universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

Transcription Factors in Myeloid Development – the Role of Kruppel-like Factor 4 (KLF4)

angestrebter akademischer Grad

Magister/Magistra der Naturwissenschaften (Mag. rer.nat.)

Verfasserin / Verfasser:	Christina Mühlbacher
Matrikel-Nummer:	0130408
Studienrichtung /Studienzweig (lt. Studienblatt):	Genetik/Mikrobiologie
Betreuerin / Betreuer:	Prof. Thomas Decker

Wien, im September 2009

Inmitten der Schwierigkeiten liegt die Möglichkeit

- Albert Einstein -

ABSTRACT

Hematopoiesis is a well coordinated process which is regulated by the fine tuned interplay of several transcription factors. Hematopoietic stem cells (HSC) differentiate into lineage-committed progenitor cells which then give rise to distinct immune system cells. Langerhans cells (LC) are immune system cells located in the skin and their developmental pathway is absolutely dependent on the cytokine TGF-β1. However, still very little is known on the transcriptional control of LC differentiation. We identified the transcription factor Kruppel-like factor 4 (KLF4) to be inhibited by TGF-β1 in myeloid progenitor cells undergoing LC lineage commitment. Since LC share a common progenitor cell with monocytes/macrophages (Mo) and previous studies implicated KLF4 as a positive regulator of Mo development and CD14 expression, we asked whether KLF4 is a critical factor in the control of LC differentiation.

We used a well-defined human in vitro model of myelopoiesis and LC/DC subset differentiation to study KLF4 function. We found that LC lack KLF4 protein expression, whereas Mo or Mo-derived interstitial-type DC (DC) are KLF4 positive. Furthermore, our data reveals that ectopically expressed KLF4 significantly inhibits the surface expression of CD1a which is a LC/DC marker whereas it increases the Mo/DC marker CD11b. Ectopic KLF4 significantly decreases in vitro generation of LC from HSC, whereas it facilitates monocyte generation. Under DC culture condition KLF4 promotes an interstitial-type DC characterized by abundant CD11b and low CD1a expression. Moreover, ectopic KLF4 increases expression of the monocytic CD14 marker by cells cultured under LC generation conditions. In line with endogenous KLF4 in Mo, ectopic KLF4 failed to influence cell differentiation under optimal Mo culture condition. However, under suboptimal conditions, in the absence of M-CSF, KLF4 is able to restore Mo development by up-regulating the expression of CD11b as well as CD14. Since previous studies showed that LC and DC can be generated from $CD14^+$ peripheral blood Mo, we also studied KLF4 in these cells. A combination of Delta-1, which is a ligand of the Notch signalling pathway, and TGF-β1 is absolutely necessary to obtain LC characteristics from Mo. These Mo-derived LC lacked KLF4 protein whereas moDC generated in the presence of GM-CSF plus IL-4 showed abundant KLF4 protein expression. In these cultures TGF-β1 plus Delta-1 were strictly required for the repression of KLF4. In conclusion data suggest that KLF4 repression is a prerequisite for LC differentiation from monocytic cells.

ZUSAMMENFASSUNG

Hämatopoiese ist ein Prozess, der durch das Zusammenspiel verschiedener Transkriptionsfaktoren und deren Expressions-Intensität, reguliert und gesteuert wird. Aus hämatopoietischen Stammzellen (HSC) entwickeln sich Vorläuferzellen, die dann wiederum zu bestimmten Immunsystemzellen ausdifferenzieren. Langerhans Zellen (LC) sind wichtige Immunsystemzellen in der Haut, welche in ihrer Entwicklung absolut anhängig von der Existenz des Zytokines TGF-β1 sind. Es ist allerdings noch nicht bekannt welche Transkriptionsfaktoren wichtig für deren Entwicklungsprozess sind. Wir untersuchten Transkriptionsfaktoren, die downstream von TGF-β1 reguliert werden und fanden Kruppel-like Faktor 4 (KLF4) als signifikant inhibiert in myeloiden Vorläuferzellen. Da LC und Monozyten (Mo) einen gemeinsamen Vorläufer haben und von vorherigen Studien bekannt ist, dass KLF4 eine positive Auswirkung auf die Entwicklung von Mo hat, stellten wir uns die Frage ob KLF4 einen wichtigen Faktor in der LC Differenzierung darstellt.

Um die Auswirkungen von KLF4 zu testen, verwendeten wir ein in vitro Model zur myeloiden Differenzierung der verschiedenen Zelltypen. Wir stellten fest, dass LC KLF4 negative sind, wohingegen Mo und DC eine Expression von KLF4 aufwiesen. Ebenso zeigten unsere Versuche, dass ektopes KLF4 die Expression des LC/DC Markers CD1a signifikant inhibiert, wohingegen CD11b, ein wichtiger Marker für den DC/Mo Phänotypen, positive reguliert ist. Ebenso beobachten wir eine starke Reduktion der in vitro generierten LC Population, zu Gunsten eines Mo Phänotypen. Befanden sich die Zellen in DC Konditionen änderte sich deren Phänotyp, welcher eine starke CD11b und schwache CD1a Marker Expression zeigte. Weiteres stellten wir keine Änderung fest, wenn die Zellen unter optimalen Mo Bedingungen kultiviert wurden. Wird allerdings ein essentielles Zytokin für Mo, das M-CSF, weggelassen, konnten wir zeigen, dass ektopes KLF4 die Entwicklung der Mo wiederherstellen konnte. Da aus vorherigen Studien bekannt ist, dass man LC und DC aus $CD14^+$ Mo generieren kann, analysierten wir auch hier die KLF4 Protein Levels. Wir beobachteten unter Delta-1, ein Ligand des Notch-Signaltransduktionsweges, und TGF-β1 Einfluss die Generierung von charakteristischen LC Merkmalen. Die Analysierung der Protein Levels in diesen Zellen zeigte, dass aus Mo generierte LC (moLC) KLF4 negativ sind, wogegen die moDC, welche mit GM-CSF und IL-4 generiert wurden, KLF4 positive sind. In diesen Generierungsmodel ist ein Vorhandensein von Delta-1 und TGF-β1 absolut notwendig für die effiziente Inhibierung des KLF4. Letztendlich schließen wir aus unseren Daten, das KLF4 Inhibierung notwendig ist um LC aus Mo Vorläuferzellen zu generieren.

1) Introduction

1.1) Progenitor Hierarchy from Hematopoietic Stem Cells – Myeloid Lineage Commitment

Hematopoiesis is a developmental process which is used to describe the state of proliferation and differentiation of progenitor cells with ability of self-renewal into distinct cell types. These differentiated cells are called hematopoietic stem cells (HSC) and they loose the ability for self-renewal once differentiate further into specialized cell types (Nerlov, Tenen and Graf, 2001). This process is controlled by various factors which activate lineage-specific genes and, therefore, restricting lineage commitment (Tenen et al, Blood 1997) which is explained later. There are two separate lineages of mature blood cells: the lymphoid and the myeloid. The lymphoid lineage gives rise to B-, T- and natural killer-cells (NK) whereas the myeloid lineage leads to differentiation into various granulocyte (Gr) subsets (neutrophils, basophils and esinophils), erythrocytes, mast cells, megakarytrocytes and monocytes (Mo), which later differentiate into macrophages (Mac; Figure 1). Dendritic cells (DC) can be produced from both the lymphoid and the myeloid lineage (Manz et al, Blood 2001). It was shown experimentally that the common lymphoid progenitors (CLP) are restricted in their ability to differentiate into any lymphoid lineage cells, whereas the common myeloid progenitors (CMP) are able to give rise to any of the myeloid lineage cell types (Kondo et al, Cell 1997; Akashi et al, Nature 2000).

DC have a crucial role as antigen presenting cells (APC), which is a specific category of cells of the immune system specializing in capture, processing and presenting of antigens to T cells. DC are very important during bacterial or viral infections as well as for T-cell immunity against tumors. DC are also thought to play an important role in controlling the balance between immunity and immunological tolerance. Upon encounter with any microbial product immature DC initiate maturation and migrate to lymph nodes where they present the microbial antigen to T-cells. Apart from T cells, DC can also

interact with other cell types like B- or NK-cells as well as remain in the tissue as inflammatory mediators than migrate to the lymph nodes. It is conceivable, therefore, that there are different subsets of DC to account for such range in their functions (Shortman et al, Nat Rev Immunol. 2002). In human there are three known main DC subtypes with various functions under steady state conditions: epidermal Langerhans cells (LC) which represent the classical DC life and are present in the skin as “first” immunological barrier (Romani et al, APMIS. 2003); tissue/interstitial/dermal DC which are found in the tissues such as epithelia of the intestinal, respiratory and reproductive tract and will be called only DC; and plasmacytoid DC (pDC) which are present in the blood, able to produce large amounts of type I interferons and play an important role in the immune response to viruses (Liu YJ, Annu Rev Immunol. 2005). During inflammation an additional DC subset appears which is called myeloid dermal “inflammatory” DC (Shortman et al, Nat Rev Immunol 2007).

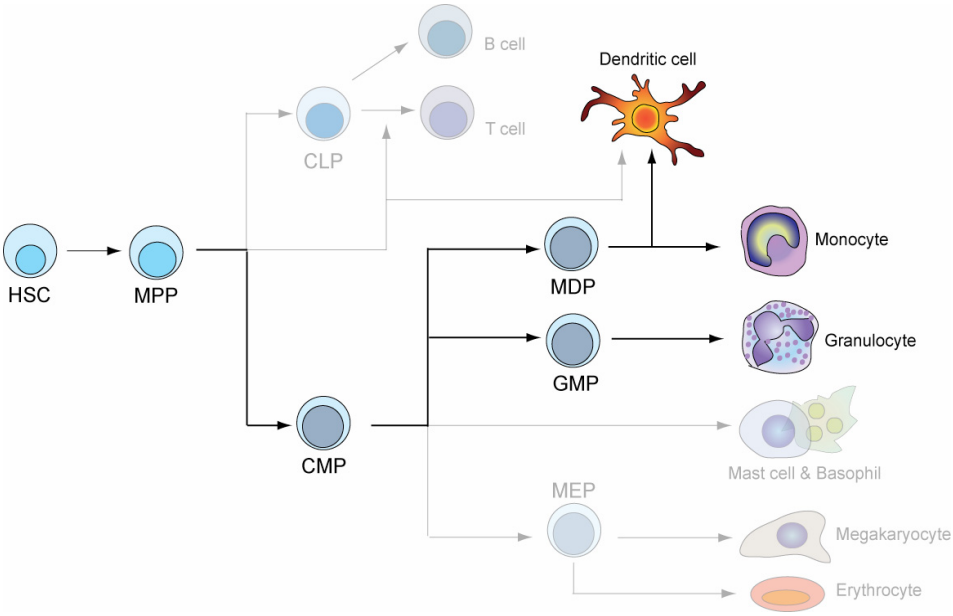


Figure 1 : **Schematic model of lineage differentiation (according to the model by Rosenbauer et al, Nature 2007).**
Hematopoietic stem cells (HSC) are cells with self renewal potential and give rise to multipotential progenitors (MPP). Those progenitors lose the self-renewal potential but still retrain the ability to differentiate into various different cell lineages. There are two main common progenitors described: The common lymphoid progenitors (CLP) are thought to develop B and T cells whereas the common myeloid progenitors (CMP) produce granulocyte/macrophage progenitors (GMP), megakaryocyte/erythroid progenitors (MEP) and also a macrophage and dendritic-cell progenitor (MDP). In our study the focus is set on the myeloid lineage commitment with the development of granulocytes, monocytes and dendritic cells (indicated by the black arrows).

1.1.1) Different DC Subsets in Steady State and Inflammation

LC are characterised by the expression of Langerin (CD207), E-cadherin and cluster of differentiation 1a (CD1a). Langerin is a membranous C-type lectin (Valladeau et al, Immunity. 2000) that is responsible for the formation of Birbeck granules. CD1a represents a major histocompatibility complex I-like molecule that presents antigens to T-cells (Barral et al, Nat Rev Immunol. 2007). LC have a long lifespan in the skin where they are in close contact with keratinocytes and they migrate to the lymph nodes under steady state conditions only at a base rate. The migration rate of LC is increased during inflammation or in the presence of other microbial stimuli (Kissenpfennig et al, Immunity. 2005). In mouse Mo ("inflammatory" Mo, see chapter 1.2.2) seem to be effective as LC precursors after LC depletion in the skin (Ginhoux et al, Nat Immunol. 2006). An important requirement is the expression of the chemokine receptor 2 (CCR2) for the recruitment of Mo from the bone marrow to the skin (Serbina et al, Nat Immunol. 2006). In humans there is also a distinct Mo subset which has been shown to be an effective LC precursor and it expresses CCR2 (Schaerli et al, Immunity 2005). Up to date the function of LC was closely studied following the induction of inflammation, leaving open the question how the steady state LC develop. It is important to note that in mice lacking the macrophage colony-stimulating factor receptor (M-CSFR), LC are completely absent which leads to the strong indication that Mo might be the real precursors for LC even under steady state conditions (Ginhoux et al, Nat Immunol. 2006).

