38. Poulin LF, Reyal Y, Uronen-Hansson H, Schraml BU, Sancho D, Murphy KM, et al. DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues. Blood. 2012;119(25):6052–62.
39. Sancho D, Joffre OP, Keller AM, Rogers NC, Martinez D, Hernanz-Falcon P, et al. Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature [Internet]. 2009;458(7240):899–903. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19219027
40. Ebner S, Nguyen VA, Forstner M, Wang Y-H, Wolfram D, Liu Y-J, et al. Thymic stromal lymphopoietin converts human epidermal Langerhans cells into antigen-presenting cells that induce proallergic T cells. J Allergy Clin Immunol. 2007;119(4):982–90.
41. Lauterbach H, Bathke B, Gilles S, Traidl-Hoffmann C, Luber CA, Fejer G, et al. Mouse CD8alpha+ DCs and human BDCA3+ DCs are major producers of IFN-lambda in response to poly IC. J Exp Med [Internet]. 2010;207(12):2703–17. Available from: http://jem.rupress.org/content/207/12/2703.full
42. Kaplan DH. In vivo Function of Langerhans Cells and Dermal DC. Trends Immunol. 2010;31(12):446–51.
43. Birbeck MS, Breathnach AS, Everall JD. An electron microscope study of basal melanocytes and high-level clear cells (Langerhans cells) in vitiligo. J Investig Dermatol Dermatol. 1961;37:51–64.
44. Valladeau J, Dezutter-Dambuyant C, Saeland S. Langerin/CD207 sheds light on formation of birbeck granules and their possible function in Langerhans cells. Immunol Res. 2003;28(2):93–107.
45. Kissenpfennig A, Ait-Yahia S, Claire-Moniot V, Stössel H, Badell E, Bordat Y, et al. Disruption of the langerin/CD207 gene abolilshes Birbeck granules without a marked loss of Langerhans cell function. Mol Cell Biol. 2005;25(1):88–99.
46. Tang A, Amagai M, Granger LG, Stanley JR, Udey MC. Adhesion of epidermal Langerhans cells to keratinocytes mediated by E-cadherin. Nature [Internet]. 1993;361(6407):82–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8421498
47. Jiang A, Bloom O, Ono S, Cui W, Unternaehrer J, Jiang S, et al. Disruption of E-Cadherin-mediated adhesion induces a functionally distinct pathway of dendritic cell maturation. Immunity. 2007;27(4):610–24.
48. Riedl E, Stöckl J, Majdic O, Scheinecker C, Knapp W, Sto J. Ligation of E-cadherin on in vitro–generated immature Langerhans-type dendritic cells inhibits their maturation. Blood. 2000;96(13):4276–84.
49. Riedl E, Stockl J, Majdic O, Scheinecker C, Rappersberger K, Knapp W, et al. Functional Involvement of E-Cadherin in TGF- 1-Induced Cell Cluster Formation of In Vitro Developing Human Langerhans-Type Dendritic Cells. J Immunol [Internet]. 2000;165(3):1381–6. Available from: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.165.3.1381
50. Geissmann F, Prost C, Monnet JP, Dy M, Brousse N, Hermine O. Transforming growth factor beta1, in the presence of granulocyte/macrophage colony-stimulating factor and interleukin 4, induces differentiation of human peripheral blood monocytes into dendritic Langerhans cells. J Exp Med [Internet]. 1998;187(6):961–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2212193&tool=pmcentrez&rendertype=abstract
51. Strobl H, Riedl E, Scheinecker C, Bello-Fernandez C, Pickl WF, Rappersberger K, et al. TGF-beta 1 promotes in vitro development of dendritic cells from CD34+ hemopoietic progenitors. J Immunol. 1996;157(4):1499–507.
52. Riedl E, Stöckl J, Majdic O, Scheinecker C, Rappersberger K, Knapp W, et al. Functional involvement of E-cadherin in TGF-beta 1-induced cell cluster formation of in vitro developing human Langerhans-type dendritic cells. J Immunol [Internet]. 2000;165(3):1381–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10903741
53. Nishibu A, Ward BR, Jester J V, Ploegh HL, Boes M, Takashima A. Behavioral responses of epidermal Langerhans cells in situ to local pathological stimuli. J Invest Dermatol [Internet]. 2006;126(4):787–96. Available from: http://dx.doi.org/10.1038/sj.jid.5700107
54. Schuler G, Romani N, Steinman RM. A comparison of murine epidermal Langerhans cells with spleen dendritic cells. J Invest Dermatol. 1985;85(1 Suppl.):99s–106s.
55. Ohl L, Mohaupt M, Czeloth N, Hintzen G, Kiafard Z, Blankenstein T, et al. CCR7 Governs Skin Dendritic Cell Migration under Inflammatory and Steady-State Conditions Max-Delbruck-Center for Molecular Medicine. Immunity. 2004;21:279–88.

56. Romani N, Inaba K, Puré E, Crowley M, Witmer-Pack M, Steinman RM. A small number of anti-CD3 molecules on dendritic cells stimulate DNA synthesis in mouse T lymphocytes. J Exp Med [Internet]. 1989;169(3):1153–68. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2189261&tool=pmcentrez&rendertype=abstract
57. Reis e Sousa C, Stahl PD, Austyn JM. Phagocytosis of antigens by Langerhans cells in vitro. J Exp Med [Internet]. 1993;178(2):509–19. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2191126&tool=pmcentrez&rendertype=abstract
58. Seneschal J, Clark RA, Gehad A, Baecher-Allan CM, Kupper TS. Human Epidermal Langerhans Cells Maintain Immune Homeostasis in Skin by Activating Skin Resident Regulatory T Cells. Immunity. 2012;36(5):873–84.
59. Lenz A, Heine M, Schuler G, Romani N. Human and murine dermis contain dendritic cells: Isolation by means of a novel method and phenotypical and functional characterization. J Clin Invest. 1993;92(6):2587–96.
60. Nestle FO, Turka LA, Nickoloff BJ. Characterization of dermal dendritic cells in psoriasis: Autostimulation of T lymphocytes and induction of Th1 type cytokines. J Clin Invest. 1994;94(1):202–9.
61. Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. Nat Rev Immunol [Internet]. 2007;7(1):19–30. Available from: http://www.nature.com/doifinder/10.1038/nri1996
62. Dubois PM, Pihlgren M, Tomkowiak M, Van Mechelen M, Marvel J. Tolerant CD8 T cells induced by multiple injections of peptide antigen show impaired TCR signaling and altered proliferative responses in vitro and in vivo. J Immunol [Internet]. 1998;161(10):5260–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9820498
63. Kissenpfennig A, Henri S, Dubois B, Laplace-Builh?? C, Perrin P, Romani N, et al. Dynamics and function of langerhans cells in vivo: Dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity. 2005;22(5):643–54.
64. Angel CE, Lala A, Chen CJJ, Edgar SG, Ostrovsky LL, Dunbar PR. CD14+ antigen-presenting cells in human dermis are less mature than their CD1a+ counterparts. Int Immunol. 2007;19(11):1271–9.
65. Morelli AE, Rubin JP, Erdos G, Tkacheva OA, Mathers AR, Zahorchak AF, et al. CD4+ T cell responses elicited by different subsets of human skin migratory dendritic cells. J Immunol [Internet]. 2005;175(12):7905–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16339526%5Cnhttp://www.jimmunol.org/content/175/12/7905.full.pdf
66. Haniffa M, Ginhoux F, Wang X, Bigley V, Abel M, Dimmick I, et al. Differential rates of replacement of human dermal dendritic cells and macrophages during hematopoietic stem cell transplantation. J Exp Med [Internet]. 2009;206(2):371–85. Available from: papers://83acc47a-068c-4c1c-ab9f-591275e2fa6d/Paper/p4681
67. Puig-Kröger A, Corbí A. RUNX3: A new player in myeloid gene expression and immune response. J Cell Biochem. 2006;98(4):744–56.
68. Klechevsky E, Morita R, Liu M, Cao Y, Coquery S, Thompson-Snipes L, et al. Functional Specializations of Human Epidermal Langerhans Cells and CD14+ Dermal Dendritic Cells. Immunity. 2008;29(3):497–510.
69. Larregina a T, Morelli a E, Spencer L a, Logar a J, Watkins SC, Thomson a W, et al. Dermal-resident CD14+ cells differentiate into Langerhans cells. Nat Immunol. 2001;2(12):1151–8.
70. Langlet C, Tamoutounour S, Henri S, Luche H, Ardouin L, Gregoire C, et al. CD64 Expression Distinguishes Monocyte-Derived and Conventional Dendritic Cells and Reveals Their Distinct Role during Intramuscular Immunization. J Immunol [Internet]. 2012;188(4):1751–60. Available from: http://dx.doi.org/10.4049/jimmunol.1102744%5Cnhttp://www.jimmunol.org/cgi/doi/10.4049/jimmunol.1102744
71. McGovern N, Schlitzer A, Gunawan M, Jardine L, Shin A, Poyner E, et al. Human dermal CD14+ cells are a transient population of monocyte-derived macrophages. Immunity [Internet]. 2014;41(3):465–77. Available from: http://dx.doi.org/10.1016/j.immuni.2014.08.006
72. Naik SH, Proietto AI, Wilson NS, Dakic A, Schnorrer P, Fuchsberger M, et al. Cutting edge: generation of splenic CD8+ and CD8- dendritic cell equivalents in Fms-like tyrosine kinase 3 ligand bone marrow cultures. J Immunol. 2005;174(11):6592–7.
73. Zaba LC, Cardinale I, Gilleaudeau P, Sullivan-Whalen M, Suárez-Fariñas M, Fuentes-Duculan J, et al. Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. J Exp Med. 2007;204(13):3183–94.
74. Serbina N V, Salazar-mather TP, Biron C a, Kuziel W a, Pamer EG. TNF / iNOS-Producing