In contrast to LC interstitial DC (also known as dermal DC) lack the expression of Langerin, they do not form Birbeck granules and are obtained from $CCR2^-$ Mo. Upon the uptake of antigens they mature and migrate to the draining lymph nodes to present these antigens to B- and T-cells (Nestle et al, J Invest Dermatol. 1998). These dermal DC can be further divided into "resident" dermal DC and a second population which appears after inflammation, the so called "inflammatory" dermal DC (Shortman et al, Nat Rev Immunol 2007). Under steady state these "resident" dermal DC are immature and exhibit modest T-cell stimulatory activity (Zaba et al, J Clin

Invest. 2007). Their potential to migrate to the lymph nodes might be explained by the fact that they express CCR7 and are, therefore, responsive to the lymph node chemokine CC chemokine ligand 19 (CCL19; Angel et al, J Immunol. 2006). Circulating DC precursors may give rise to these "inflammatory" dermal DC after migration into the skin because of inflammatory signals. In certain cases of skin diseases (e.g. psoriasis) these DC have been termed tumor necrosis factor and inducible nitric oxide synthase-producing DC (Tip-DC) because of their potential to produce inflammatory mediators like TNFα and iNOS (Lowes et al, Proc Natl Acad Sci USA 2005). In human these Tip-DC are found in the lamina propria of the gut. They seem to be important for the production of Immunoglobulin A (IgA; Tezuka et al, Nature 2007) and the stimulation and differentiation of Th17 cells which show an association in many autoimmune inflammations (Zaba et al, J Invest Dermatol. 2009). These DC seem to act as a source of inflammatory mediators rather than following the classical role of a DC as an APC. They are also involved in autoimmune inflammation and there is a strong interest in understanding the developmental and activation process of these cells.

Plasmacytoid DC (pDC) share many similarities with B-cells. For instance, pDC also depend on certain B cell transcription factors (e.g. Spi-B). pDC are characterized by their production of large amounts of type I interferons (i.e. IFNα, β, ω) during viral infections (Liu et al, Annu Rev Immunol 2005). IFNα modulates the differentiation of various cell types, including T-cells and myeloid DC. In the normal skin the numbers of pDC are low, whereas markedly elevated cell numbers of pDC are found in psoriasis. Blocking of IFNα has been shown to prevent psoriasis, which leads to the suggestion that pDC might be causally implicated in the pathogenesis of psoriasis (Nestle et al, J Exp Med. 2005). The keratinocytes peptide LL37 is able to induce IFNα production in pDC (Lande et al, Nature. 2007) and it has notable higher expression levels during inflammation (e.g. psoriasis). It is, therefore, plausible that in psoriasis these pDC are extra sensitive and tend to respond to inflammation by overproduction of IFNα.

1.1.2) Master Transcription Factors and Cytokines in the Development of Dendritic Cells and Monocytes

Transcription factors play an important role in hematopoiesis and development of distinct cell types. They lead to the expression of characteristic lineage-specific target genes driving the precursor cell into a specific differentiation program. The interplay of these transcription factors and their expression levels are important for the determination of cell fate during their differentiation process (Orkin SH, Nat Rev Genet. 2000).

Early myeloid progenitors and common lymphoid progenitors are dependent on PU.1 (Tenen et al, Blood 1997). PU.1 knockout mice reveal a complete absence of B-cells and Mac (McKercher et al, EMBO J 1996). Interestingly, high levels of PU.1 support Mac development whereas low levels support the production of Gr (Dahl et al, Nature Immunol 2003). CCAAT/enhancer binding protein α (C/EBPα) is required for the production of granulocyte/macrophage progenitors (GMP) from common myeloid progenitors and, therefore, for granulopoiesis in general (Radomska et al, Mol Cell Biol 1998). The differentiation into Mac or Gr from the GMP is controlled by PU.1 and one of its protein binding partners, the interferon-γ (IFNγ)-responsive transcription factor also called IRF8. IRF8 is expressed by B-cells, DC and Mac but not by Gr (Tamura et al, Immunity 2000). GFI1 and C/EBPε are required for Gr development after reaching the GMP state (Laslo et al, Cell 2006; Yamanaka et al, Proc Natl Acad Sci USA 1997). GFI1-deficient mice show a defect in lymphoid progenitor development and they lack neutrophilic Gr (Hock et al, Immunity 2003). Retinoic acid receptor (RAR) is also implicated in granulopoiesis since its inhibition leaves the cells at the promyelocyte stage (Tsai et al, Proc Natl Acad Sci USA. 1993). RAR heterodimerizes with the retinoid X receptor (RXR) proteins. Addition of a combination of retinoic acid (RA) and vitamin D induces monocytic development by activation of vitamin D receptor (VDR):RXR complexes which bind and repress RAR:RXR responsive elements (Bastie et al, Mol Endocrinol 2004). It is the interplay of GFI1, IRF8 and the expression level of PU.1 as well as the complex formation

with either VDR or RAR that determine the developmental pathway towards either Mac or Gr.

STAT3 which is a transcription factor downstream of Fms-like tyrosine kinase 3 (FLT3) signaling seems to be an important factor for DC since its deletion leads to reduced DC development (Laouar et al, Immunity 2003). On the other hand, Ikaros is shown to be a necessary factor for DC generated from common lymphoid progenitors. *Ikaros*$^{-/-}$ mice lack B-, T-, NK-cells and their DC subsets, whereas LC and cells from the erythroid and myeloid lineage are unaffected (Wu et al, Immunity 1997). PU.1 is highly expressed in DC and is required for DC development especially for monocyte-derived DC (Guerriero et al, Blood 2000). Additionally relB seems to have an important function in developing LC/DC/Mo progenitors which differentiate further into DC. Blocking this pathway results in impaired moDC development but has no effect on the LC pathway (Platzer et al, Blood 2004). A strong influence on DC development is exerted by IFN-regulatory factors (IRFs). IRF8$^{-/-}$ mice show a severely reduced number of pDC whereas IRF4$^{-/-}$ mice have only some reduction in the pDC counts (Tamura et al, J Immunol 2005). In IRF8- and IRF2-deficient mice are reported to have a decreased number of LC (Schiavoni et al, Blood 2004). Another factor necessary for pDC development is the Ets-domain transcription factor Spi-B. This has been shown by RNA interference-mediated gene knockdown of Spi-B mRNA which results in diminished pDC and enhanced B-cell and myeloid cell development (Schotte et al, J Exp Med 2004). Additionally, the inhibitor of DNA binding Id2 which is a member of the inhibitory helix-loop-helix (HLH) factors is essential for DC and LC development. Id2 inactivation shows increased numbers of pDC (Hacker et al, Nat Immunol 2003). On the other hand, activation of HLH factors such as E2A or HEB stimulates pDC development (Schotte et al, J Exp Med 2004) leading to the conclusion that multiple factors and their interactions are important for determination of cell fate and pDC differentiation. The LC pathway and its developmental origin are more complicated, although, much is known about activation of LC and their trafficking towards the lymph nodes. It is known that LC differentiation is dependent on transforming growth factor (TGF) β1 (Strobl et al, J Immunol

1996). TGF-β1 induces Id2 which antagonizes the activating HLH factors like E2A and, therefore, represses B-cell genes (Hacker et al, Nat Immunol 2003). Removal of either TGF-β1 or Id2 leads to a loss of LC. RunX3 expressed in mature DC is another important player as a mediator of TGF-β1 response. RunX3$^{-/-}$ mice lacks LC (Fainaru et al, EMBO J 2004). As mentioned earlier, LC numbers are decreased in IRF8$^{-/-}$ and are notably increased in Gfi1$^{-/-}$ mice (Rathinam et al, Immunity 2005). A short overview of the transcription factors in the different lineages is shown in Figure 2A.

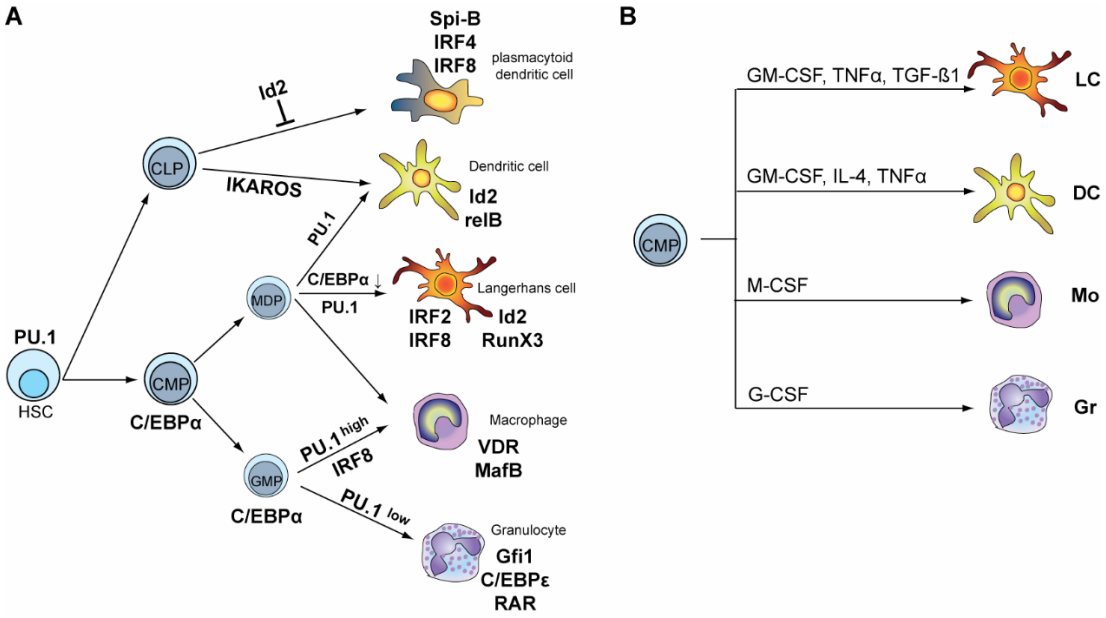


Figure 2: **Important transcription factors and cytokines in the development of different myeloid cells as well as DC from lymphoid progenitors.**
A) As indicated in the text PU.1 is absolutely necessary for early progenitor cell development in the myeloid as well as the lymphoid lineages (Tenen et al, Blood 1997). The different expression levels of PU.1 play an important role in subsequent differentiation steps: low PU.1 levels lead to Gr differentiation whereas high levels are obtained in Mac and DC. Also important for Gr is C/EBPε, conversely IRF8 forces Mac development from the GMP state. Id2 is necessary for DC and LC whereas it inhibits pDC development. An essential factor for pDC is Spi-B. DC differentiation from CLP cells is dependent on Ikaros. The interplay of different factors and their expression level are important for determining cell fate and decision. B) There are different important cytokines to obtain the various lineages from hematopoietic progenitors. TGF-β1 is an absolutely necessary cytokine for LC differentiation whereas M-CSF induces Mac and G-CSF induces differentiation from GMP into Gr.

An important cytokine for DC development from hematopoietic precursors or Mo in vivo is granulocyte-macrophage colony-stimulating factor (GM-CSF; Caux et al, Nature 1992; Sallusto et al, J Exp Med 1994), despite the fact that mice lacking GM-CSF or its receptor (GM-CSFR) display no effects on the

developmental pathway of DC (Vremec et al, Eur J Immunol 1997). GM-CSF is not detectable in steady state conditions but increases under inflammatory circumstances (Hamilton JA, Nat Rev Immunol 2008) which lead to the suggestion that it might be a crucial factor for DC differentiation during inflammation. Also important to mention is that GM-CSF leads to STAT3 activation and IRF4 expression (Esashi et al, Immunity 2008) which are required for DC differentiation. Fms-like tyrosine kinase 3 ligand (Flt3L) plays a key role in the DC differentiation because its inhibition results in a decrease of DC and pDC numbers but interestingly reveals no affects on LC (McKenna et al, Blood 2000, Merad M. and Manz MG, unpublished data, January 2009). Tumor necrosis factor-α (TNFα) in combination with GM-CSF is necessary to obtain DC in vitro. When these cells are further cultured with TGF-β1 they develop LC characteristics, whereas Interleukin 4 (IL-4) induces DC (Caux et al. J. Exp. Med. 1996). An important finding was that TGF-β1 is one of the un-redundant cytokines necessary for LC differentiation in human as well as in mice (Strobl et al, J Immunol. 1996; Borkowski et al, J Exp Med 1996) and delivers the upstream signal for Id2 which is an important factor for LC development as referred to before (Hacker et al, Nat Immunol 2003). Macrophage colony-stimulating factor (M-CSF) is an important cytokine for several Mac populations as it is exhibited in mice lacking M-CSF or its receptor (M-CSFR). Those mice reveal a total absence of osteoclasts and therefore develop osteoporosis (Dai et al, Blood 2000). M-CSF is detectable in steady state condition but increases after inflammation (Hamilton JA, Nat Rev Immunol 2008). As mentioned before it is interesting that LC differentiation seems to be indispensable on M-CSFR because mice lacking M-CSFR show an absence of LC and it is indicated that a special Mo subset repopulates LC in inflamed skin in a M-CSFR manner (Ginhoux et al, Nat Immunol 2006). This and all the possible generation models to obtain DC or LC from a $CD14^+$ precursor cell will be discussed later (chapter 1.2.2). Last but not least an important cytokine for Gr differentiation in vivo is the granulocyte colony-stimulating factor (G-CSF). A short overview on cytokines that determine myeloid/DC sublineage differentiation from human $CD34^+$ progenitor cells is shown in Figure 2B.

1.1.3) Characteristic Marker Expression of the Different Lineages

The diverse cell types can be distinguished by their expression of various markers. There are many different markers extra- as well as intracellular. Here only those markers will be mentioned which are relevant to the following experiments. As mentioned before LC are characterized by their surface expression of CD207, CD1a and E-cadherin. DC by contrast co-express CD11b and CD1a surface marker in the absence of E-cadherin. Mo are identified by high CD14 surface expression and CD11b, whereas Gr show expression of intracellular Lactoferrin (LF) and Myeloperoxidase (MPO) expression as well as surface expression of CD15 and CD11b (Figure 3).

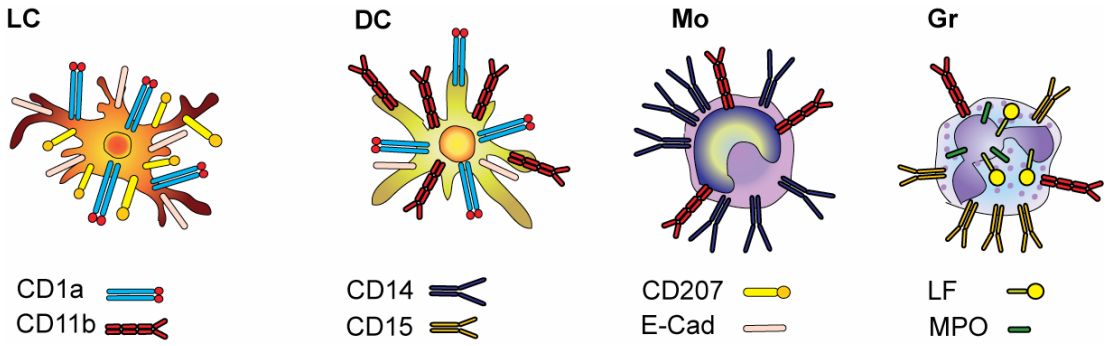


Figure 3: **Marker expression of the different lineages.**
LC are characterized by CD207 expression whereas this molecule is absent from all other lineages. Mo are indicated by high CD14 expression and Gr show intracellular LF and MPO as well as surface expression of CD15. E-Cadherin (E-Cad); Lactoferrin (LF); Myeloperoxidase (MPO).

1.2) Monocyte Heterogeneity

About 5 to 10% of the peripheral blood leukocytes in mouse and human represent monocytes. They are short-lived and believed to continuously repopulate Mac and DC populations. Therefore they fulfil critical roles in innate and adaptive immunity in steady-state as well as during inflammation (Ziegler-Heitbrock, J Leukoc Biol 2000). They are characterized by their expression of CD11b, CD11c and the lipopolysaccharide (LPS) receptor

(CD14) in human and CD11b and F4/80 in mice. Moreover they lack the expression of B, T, NK and DC markers.

1.2.1) Two Different Main Monocyte Subsets in Human and Mouse

Human Mo subsets can be divided into “classical” with high CD14 and no expression of the Fc receptor CD16 (~90% of the total human Mo) and into “proinflammatory” Mo which are CD14 and CD16 positive (Passlick et al, Blood 1989). It has been shown that the expression of CD16 can be induced by the cytokines M-CSF, IL10 and TGF-β1 (Wahl et al, J Immunol 1992; Calzada-Wack et al, J Inflamm 1996). Conversely, CD14 expression seems to be reduced by TGF-β1, but up-regulated by IL-10. In addition to the expression of CD16 the “pro-inflammatory” Mo subset lacks the expression of receptors for inflammatory cytokines [CC chemokine receptor 1 (CCR1), CXC chemokine receptors 1 (CXCR1) and CXCR2]. However they express high amounts of major histocompatibility complex (MHC) class II [is found on antigen presenting cells (APC)] and CD32 which acts to modulate B cell signalling (Geissmann et al, Immunity 2003; Weber et al, J Leukoc Biol 2000; Gordon et al, Nat Rev Immunol. 2005).

It has been shown that $CD14^+/CD16^+$ proinflammatory Mo produce high levels of the proinflammatory cytokine TNFα and low levels of anti-inflammatory IL-10 after LPS stimulation (Frankenberger et al, Blood 1996). Furthermore they can rapidly migrate to the site of inflammation and differentiate into tissue Mac there (Ziegler-Heitbröck et al, Eur J Immunol. 1993). However both subsets are able to differentiate into DC; the $CD16^+$ subset shows a higher predisposition for DC (Grage-Griebenow et al., Eur. J. Immunol. 2001; Randolph et al., J. Exp. Med. 2002) whereas the $CD16^-$ subset has a propensity to develop into LC (Schaerli et al, Immunity 2005). Surface marker molecules expressed by Mo subsets are depicted in Table 1.

Table 1: **Surface expression markers on the two different Mo subsets in human.** Adapted from Strauss-Ayali et al, J Leukoc Biol. 2007 and Tacke et al, Immunobiology. 2006. ND, not determined

surface marker expression	Human		Mouse	
	$CD14^+$ $CD16^+$	**$CD14^{++}$ $CD16^-$**	**$Gr1^{low}$**	**$Gr1^{high}$**
CD11b	+	+	+	+
CD14	+	High	ND	ND
CD16 (FcγRIII)	+	-	+	+
CD32	+	High	+	+
CD43	High	+	+	-
CD49b	ND	ND	+	-
CD62L (L-Selectin)	-	+	-	+
CD64 (FcγRI)	-	+	ND	ND
CD163	-	++	ND	ND
CCR1	-	+	ND	ND
CCR2	-	+	-	+
CCR4	-	+	ND	ND
CCR5	High	-	+	-
CCR6	?	?	?	?
CCR7	-	+	ND	ND
CXCR1 and 2	-	+	ND	ND
CX_3CR1	High	Low	++	+
F4/80	ND	ND	+	+
Ly6C(Gr1)	ND	ND	-	+
MHC Class II	High	+	-	-

For the selective recruitment of leukocytes to the site of inflammation, chemokines and their receptors are necessary (Baggiolini et al, Nature 1996). The $CD16^+$ population shows impaired transendothelial chemotaxis and migration after monocyte chemotactic protein-1 (MCP-1) stimulation. In contrast to classical Mo, this subset fails to up-regulate CD11b in response to MCP-1 due to the fact that they have no expression of the receptor CCR2. CD11b is an essential molecule for Mo adhesion to endothelial cells. However, they show more pronounced up-regulation of CD11b in response to macrophage inflammatory protein-1α (MIP-1α) which binds the receptor CCR5 (Weber et al, J. Leukoc. Biol. 2000). In addition to this, the $CD16^+$ subset expresses higher levels of CX_3CR1, a receptor that is important for mediating both leukocyte migration and leukocyte firm adhesion (Imai et al, Cell 1997). Fractalkine (FKN) is the ligand for CX_3CR1 and is activated by proinflammatory cytokines such as TNFα (Imaizumi et al, J Atheroscler Thromb. 2004). Interestingly, CX_3CR1 seems to be up-regulated by IL-10

(Yano et al, Acta Med Okayama. 2007) as is CD16 (which additionally needs TGF-β1). Interestingly both of these cytokines are present for example in the inflamed joints of rheumatoid arthritis (RA). It is shown that in many inflammatory diseases, like RA (Kawanaka et al, Arthritis Rheum. 2002), atherosclerosis (Hakkinen et al, Virchows Arch. 2000), asthma (Rivier et al, Exp. Immunol. 1995), peridontitis (Nagasawa et al, J. Periodontal Res. 2004) and others the $CD16^+$ subset is significantly increased. Also in bacterial and HIV infections as well as in cancer the $CD16^+$ $CD14^+$ subset is increased (Fingerle et al, Blood 1993; Pulliam et al, Lancet 1997; Saleh et al, Blood 1995).

The $CD16^+/CD14^+$ proinflammatory Mo subset has further been divided into two populations; one is $CD14^{high}$ $CD16^+$ and the other is $CD14^{dim}$ $CD16^+$ (Passlick et al, Blood 1989). These two populations seem to have different phenotypes and functions: The $CD14^{high}$ subset shows higher CD11b and TLR4 expression as well as a higher IL-10 production after LPS stimulation than the $CD14^+$ $CD16^+$ and $CD14^+$ $CD16^-$ subsets although the TNFα production is the same compared with the $CD14^+$ $CD16^+$ subset. Therefore this $CD14^{high}$ $CD16^+$ subset seems to be rather an anti-inflammatory than a proinflammatory subset (Skrzeczyn´ska-Moncznik et al, Scand J Immunol. 2008).

Also in mouse two different Mo subsets are described. They were first divided based on the different expression of CX3CR1 (Palframan et al, J Exp Med 2001) and later by the expression of Ly6C (Gr-1) (Geissmann et al, Immunity 2003). Similar to the "classical" human Mo the $Ly6C^+$ subset is $CCR2^+$ $CX3CR1^+$ $CD62L^+$, whereas the $Ly6C^-$ Mo, that equal the human the "proinflammatory" subset, are $CCR2^-$ $CX3CR1^{++}$ $CD62L^-$ (Palframan et al., J Exp Med 2001; Geissmann et al., Immunity 2003; Sunderkotter et al., J. Immunol. 2004). Geissmann et al. defined the CX_3CR1^{low} $Ly6C^+$ as "inflammatory" Mo subset which is short-lived and represents a circulating precursor for dendritic and myeloid cells at inflammatory sites. The second CX_3CR1^{high} $Ly6C^-$ subset, the so called "resident" Mo was proposed to persist longer and represent a precursor in noninflamed tissue. In strong contrast to

the human system where the $CD14^+$ $CD16^+$ subset is expanded during infections in the mouse it is the $Ly6C^{high}$ population which is elevated during an inflammation process. In Figure 4A-C a short summary of the different human and mouse subsets is shown.