Dendritic Cells Mediate Innate Immune Defense against Bacterial Infection. Immunity. 2003;19:59–70.
75. Randolph GJ, Sanchez-Schmitz G, Liebman RM, Schäkel K. The CD16(+) (FcgammaRIII(+)) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting. J Exp Med. 2002;196(4):517–27.
76. Tacke F, Randolph GJ. Migratory fate and differentiation of blood monocyte subsets. Immunobiology. 2006;211(6–8):609–18.
77. Piccioli D, Tavarini S, Borgogni E, Steri V, Nuti S, Sammicheli C, et al. Functional specialization of human circulating CD16 and CD1c myeloid dendritic-cell subsets. Blood. 2007;109(12):5371–9.
78. Lowes MA, Chamian F, Abello MV, Fuentes-Duculan J, Lin S-L, Nussbaum R, et al. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). Proc Natl Acad Sci U S A [Internet]. 2005;102(52):19057–62. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1323218&tool=pmcentrez&rendertype=abstract
79. Hänsel A, Günther C, Ingwersen J, Starke J, Schmitz M, Bachmann M, et al. Human slan (6-sulfo LacNAc) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong Th17/Th1 T-cell responses. J Allergy Clin Immunol. 2011;127(3):787–94.
80. Günther C, Starke J, Zimmermann N, Schäkel K. Human 6-sulfo LacNAc (slan) dendritic cells are a major population of dermal dendritic cells in steady state and inflammation. Clin Exp Dermatol. 2012;37(2):169–76.
81. Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, et al. Human CD14dim Monocytes Patrol and Sense Nucleic Acids and Viruses via TLR7 and TLR8 Receptors. Immunity. 2010;33(3):375–86.
82. Schäkel K, von Kietzell M, Hänsel A, Ebling A, Schulze L, Haase M, et al. Human 6-Sulfo LacNAc-Expressing Dendritic Cells Are Principal Producers of Early Interleukin-12 and Are Controlled by Erythrocytes. Immunity. 2006;24:767–77.
83. Wollenberg A, Kraft S, Hanau D, Bieber T. Immunomorphological and Ultrastructural Characterization of Langerhans Cells and a Novel, Inflammatory Dendritic Epidermal Cell (IDEC) Population in Lesional Skin of Atopic Eczema. J Invest Dermatol [Internet]. 1996;106(3):446–53. Available from: http://dx.doi.org/10.1111/1523-1747.ep12343596
84. Guttman-Yassky E, Lowes MA, Fuentes-Duculan J, Whynot J, Novitskaya I, Cardinale I, et al. Major differences in inflammatory dendritic cells and their products distinguish atopic dermatitis from psoriasis. J Allergy Clin Immunol. 2007;119(5):1210–7.
85. Romani N, Gruner S, Brang D, Kämpgen E, Lenz A, Trockenbacher B, et al. Proliferating dendritic cell progenitors in human blood. J Exp Med [Internet]. 1994;180(1):83–93. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2191538&tool=pmcentrez&rendertype=abstract
86. Ferrero I, Held W, Wilson A, Tacchini-Cottier F, Radtke F, Robson MacDonald H. Mouse CD11c+ B220+ Gr1+ plasmacytoid dendritic cells develop independently of the T-cell lineage. Blood. 2002;100(8):2852–7.
87. Brawand P, Fitzpatrick DR, Greenfield BW, Brasel K, Maliszewski CR, De Smedt T. Murine plasmacytoid pre-dendritic cells generated from Flt3 ligand-supplemented bone marrow cultures are immature APCs. J Immunol [Internet]. 2002;169(12):6711–9. Available from: files/225/Brawand (2002) Murine Plasmacytoid pre-dcs generated from Flt3l Ligand supplemted bone marrow cultures are immature APCs.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/12471102%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/12471102
88. Gilliet M, Boonstra A, Paturel C, Antonenko S, Xu XL, Trinchieri G, et al. The development of murine plasmacytoid dendritic cell precursors is differentially regulated by FLT3-ligand and granulocyte/macrophage colony-stimulating factor. J Exp Med. 2002;195(7):953–8.
89. Manfra DJ, Chen S-C, Jensen KK, Fine JS, Wiekowski MT, Lira S a. Conditional expression of murine Flt3 ligand leads to expansion of multiple dendritic cell subsets in peripheral blood and tissues of transgenic mice. J Immunol. 2003;170:2843–52.
90. Asselin-Paturel C, Brizard G, Pin J-J, Brière F, Trinchieri G. Mouse strain differences in plasmacytoid dendritic cell frequency and function revealed by a novel monoclonal antibody. J Immunol. 2003;171(12):6466–77.
91. Helft J, Ginhoux F, Bogunovic M, Merad M. Origin and functional heterogeneity of non-lymphoid tissue dendritic cells in mice. Immunol Rev. 2010;234(1):55–75.
92. del Rio M-L, Bernhardt G, Rodriguez-Barbosa J-I, Förster R. Development and functional

specialization of CD103+ dendritic cells. Immunol Rev. 2010;234(1):268–81.
93. Bogunovic M, Ginhoux F, Helft J, Shang L, Greter M, Liu K, et al. Origin of the Lamina Propria Dendritic Cell Networkc Access. Immunity. 2009;31(3):513–25.
94. Varol C, Vallon-Eberhard A, Elinav E, Aychek T, Shapira Y, Luche H, et al. Intestinal Lamina Propria Dendritic Cell Subsets Have Different Origin and Functions. Immunity. 2009;31(3):502–12.
95. Merad M, Sathe P, Helft J, Miller J, Mortha A. The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed Setting. Annu Rev iImunology. 2013;31(9):15–25.
96. Merad M, Ginhoux F, Collin M. Origin, homeostasis and function of Langerhans cells and other langerin-expressing dendritic cells. Nat Rev Immunol [Internet]. 2008;8(12):935–47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19029989
97. Ginhoux F, Liu K, Helft J, Bogunovic M, Greter M, Hashimoto D, et al. The origin and development of nonlymphoid tissue CD103+ DCs. J Exp Med [Internet]. 2009;206(13):3115–30. Available from: http://www.jem.org/cgi/doi/10.1084/jem.20091756%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/20008528%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2806447
98. Förster R, Davalos-Misslitz AC, Rot A. CCR7 and its ligands: balancing immunity and tolerance. Nat Rev Immunol [Internet]. 2008;8(5):362–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18379575
99. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature [Internet]. 1998;392(6673):245–52. Available from: http://www.nature.com/nature/journal/v392/n6673/full/392245a0.html
100. Shortman K. Ralph Steinman and dendritic cells. Immunol Cell Biol [Internet]. 2012;90(1):1–2. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22024620
101. Aliberti J, Schulz O, Pennington DJ, Tsujimura H, Reis e Sousa C, Ozato K, et al. Essential role for ICSBP in the in vivo development of murine CD8alpha + dendritic cells. Blood. 2003;101(1):305–10.
102. Hacker C, Kirsch RD, Ju XS, Hieronymus T, Gust TC, Kuhl C, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. Nat Immunol [Internet]. 2003;4(4):380–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12598895%5Cnhttp://www.nature.com/ni/journal/v4/n4/pdf/ni903.pdf
103. Hildner K, Edelson BT, Purtha WE, Diamond M, Matsushita H, Kohyama M, et al. Batf3 deficiency reveals a critical role for CD8α+ dendritic cells in cytotoxic T cell immunity. Science (80-) [Internet]. 2008;322(5904):1097–100. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19008445
104. McKenna HJ, Stocking KL, Miller RE, Brasel K, De Smedt T, Maraskovsky E, et al. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. Blood. 2000;95(11):3489–97.
105. Waskow C, Liu K, Darrasse-jèze G, Guermonprez P, Merad M, Shengelia T, et al. FMS-like tyrosine kinase 3 is required for dendritic cell development in peripheral lymphoid tissues. Nat Immunol. 2008;9(6):676–83.
106. Wu L, D'Amico A, Winkel KD, Suter M, Lo D, Shortman K. RelB is essential for the development of myeloid-related CD8a- dendritic cells but not of lymphoid-related CD8a+ dendritic cells. Immunity. 1998;9(6):839–47.
107. Lewis KL, Caton ML, Bogunovic M, Greter M, Lucja T, Ng D, et al. Notch2 receptor signaling controls functional differentiation of dendritic cells in the spleen and intestine. Immunity. 2011;35(5):780–91.
108. Ichikawa E, Hida S, Omatsu Y, Shimoyama S, Takahara K, Miyagawa S, et al. Defective development of splenic and epidermal CD4+ dendritic cells in mice deficient for IFN regulatory factor-2. Proc Natl Acad Sci U S A. 2004;101(11):3909–14.
109. Suzuki S, Honma K, Matsuyama T, Suzuki K, Toriyama K, Akitoyo I, et al. Critical roles of interferon regulatory factor 4 in CD11bhigh CD8alpha- dendritic cell development. Proc Natl Acad Sci ences United States Am. 2004;101(24):8981–6.
110. Vander Lugt B, Khan A a, Hackney J a, Agrawal S, Lesch J, Zhou M, et al. Transcriptional programming of dendritic cells for enhanced MHC class II antigen presentation. Nat Immunol [Internet]. 2014;15(2):161–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24362890
111. Orkin SH. Diversification of haematopoietic stem cells to specific lineages. Nat Rev Genet [Internet]. 2000;1(1):57–64. Available from: http://www.nature.com.proxy-ub.rug.nl/nrg/journal/v1/n1/full/nrg1000_057a.html
112. Graf T. Differentiation plasticity of hematopoietic cells Review article Differentiation plasticity of

hematopoietic cells. Blood. 2002;99(9):3089–101.
113. Zenke M, Hieronymus T. Towards an understanding of the transcription factor network of dendritic cell development. Trends Immunol. 2006;27(3):140–5.
114. Guerriero a, Langmuir PB, Spain LM, Scott EW. PU.1 is required for myeloid-derived but not lymphoid-derived dendritic cells. Blood [Internet]. 2000;95(3):879–85. Available from: http://www.bloodjournal.org/content/95/3/879.abstract
115. Hromas R, Orazi A, Neiman RS, Maki R, Van Beveran C, Moore J, et al. Hematopoietic lineage- and stage-restricted expression of the ETS oncogene family member PU.1. Blood [Internet]. 1993;82(10):2998–3004. Available from: http://www.bloodjournal.org/content/82/10/2998.abstract
116. Spain LM, Guerriero A, Kunjibettu S, Scott EW. T cell development in PU.1-deficient mice. J Immunol [Internet]. 1999;163(5):2681–7. Available from: http://www.ncbi.nlm.nih.gov/htbin-post/Entrez/query?db=m&form=6&dopt=r&uid=10453009%0Ahttp://www.jimmunol.org/v163n5/2681/2681-abs-frame.html
117. Back J, Dierich A, Bronn C, Kastner P, Chan S, Dc W. PU . 1 determines the self-renewal capacity of erythroid progenitor cells Plenary paper PU . 1 determines the self-renewal capacity of erythroid progenitor cells. Blood. 2004;103(10):3615–23.
118. Nutt SL, Metcalf D, D'Amico A, Polli M, Wu L. Dynamic regulation of PU.1 expression in multipotent hematopoietic progenitors. J Exp Med [Internet]. 2005;201(2):221–31. Available from: http://jem.rupress.org/content/201/2/221%5Cnhttp://jem.rupress.org/content/201/2/221.full.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/15657291
119. Dakic A, Metcalf D, Di Rago L, Mifsud S, Wu L, Nutt SL. PU.1 regulates the commitment of adult hematopoietic progenitors and restricts granulopoiesis. J Exp Med [Internet]. 2005;201(9):1487–502. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2213186&tool=pmcentrez&rendertype=abstract
120. Iwasaki H, Somoza C, Shigematsu H, Duprez E a, Iwasaki-Arai J, Mizuno S-I, et al. Distinctive and indispensable roles of PU. 1 in maintenance of hematopoietic stem cells and their differentiation. Blood [Internet]. 2005;106(5):1590–600. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1895212&tool=pmcentrez&rendertype=abstract
121. Rosenbauer F, Wagner K, Kutok JL, Iwasaki H, Le Beau MM, Okuno Y, et al. Acute myeloid leukemia induced by graded reduction of a lineage-specific transcription factor, PU.1. Nat Genet [Internet]. 2004;36(6):624–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15146183%5Cnhttp://www.nature.com/doifinder/10.1038/ng1361
122. Anderson KL, Perkin H, Surh CD, Venturini S, Maki RA, Torbett BE. Transcription Factor PU.1 Is Necessary for Development of Thymic and Myeloid Progenitor-Derived Dendritic Cells. J Immunol [Internet]. 2000;164(4):1855–61. Available from: http://www.jimmunol.org/content/164/4/1855.full
123. Bakri Y, Sarrazin S, Mayer UP, Tillmanns S, Nerlov C, Boned A, et al. Balance of MafB and PU.1 specifies alternative macrophage or dendritic cell fate. Blood. 2005;105(7):2707–16.
124. Heinz LX, Platzer B, Reisner PM, J??rgl A, Taschner S, G??bel F, et al. Differential involvement of PU.1 and Id2 downstream of TGF-β1 during Langerhans-cell commitment. Blood. 2006;107(4):1445–53.
125. Iwama A, Osawa M, Hirasawa R, Uchiyama N, Kaneko S, Onodera M, et al. Reciprocal roles for CCAAT/enhancer binding protein (C/EBP) and PU.1 transcription factors in Langerhans cell commitment. J Exp Med. 2002;195(5):547–58.
126. Dahl R, Walsh JC, Lancki D, Laslo P, Iyer SR, Singh H, et al. Regulation of macrophage and neutrophil cell fates by the PU.1:C/EBPalpha ratio and granulocyte colony-stimulating factor. Nat Immunol. 2003;4(10):1029–36.
127. Nerlov C, Querfurth E, Kulessa H, Graf T. GATA-1 interacts with the myeloid PU. 1 transcription factor and represses PU. 1-dependent transcription. Blood. 2000;95(8):2543–51.
128. Reddy V a, Iwama A, Iotzova G, Schulz M, Elsasser A, Vangala K, et al. Granulocyte inducer C/EBPa inactivates the myeloid master regulator PU.1: possible role in lineage commitment decisions. Blood. 2002;100(2):483–90.
129. Xie H, Ye M, Feng R. Stepwise reprogramming of B cells into macrophages. Cell [Internet]. 2004;117:1994–5. Available from: http://www.sciencedirect.com/science/article/pii/S0092867404004192
130. Hacker C, Kirsch RD, Ju X-S, Hieronymus T, Gust TC, Kuhl C, et al. Transcriptional profiling identifies Id2 function in dendritic cell development. Nat Immunol. 2003;4(4):380–6.
131. Kusunoki T, Sugai M, Katakai T, Omatsu Y, Iyoda T, Inaba K, et al. TH2 dominance and defective