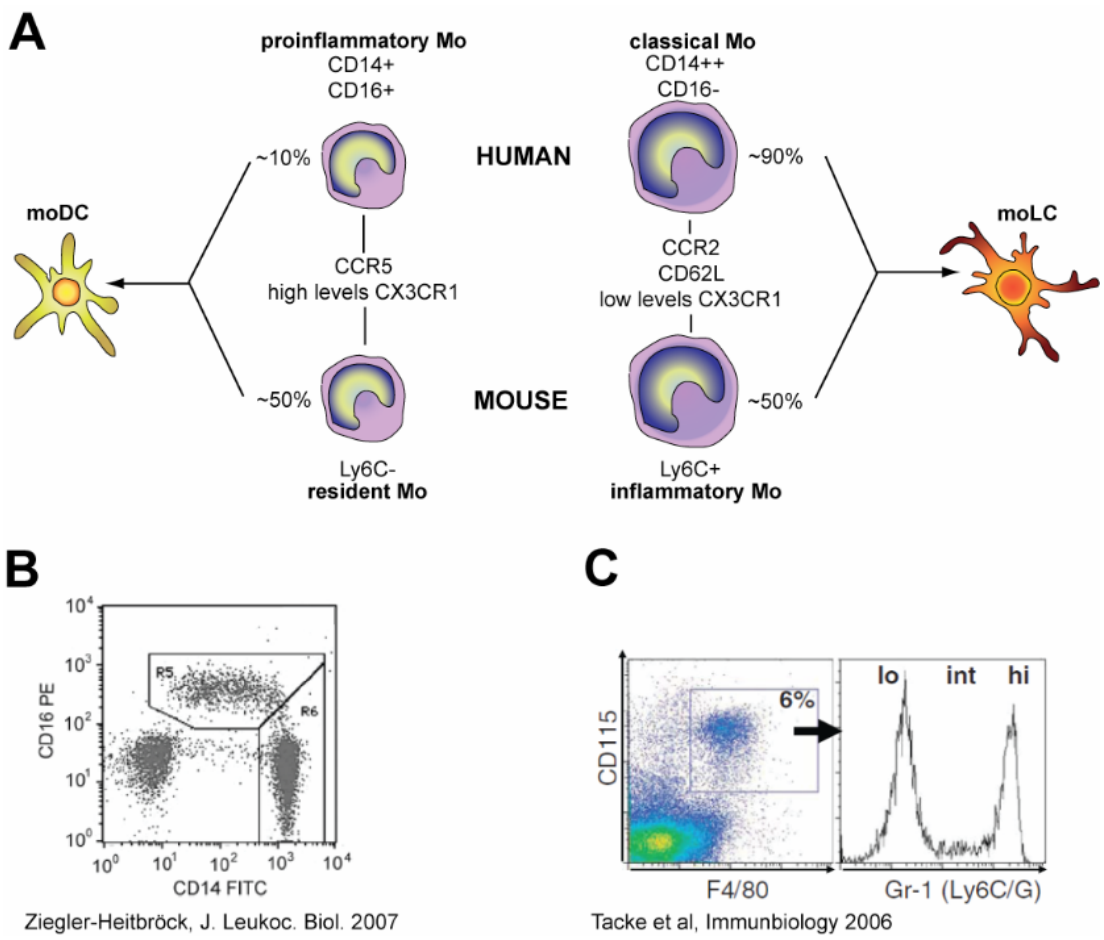


Figure 4: **Human and mouse Mo subsets.**
There exist two different Mo subsets in human and mouse. They show different surface receptor expression and migratory behaviour. A) The human proinflammatory subset, characterized by the expression of CD16, shares many features with the mouse resident subset that lacks the expression of Ly6C. They are smaller in size, less granular and both express CCR5 but lack the expression of the inflammatory chemokine receptors CCR1, CXCR1 and CXCR2. Due to the higher CX_3CR1 expression the $CD16^+$ subset can migrate easier across endothelial monolayer (Randolph et al, J. Exp. Med 2002). It furthermore seems to be a survival signal for the $Ly6C^-$ subset. The second subset also called classical (human) or inflammatory (mouse) Mo, expresses CCR2 and therefore is rapidly recruited to inflamed tissue. These cells seem to be involved in innate inflammatory response in contrast to the first subset, which has been implicated in tissue homeostasis. In human the classical Mo give rise to moLC (Larregina et al, Nat Immunol. 2001) whereas in mouse the inflammatory Mo seem to be direct LC precursors (Ginhoux et al, Nat Immunol. 2006). B) A FACS analysis of the human Mo subsets. The FACS diagram shows the different expression pattern of CD16 and CD14 by Ziegler-Heitbröck, J.Leukoc. Biol. 2007. C) Ly6C expression in murine Mo subsets as shown by Tacke et al, Immunbiology 2006.

1.2.2) Are Monocytes Potential Progenitors of Langerhans Cells?

In human LC are characterised by the expression of CD207, E-cadherin and CD1a and lack of CD14 expression. The mechanisms how LC precursors migrate to the epidermis are not well understood but it is thought that a bone marrow-derived myeloid precursor traffics somehow into the overlaying epidermis. Macrophage inhibitory protein-3α (MIP-3α) also known as CCL20 which is a ligand for CCR6 seems to be important for that (Charbonnier et al, J Exp Med. 1999). In normal human skin CCL20 is low. However under inflammatory conditions it is highly up-regulated (Moser et al, Trends Immunol. 2004). Interestingly it has been shown that IL-10 induces the expression of CCR6 whereas the moDC cytokine IL-4 represses it (Dieu-Nosjean et al, J Immunol 2001). Furthermore it was shown that the expression of the CCR6 ligand MIP-3α is provoked by TNFα and produced by keratinocytes (Tohyama et al, J Dermatol Sci 2001). Both, TNFa and MIP-3a can also be produced by the human $CD14^{high}$ $CD16^{+}$ proinflammatory Mo subset (Skrzeczyn´ska-Moncznik et al, Scand J Immunol 2008).

Studies showed that there exists a population of human $CD14^{+}$ cells which actively migrates in response to MIP-3α and which exhibits immature LC characteristics after short term cultivation with TGF-β1 (Larregina et al, Nat Immunol 2001). When these migratory $CD14^{+}$ ($miCD14^{+}$) cells are cultured with M-CSF they undergo cell death whereas peripheral blood Mo differentiate into Mac (Larregina et al, Immunology 1997). The $miCD14^{+}$ cells express CCR6 which is the receptor for the ligand MIP-3α and which is also present on LC. Larregina et al. postulated the suggestion that these $miCD14^{+}$ cells migrate into the epidermis in response to MIP-3α and differentiate to immature LC under the influence of TGF-β1; under inflammatory conditions containing GM-CSF with or without IL-4 the cells further differentiate into mature LC which have an enhanced potential for T cell stimulation. However it has not been shown yet that this $miCD14^{+}$ cells represent the $CD16^{+}$ or $CD16^{-}$ proinflammatory Mo subset.
Schaerli et al. postulated that CXC motif chemokine ligand-14 (CXCL14) seems to be important for LC differentiation because unlike CCL20 it is

present in the skin under steady state conditions and is not up-regulated further in skin diseases. In the skin it is selectively expressed in blood vessels of the superficial dermal plexus and in the epidermis. It has been shown that CXCL14 selectively attracts $CD14^+$ cells but no other myeloid cells like moDC or LC. In this study it was furthermore indicated that the $CD16^+$ inflammatory Mo subset shows limited chemotaxis towards CXCL14. Therefore under steady state conditions it is more likely that the classical $CD14^{high}$ $CD16^-$ Mo subset is the one which migrates into the skin and acquires LC characteristics (Schaerli et al, Immunity 2005).

In a mouse model it was, however, shown that the CXCL14 is dispensable for homeostatic recruitment of DC-like cells to the skin (Meuter et al, Mol Cell Biol 2007). It was furthermore demonstrated that in mouse the recruitment of LC precursors after UV-light induced skin inflammation is absolutely dependent on the expression of CCR2 (Merad et al, Nat Immunol 2002). In accordance with this, Ginhoux et al. demonstrated that in mouse the $Ly6C^+$ Mo subset which also expresses CCR2 is a direct precursor for LC. They furthermore could show that this process is depended on colony-stimulating factor-1 receptor (CSF-1R=M-CSFR; Ginhoux et al, Nat Immunol 2006). Therefore in mouse it is rather the “inflammatory” subset which gives rise to LC, whereas in humans is rather “classical” Mo subset that develops to LC (Figure 5).

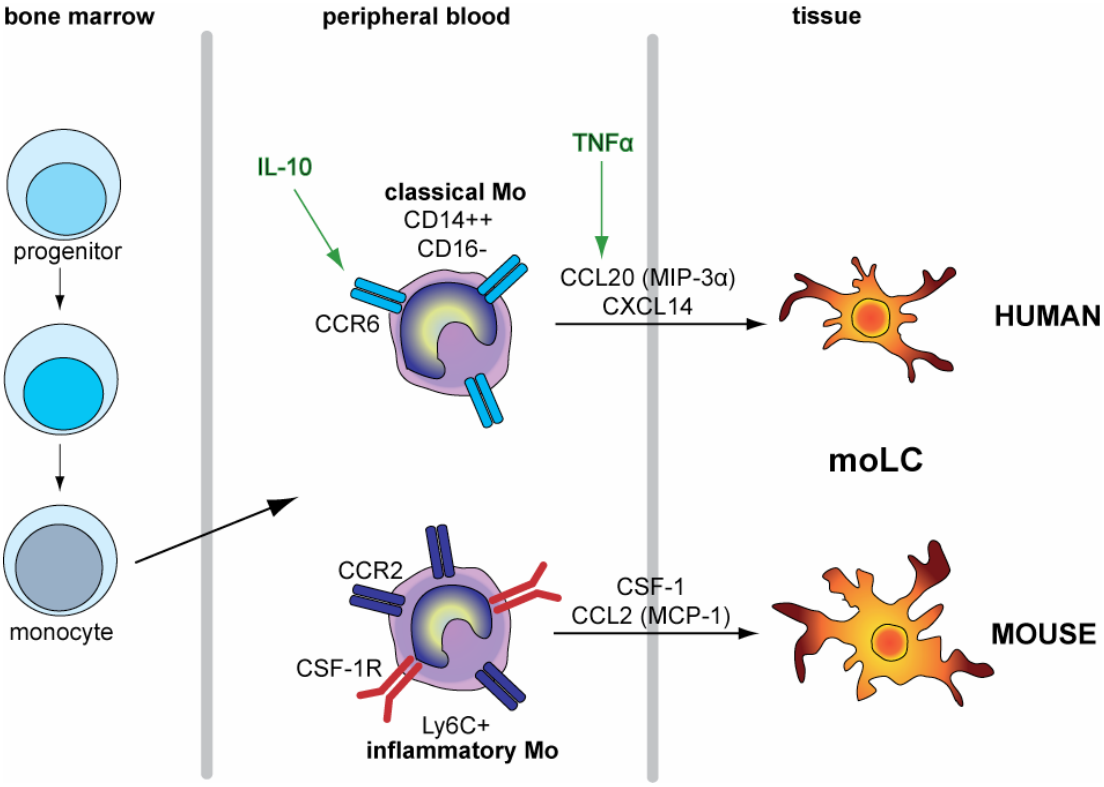


Figure 5: **Schematic representation of the differentiation from Mo into LC.**
In human as well as in mouse the Mo population is heterogeneous (Passlick et al, Blood 1989; Geißman et al, Immunity 2003). It is indicated that these subsets differ in their migratory behaviour as well as in their surface marker expression. In human it is has been shown that MIP-3α is released by epithelial cells. It was therefore suggested that it is one of the signals that recruits LC precursors to the skin (Charbonnier et al, J Exp Med 1999). Larregina et al. shows that miCD14$^+$ cells express CCR6 the receptor for MIP-3α and therefore traffic into the epidermis where they develop LC characteristics. Schaerli et al. however postulated that CXCL14 is also required for this process. They furthermore could show that only the CD14$^+$ and not so much the CD16$^+$ Mo subset responds to this ligand. In contrast to the human system CXCL14 shows no relevance in regulating this recruitment in mouse (Meuter et al, Mol Cell Biol 2007). Here CCR2 as well as M-CSFR (CSF-1R) are believed to be absolutely necessary for the recruitment of LC progenitors into the skin (Merad et al, Nat Immunol 2002; Ginhoux et al, Nat Immunol. 2006). Consequently the mouse Mo subset which represents the counterpart of the human Mo subset does not only show similar surface expression but also seems to possess same LC precursor potential. Interleukin-10 (IL-10); tumor necrosis factor (TNF); CC chemokine receptor (CCR); CC chemokine ligand (CCL); macrophage inhibitory protein-3α (MIP-3α); CXC motif chemokine ligand-14 (CXCL14); monocyte chemoattractant protein-1 (MCP-1); colony-stimulating factor-1 receptor (CSF-1R) is equivalent to M-CSFR.

1.2.3) Generation Model for Monocyte-derived Dendritic Cell Types

A generation model to obtain monocyte-derived DC (moDC) in vitro is well established. This model uses the cytokines GM-CSF and IL-4 to induce DC from monocytic precursors (Chapuis et al, Eur J Immunol 1997). Also other cytokines are now discovered to induce DC differentiation from a $CD14^+$ Mo.

TNFα gives rise to a special moDC-like phenotype. This moDC like cells have monocytic characteristics but additionally express the DC marker CD1a. It has been shown that this moDC are able to evoke T helper cells 1 (Th1) and Th17 response (Iwamoto et al, J Immunol. 2007). It has also been shown that addition of IL-15 to such monocytic cells leads to the development of a CD1a positive cell population that looses CD14. In addition half of the population gains CD11b. Interestingly these cells also became CD207 positive (Mohamadzadeh et al, J Exp Med 2001). Furthermore IFNα stimulation also induces the development of CD1a positive cells (Santini et al, J Exp Med 2000).

In addition to the generation of moDC from Mo, prior studies from Geissmann et al. indicate that it is also possible to generate cells with LC characteristics from $CD14^+$ Mo under GM-CSF, IL-4 and TGF-β1 conditions (Geismann et al, J Exp Med 1998). Both cytokine, TGF-β1 and GM-CSF are also present in the skin in vivo. TGF-β1 is produced by keratinocytes in the epidermis (Sporn et al, J. Cell Biol 1992) and GM-CSF by a variety of cells which populate the skin (Pastore et al, Clin Invest 1997). Relating to Geismann's study it was furthermore shown by another group (Hoshino et al, J Leukoc Biol 2005) that GM-CSF, TGF-β1 and Notch ligand Delta-1 are required for LC differentiation from $CD14^+$ Mo (Figure 6). The group could furthermore show that Delta1, which is involved in Notch signalling, and is as well as TGF-β1 and GM-CSF present in the skin environment in vivo (Hoshino et al, J Leukoc Biol 2005; Lowell et al, Curr Biol 2000).