development of a CD8+ dendritic cell subset in Id2-deficient mice. J Allergy Clin Immunol. 2003;111(1):136–42.
132. Borkowski TA, Letterio JJ, Farr AG, Udey MC. A role for endogenous transforming growth factor beta 1 in Langerhans cell biology: the skin of transforming growth factor beta 1 null mice is devoid of epidermal Langerhans cells. J Exp Med [Internet]. 1996;184(6):2417–22. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2196398&tool=pmcentrez&rendertype=abstract
133. Seré K, Baek JH, Ober-Blöbaum J, Müller-Newen G, Tacke F, Yokota Y, et al. Two Distinct Types of Langerhans Cells Populate the Skin during Steady State and Inflammation. Immunity. 2012;37(5):905–16.
134. Chopin M, Seillet C, Chevrier S, Wu L, Wang H, Morse 3rd HC, et al. Langerhans cells are generated by two distinct PU.1-dependent transcriptional networks. J Exp Med [Internet]. 2013;210(13):2967–80. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3865480/pdf/JEM_20130930.pdf
135. Fainaru O, Woolf E, Lotem J, Yarmus M, Brenner O, Goldenberg D, et al. Runx3 regulates mouse TGF-beta-mediated dendritic cell function and its absence results in airway inflammation. EMBO J [Internet]. 2004;23(4):969–79. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=380997&tool=pmcentrez&rendertype=abstract
136. Tamura T, Tailor P, Yamaoka K, Kong HJ, Tsujimura H, O'Shea JJ, et al. IFN regulatory factor-4 and -8 govern dendritic cell subset development and their functional diversity. J Immunol [Internet]. 2005;174(5):2573–81. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15728463%5Cnhttp://www.jimmunol.org/content/174/5/2573.full.pdf
137. Tsujimura H, Tamura T, Ozato K. Cutting Edge: IFN Consensus Sequence Binding Protein/IFN Regulatory Factor 8 Drives the Development of Type I IFN-Producing Plasmacytoid Dendritic Cells. J Immunol [Internet]. 2003;170(3):1131–5. Available from: http://www.jimmunol.org/content/170/3/1131.full
138. Schiavoni G, Mattei F, Borghi P, Sestili P, Venditti M, Morse HC, et al. ICSBP is critically involved in the normal development and trafficking of Langerhans cells and dermal dendritic cells. Blood. 2004;103(6):2221–8.
139. Savitsky D, Tamura T, Yanai H, Taniguchi T. Regulation of immunity and oncogenesis by the IRF transcription factor family. Cancer Immunol Immunother. 2010;59(4):489–510.
140. Marecki S, Atchison ML, Fenton MJ. Differential Expression and Distinct Functions of IFN Regulatory Factor 4 and IFN Consensus Sequence Binding Protein in Macrophages. J Immunol. 1999;163:2713–22.
141. Rosenbauer F, Waring JF, Foerster J, Wietstruk M, Philipp D, Horak I. Interferon consensus sequence binding protein and interferon regulatory factor-4/Pip form a complex that represses the expression of the interferon-stimulated gene-15 in macrophages. Blood. 1999;94(12):4274–81.
142. Holtschke T, Löhler J, Kanno Y, Fehr T, Giese N, Rosenbauer F, et al. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell. 1996;87(2):307–17.
143. Laricchia-Robbio L, Tamura T, Karpova T, Sprague BL, McNally JG, Ozato K. Partner-regulated interaction of IFN regulatory factor 8 with chromatin visualized in live macrophages. Proc Natl Acad Sci U S A [Internet]. 2005;102(40):14368–73. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1242294&tool=pmcentrez&rendertype=abstract
144. Tsujimura H, Tamura T, Kong HJ, Nishiyama a., Ishii KJ, Klinman DM, et al. Toll-Like Receptor 9 Signaling Activates NF- B through IFN Regulatory Factor-8/IFN Consensus Sequence Binding Protein in Dendritic Cells. J Immunol [Internet]. 2004;172(11):6820–7. Available from: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.172.11.6820
145. Zhang DE, Zhang P, Wang ND, Hetherington CJ, Darlington GJ, Tenen DG. Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein alpha-deficient mice. Proc Natl Acad Sci U S A [Internet]. 1997;94(2):569–74. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=19554&tool=pmcentrez&rendertype=abstract
146. Sieweke MH, Tekotte H, Frampton J, Graf T. MafB is an interaction partner and repressor of Ets-1 that inhibits erythroid differentiation. Cell. 1996;85(1):49–60.
147. Kelly LM, Englmeier U, Lafon I, Sieweke MH, Graf T. MafB is an inducer of monocytic differentiation. EMBO J [Internet]. 2000;19(9):1987–1897. Available from:

http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=305687&tool=pmcentrez&rendertype=abstract
148. Meredith MM, Liu K, Darrasse-Jeze G, Kamphorst AO, Schreiber H a., Guermonprez P, et al. Expression of the zinc finger transcription factor zDC (Zbtb46, Btbd4) defines the classical dendritic cell lineage. J Exp Med [Internet]. 2012;209(6):1153–65. Available from: http://jem.rupress.org/cgi/content/long/209/6/1153
149. Satpathy AT, Kc W, Albring JC, Edelson BT, Kretzer NM, Bhattacharya D, et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. J Exp Med [Internet]. 2012;209(6):1135–52. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3371733&tool=pmcentrez&rendertype=abstract
150. Baeuerle P a, Baltimore D. NF-KB : Ten Years After Meeting Review. Cell. 1996;87:13–20.
151. Traenckner EB, Pahl HL, Henkel T, Schmidt KN, Wilk S, Baeuerle PA. Phosphorylation of human IKB-ox on serines 32 and 36 controls IKB-a proteolysis and NF-KB activation in response to diverse stimuli. EMBO J. 1995;14(12):2876–83.
152. Chen ZJ, Parent L, Maniatis T. Site-specific phosphorylation of I kappa B alpha by a novel ubiquitination-dependent protein kinase activity. Cell. 1996;84(6):853–62.
153. Weih F, Carrasco D, Durham SK, Barton DS, Rizzo CA, Ryseck RP, et al. Multiorgan Inflammation and Hematopoietic Abnormalities in Mice With a Targeted Disruption of Relb, a Member of the Nf-Kappa-B/Rel Family. Cell. 1995;80(2):331–40.
154. Platzer B, Jörgl A, Taschner S, Höcher B, Strobl H. RelB regulates human dendritic cell subset development by promoting monocyte intermediates. Blood. 2004;104(12):3655–63.
155. Carrasco D, Ryseck RP, Bravo R. Expression of relB transcripts during lymphoid organ development: specific expression in dendritic antigen-presenting cells. Development [Internet]. 1993;118(4):1221–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8269849
156. Clark GJ, Gunningham S, Troy A, Vuckovic S, Hart DNJ. Expression of the RelB transcription factor correlates with the activation of human dendritic cells. Immunology. 1999;98(2):189–96.
157. Pettit AR, MacDonald KPA, O'Sullivan B, Thomas R. Differentiated dendritic cells expressing nuclear RelB are predominantly located in rheumatoid synovial tissue perivascular mononuclear cell aggregates. Arthritis Rheum. 2000;43(4):791–800.
158. Ammon C, Mondal K, Andreesen R, Krause SW. Differential expression of the transcription factor NF-kappaB during human mononuclear phagocyte differentiation to macrophages and dendritic cells. Biochem bBophysical Res Commun [Internet]. 2000;268(1):99–105. Available from: http://www.sciencedirect.com/science/article/pii/S0006291X99920831%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/10652220
159. Hofer S, Rescigno M, Granucci F, Citterio S, Francolini M. Differential activation of NF- j B subunits in dendritic cells in response to Gram-negative bacteria and to lipopolysaccharide. Microbes Infect. 2001;3:259–65.
160. Feinberg MW, Cao Z, Wara AK, Lebedeva MA, SenBanerjee S, Jain MK. Kruppel-like factor 4 is a mediator of proinflammatory signaling in macrophages. J Biol Chem. 2005;280(46):38247–58.
161. Garrett-Sinha LA, Eberspaecher H, Seldin MF, de Crombrugghe B. A gene for a novel zinc-finger protein expressed in differentiated cells and transiently in certain mesechymal cells. J Biol Chem. 1996;271(49):31384–90.
162. Feinberg MW, Wara AK, Cao Z, Lebedeva MA, Rosenbauer F, Iwasaki H, et al. The Kruppel-like factor KLF4 is a critical regulator of monocyte differentiation. EMBO J [Internet]. 2007;26(18):4138–48. Available from: http://emboj.embopress.org/content/26/18/4138.abstract
163. Alder JK, Iii RWG, Hildreth RL, Kaplan IM, Morisot S, Yu X, et al. Kruppel-Like Factor 4 Is Essential for In ammatory Monocyte Differentiation In Vivo 1. J Immunol. 2008;180(20):5645–52.
164. Takahashi K, Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell. 2006;126(4):663–76.
165. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature [Internet]. 2007;448(July):313–8. Available from: http://dx.doi.org/10.1038/nature05934%0Ahttp://www.nature.com/nature/journal/v448/n7154/suppinfo/nature05934_S1.html
166. Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K, et al. In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature [Internet]. 2007;448(7151):318–24. Available from: papers3://publication/doi/10.1038/nature05944
167. Kurotaki D, Osato N, Nishiyama A, Yamamoto M, Ban T, Sato H, et al. Essential role of the IRF8-KLF4 transcription factor cascade in murine monocyte differentiation. Blood. 2013;121(10):1839–49.

168. Artavanis-Tsakonas S, MD R, Lake RJ. Notch Signaling: Cell Fate Control and Signal Integration in Development. Science (80-) [Internet]. 1999;284(5415):770–6. Available from: https://www.pubchase.com/article/10221902%5Cnhttp://www.sciencemag.org/cgi/doi/10.1126/science.284.5415.770
169. Radtke F, Fasnacht N, MacDonald HR. Notch Signaling in the Immune System. Immunity [Internet]. 2010;32(1):14–27. Available from: http://dx.doi.org/10.1016/j.immuni.2010.01.004
170. Kao H, Ordentlich P, Koyano-nakagawa N, Tang Z. A histone deacetylase corepressor complex regulates the Notch signal transduction ? pathway A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. Genes Dev. 1998;2:2269–77.
171. Poellinger L, Lendahl U. Modulating Notch signaling by pathway-intrinsic and pathway-extrinsic mechanisms. Curr Opin Genet Dev. 2008;18(5):449–54.
172. Samon JB, Champhekar A, Minter LM, Telfer JC, Miele L, Fauq A, et al. Notch1 and TGF Beta1 cooperatively regulate Foxp3 expression and the maintenance of peripheral regulatory T cells. Blood. 2008;112(5):1813–21.
173. Maeng YS, Choi YJ, Kim EK. TGFBIp regulates differentiation of EPC (CD133+c-kit+lin- cells) to EC through activation of the notch signaling pathway. Stem Cells. 2015;33(6):2052–62.
174. Hurlbut GD, Kankel MW, Lake RJ, Artavanis-Tsakonas S. Crossing paths with Notch in the hyper-network. Curr Opin Cell Biol. 2007;19(2):166–75.
175. Kopan R, Ilagan MXG. The Canonical Notch Signaling Pathway: Unfolding the Activation Mechanism. Cell. 2009;137(2):216–33.
176. Hoyne GF. Notch signaling in the immune system Abstract : Notch signaling plays a preeminent. J Leukoc Biol. 2003;74(December):971–81.
177. Kumano K, Chiba S, Kunisato A, Sata M, Saito T, Nakagami-Yamaguchi E, et al. Notch1 but not Notch2 is essential for generating hematopoietic stem cells from endothelial cells. Immunity. 2003;18(5):699–711.
178. Robert-Moreno A, Espinosa L, de la Pompa JL, Bigas A. RBPjkappa-dependent Notch function regulates Gata2 and is essential for the formation of intra-embryonic hematopoietic cells. Development [Internet]. 2005;132(5):1117–26. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15689374%5Cnhttp://dev.biologists.org/cgi/reprint/132/5/1117.pdf
179. Dahlberg A, Delaney C, Bernstein ID. Review article Ex vivo expansion of human hematopoietic stem and progenitor cells. Blood. 2011;117(23):6083–90.
180. Delaney C, Varnum-Finney B, Aoyama K, Brashem-Stein C, Bernstein ID. Dose-dependent effects of the Notch ligand Delta1 on ex vivo differentiation and in vivo marrow repopulating ability of cord blood cells. Blood. 2005;106(8):2693–9.
181. Varnum-Finney B, Purton LE, Yu M, Brashem-Stein C, Flowers D, Staats S, et al. The Notch ligand, Jagged-1, influences the development of primitive hematopoietic precursor cells. Blood. 1998;91(11):4084–91.
182. Jones P, May G, Healy L, Brown J, Hoyne G, Delassus S, et al. Stromal expression of Jagged 1 promotes colony formation by fetal hematopoietic progenitor cells. Blood [Internet]. 1998;92(5):1505–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9716576
183. Kunisato A, Kunisato A, Chiba S, Chiba S, Nakagami-Yamaguchi E, Nakagami-yamaguchi E, et al. HES-1 preserves puri ed hematopoietic stem cells ex vivo and accumulates side population cells in vivo. Blood. 2003;101(5):1777–83.
184. Stier S, Cheng T, Dombkowski D, Carlesso N, Scadden DT. Notch1 activation increases hematopoietic stem cell self-renewal in vivo and favors lymphoid over myeloid lineage outcome. Blood. 2002;99(7):2369–78.
185. Sallusto BF, Lanzavecchia A. Efficient Presentation of Soluble Antigen by Cultured Human Dendritic Cells is maintained by Granulocyte/Macrophage Colony-stimulating Factor plus Interleukin 4 and Downregulated by Tumor Necrosis Factor a. J Exp Med. 1994;179:1109–18.
186. Caux C, Massacrier C, Vanbervliet B, Dubois B, Durand I, Cella M, et al. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to granulocyte-macrophage colony-stimulating factor plus tumor necrosis factor alpha: II. Functional analysis. Blood [Internet]. 1997;90(4):1458–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9269763
187. Gilliet M, Boonstra A, Paturel C, Antonenko S, Xu XL, Trinchieri G, et al. The development of murine plasmacytoid dendritic cell precursors is differentially regulated by FLT3-ligand and granulocyte/macrophage colony-stimulating factor. J Exp Med. 2002;195(7):953–8.
188. Maraskovsky E, Brasel K, Teepe M, Roux ER, Lyman SD, Shortman K, et al. Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell

subpopulations identified. J Exp Med [Internet]. 1996;184(5):1953–62. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2192888&tool=pmcentrez&rendertype=abstract

189. Pulendran B, Lingappa J, Kennedy MK, Smith J, Teepe M, Rudensky A, et al. Developmental pathways of dendritic cells in vivo: distinct function, phenotype, and localization of dendritic cell subsets in FLT3 ligand-treated mice. J Immunol [Internet]. 1997;159(5):2222–31. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=9278310
190. Strobl H, Bello-Fernandez C, Riedl E, Pickl WF, Majdic O, Lyman SD, et al. flt3 ligand in cooperation with transforming growth factor-beta1 potentiates in vitro development of Langerhans-type dendritic cells and allows single-cell dendritic cell cluster formation under serum-free conditions. Blood [Internet]. 1997;90(4):1425–34. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9269760
191. Blom B, Ho S, Antonenko S, Liu YJ. Generation of Interferon alpha-producing Predendritic Cell (Pre-DC)2 from Human CD34+ Hematopoietic Stem Cells. J Exp Med [Internet]. 2000;192(12):1785–96. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11120775%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2213503
192. Chicha L. Clonal Type I Interferon-producing and Dendritic Cell Precursors Are Contained in Both Human Lymphoid and Myeloid Progenitor Populations. J Exp Med [Internet]. 2004;200(11):1519–24. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2211954&tool=pmcentrez&rendertype=abstract
193. Onai N, Obata-Onai A, Schmid M a, Ohteki T, Jarrossay D, Manz MG. Identification of clonogenic common Flt3+M-CSFR+ plasmacytoid and conventional dendritic cell progenitors in mouse bone marrow. Nat Immunol [Internet]. 2007;8(11):1207–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17922016
194. Adolfsson J, Borge OJ, Bryder D, Theilgaard-Mönch K, Åstrand-Grundström I, Sitnicka E, et al. Upregulation of Flt3 expression within the bone marrow Lin-Sca1+c-kit+ stem cell compartment is accompanied by loss of self-renewal capacity. Immunity. 2001;15(4):659–69.
195. Karsunky H, Merad M, Cozzio a., Weissman IL, Manz MG. Flt3 Ligand Regulates Dendritic Cell Development from Flt3+ Lymphoid and Myeloid-committed Progenitors to Flt3+ Dendritic Cells In Vivo. J Exp Med [Internet]. 2003;198(2):305–13. Available from: http://jem.rupress.org/content/198/2/305.long%5Cnhttp://www.jem.org/cgi/doi/10.1084/jem.20030323
196. Fogg DK. A Clonogenic Bone Marrow Progenitor Specific for Macrophages and Dendritic Cells. Science (80-) [Internet]. 2006;311(5757):83–7. Available from: http://www.sciencemag.org/cgi/doi/10.1126/science.1117729
197. Naik SH, Metcalf D, van Nieuwenhuijze A, Wicks I, Wu L, O'Keeffe M, et al. Intrasplenic steady-state dendritic cell precursors that are distinct from monocytes. Nat Immunol [Internet]. 2006;7(6):663–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16680143
198. Liu K, Victora G, Schwickert T. In vivo analysis of dendritic cell development and homeostasis. Science (80-) [Internet]. 2009;324(5925):392–7. Available from: http://www.sciencemag.org/content/324/5925/392.short
199. Pixley FJ, Stanley ER. CSF-1 regulation of the wandering macrophage: Complexity in action. Trends Cell Biol. 2004;14(11):628–38.
200. Ginhoux F, Tacke F, Angeli V, Bogunovic M, Loubeau M, Dai X-M, et al. Langerhans cells arise from monocytes in vivo. Nat Immunol [Internet]. 2006;7(3):265–73. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16444257%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4727824
201. Dai X, Ryan GR, Hapel a J, Dominguez MG, Russell RG, Kapp S, et al. Targeted disruption of the mouse CSF-1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primititive progenitor cell frequencies and reproductive defects. Blood. 2002;99(1):111–20.
202. Wang NS, McHeyzer-Williams LJ, Okitsu SL, Burris TP, Reiner SL, McHeyzer-Williams MG. Divergent transcriptional programming of class-specific B cell memory by T-bet and RORα. Nat Immunol [Internet]. 2012;13(6):604–11. Available from: http://dx.doi.org/10.1038/ni.2294
203. Hieronymus T, Zenke M, Baek JH, Ser?? K. The clash of Langerhans cell homeostasis in skin: Should I stay or should I go? Semin Cell Dev Biol [Internet]. 2015;41:30–8. Available from: http://dx.doi.org/10.1016/j.semcdb.2014.02.009
204. Moustakas A, Heldin C-H. The regulation of TGFbeta signal transduction. Development [Internet].