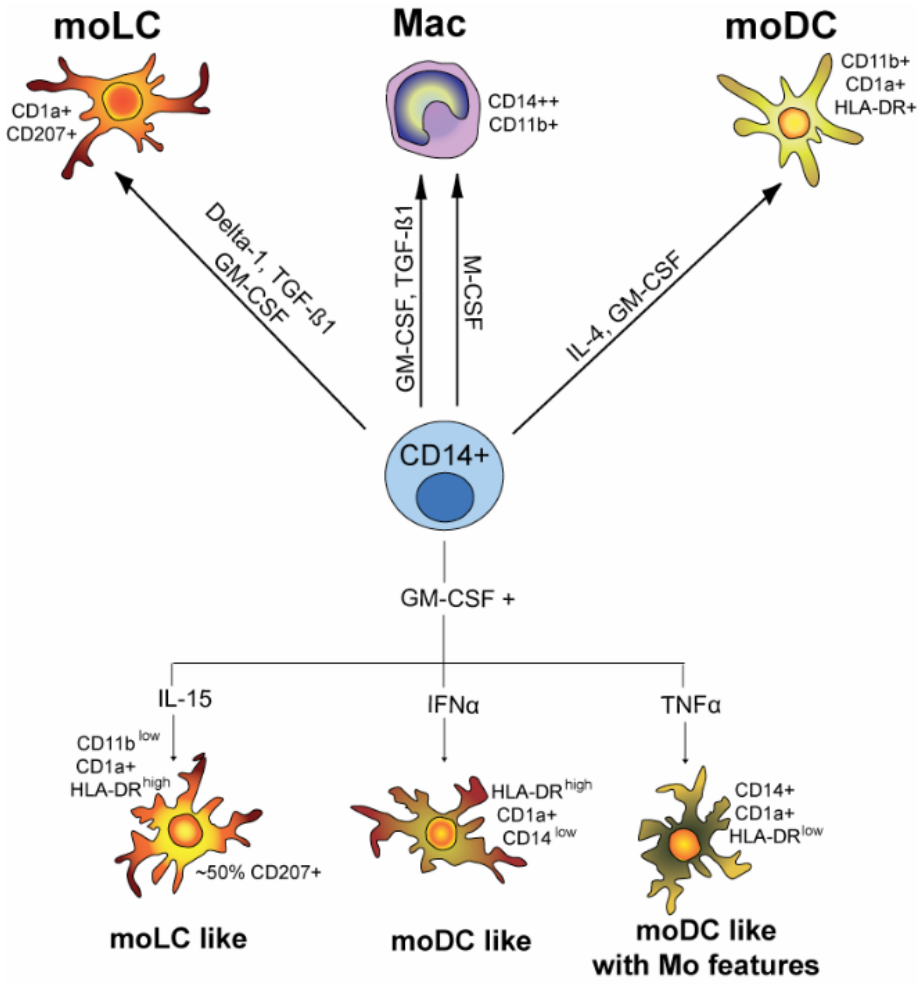


Figure 6: **Generation model of monocyte-derived dendritic cell types under different conditions.**
When isolated Mo are cultivated in special conditions they acquire DC characteristics. The best established model is the generation of moDC with GM-CSF and IL-4 (Chapuis et al, Eur J Immunol 1997) but until now also other cytokines were shown to induce DC differentiation from Mo. Those cytokines include TNFα (Iwamoto et al, J Immunol 2007), which was shown to induce moDC with monocytic features, IL-15 which also is able to induce CD207 expressing moLC (Mohamadzadeh et al, J Exp Med 2001) and IFNα, which was shown to induce moDC like cells (Santini et al, J Exp Med 2000). Prior studies also indicated that cells with LC characteristics can be generated from Mo using GM-CSF, IL-4 and TGF-β1 (Geismann et al, J Exp Med 1998). These findings were extended by the finding that Delta-1, TGF-β1 and GM-CSF lead to the differentiation of moLC with the expression of CD1a and CD207 (Hoshino et al, J Leukoc Biol 2005; Lowell et al, Curr Biol 2000).

Notch signalling regulates a broad spectrum of cell fate decisions (Artavanis-Tsakonas et al, Science 1999). The Notch family consists of four receptors, Notch-1 to 4, and five ligands, Jagged-1 and -2 and Delta-1, -3 and -4. It is indicated that activation of Notch signaling regulates proliferation and differentiation of hematopoietic stem cells and that in the hematopoietic system especially Delta-1 family are the predominant ligands involved (Ohishi et al, J Clin Invest 2002). Interestingly it is shown that Mo express high

amounts of the Notch receptors. Signalling via those receptors inhibits the survival of Mo in presence of M-CSF but not when GM-CSF is available and permits their differentiation into DC (Figure 7). For this reason it was proposed that Notch signalling inhibits Mac differentiation, but promotes DC development from monocytic precursors (Ohishi et al, Blood 2000 and 2001). It was furthermore shown that LC generated with Delta-1, TGF-β1 and GM-CSF from $CD14^+$ Mo seem to correspond to immature LC. Those cells show phagocytic activity and migrate in response to MIP-3α, which are functional properties of immature LC. (Hoshino et al, J Leukoc Biol 2005). As already mentioned before, TGF-β1 is sufficient to induce LC development from haematopoietic progenitors in vitro. However for the differentiation of LC from Mo additional signalling via Delta1-Notch is needed. It is interesting in this respect that several reports have shown that TGF-β1 and Notch synergistically regulate common target genes in various cell types. It is therefore interesting to speculate that while TGF-β1 alone is sufficient to induce LC differentiation from mulitpotent progenitors, TGF-β1 and Notch signalling have to act synergistically to induce the development of LC from fully differentiated Mo.

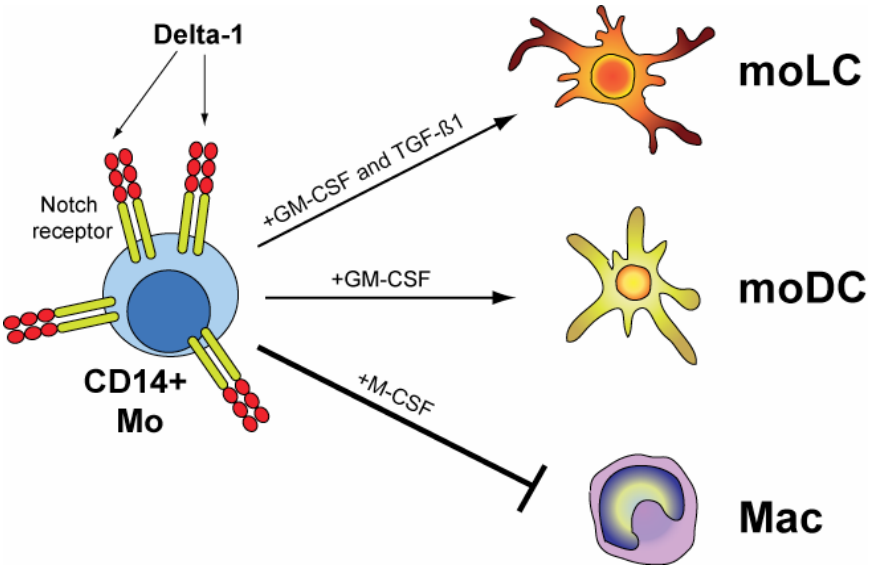


Figure 7: **Notch signalling via Delta-1 is important for LC differentiation.** Mo express a high amount of Notch receptors. In response to Delta-1 stimulation they develop DC-like characteristics under GM-CSF influence whereas under M-CSF they undergo cell death. If additionally TGF-β1 is present they are able to differentiate into LC (Hoshino et al, J Leukoc Biol 2005).

1.3) Kruppel-like Transcription Factors

The gene regulatory protein family Kruppel-like factor (KLF) is named after the embryonic pattern regulator Kruppel in *Drosophila* because they show homology with the DNA binding domains. This family of transcription factors is implicated in a wide variety of different biological processes ranging from proliferation to differentiation and apoptosis (Dang et al, Int J Biochem Cell Biol 2000). They belong to the family of zinc-finger containing transcription factors which are containing three highly conserved C-terminal C_2H_2 zinc fingers which bind DNA and recognize very similar “GT-box” or “CACCC element” consensus sequences (Bieker JJ, J Biol Chem 2001) and phylogenetic analyses show their close relation to Sp1 and Krox zinc finger proteins. The structural component of this protein family is a single zinc atom which is tetrahedrally coordinated by amino acids, such as cysteine and histidine. This zinc holds together two β-pleaded sheets in the amino terminal half and an α-helix in the carboxyl terminal half of each finger (Figure 8). A seven-amino acid spacer TGEKP(Y/F)X also called Kruppel-link connects the fingers and is highly conserved between the family members (Dang et al, Int J Biochem Cell Biol 2000)

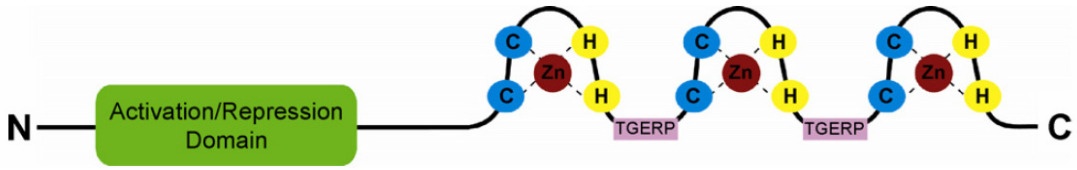


Figure 8: **Schematic representation of the zinc fingers from the KLF molecule.**
The three C-terminal zinc fingers including zinc ions which are able to bind target DNA. At the N-terminus an activation/repression domain is found (taken from Pearson et al, Int J Biochem Cell Biol 2008).

Till now 17 members have been identified which are referred to as KLF1-KLF17 (Suske et al, Genomics 2005) and which show either a tissue restricted or widely expression pattern. In the regulation of the same cellular process more than one KLF protein can be involved and various KLFs may result in opposing effects. It is also shown that KLF expression seems to be altered upon various cytokines.

KLF1 (EKLF) expression is restricted to erythroid cells where it activates β-globin transcription and consequently plays an essential role in erythropoiesis (Dang et al, Int J Biochem Cell Biol 2000). KLF1 null mice die at E14.5 because fatal anemia occurs when the switch from fetal to adult globin expression takes place (Perkins et al, Nature 1995).

KLF2 (LKLF) is highly expressed in the lung but can also be found in erythroid, lymphoid and other tissues (Dang et al, Int J Biochem Cell Biol 2000). KLF2 null mice show an embryonic lethality due to defective regulation of cardiac output and heart failure although lung development seems to be unaffected (Wani et al, Transgenic Res 1998). Loss of KLF2 also leads to a massive down-regulation of the peripheral T-cell pool suggesting that KLF2 might play a role in the survival of these cells (Carlson et al, Nature 2006).

KLF3 (BKLF) seems to play a role in controlling adipogenesis because KLF3 knockout mice show less white adipose tissue and even in their fat pads are smaller and fewer cells (Sue et al, Mol Cell Biol 2008).

KLF4 (GKLF) is highly expressed in post-mitotic cells of the gut and skin epithelium (Dang et al, Int J Biochem Cell Biol 2000). KLF4 knockout mice die shortly after birth due to a loss of normal skin barrier function because the late stage differentiation in the epidermis is abnormal (Segre et al, Nat Genet 1999). The absence of KLF4 also leads to an impaired formation of the goblet cells (Katz et al, Development 2002). It should also be mentioned that KLF4 is expressed in embryonic stem (ES) cells and is one of four factors which are able to reprogram somatic cells and therefore induce pluripotent stem cells (iPS) in mouse as well as in human (Takahashi et al, Cell 2006; Loh et al, Blood 2009).

KLF5 (IKLF/Bteb2) expression seems to be complementary to KLF4 expression. KFF5 knockout mice die before E8.5 and heterozygote mice reveal defects in angiogenesis (Shindo et al, Nat Med 2002) as well as affected gut differentiation. It is also shown that KLF5 plays a role in the differentiation of white adipose tissue and thereby PPARγ appeared to be a target gene of KLF5 (Oishi et al, Cell Metab 2005).

KLF6 (BCD1) is widely expressed in several embryo tissues and knockout of the KLF6 gene leads to embryonic death at E12.5 because of failure of yolk

sac vascularisation and malfunction of erythropoiesis (Matsumoto et al, Blood 2006).

KLF7 (UKLF) high level expression is restricted to neuronal tissue reflecting its importance for neuronal differentiation. KLF7 knockout mice die after birth because of defects in the development of axonal pathways which leads to widespread neurological defects (Laub et al, Mol Cell Biol 2005). The loss of KLF7 also leads to an increased apoptosis rate of sensory neurons.

KLF8 (BKLF3) is important in the epithelial to mesenchymal transition (Wang et al, Cancer Res 2007 and J Biol Chem 2008) and plays a role in oncogenic transformation (Wei et al, Biol Chem 2006). It is important in the regulation of cell cycle progression and over expressed in several cancers (Wang et al, Oncogene 2007).

KLF9 (Bteb1) shows a high level expression in the cerebellum and hippocampus (Morita et al, Mol Cell Biol 2003) and KLF9 knockout mice show mild defects in behavior, have a normal lifespan however a reduced fertility is proven in females du to the fact that normal uterine development and embryo implantation fails (Simmen et al, J Biol Chem 2004).