2009;136(22):3699–§714. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19855013
205. Meng X-M, Chung ACK, Lan HY. Role of the TGF-β/BMP-7/Smad pathways in renal diseases. Clin Sci [Internet]. 2013;124(4):243–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23126427
206. Kulkarni AB, Huh CG, Becker D, Geiser A, Lyght M, Flanders KC, et al. Transforming growth factor beta 1 null mutation in mice causes excessive inflammatory response and early death. Proc Natl Acad Sci U S A [Internet]. 1993;90(2):770–4. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=45747&tool=pmcentrez&rendertype=abstract
207. Shull M, Ormsby I, Kier A, Pawlowskr S, Diebold R, Yin M, et al. Targeted disruption of the mouse transforming growth factor-β1 gene results in multifocal inflamatory disease. Nature. 1992;359(6397):693–9.
208. Kaplan DH, Li MO, Jenison MC, Shlomchik WD, Flavell RA, Shlomchik MJ. Autocrine/paracrine TGFβ1 is required for the development of epidermal Langerhans cells. J Exp Med [Internet]. 2007;204(11):2545–52. Available from: http://jem.rupress.org/cgi/content/full/204/11/2545%5Cnpapers2://publication/doi/10.1084/jem.20071401
209. Caux C, Massacrier C, Dubois B, Valladeau J, Dezutter-Dambuyant C, Durand I, et al. Respective involvement of TGF-beta and IL-4 in the development of Langerhans cells and non-Langerhans dendritic cells from CD34+ progenitors. J Leukoc Biol. 1999;66(November):781–91.
210. Travis MA, Sheppard D. TGF-β activation and function in immunity. Annu Rev Immunol. 2014;32:51–82.
211. Mohammed J, Beura LK, Bobr A, Astry B, Chicoine B, Kashem SW, et al. Stromal cells control the epithelial residence of DCs and memory T cells by regulated activation of TGF-β. Nat Immunol [Internet]. 2016;17(4):414–21. Available from: http://www.nature.com/doifinder/10.1038/ni.3396
212. Yasmin N, Bauer T, Modak M, Wagner K, Schuster C, Köffel R, et al. Identification of bone morphogenetic protein 7 (BMP7) as an instructive factor for human epidermal Langerhans cell differentiation. J Exp Med [Internet]. 2013;210(12):2597–610. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3832935&tool=pmcentrez&rendertype=abstract
213. Zouvelou V, Passa O, Segklia K, Tsalavos S, Valenzuela DM, Economides AN, et al. Generation and functional characterization of mice with a conditional BMP7 allele. Int J Dev Biol. 2009;53(4):597–603.
214. Helder N, Ozkaynak E, Sampath K, Luyten F, Latin V, Oppermann H, et al. Expression pattern of osteogenic protein-1 (bone morphogenetic protein-7) in human and mouse development. J Histochem Cytochem. 1995;43(10):1035–44.
215. Elbe A, Tschachler E, Steiner G, Binder A, Wolff K, Stingl G. Maturational steps of bone marrow-derived dendritic murine epidermal cells . Phenotypic and functional studies on Langerhans cells and Thy-1 + dendritic epidermal cells in the perinatal period . Information about subscribing. J Immunol. 1989;143:2431–8.
216. Chang-Rodriguez S, Hoetzenecker C, Schwärzler C, Biedermann T, Saeland S, Elbe-Bürger E. Fetal and neonatal murine skin harbors Langerhans cell precursors. J Leukoc Biol. 2005;77(3):352–60.
217. Chorro L, Sarde A, Li M, Woollard KJ, Chambon P, Malissen B, et al. Langerhans cell (LC) proliferation mediates neonatal development, homeostasis, and inflammation-associated expansion of the epidermal LC network. J Exp Med [Internet]. 2009;206(13):3089–100. Available from: http://jem.rupress.org/content/206/13/3089
218. Martínez VG, Hernández-López C, Valencia J, Hidalgo L, Entrena A, Zapata AG, et al. The canonical BMP signaling pathway is involved in human monocyte-derived dendritic cell maturation. Immunol Cell Biol [Internet]. 2011;89(5):610–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21102536
219. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science (80-) [Internet]. 2010;327(5966):656–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20133564
220. Naik SH, Sathe P, Park H-Y, Metcalf D, Proietto AI, Dakic A, et al. Development of plasmacytoid and conventional dendritic cell subtypes from single precursor cells derived in vitro and in vivo. Nat Immunol [Internet]. 2007;8(11):1217–26. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17922015
221. Lee J, Breton G, Oliveira TYK, Zhou YJ, Aljoufi A, Puhr S, et al. Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow. J Exp Med [Internet]. 2015;212(3):385–99. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25687283
222. Breton G, Lee J, Zhou YJ, Schreiber JJ, Keler T, Puhr S, et al. Circulating precursors of human

CD1c+ and CD141+ dendritic cells. J Exp Med [Internet]. 2015;212(3):401–13. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=25687281&retmode=ref&cmd=prlinks%5Cnpapers2://publication/doi/10.1084/jem.20141441%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/25687281%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fc

223. Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FM V. Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nat Neurosci [Internet]. 2007;10(12):1538–43. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18026097
224. Auffray C, Fogg DK, Narni-Mancinelli E, Senechal B, Trouillet C, Saederup N, et al. CX3CR1+ CD115+ CD135+ common macrophage/DC precursors and the role of CX3CR1 in their response to inflammation. J Exp Med [Internet]. 2009;206(3):595–606. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=19273628&retmode=ref&cmd=prlinks%5Cnhttp://www.ncbi.nlm.nih.gov/pmc/articles/PMC2699130/pdf/JEM_20081385.pdf
225. Breton G, Lee J, Liu K, Nussenzweig MC. Defining human dendritic cell progenitors by multiparametric flow cytometry. Nat Protoc [Internet]. 2015;10(9):1407–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26292072%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4607256
226. Varol C, Landsman L, Fogg DK, Greenshtein L, Gildor B, Margalit R, et al. Monocytes give rise to mucosal, but not splenic, conventional dendritic cells. J Exp Med. 2007;204(1):171–80.
227. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science (80-) [Internet]. 2010;327(5966):656–61. Available from: http://www.sciencemag.org/content/327/5966/656.short%5Cnpapers://373b69f9-0281-4879-8e6d-d1a4fe665b92/Paper/p7758
228. Diao J, Winter E, Cantin C, Chen W, Xu L, Kelvin D, et al. In situ replication of immediate dendritic cell (DC) precursors contributes to conventional DC homeostasis in lymphoid tissue. J Immunol [Internet]. 2006;176(12):7196–206. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=16751363&retmode=ref&cmd=prlinks%5Cnpapers2://publication/uuid/E3F439E1-7E58-4B59-81EB-FDCB5DB20FCE
229. Akashi K, Traver D, Miyamoto T, Weissman IL. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature. 2000;404(6774):193–7.
230. Kondo M, Weissman IL, Akashi K, Akashi K, Weissman IL, Akashi K, et al. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell [Internet]. 1997;91(5):661–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9393859
231. Cheong C, Matos I, Choi J-H, Dandamundi DB, Shrestha E, Longhi MP, et al. Microbial stimulation fully differentiates monocytes to DC-SIGN/CD209+ dendritic cells for immune T cell areas. Cell. 2010;143(3):416–29.
232. Dcs CCD, Breton G, Zheng S, Valieris R, Tojal I, Satija R, et al. Human dendritic cells (DCs) are derived from distinct circulating precursors that are precommitted to become. J Exp Med. 2016;18:1–10.
233. Terry RL, Miller SD. Molecular control of monocyte development. Cell Immunol [Internet]. 2014;291(1–2):16–21. Available from: http://dx.doi.org/10.1016/j.cellimm.2014.02.008
234. Schönheit J, Kuhl C, Gebhardt ML, Klett FF, Riemke P, Scheller M, et al. PU.1 Level-Directed Chromatin Structure Remodeling at the Irf8 Gene Drives Dendritic Cell Commitment. Cell Rep. 2013;3(5):1617–28.
235. Wang H, Lee CH, Qi C, Tailor P, Feng J, Abbasi S, et al. IRF8 regulates B-cell lineage specification, commitment, and differentiation. Blood. 2008;112(10):4028–38.
236. Koenigsmann J, Rudolph C, Sander S, Kershaw O, Gruber AD, Bullinger L, et al. Nf1 haploinsufficiency and Icsbp deficiency synergize in the development of leukemias. Blood. 2009;113(19):4690–701.
237. Scheller M, Foerster J, Heyworth CM, Waring JF, Löhler J, Gilmore GL, et al. Altered development and cytokine responses of myeloid progenitors in the absence of transcription factor, interferon consensus sequence binding protein. Blood. 1999;94(11):3764–71.
238. Tsujimura H, Nagamura-Inoue T, Tamura T, Ozato K. IFN Consensus Sequence Binding Protein/IFN Regulatory Factor-8 Guides Bone Marrow Progenitor Cells Toward the Macrophage Lineage. J Immunol [Internet]. 2002;169(3):1261–9. Available from: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.169.3.1261
239. Sunderkötter C, Nikolic T, Dillon MJ, van Rooijen N, Stehling M, Drevets DA, et al. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J Immunol. 2004;172:4410–7.
240. Ingersoll M, Spanbroek R, Lottaz C, Gautier E, Frankenberger M, Hoffmann R, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. Blood [Internet].

2010;115(3):10–20. Available from: http://bloodjournal.hematologylibrary.org/content/115/3/e10.short
241. Lin H, Lee E, Hestir K, Leo C, Huang M, Bosch E, et al. Discovery of a Cytokine and Its Receptor by Functional Screening of the Extracellular Proteome. Science (80-). 2008;320(1996):807–11.
242. Doherty TM, Coffman RL. Leishmania antigens presented by GM-CSF-derived macrophages protect susceptible mice against challenge with Leishmania major. J Immunol. 1993;150(12):5476–83.
243. Hamilton JA. GM-CSF in inflammation and autoimmunity. Trends Immunol. 2002;23(8):403–8.
244. Lieschke GJ, Grail D, Hodgson G, Metcalf D, Stanley E, Cheers C, et al. Mice lacking granulocyte colony-stimulating factor have chronic neutropenia, granulocyte and macrophage progenitor cell deficiency, and impaired neutrophil mobilization. Blood. 1994;84(6):1737–46.
245. Stanley E, Lieschke GJ, Grail D, Metcalf D, Hodgson G, Gall JA, et al. Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. Proc Natl Acad Sci U S A [Internet]. 1994;91(12):5592–6. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=44042&tool=pmcentrez&rendertype=abstract
246. Burgess AW, Metcalf D. The nature and action of granulocyte-macrophage colony stimulating factors. Blood [Internet]. 1980;56(6):947–58. Available from: http://www.bloodjournal.org/content/bloodjournal/56/6/947.full.pdf?sso-checked=true
247. Gasson JC. Molecular Physiology of Granulocyte-Macrophage Colony-Stimulating Factor. Blood. 1991;77(6):1131–46.
248. Campbell IK, van Nieuwenhuijze A, Segura E, O'Donnell K, Coghill E, Hommel M, et al. Differentiation of inflammatory dendritic cells is mediated by NF-κB1-dependent GM-CSF production in CD4 T cells. J Immunol [Internet]. 2011;186(9):5468–77. Available from: http://www.jimmunol.org/content/186/9/5468.full
249. Codarri L, Gyülvészi G, Tosevski V, Hesske L, Fontana A, Magnenat L, et al. RORγt drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. Nat Immunol [Internet]. 2011;12(6):560–7. Available from: http://dx.doi.org/10.1038/ni.2027
250. El-Behi M, Bogoljub C, Hong D, Yaping Y, Melissa C, Farinaz S, et al. The Encephalitogenicity of T H17 Cells Is Dependent on IL-1- And IL-23-induced Production of the Cytokine GM-CSF. Nat Immunol [Internet]. 2011;12(6):568–75. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3116521/pdf/nihms-301291.pdf
251. Mitchell AJ, Roediger B, Weninger W. Monocyte homeostasis and the plasticity of inflammatory monocytes. Cell Immunol [Internet]. 2014;291(1–2):22–31. Available from: http://dx.doi.org/10.1016/j.cellimm.2014.05.010
252. Ziegler-Heitbrock HWLW. Heterogeneity of human blood monocytes: the CD14+ CD16+ subpopulation. Immunol Today [Internet]. 1996;17(9):424–8. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8854561
253. Geissmann F, Jung S, Littman DR. Blood Monocytes Consist of Two Principal Subsets with Distinct Migratory Properties. Immunity [Internet]. 2003;19:71–82. Available from: papers2://publication/uuid/EE9FACA2-73CA-4A73-A8F3-98BE7571C2BA
254. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. Vol. 116, Blood. 2010.
255. Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. J Leukoc Biol [Internet]. 2007;81(3):584–92. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17135573
256. Passlick B, Flieger D, Ziegler-Heitbrock H.W. L. Identification and Characterization of a Novel Monocyte Subpopulation in Human Peripheral Blood. Blood [Internet]. 1989;74(7):2527–34. Available from: http://www.bloodjournal.org/content/bloodjournal/74/7/2527.full.pdf
257. Wong KL, Tai JJ-Y, Wong W-C, Han H, Sem X, Yeap W-H, et al. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. Blood [Internet]. 2011;118(5):e16-31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21653326
258. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Derek N, et al. Nomenclature of monocytes and dendritic cells in blood Nomenclature of monocytes and dendritic cells in blood. e-Blood. 2010;116(16):e74–80.
259. Steppich B, Dayyani F, Gruber R, Lorenz R, Mack M, Ziegler-Heitbrock HWL. Selective mobilization of CD14+CD16+ monocytes by exercise. Am J Physiol - Cell Physiol [Internet]. 2000;279(3):C578–86. Available from:

http://ajpcell.physiology.org/content/279/3/C578%5Cnhttp://ajpcell.physiology.org/content/279/3/C578.full.pdf%5Cnhttp://ajpcell.physiology.org/content/279/3/C578.short%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/10942707
260. Belge K-U, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, et al. The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. J Immunol. 2002;168(7):3536–42.
261. Szaflarska A, Baj-Krzyworzeka M, Siedlar M, Weglarczyk K, Ruggiero I, Hajto B, et al. Antitumor response of CD14+/CD16+ monocyte subpopulation. Exp Hematol [Internet]. 2004;32(8):748–55. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15308326
262. Ancuta P, Rao R, Moses A, Mehle A, Shaw SK, Luscinskas FW, et al. Fractalkine preferentially mediates arrest and migration of CD16+ monocytes. J Exp Med [Internet]. 2003;197(12):1701–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2193954&tool=pmcentrez&rendertype=abstract
263. Weber C, Belge K, Hundelshausen P Von, Draude G, Steppich B, Mack M, et al. Differential chemokine receptor expression and function in human monocyte subpopulations. J Leukocytes Biol. 2000;67(5):699–704.
264. Segura E, Amigorena S. Inflammatory dendritic cells in mice and humans. Trends Immunol [Internet]. 2013;34(9):440–5. Available from: http://dx.doi.org/10.1016/j.it.2013.06.001
265. Nakano H, Lin KL, Yanagita M, Charbonneau C, Cook DN, Kakiuchi T, et al. Blood-derived inflammatory dendritic cells in lymph nodes stimulate acute T helper type 1 immune responses. Nat Immunol [Internet]. 2009;10(4):394–402. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19252492%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2668134
266. Martinez F, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. Front Biosci [Internet]. 2008;13:453–61. Available from: http://dx.doi.org/10.2741/2692
267. Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. J Clin Invest. 2012;122(3):787–95.
268. Domínguez PM, Ardavín C, Dom PM, Ardav C. Differentiation and function of mouse monocyte-derived dendritic cells in steady state and inflammation. Immunol Rev [Internet]. 2010;234(1):90–104. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20193014
269. Lauvau G, Chorro L, Spaulding E, Soudja SMH. Inflammatory monocyte effector mechanisms. Cell Immunol [Internet]. 2014;291(1–2):32–40. Available from: http://dx.doi.org/10.1016/j.cellimm.2014.07.007
270. Romani N, Tripp CH, Stoitzner P. Langerhans Cells Come in Waves. Immunity. 2012;37(5):766–8.
271. Stary G, Klein I, Brüggen M-C, Kohlhofer S, Brunner PM, Spazierer D, et al. Host Defense Mechanisms in Secondary Syphilitic Lesions. Am J Pathol [Internet]. 2010;177(5):2421–32. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0002944010602945
272. Ratzinger G, Baggers J, de Cos M a, Yuan J, Dao T, Reagan JL, et al. Mature human Langerhans cells derived from CD34+ hematopoietic progenitors stimulate greater cytolytic T lymphocyte activity in the absence of bioactive IL-12p70, by either single peptide presentation or cross-priming, than do dermal-interstitial or monoc. J Immunol. 2004;173(4):2780–91.
273. Caux C, Massacrier C, Jacquet C, Imamurafl Daniel Schmitt S, Banchereau J. CD34 + Hematopoietic Progenitors from Human Cord Blood Differentiate Along Two Independent Dendritic Cell Pathways in Response to GM-CSF+TNFa. J Exp Med. 1996;184(August):695–706.
274. Varnum-Finney B, Wu L, Yu M, Brashem-Stein C, Staats S, Flowers D, et al. Immobilization of Notch ligand, Delta-1, is required for induction of notch signaling. J Cell Sci. 2000;113 Pt 23:4313–8.
275. Gold D, Coombes K, Medhane D, Ramaswamy A, Ju Z, Strong L, et al. A comparative analysis of data generated using two different target preparation methods for hybridization to high-density oligonucleotide microarrays. BMC Genomics. 2004;5(1):2.
276. Hutter C, Kauer M, Simonitsch-Klupp I, Jug G, Schwentner R, Leitner J, et al. Notch is active in Langerhans cell histiocytosis and confers pathognomonic features on dendritic cells. Blood. 2012;120(26):5199–208.
277. Irizarry RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res. 2003;31(4):e15.
278. Gentleman VJ, Carey DM, Bates B, Bolstad M, Dettling S, Dudoit B, et al. Bioconductor: Open software development for computational biology and bioinformatics R. Genome Biol. 2004;(5):R80.
279. Platzer B, Jörgl A, Taschner S, Höcher B, Strobl H, Platzer B, et al. RelB regulates human dendritic cell subset development by promoting monocyte intermediates RelB regulates human dendritic cell

subset development by promoting monocyte intermediates. Blood. 2014;104(12):3655–63.
280. Jörgl A, Platzer B, Taschner S, Heinz LX, Hocher B, Reisner PM, et al. Human Langerhans-cell activation triggered in vitro by conditionally expressed MKK6 is counterregulated by the downstream effector RelB. Blood. 2007;109(1):185–93.
281. Platzer B, Jörgl A, Taschner S, Höcher B, Strobl H, Platzer B, et al. RelB regulates human dendritic cell subset development by promoting monocyte intermediates RelB regulates human dendritic cell subset development by promoting monocyte intermediates. Blood. 2004;104(12):3655–63.
282. Schuster C, Vaculik C, Fiala C, Meindl S, Brandt O, Imhof M, et al. HLA-DR+ leukocytes acquire CD1 antigens in embryonic and fetal human skin and contain functional antigen-presenting cells. J Exp Med [Internet]. 2009;206(1):169–81. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2626673&tool=pmcentrez&rendertype=abstract
283. Stöckl J, Vetr H, Majdic O, Zlabinger G, Kuechler E, Knapp W. Human major group rhinoviruses downmodulate the accessory function of monocytes by inducing IL-10. J Clin Invest. 1999;104(7):957–65.
284. Lansdorp BPM, Sutherland H, Eaves C. Selective Expression of CD45 Isoforms on Functional Subpopulations of CD34 + Hemopoietic Cells from Human Bone Marrow By P.M. Lansdorp, Hj. Sutherland, and Cj. Eaves. Cytometry. 1990;172(July).
285. Canque B, Camus S, Dalloul A, Kahn E, Yagello M, Dezutter-Dambuyant C, et al. Characterization of dendritic cell differentiation pathways from cord blood CD34(+)CD7(+)CD45RA(+) hematopoietic progenitor cells. Blood. 2000;96(12):3748–56.
286. Manz MG, Miyamoto T, Akashi K, Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. Proc Natl Acad Sci U S A. 2002;99(18):11872–7.
287. Haddad R, Guardiola P, Izac B, Thibault C, Radich J, Delezoide A, et al. Molecular characterization of early human T / NK and B-lymphoid progenitor cells in umbilical cord blood. Blood. 2004;104(13):3918–26.
288. Pearson R, Fleetwood J, Eaton S, Crossley M, Bao S. Kruppel-like transcription factors: A functional family. Int J Biochem Cell Biol. 2008;40(10):1996–2001.
289. Spittau B, Krieglstein K. Klf10 and Klf11 as mediators of TGF-beta superfamily signaling. Vol. 347, Cell and Tissue Research. 2012. p. 65–72.
290. Guironnet G, Dezutter-Dambuyant C, Vincent C, Bechetoille N, Schmitt D, Péguet-Navarro J. Antagonistic effects of IL-4 and TGF-β1 on Langerhans cell-related antigen expression by human monocytes. J Leukoc Biol [Internet]. 2002;71(5):845–53. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11994510
291. Singh TP, Zhang HH, Borek I, Wolf P, Hedrick MN, Singh SP, et al. Monocyte-derived inflammatory Langerhans cells and dermal dendritic cells mediate psoriasis-like inflammation. Nat Commun [Internet]. 2016;7(May):13581. Available from: http://www.nature.com/doifinder/10.1038/ncomms13581
292. Jin S, Mutvei a P, Chivukula I V, Andersson ER, Ramsköld D, Sandberg R, et al. Non-canonical Notch signaling activates IL-6/JAK/STAT signaling in breast tumor cells and is controlled by p53 and IKKα/IKKβ. Oncogene [Internet]. 2013;32(41):4892–902. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3795477&tool=pmcentrez&rendertype=abstract
293. Hirata N, Yamada S, Shoda T, Kurihara M, Sekino Y, Kanda Y. Sphingosine-1-phosphate promotes expansion of cancer stem cells via S1PR3 by a ligand-independent Notch activation. Nat Commun [Internet]. 2014;5:4806. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25254944
294. Bray S. Notch signalling: a simple pathway becomes complex. Nat Rev Mol Cell Biol. 2006;7(September):678–89.
295. Caux C, Massacrier C, Vanbervliet B, Dubois B, Durand I, Cella M, et al. CD34+ Hematopoietic Progenitors From Human Cord Blood Differentiate Along From Independent Dendritic Cell Pathways in Response to Granulocyte-Macrophage Colony-Stimulating Factor Plus Tumor Necrosis Factor a: II. Functional Analysis. Blood. 1997;90(4):1458–70.
296. Yusuf I, Kharas MG, Chen J, Peralta RQ, Maruniak A, Sareen P, et al. KLF4 is a FOXO target gene that suppresses B cell proliferation. Int Immunol. 2008;20(5):671–81.
297. Ueno H, Klechevsky E, Morita R, Aspord C, Cao T, Matsui T, et al. Dendritic cell subsets in health and disease. Vol. 219, Immunological Reviews. 2007. p. 118–42.
298. Milne P, Bigley V, Gunawan M, Haniffa M, Collin M. CD1c 1 blood dendritic cells have Langerhans cell potential. Blood. 2015;125(3):470–4.
299. Yin X, Yu H, Jin X, Li J, Guo H, Shi Q, et al. Human Blood CD1c $^{+}$ Dendritic Cells Encompass CD5 ^{high} and CD5 ^{low} Subsets That Differ Significantly in Phenotype, Gene Expression, and Functions. J

Immunol [Internet]. 2017;1600193. Available from: http://www.jimmunol.org/lookup/doi/10.4049/jimmunol.1600193
300. Cheng P, Nefedova Y, Corzo CA, Gabrilovich DI. Regulation of dendritic-cell differentiation by bone marrow stroma via different Notch ligands. Blood. 2007;109(2):507–15.
301. Romani N, Brunner PM, Stingl G. Changing Views of the Role of Langerhans Cells. J Invest Dermatol [Internet]. 2012;132(3):872–81. Available from: http://dx.doi.org/10.1038/jid.2011.437
302. Wollenberg A, Mommaas M, Oppel T, Schottdorf EM, Günther S, Moderer M. Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases. J Invest Dermatol. 2002;118(2):327–34.
303. Jaksits S, Kriehuber E, Charbonnier a S, Rappersberger K, Stingl G, Maurer D. CD34+ cell-derived CD14+ precursor cells develop into Langerhans cells in a TGF-beta 1-dependent manner. J Immunol. 1999;163:4869–77.
304. Mildner A, Jung S. Development and function of dendritic cell subsets. Vol. 40, Immunity. 2014. p. 642–56.
305. Tussiwand R, Everts B, Grajales-Reyes GE, Kretzer NM, Iwata A, Bagaitkar J, et al. Klf4 expression in conventional dendritic cells is required for T helper 2 cell responses. Immunity. 2015;42(5):916–28.
306. Xu Y, Xue S, Zhou J, Voorhees JJ, Fisher GJ. Notch and TGF-β pathways cooperatively regulate receptor protein tyrosine phosphatase-κ (PTPRK) gene expression in human primary keratinocytes. Mol Biol Cell [Internet]. 2015;26(6):1199–206. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4357517&tool=pmcentrez&rendertype=abstract
307. Zheng H, Pritchard DM, Yang X, Bennett E, Liu G, Liu C, et al. KLF4 gene expression is inhibited by the notch signaling pathway that controls goblet cell differentiation in mouse gastrointestinal tract. Am J Physiol Gastrointest Liver Physiol [Internet]. 2009;296(3):G490-8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19109406%5Cnhttp://ajpgi.physiology.org/content/296/3/G490.full.pdf
308. Ghaleb AM, Aggarwal G, Bialkowska AB, Nandan MO, Yang VW. Notch Inhibits Expression of the Krüppel-like Factor 4 Tumor Suppressor in the Intestinal Epithelium. Mol Cancer Res. 2008;6(12):1920–7.
309. Van Es JH, Gijn ME Van, Riccio O, Born M Van Den, Vooijs M, Begthel H, et al. Notch / y-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. Nature. 2005;435(June):959–63.
310. Veenendaal LM, Kranenburg O, Smakman N, Klomp A, Rinkes IHMB, van Diest PJ. Differential Notch and TGFbeta signaling in primary colorectal tumors and their corresponding metastases. Cell Oncol [Internet]. 2008;30(1):1–11. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=AbstractPlus&list_uids=18219106
311. Hu D, Wan Y. Regulation of Krüppel-like factor 4 by the anaphase promoting complex pathway is involved in TGF-b signaling. J Biol Chem. 2011;286(9):6890–901.
312. Senechal B, Elain G, Jeziorski E, Grondin V, De Serre NPM, Jaubert F, et al. Expansion of regulatory T cells in patients with langerhans cell histiocytosis. PLoS Med. 2007;4(8):1374–84.
313. van der Aar AMG, Sylva-Steenland RMR, Bos JD, Kapsenberg ML, de Jong EC, Teunissen MBM. Loss of TLR2, TLR4, and TLR5 on Langerhans cells abolishes bacterial recognition. J Immunol [Internet]. 2007;178(4):1986–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/17277101
314. Bobr A, Olvera-Gomez I, Igyarto BZ, Haley KM, Hogquist K a, Kaplan DH. Acute ablation of Langerhans cells enhances skin immune responses. J Immunol. 2010;185(8):4724–8.
315. Kautz-Neu K, Noordegraaf M, Dinges S, Bennett CL, John D, Clausen BE, et al. Langerhans cells are negative regulators of the anti-Leishmania response. J Exp Med [Internet]. 2011;208(5):885–91. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21536741%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3092359
316. Stary G, Klein I, Bauer W, Koszik F, Reininger B, Kohlhofer S, et al. Glucocorticosteroids modify Langerhans cells to produce TGF-β and expand regulatory T cells. J Immunol [Internet]. 2011;186(1):103–12. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21135170
317. Li H, Han M, Bernier M, Zheng B, Sun S, Su M, et al. Krüppel-like Factor 4 Promotes Differentiation by Transforming Growth Factor-b Receptor-mediated Smad and p38 MAPK Signaling in Vascular Smooth Muscle Cells. J Biol Chem. 2010;285(23):17846–56.
318. Ohishi K, Varnum-Finney B, Serda RE, Anasetti C, Bernstein ID. The Notch ligand, Delta-1, inhibits the differentiation of monocytes into macrophages but permits their differentiation into dendritic

cells. Blood. 2001;98(5):1402–7.
319. Lowell S, Jones P, Le Roux I, Dunne J, Watt FM. Stimulation of human epidermal differentiation by Delta-Notch signalling at the boundaries of stem-cell clusters. Curr Biol. 2000;10(9):491–500.
320. Jaksits S, Kriehuber E, Charbonnier a S, Rappersberger K, Stingl G, Maurer D. CD34+ cell-derived CD14+ precursor cells develop into Langerhans cells in a TGF-beta 1-dependent manner. J Immunol. 1999;163:4869–77.
321. Göbel F, Taschner S, Jurkin J, Konradi S, Vaculik C, Richter S, et al. Reciprocal role of GATA-1 and vitamin D receptor in human myeloid dendritic cell differentiation. Blood [Internet]. 2009;114(18):3813–21. Available from: http://www.scopus.com/inward/record.url?eid=2-s2.0-70449700022&partnerID=40&md5=957c5d689aec736d71eb5daa843baa56
322. Luo S, Skerka C, Kurzai O, Zipfel PF. Complement and innate immune evasion strategies of the human pathogenic fungus Candida albicans. Mol Immunol [Internet]. 2013;56(3):161–9. Available from: http://dx.doi.org/10.1016/j.molimm.2013.05.218
323. Jang SH, Chen H, Gregersen PK, Diamond B, Kim SJ. Kruppel-like factor 4 regulates PRDM1 expression through binding to an autoimmune risk allele. JCI Insight. 2017;2(1):1–14.
324. Lemke G, Burstyn-Cohen T. TAM receptors and the clearance of apoptotic cells. Ann N Y Acad Sci. 2010;1209:23–9.
325. Bauer T, Zagórska A, Jurkin J, Yasmin N, Köffel R, Richter S, et al. Identification of Axl as a downstream effector of TGF-β1 during Langerhans cell differentiation and epidermal homeostasis. J Exp Med [Internet]. 2012;209(11):2033–47. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3478937&tool=pmcentrez&rendertype=abstract
326. Yasmin N, Konradi S, Eisenwort G, Schichl YM, Seyerl M, Bauer T, et al. β-Catenin promotes the differentiation of epidermal Langerhans dendritic cells. J Invest Dermatol [Internet]. 2013;133:1250–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23303458
327. Evans PM, Chen X, Zhang W, Liu C. KLF4 interacts with beta-catenin/TCF4 and blocks p300/CBP recruitment by beta-catenin. Mol Cell Biol [Internet]. 2010;30(2):372–81. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2798472&tool=pmcentrez&rendertype=abstract
328. Hecht a, Vleminckx K, Stemmler MP, van Roy F, Kemler R. The p300/CBP acetyltransferases function as transcriptional coactivators of β-catenin in vertebrates. EMBO J. 2000;19(8):1839–50.
329. Takemaru K-I, Moon RT. The transcriptional coactivator Cbp interacts with \textbeta-catenin to activate gene expression. J Cell Biol [Internet]. 2000;149(2):249–54. Available from: http://jcb.rupress.org/content/149/2/249.abstract
330. Hewison M, Freeman L, Hughes S V, Evans KN, Bland R, Eliopoulos AG, et al. Differential regulation of vitamin D receptor and its ligand in human monocyte-derived dendritic cells. J Immunol. 2003;170:5382–90.
331. Li Q, Gao Y, Jia Z, Mishra L, Guo K, Li Z, et al. Dysregulated KLF4 and Vitamin D Receptor Signaling Promotes Hepatocellular Carcinoma Progression. Gastroenterology. 2012;143(3):799–810.
332. Blokzijl A, Dahlqvist C, Reissmann E, Falk A, Moliner A, Lendahl U, et al. Cross-talk between the Notch and TGF-β signaling pathways mediated by interaction of the Notch intracellular domain with Smad3. J Cell Biol. 2003;163(4):723–8.
333. Massagúe J. TGF-beta signal transduction. Annu Rev Biochem. 1998;67:753–91.
334. Botchkarev VA. Bone morphogenetic proteins and their antagonists in skin and hair follicle biology. J Invest Dermatol [Internet]. 2003;120(1):36–47. Available from: http://dx.doi.org/10.1046/j.1523-1747.2003.12002.x
335. Dahlqvist C, Blokzijl A, Chapman G, Falk A, Dannaeus K, Ibâñez CF, et al. Functional Notch signaling is required for BMP4-induced inhibition of myogenic differentiation. Development. 2003;130(24):6089–99.
336. Martínez-Cingolani C, Grandclaudon M, Jeanmougin M, Jouve M, Zollinger R, Soumelis V. Human blood BDCA-1 dendritic cells differentiate into Langerhans-like cells with thymic stromal lymphopoietin and TGF-β. Blood. 2014;124(15):2411–20.
337. Reche PA, Soumelis V, Gorman DM, Clifford T, Liu M -r., Travis M, et al. Human Thymic Stromal Lymphopoietin Preferentially Stimulates Myeloid Cells. J Immunol [Internet]. 2001;167(1):336–43. Available from: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.167.1.336
338. Arima K, Watanabe N, Hanabuchi S, Chang M, Sun S-C, Liu Y-J. Distinct Signal Codes Generate Dendritic Cell Functional Plasticity. Sci Signal. 2012;3(105):19–22.
339. Liu Y, Soumelis V, Watanabe N, Ito T, Wang Y, Malefyt Rde W, et al. TSLP: an epithelial cell cytokine that regulates T cell differentiation by conditioning dendritic cell maturation. Annu Rev Immunol. 2007;25:193–219.