KLF10 (Tieg1) knockout mice show defects in the development of bones and older mice develop cardiac hypertrophy. They have high osteoblast numbers with impaired ability to differentiate and mineralize and their bones are weakened and display reduced osteocyte number, density and size and thus leading to impaired skeletal development (Subramaniam et al, Mol Cell Biol 2005).

KLF11 (FKLF/Tieg2) knockout mice are phenotypically normal (Song et al, Blood Cells Mol. Dis. 2005) and show no effects in erythropoiesis although it is demonstrated that KLF11 is expressed in erythroid cells regulating embryonic globin expression *in vitro* (Emery et al, J Cell Biochem. 2007).

KLF12 (AP-2rep) seems to be an important gene in maturation process of the collecting ducts after birth (Suda et al, Biochem Biophys Res Commun 2006) and is able to repress the expression of the activating enhancer-binding protein 2 α (AP-2α; Imhof et al, Mol Cell Biol 1999).

KLF13 (FKLF2/Bteb3) knockout mice reveal a down-regulation of RANTES (CCL5) expression which is an important chemokine involved in the activated T cell response (Zhou et al, J Immunol 2007) and because of the decreased T

cell apoptosis these mice show enlarged thymus and spleen. In *Xenopus* KLF13 interacts with GATA-4 and therefore seems to be required for heart development (Lavallée et al, EMBO J 2006).

KLF14 (Bteb5) is shown to repress the promoter of transforming growth factor-beta receptor II (TGF-βRII; Truty et al, J Biol Chem 2009).

KLF15 (KKLF) is widely expressed and shows strongest expression in kidney and liver (Gray et al., J Biol Chem 2002). KLF15 knock out mice are viable and fertile but are susceptible for cardiac hypertrophy because of abnormal heart morphology (Fisch et al, Proc Natl Acad Sci USA 2007). If these KLF15 knockout mice are fasted over night (18h) they exhibit severe hypoglycaemia (Gray et al, Cell Metab 2007). It is also shown that KLF15 regulates the expression of genes involved in adipocyte differentiation and glucose uptake (Gray et al., J Biol Chem 2002; Mori et al., J Biol Chem 2005).

KLF16 (DRRF/Bteb4) highest expression is found in the brain and seems to be important for the modulation of dopaminergic transmission in the brain (Hwang et al, Proc Natl Acad Sci USA 2001).

KLF17 is not well examined until now except its relation to the KLF family (van Vliet et al, Genomics 2006).

The different effects of the various KLF knockouts are indicated in Table 2.

Table 2: **Different KLF4 transcription factors and there knockout outcome if available:**

Transcription factor	**Knockout existent**	**outcome of the knockout**		
		embryo lethality	die after birth	Viable
KLF1	√	√		
KLF2	√	√		
KLF3	√	No	No	√
KLF4	√	No	√	
KLF5	√	√		
KLF6	√	√		
KLF7	√	No	√	
KLF8	**NO**			
KLF9	√	No	No	√
KLF10	√	No	No	√
KLF11	√	No	No	√
KLF12	**NO**			
KLF13	√	No	No	√
KLF14	**NO**			
KLF15	√	No	No	√
KLF16	**NO**			
KLF17	**NO**			

1.3.1) KLF4 and its Importance in Various Different Physiologic Processes

As mentioned before KLF4 is a transcription factor which is highly expressed in differentiated, post-mitotic epithelial cells of the skin and gastrointestinal tract (Garrett-Sinha et al, J Biol Chem 1996) and KLF4 knockout mice indicate that it may function as a proliferation-differentiation switch because these mice show defects in skin differentiation (Segre et al, Nat Genet 1999) as well as less secretory goblet cells in the colon (Katz et al, Development 2002).

It has been shown that KLF4 is inhibited by TGF-β1 which is an absolutely necessary cytokine for LC development (see chapter 1.1.2) and that KLF4 seems to be critical for the development of Mo (Feinberg et al, J Biol Chem 2005 and EMBO J 2007). Certain cytokines like IFN-γ, LPS or TNFα are able to induce KLF4 expression and after LPS activation of Mac KLF4 up-regulates the expression of IL-10 by binding to its promoter (Liu et al, Biochem Biophys Res Commun 2007). In Mac KLF4 appears to be critical in mediating profinflammatory responses because after LPS or IFN-γ stimulation it interacts with p65 (RelA) and therefore activates Mac by induction of the iNOS promoter. On the other hand KLF4 itself is able to interfere with the TGF-β1-pathway; it can inhibit the Smad3 signalling by capturing the p300 away from the Smad3 in certain cases. Therefore target genes of Smad3, like Plasminogen-Activator-Inhibitor-1-protein (PAI-1), are repressed by KLF4. Smad3 is known to play a critical role in suppressing important Mac markers of inflammation [iNOS, monocyte chemoattractant protein-1 (MCP-1); Werner et al, J Biol Chem 2000; Feinberg et al, Circ Res 2004; J Biol Chem 2005 and EMBO J 2007] whereas KLF4 is an activator of them. Interestingly Notch signalling seems to repress KLF4 expression (Ghaleb et al, Mol Cancer Res 2008). As discussed before the Notch ligand Delta-1 plus TGF-β1, both are inhibitors of KLF4, are important factors to obtain moLC (1.2.3). KLF4 down-regulation seems to be an important part of this differentiation process which is performed by cytokines and products important for LC development.

Depending on the cellular context, KLF4 can act either as an activator or a repressor. It is revealed that *β-catenin* signalling is inhibited by interaction with KLF4 which therefore leads to reduced tumorigenic activity (Zhang et al, Mol Cell Biol 2006). *β-catenin* is encoded by an oncogene and is a component of the *wnt*-pathway which activates cell proliferation. Because of the high expression in terminally differentiated cells a link to growth arrest is suggested and it is proven that KLF4 activates genes encoding negative regulators of the cell cycle whereas genes promoting the cell cycle are repressed by KLF4 (Chen et al, J Mol Biol 2003). It is also indicated that KLF4 is able to recruit p53 to the $p21^{Cip1/Waf1}$ promoter which thus drives its transcription and causes cell cycle arrest (Yoon et al, J Biol Chem 2004). Important to notice is that KLF4 is also implicated in regulating apoptosis by inhibiting the transactivation of the proapoptotic protein *Bax* after DNA damage due to p53. Therefore it is able to prevent apoptosis by inhibiting the expression of Bax as well as by the up-regulation of p21 (Ghaleb et al, Oncogene 2007).

Because of all these data it is logical to suggest that KLF4 might function as a tumor suppressor. Many studies reveal a loss of KLF4 expression in human tumors like colorectal, stomach, esophageal and bladder cancer (McConnell et al, Bioessays 2007) and over expression of KLF4 in the colon cancer cell line RKO leads to a reduction of colony formation and cell migration (Dang et al, Oncogene 2003). But there also exist studies which reveal KLF4 as a putative oncogene rather than a tumor suppresser: elevated levels of KLF4 are found in many mammary carcinomas (Foster et al, Cancer Res 2000) and it is also over expressed in squamous-cell carcinomas of the oropharynx (Foster et al, Cell Growth Differ 1999). Taken together KLF4 reveals cell-cycle-checkpoint functions after DNA damage which contributes to its activity as a tumor suppressor but on the other hand it also exhibits anti-apoptotic activity which under certain circumstances can drive cellular transformation. A short overview is given in Figure 9.

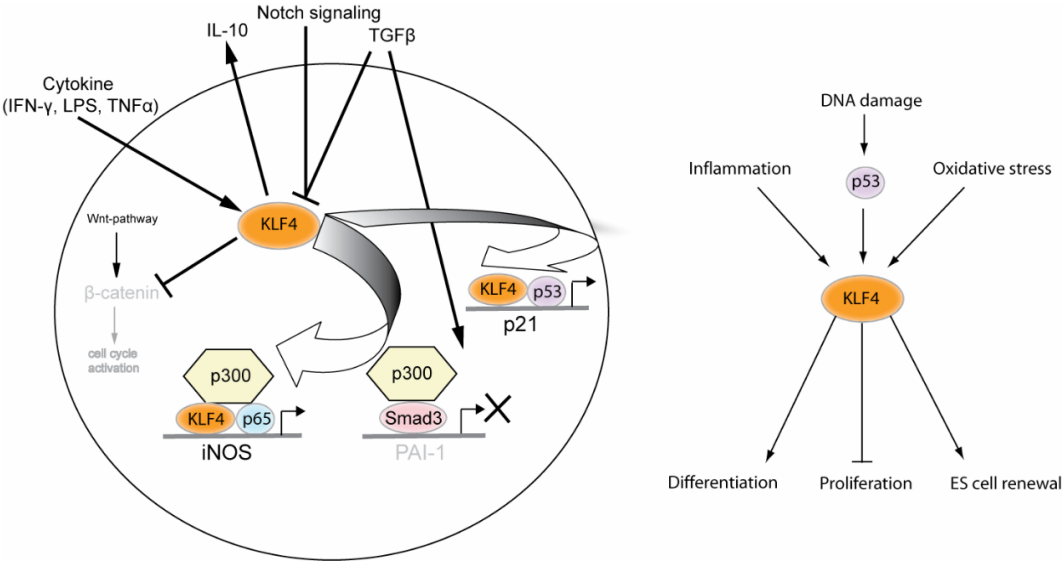


Figure 9: **KLF4 and its contribution to different physiological processes.** KLF4 is activated by different cytokines like IFN-γ, LPS or TNFα whereas TGF-β1 and Notch signalling represses it. KLF4 itself negatively regulates the TGF-β1 pathway by capturing away the p300 from Smad3. It is also able to interact with p65 (RelA) and therefore mediating profinflammatory responses like activating iNOS (Feinberg et al, EMBO J 2007; Ghaleb et al, Mol Cancer Res 2008)

1.3.2) Mouse Inflammatory Monocytes show a Dependency on KLF4

It has been postulated that KLF4 is an important downstream target of PU.1 which is necessary to drive Mo instead of Gr development in human as well as in mice. Moreover, KLF4 is a direct transcriptional regulator of the human monocytic marker CD14 (Feinberg et al, EMBO J 2007; Alder et al, J Immunol 2008).

Mouse Mo are distinguished into “inflammatory” ($Ly6C^{high}$) and “resident” ($Ly6C^{-}$) Mo whereas the “inflammatory” Mo are short lived and migrate to inflammation sites and the “resident” Mo persist longer and seem to be precursors for DC and Mac (Suderkotter et al, J Immunol 2004). Alder et al. showed that “resident” Mo are decreased whereas “inflammatory” Mo are absolutely absent when KLF4 is missing thus demonstrating an important role of KLF4 for the production or survival of the monocytic precursor cells in the bone marrow. $KLF4^{-/-}$ cells are twice as apoptotic as $KLF4^{+/+}$ cells and induction of KLF4 leads to higher survival rate. Because of these data and the

rapid down-regulation of Ly6C KLF4 seems to be an important factor for the differentiation, development and/or survival of inflammatory Mo (Alder et al, J Immunol 2008). Suderkotter et al. supposed that first “inflammatory” Mo arise and that “resident” Mo develop out of them (Suderkotter et al, J Immunol 2004). The new findings from Alder et al. reveal the possibility of a common precursor which gives rise to either “resident” or “inflammatory” Mo whereas KLF4 seems to play an important role in the differentiation process and/or survival of “inflammatory” Mo (Figure 10).

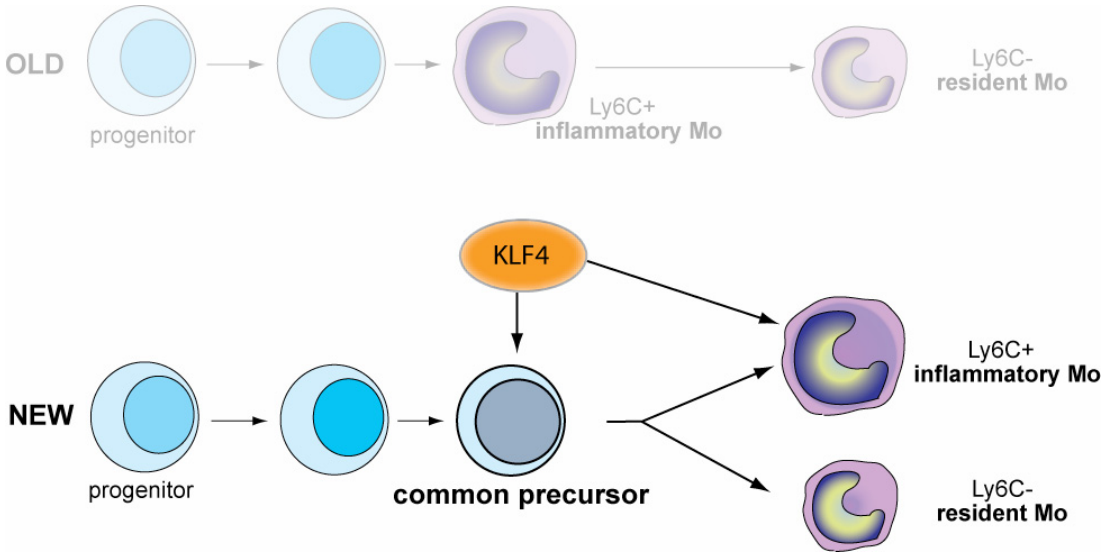


Figure 10: **Myeloid development pathway of “resident” and “inflammatory” Mo in mouse.**
Before it was thought that first “inflammatory” Mo arise which then become precursors for “resident” Mo (Suderkotter et al, J Immunol 2004). New studies reveal a common precursor for both under the importance of KLF4. When KLF4 is absent no “inflammatory” Mo develop and the number of “resident” Mo is decreased which is consistent with the existence of a common precursor (Alder et al, J Immunol. 2008).