340. Merad M, Sathe P, Helft J, Millfer J, Mortha A. The Dendritic Cell Lineage: Ontogeny and Function of Dendritic Cells and Their Subsets in the Steady State and the Inflamed setting. Annu Rev Immunol. 2013;31(9):15–25.
341. Breton G, Zheng S, Valieris R, Tojal I, Satija R, Nussenzweig MC. Human dendritic cells (DCs) are derived from distinct circulating precursors that are precommitted to become CD1c+ or CD141+ DCs. J Exp Med. 2016;213(13):2861–70.
342. Wu X, Briseño CG, Durai V, Albring JC, Haldar M, Bagadia P, et al. Mafb lineage tracing to distinguish macrophages from other immune lineages reveals dual identity of Langerhans cells. J Exp Med. 2016;
343. Robbins SH, Walzer T, Dembélé D, Thibault C, Defays A, Bessou G, et al. Novel insights into the relationships between dendritic cell subsets in human and mouse revealed by genome-wide expression profiling. Genome Biol [Internet]. 2008;9(1):R17. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2395256&tool=pmcentrez&rendertype=abstract
344. Jongbloed SL, Kassianos AJ, McDonald KJ, Clark GJ, Ju X, Angel CE, et al. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. J Exp Med [Internet]. 2010;207(6):1247–60. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2882828&tool=pmcentrez&rendertype=abstract
345. Schlitzer A, McGovern N, Teo P, Zelante T, Atarashi K, Low D, et al. IRF4 Transcription Factor-Dependent CD11b+ Dendritic Cells in Human and Mouse Control Mucosal IL-17 Cytokine Responses. Immunity [Internet]. 2013;38(5):970–83. Available from: http://dx.doi.org/10.1016/j.immuni.2013.04.011
346. Yu CI, Becker C, Wang Y, Marches F, Helft J, Leboeuf M, et al. Human CD1c+ dendritic cells drive the differentiation of CD103+CD8+ mucosal effector T cells via the cytokine TGF-b. Immunity. 2013;38(4):818–30.
347. Breton G, Lee J, Liu K, Nussenzweig MC. Defining human dendritic cell progenitors by multiparametric flow cytometry. Nat Protoc. 2015;10(9):1407–22.
348. Kabashima K, Banks TA, Ansel KM, Lu TT, Ware CF, Cyster JG. Intrinsic lymphotoxin-b receptor requirement for homeostasis of lymphoid tissue dendritic cells. Immunity. 2005;22(4):439–50.
349. KC W, Satpathy AT, Rapaport AS, Briseño CG, Wu X, Albring JC, et al. L-Myc expression by dendritic cells is required for optimal T-cell priming. Nature [Internet]. 2014;507(7491):243–7. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3954917&tool=pmcentrez&rendertype=abstract
350. Worah K, Mathan TSM, Vu Manh TP, Keerthikumar S, Schreibelt G, Tel J, et al. Proteomics of Human Dendritic Cell Subsets Reveals Subset-Specific Surface Markers and Differential Inflammasome Function. Cell Rep. 2016;16(11):2953–66.
351. Fasnacht N, Huang H-Y, Koch U, Favre S, Auderset F, Chai Q, et al. Specific fibroblastic niches in secondary lymphoid organs orchestrate distinct Notch-regulated immune responses. J Exp Med [Internet]. 2014;211(11):2265–79. Available from: http://jem.rupress.org/content/211/11/2265.abstract?sid=f56e9a4b-f63c-4c15-82be-a8f1f5bf0970
352. Dudziak D, Kamphorst AO, Heidkamp GF, Buchholz VR, Trumpfheller C, Yamazaki S, et al. Differential antigen processing by dendritic cell subsets in vivo. Science (80-) [Internet]. 2007;315(5808):107–11. Available from: http://www.sciencemag.org/cgi/content/full/315/5808/107%5Cnpapers2://publication/doi/10.1126/science.1136080
353. Kanno Y, Levi BZ, Tamura T, Ozato K. Immune cell-specific amplification of interferon signaling by the IRF-4/8-PU.1 complex. J Interf Cytokine Res [Internet]. 2005;25(12):770–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16375605%5Cnhttp://online.liebertpub.com/doi/abs/10.1089/jir.2005.25.770%5Cnhttp://online.liebertpub.com/doi/abs/10.1089/jir.2005.25.770?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub=pubmed%5Cnhttp://onl
354. Hambleton S, Salem S, Bustamante J, Bigley V, Boisson-Dupuis S, Azevedo J, et al. Mutations in IRF8 and Human Dendritic Cell Immunodeficiency. N Engl J Med. 2011;365(2):127–38.
355. Hashimoto D, Miller J, Merad M. Dendritic Cell and Macrophage Heterogeneity In Vivo. Immunity. 2011;35(3):323–35.
356. Bartel DP. MicroRNA Target Recognition and Regulatory Functions. Cell. 2009;136(2):215–33.
357. Kuipers H, Schnorfeil FM, Fehling H-J, Bartels H, Brocker T. Dicer-dependent microRNAs control maturation, function, and maintenance of Langerhans cells in vivo. J Immunol. 2010;185(1):400–9.
358. Jurkin J, Schichl YM, Koeffel R, Bauer T, Richter S, Konradi S, et al. miR-146a Is Differentially

Expressed by Myeloid Dendritic Cell Subsets and Desensitizes Cells to TLR2-Dependent Activation. J Immunol [Internet]. 2010;184(9):4955–65. Available from: http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.0903021

359. Mi Q-S, Xu Y-P, Qi R-Q, Shi Y-L, Zhou L. Lack of microRNA miR-150 reduces the capacity of epidermal Langerhans cell cross-presentation. Exp Dermatol. 2012;21(11):876–7.
360. Zhou L, Chong MMW, Littman DR. Plasticity of CD4+ T Cell Lineage Differentiation. Immunity [Internet]. 2009;30(5):646–55. Available from: http://dx.doi.org/10.1016/j.immuni.2009.05.001
361. Hashimi ST, Hashimi ST, Fulcher J a, Fulcher J a, Chang MH, Chang MH, et al. MicroRNA pro ling identi es miR-34a and miR-21 and their target genes. Differentiation. 2009;114(2):404–14.
362. Ichihara A, Jinnin M, Yamane K, Fujisawa A, Sakai K, Masuguchi S, et al. microRNA-mediated keratinocyte hyperproliferation in psoriasis vulgaris. Br J Dermatol. 2011;165(5):1003–10.
363. Joyce CE, Zhou X, Xia J, Ryan C, Thrash B, Menter A, et al. Deep sequencing of small RNAs from human skin reveals major alterations in the psoriasis miRNAome. Hum Mol Genet. 2011;20(20):4025–40.
364. Rosa A, Ballarino M, Sorrentino A, Sthandier O, De Angelis FG, Marchioni M, et al. The interplay between the master transcription factor PU.1 and miR-424 regulates human monocyte/macrophage differentiation. Proc Natl Acad Sci U S A [Internet]. 2007;104(50):19849–54. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2148386&tool=pmcentrez&rendertype=abstract
365. Fingerle G, Pforte A, Passlick B, Blumenstein M, Ströbel M, Ziegler-Heitbrock HW. The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. Blood [Internet]. 1993;82(10):3170–6. Available from: http://www.bloodjournal.org/content/82/10/3170.abstract
366. Grage-Griebenow E, Flad HD, Ernst M. Heterogeneity of human peripheral blood monocyte subsets. J Leukoc Biol. 2001;69(1):11–20.
367. Ziegler-Heitbrock L. Monocyte subsets in man and other species. Cell Immunol [Internet]. 2014;291(1–2):11–5. Available from: http://dx.doi.org/10.1016/j.cellimm.2014.06.008
368. Wong KL, Yeap WH, Tai JJY, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: Implications for health and disease. Immunol Res. 2012;53(1–3):41–57.
369. Frankenberger M, Sternsdorf T, Pechumer H, Ziegler-heitbrock HWL. Differential Cytokine Expression in Human Blood Monocyte Supopulations: A Polymerase Chain Reaction Analysis. Blood. 1996;87(1):373–7.
370. Geissmann BF, Prost C, Monnet J, Dy M, Brousse N, Hermine O. Transforming Growth Factor ␤1, in the Presence of Granulocyte/Macrophage Colony-stimulating Factor and Interleukin 4, Induces Differentiation of Human Peripheral Blood Monocytes into Dendritic Langerhans Cells. J Exp Med. 1998;187(6):961–6.
371. Hoshino N, Katayama N, Shibasaki T, Ohishi K, Nishioka J, Masuya M, et al. A novel role for Notch ligand Delta-1 as a regulator of human Langerhans cell development from blood monocytes. J Leukoc Biol [Internet]. 2005;78(4):921–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16037408
372. Zawada AM, Rogacev KS, Rotter B, Winter P, Marell RR, Fliser D, et al. SuperSAGE evidence for CD14 ++CD16 + monocytes as a third monocyte subset. Blood. 2011;118(12):50–62.
373. Schäkel K, Kannagi R, Kniep B, Goto Y, Mitsuoka C, Zwirner J, et al. 6-Sulfo LacNAc, a Novel Carbohydrate Modification of PSGL-1, Defines an Inflammatory Type of Human Dendritic Cells. Immunity. 2002;17:289–301.
374. Hänsel A, Günther C, Ingwersen J, Starke J, Schmitz M, Bachmann M, et al. Human slan (6-sulfo LacNAc) dendritic cells are inflammatory dermal dendritic cells in psoriasis and drive strong TH17/TH1 T-cell responses. J Allergy Clin Immunol. 2011;127(3):787–94.
375. Sagiv JY, Michaeli J, Assi S, Mishalian I, Kisos H, Levy L, et al. Phenotypic diversity and plasticity in circulating neutrophil subpopulations in cancer. Cell Rep [Internet]. 2015;10(4):562–74. Available from: http://dx.doi.org/10.1016/j.celrep.2014.12.039
376. Köffel R, Meshcheryakova A, Warszawska J, Hennig A, Wagner K, Jörgl A, et al. Monocytic cell differentiation from band-stage neutrophils under inflammatory conditions via MKK6 activation. Blood. 2014;124(17):2713–24.
377. Singhal S, Bhojnagarwala PS, O'Brien S, Moon EK, Garfall AL, Rao AS, et al. Origin and Role of a Subset of Tumor-Associated Neutrophils with Antigen-Presenting Cell Features in Early-Stage Human Lung Cancer. Cancer Cell. 2016;30(1):120–35.
378. Takashima A, Yao Y. Neutrophil plasticity: acquisition of phenotype and functionality of antigen-presenting cell. J Leukoc Biol [Internet]. 2015;98(October):1–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25632045
379. Oehler L, Majdic O, Pickl WF, Stöckl J, Riedl E, Drach J, et al. Neutrophil granulocyte-committed

cells can be driven to acquire dendritic cell characteristics. J Exp Med [Internet]. 1998;187(7):1019–28. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2212207&tool=pmcentrez&rendertype=abstract

380. Wright HL, Moots RJ, Edwards SW. The multifactorial role of neutrophils in rheumatoid arthritis. Nat Rev Rheumatol [Internet]. 2014;10(10):593–601. Available from: http://dx.doi.org/10.1038/nrrheum.2014.80
381. Semerad CL, Liu F, Gregory AD, Stumpf K, Link DC. G-CSF is an essential regulator of neutrophil trafficking from the bone marrow to the blood. Immunity. 2002;17(4):413–23.
382. Reddy RC, Narala VR, Keshamouni VG, Milam JE, Newstead MW, Standiford TJ. Sepsis-induced inhibition of neutrophil chemotaxis is mediated by activation of peroxisome proliferator-activated receptor- y. Blood. 2008;112(10):4250–8.
383. Geng S, Matsushima H, Okamoto T, Yao Y, Lu R, Page K, et al. Emergence, origin and function of neutrophil-dendritic cell hybrids in experimentally induced inflammatory lesions in mice. Blood. 2013;121(10):1690–700.