1.3.3) KLF4 and its Implication in Cell Fate Decision

As mentioned before KLF4 is an important downstream target of PU.1 which therefore is important for Mo development (Feinberg et al, EMBO J 2007). Other factors which repress the differentiation into Mo or favour another cell type (TGF-β1 or Delta-1) are inhibitors of KLF4 expression (Ghaleb et al, Mol Cancer Res 2008; Feinberg et al, J Biol Chem 2005). There seems to be an important transcription factor network in myeloid progenitors which includes

the expression or inhibition of KLF4 to determine cell fate and decision. Another interesting finding is that KLF4 plays a necessary role in the differentiation process of mouse “inflammatory” Mo which are thought to be direct precursors for moLC in inflamed skin (Alder et al, J Immunol 2008; Ginhoux et al, Nat Immunol 2006). On the one hand KLF4 seems to be an absolutely necessary factor for LC precursors but on the other hand KLF4 down-regulation is definitely required to obtain a moLC phenotype (Figure 11).

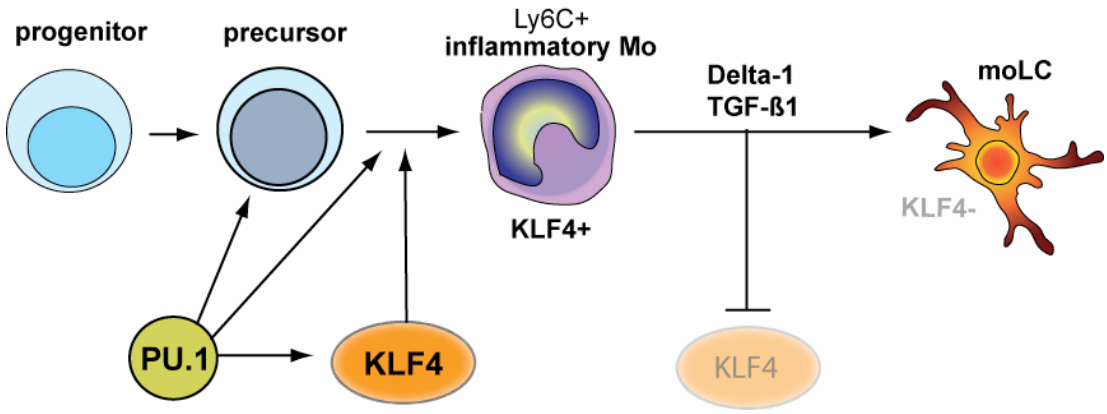


Figure 11: **KLF4 is necessary for the moLC precursors but its down-regulation is required to obtain LC characteristics.**
PU.1 is an important factor for Mo and it activates KLF4 expression which is necessary for the differentiation and survival of inflammatory Mo which are direct precursors of LC (Feinberg et al, EMBO J 2007; Alder et al, J Immunol 2008; Ginhoux et al, Nat Immunol 2006). To gain a LC phenotype it seems to be absolutely essential to inhibit KLF4 expression. This acts via diverse cytokines and signalling pathways which are important for LC differentiation.

2) Material and Methods

2.1) Self-made Buffers and Solutions

CellGroDC + Pen/Strep + Glutamax (P/S/GMX)

CellGroDC medium (BioWhittaker) + GlutaMAX (2.5mM; Gibco/ Invitrogen) + penicillin/streptomycin (125U/mL each; Sigma)

X-vivo + Pen/Strep + Glutamax (P/S/GMX)

X-vivo15 medium (BioWhittaker) + GlutaMAX (2.5mM; Gibco/ Invitrogen) + penicillin/streptomycin (125U/mL each; Sigma)

DMEM + 10%FCS + Pen/Strep + Glutamine (P/S/G)

DMEM (Sigma) + 10% Foetal Bovine Serum (Gibco) + L-Glutamin (2mM; Sigma) + penicillin/streptomycin (125U/mL each; Sigma)

RPMI + 10%FCS + Pen/Strep + Glutamine (P/S/G)

RPMI (Sigma) 10 %Foetal Bovine Serum (Gibco) + L-Glutamin (2mM; Sigma) + penicillin/streptomycin (125U/mL each; Sigma)

dH2O

Aqua bidest. “Fresenius” (Fresenius Kabi)

2xHBS

8g NaCl; 6.5g HEPES; 105mg Na2HPO4 in 500ml dH2O (pH 7.0)

RetroNectin

90µl RetroNectin (1mg/ml; Takara Bio) in 3ml 1xPBS

Beriglobin

Beriglobin (CSL Behring) diluted 1:8 in PBS/BSA/Azid

4xSampleBuffer

0.5M Tris/HCl (pH 6.8); 40% glycerol (Roth); 4% SDS (Roth); 5µl/ml Bromphenolblue (Roth); 5% Mercartoethanol (Sigma)

2xSamleBuffer

0.5ml 4xSample Buffer; 0.5ml dH_2O

Separating gel (10%)

2667µl 30% Acrylamid/Bis (29:1) (BioRad); 2000µl Tris/HCl pH 8.8; 3203µl H2O; 40µl 20% SDS (Roth); 80µl 10% APS (Roth); 10µl TEMED (BioRad)

Stacking gel (4%)

396µl 30% Acrylamid/Bis (29:1) (BioRad); 378µl Tris/HCl pH 6.8; 2196µl H2O; 15µl 20% SDS (Roth); 20µl 10% APS (Roth); 5µl TEMED (BioRad)

10x SDS-PAGE runnig buffer

30.2g Tris (Roth); 144g Glycin (Roth); 10g SDS (Roth) in 1000ml dH2O

10x Blotting buffer

30,3g Tris (Roth); 144g Glycin in 1000ml dH2O

10x PBS

400g NaCl (Roth); 10g KCl (Roth); 72g Na2HPO4 (Roth); 10g KH2PO4 (Roth); adjust to pH 7.4; in 5000ml dH2O

1x PBS/Tween

1xPBS/ 0.05% Tween (Tween20; Roth)

1x PBS/BSA/Azid

1xPBS; 20% BSA (Roth); 0.4% Na3N (Roth)

50xTAE Buffer

242g Tris/HCl; 57.1ml acetic acid; 37.2g Na2EDTA.2H2O in 1000ml dH2O

5X KCM Buffer

0.5M KCl; 0.15M CaCl2; 0.25M MgCl2 in dH2O

SOC-medium

2g tryptone (Roth); 0.5g yeast extract; 1ml 1M NaCl (Roth); 0.25ml 1M KCl (Roth); 1ml 2M Mg^{2+} (Roth); 1ml 2M glucose (Roth) in 100ml dH2O

LB_{Amp} medium

LB medium (10g tryptone (Roth); 5g yeast extract (Roth); 5g NaCl (Roth); 100µg/ml Ampicillin (Applichem)

LB_{Amp} plates

15g agar (Roth) in 1000ml LBAmp medium

2.2) Cell Culture

2.2.1) Reagents and Cytokines

All reagents were purchased from Sigma, Fermentas, Qiagen, Promega or Roth if not stated explicitly. Foetal calf serum (FCS) was supplied by Gibco and heat inactivated (30min at 65°C) prior to use.
Interleukin-4 (IL-4), Interleukin-6 (IL-6), human stem cell factor (SCF), thrombopoietin (TPO) and tumor necrosis factor alpha (TNFα) were purchased from PeproTech (London, United Kingdom); transforming growth factor beta-1 (TGF-β1) was purchased from R&D Systems (Wiesbaden, Germany); Interleukin-15 (IL-15), fms-related tyrosine kinase 3 ligand (FL, Flt3L), granulocyte colony-stimulating factor (G-CSF) and macrophage colony-stimulating factor (M-CSF) were obtained from PeproTech (London, United Kingdom); granulocyte-macrophage colony-stimulating factor (GM-CSF) was kindly provided by Novartis Research Institute (Vienna, Austria); donkey α-human Immunoglobulin G (dkαH-IgG) was obtained from Jackson ImmunoResearch and Delta-1 IgG was a gift from Bernstein ID.

2.2.2) Cell Lines and Culture

U937Te cells were maintained in RPMI plus 10% FCS plus Penicillin (10.000U/ml)/ Streptomycin (10mg/ml)/L-Glutamin (200mM) medium (P/S/G) in cell culture flasks (Nunclon™Δ Surface, Nunc). Phoenix cell lines (Ph-GP and Ph-E) were grown in DMEM plus 10% FCS plus P/S/G on cell culture dishes (Nunclon™Δ Surface, Nunc) and were detached by Trypsin-EDTA (Sigma). All cell lines and primary cells were maintained at 37°C in the 5% CO2 incubator.

2.2.2.1) Passaging of Cells

First the medium was removed from the cell monolayer and than the cells were trypsinised using 3ml Trypsin EDTA. When the cells started to detach from the plate 8ml medium were added, to stop Trypsin EDTA function. The cell suspension was then split (for example 1:5) to a new culture tissue plate containing 10ml of the needed medium and incubated at 37°C.

2.2.2.2) Freezing and Unfreezing of Cells

After trypsinising the cells were pelleted by centrifugation and washed once with PBS. Afterwards the pellet was taken up in 1ml freezing medium (10% Dimethyl Sulfoxide (DMSO), 70% DMEM, 20% FCS) and transferred to a freezing tube. The cells got slowly frozen, first an ice then at -20°C; storage of the cells was carried out at -80°C.
For unfreezing, cells from the freezing tube were thawed on ice first, then at room temperature, resuspended in 8ml of prewarmed medium and pelleted. Following a washing step with PBS to remove the DMSO, the cell pellet was then resuspended in growth medium, transferred into a plate and incubated at 37°C.

2.2.3) Isolation of Cord Blood $CD34^+$ and $CD14^+$ Cells

Cord blood samples from healthy donors were collected during healthy full-term deliveries. Approval was obtained from the Medical University of Vienna institutional review board for these studies. Informed consent was provided according to the Declaration of Helsinki. Cord blood mononuclear cells (MNCs) were obtained by density gradient centrifugation over Lymphoprep (Axis-Shield PoC AS, Oslo, Norway). $CD34^+$ cells were isolated from mononuclear cord blood cells using MACS Direct CD34 Progenitor Cell Isolation Kit (Miltenyi Biotech, Bergisch Gladbach, Germany) according to the instructions of the manufacturer. The purity of the isolated CD34+ cells analyzed by flow cytometry was greater than 95%. $CD14^+$ cells were isolated

from mononuclear cord blood cells or mononuclear peripheral blood cells by using a CD14-PE antibody (Caltag) which is recognized by anti-PE microbeads (Miltenyi Biotec). The cells were collected by using MACS columns (Miltenyi Biotec).

2.2.4) Retroviral Vectors, Gene Transduction and Infection

For the ectopic expression study a RV-GFP plasmid (empty or including KLF4) which contains the FACS detectable marker GFP from Mukesh K. Jain was obtained.

The packaging cell lines Phoenix E (Ph-E; ecotropic retrovirus) and Phoenix-Gag-Pol (Ph-GP; amphotropic virus) were transfected by calcium phosphate precipitation according to the Nolan lab protocol for retroviral transfection (http://www.stanford.edu/group/nolan/); the Ph-GP were cotransfected with the gibbon ape leukemia virus envelope protein (GalV). For the transfection 5 to 10µg of DNA were used and mixed with 61µl 2M $CaCl_2$, 425µl dH_2O (Fresenius) and 500µl 2xHBS by vortexing 10 to 15 seconds, during an incubation time of 10 to 15min, 3µl 50mM Chloroquin (Sigma) was added to the Ph-GP or Ph-E plates. DNA precipitate was added drop wise onto the cells. After 6 to 12 hours the medium was changed and the cells were incubated for >36 hours. The virus supernatant was collected with a syringe and filtered through a 0.45µ filter (IWAKI).

Target cells ($2x10^4$ to $5x10^5$) were plated on RetroNectin (Takara Bio, Shiga, Japan)-coated nontissue suspension culture plates (o/n 4°C) coated with virus (4 to 6 hours before) in specific growth medium. Infection was repeated 2 to 3 times in an interval of 12 to 24 hours. $CD34^+$ cells were infected in expansion mix (SCF, FL, TPO; each 50ng/ml).

2.2.5) Lineage Differentiation

All are 100x mixes and from these always 10µl were used to be given in 1ml. All cells were expanded in serum free X-VIVO 15 medium +P/S/GMX plus expansion mix (50ng/ml TPO, 50ng/ml Flt3L and 50ng/ml SCF) for three days before cultivation.

To generate LC GM-CSF (200ng/ml), Flt3L (50ng/ml), SCF (20ng/ml), TNFα (2.5ng/ml), and TGF-β1 (1ng/ml) were used in serum-free Cell Grow DC supplemented with P/S/GMX. They were differentiated for 7 days; after day 4 the medium was changed. For the generation of intDC the same as for LC generation was used however without TGF-β1 but plus 10% FCS. On day 4 the medium was changed into intDC-mix 2 containing GM-CSF (200ng/ml) and IL-4 in RPMI +10% FCS and +P/S/G. The cells were harvested on day 9 or 10. For Mo differentiation M-CSF (100ng/ml), Flt3L (50ng/ml), SCF (20ng/ml) and IL-6 (20ng/ml) were used in serum-free X-VIVO 15 supplemented with P/S/GMX. After day 4 the medium got changed every 2 or 3 days and the cells were harvested on day 10. To get granulocytes G-CSF (100ng/ml) and SCF (20ng/ml) were used in serum-free X-VIVO 15 medium +P/S/GMX. Also here the medium got changed every 2 or 3 days after day 4 and the cells were harvested on day 13 or 14.

There is another differentiation method using serum-containing media. Therefore the cells were cultivated in CellGroDC supplemented with P/S/GMX and 10% FCS for 5 days in GM-CSF (200ng/ml), TNFα (2.5ng/ml), Flt3L (50ng/ml) and SCF (50ng/ml). After the five days the cells were grown in RPMI + 10%FCS with either GM-CSF plus TGF-β1 (1ng/ml) and TNFα to obtain LC, or in GM-CSF plus IL-4 (25ng/ml) and TNFα to acquire DC, or in M-CSF (100ng/ml), Flt3L (50ng/ml), SCF (20ng/ml) and IL-6 (20ng/ml) to gain a monocyte phenotype.

2.2.6) CD14 Generation and Delta-1 Coating

$CD14^+$ cells were cultivated in RPMI + 10% FCS until day seven. To obtain moLC $CD14^+$ cells were cultivated on Delta-1 coated nontissue suspension

culture plates. Therefore, the plates were coated with 10µg/ml dkαH-IgG in PBS which were incubated for one hour at 37°C, after that blocked for 45min at RT with RPMI + 10% FCS followed by an addition of 10µg/ml Delta-1 either o/n at 4°C or for three hours at 37°C. To those Delta-1 coated plates GM-CSF (200ng/ml) and TGF-β1 (10ng/ml) were added. For moDC cells got cultivated in GM-CSF and IL-4 (25ng/ml), or GM-CSF, TGF-β1 and IL-4. Also a possible moDC generation method is in GM-CSF with either TNFα (10ng/ml) or IFNα (400U/ml) or IL-15 (200ng/ml). To obtain monocytic cells they were left in GM-CSF.

2.3) Bacteria

2.3.1) Cultivation of Bacteria

Bacteria were cultivated in sterile Luria-Bertani (LB) media at 37°C. For bacteria harbouring a plasmid selective media which was supplemented with a specific antibiotic was used. For freshly transformed bacteria a SOC media was used. This media contains 5g Bacto-yeast extract , 20g Bacto-tryptone , 0.5g NaCl , 2.5ml 1M KCl , ddH_2O to final volume of 1l , pH adjusted to 7.0 using 10M NaOH, autoclaved and sterilized and after that 20ml of sterile 1M glucose was added before use. For storage bacterial overnight cultures were mixed with glycerol (30% glycerol) and stored at -80°C. For recultivation a little bit of the stock was inoculated in 3ml LB media (+/- selective antibiotics) at 37°C over night with shaking.

2.3.2) Bacterial Transformation

Therefore KCM competent cells were used. They were made by inoculating the cells in LB media until the OD_{600} is between 0.3-0.6, they were pellet at 2500g for 10min at 4°C, resuspended in 10ml TSB and incubated on ice for 10min. Aliquots were snap freeze in liquid N_2 and stored at -80°C. The TSB buffer contained 1x LB broth, 10% PEG (MW 3.350), 5% DMSO, 10mM

$MgCl_2$, 10mM $MgSO_4$ and was filled up with ddH_2O to 50ml. LB broth was autoclaved before the other ingredients were added, after that the solution got sterile filtered and was stored at 4°C.
For the transformation the KCM cells were thaw on ice for 10min. Meanwhile 20µl 5x KCM buffer and 10-20µl Ligation product (2.4.3) or the plasmid of interest were mixed and filled up with ddH_2O to 100µl. 100µl KCM cells were added, left on ice for 20min, then for 10min on room temperature, 900µl SOC medium was added and the cells were incubated at 37°C for 60min with shaking. 100-300µl of the cell suspension were transferred on LB plates (+ antibiotics) and placed at 37°C over night. After that positive clones were picked and cultivated over night to isolate the DNA which was controlled by a control restriction if the insert is inside or the right plasmid was taken up.

2.3.3) Isolation of Bacterial Plasmid DNA

For the purification of high amounts of the plasmid DNA the QIAfilter Plasmid Maxi Kit (QIAGEN) was used. A single colony got inoculated in 2 to 3ml LB media containing antibiotics (AB) at 37°C shaking over night to grow a starter culture. 300µl of this culture was transferred into fresh 150ml LB media plus AB to generate an overnight culture (37°C/shaking over night). The bacterial cells were harvested by centrifugation (5000g/15min/4°C). The plasmid DNA purification was performed according to the protocol. The bacterial pellet was resuspended in 4ml P1 Buffer, 4ml P2 Buffer were added and mixed by inverting followed by an incubation period of 5min at RT. After addition of P3 Buffer (4ml) the suspension got mixed and the lysate was applied into the QIAfilter Cartridge. During the subsequent incubation period (10min at RT) the precipitate floated and formed a layer at the top of the solution to enable a successful filtration. In the meantime a QIAGEN-tip 500 column was equilibrated by applying 10ml QBT Buffer. The cell lysate was filtered trough the QIAfilter Cartridge into the equilibrated QUIAGEN-tip and entered the column by gravity flow. The column was washed twice with 30ml QC Buffer and the plasmid DNA was eluted in 15ml QF buffer. To precipitate the DNA 10.5ml isopropanol were added followed by centrifugation (1h/5000g at 4°C).

The DNA pellet was washed with 5ml 70% EtOH and centrifuged (1h/5000g at 4°C). The pellet was air dried and dissolved in 200µl dH20. To determine the yield, DNA concentration was determined by spectrophotometry at 260nm.

2.4) DNA, RNA and proteins

2.4.1) Restriction Enzyme Digest and Gel Electrophoresis

Enzymes were obtained from Fermentas. For every enzyme there is a specific restriction buffer, for double digest 1x or 2x Tango buffer was used. The reaction mixture for the digest contains 0.5-7µg DNA, 10x or 20x reaction buffer, 1-5µl enzyme, filled up with dH_2O to 20 or 100µl and placed at 37°C for 2-3h.

The digested DNA was loaded onto a 1% agarose gel [1 g agarose (Seakem LE Agarose; Biozyme) was dissolved in 100 ml 1x TAE (Tris-Acetate-EDTA) Buffer by boiling this mixture in a microwave and after cooling down to ~50°C 10 µl Ethidium Bromide (Roth) were added]. To the samples 6x Loading Dye (Fermentas) was added before loading onto the gel. The gel was run using the Bio-Rad Sub-Cell GT system (Bio-Rad) in 1xTAE and the bands got visualized using the Lumi-ImagerTM.

2.4.2) DNA Extraction/Purification from Agarose Gel

The bands of interest can be cut out of the agarose gel and the DNA can be extracted by using the QIAquick Gel Extraction Kit protocol. According to the protocol 3x volume of QG buffer was added to 1x volume of the excised gel fragment and dissolved at 50°C for 10 min following the addition of 1x volume of isopropanol (Roth). To bind DNA, the sample was applied on the QIAquick column and centrifuged 1 min at 13000 rpm. To remove trace amounts of agarose the column was washed with 0.5 ml of QG buffer (1 min/13000 rpm). Additional washing was performed using 0.75 ml PE Buffer (prior to

centrifugation the column membrane was incubated with the applied PE buffer for 5 min at RT). To remove residual fluid the column was centrifuged for 1 min at 13000 rpm followed by elution. Therefore 30µl dH2O were added to the centre of the membrane, incubated for 1 min, and then centrifuged (1 min/13000 rpm). So the clean DNA was eluted and can be used for further experiments. The concentration of the DNA content was determined by gel electrophoresis using a molecular weight marker (DNA ladder mix from Fermentas). The intensity of the band was compared with the marker which had a know concentration.

2.4.3) Ligation

After the determination of the DNA content of the vector and the insert a vector:insert ratio of 1:1 to 1:3 is used for ligation or it is determined mathematically by following formula:

$$\frac{\text{ng vector} \times \text{kb size insert}}{\text{kb size vector}} \times 3{:}1 = \text{ng of insert}$$

The reaction mixture contained 50-400ng of the linear vector DNA, the mathematically determined amount or a 1:1 to 1:3 ratio of the insert DNA, 2µl of 10x Ligation Buffer and 0.4µl T4 DNA Ligase. This mixture was filled up to 20µl with dH_2O and was put on 16°C over night. As a control the same mixture without the T4 DNA Ligase was used. 10µl of the ligation product were used for the following transformation of bacteria (2.3.2).

2.4.4) RNA Isolation, cDNA and qRT-PCR

For RNA isolation $1x10^5$ cells were harvested, washed twice with 1xPBS and resuspended in 70µl RLT Buffer (Qiagen; RNeasy® Micro Kit) with 1/100 β-Mercaptoethanol by vortexing for 30 seconds. RNA isolation was done

according to the manufacturer's protocol (Qiagen; RNeasy® Micro Kit). After addition of 350µl of 70% EtOH to the sample it was transferred to the RNeasy column and centrifuged for 15sec at 8000g. The column was washed with 350µl RW1 buffer (15sec/8000g) followed by the addition of 80µl of a DNase solution (10µl DNase I stock solution; 70µl RDD buffer) and incubation for 15min at RT. The column was washed with 350µl RW1 buffer (15sec/8000g), 500µl RPE buffer (15sec/8000g) and PE buffer (2min/8000g). To remove the residual fluid the column was centrifuged at full speed for 1min. For RNA elution, RNasefree water (15 to 20µl) was used (1min/8000g). To increase the RNA yield the described elution procedure was repeated twice. From the isolated RNA a cDNA can be generated; therefore maximal 16.25µl RNA was taken and mixed with 1µl 25mM oligo T20 primers (Fermentas); primer annealing was performed using following PCR program: 5min/70°C; 5min/4°C. 5µl RevertAid [TM] H Minus M-MuLV RT Buffer 5x; 1.25µl dNTP mix; 0.5µl RiboLock RNase Inhibitor (40u/µl) and 1µl RevertAid [TM] H Minus M- MuLV RT (200u/µl; all these reagents are obtained from Fermentas) were added and a cDNA was prepared using a 2 step cycle; 42°C for 1 hour followed by 70°C for 15 min and cooling to 4°C. The resulting cDNA was stored at -20°C. For the qRT-PCR detection a LightCycler 2.0 was used. Reactions were carried out in LightCycler® Capillaries (20µl) obtained from Roche Diagnostics GmbH using 7.5µl of Sybr Green Mix and 0.75µl 10xBSA (Invitrogen); 0.5µl 10µM forward and reverse primers of interest and 1.5µl cDNA (1:5 dilution with dH_2O) in an end volume of 15µl. Amplification parameters were denaturation at 95°C for 5min followed by 40 cycles of 95°C for 15sec and 60°C for 60sec. All primers were designed using the Primer3 design tool and were obtained from MWG. For relative quantification, data were analyzed using the "Normalized Relative Quantification" method (LightCycler; Roche). Expression levels of target genes in cells were normalized to HPRT and shown relative to unstimulated cells (time point 0).

2.4.5) Protein Extracts and Western Blot

For Western Blot analysis $2x10^5$ or more cells were harvested, washed twice with 1xPBS, resuspended in 20µl 2xSample Buffer and put on 95°C for

10min. The probes were frozen at -20°C until usage. Minimum $1x10^5$ cells were used.

10% separating and 4% stacking gels were prepared and ran in 1x SDS PAGE Buffer. Proteins resolved by sodium dodecyl sulphate-polyacrylamid gel electrophoresis (SDS-PAGE) were transferred to a polyvenylidene difluoride membrane (Immobilon-P, Millipore) at 100 V for 90 min using a Bio-Rad Criterion Blotter (Bio-Rad) and after that these membranes were blocked with 1xPBS-Tween/5% low-fat milk for 60min at RT. Primary antibodies GKLF (Santa Cruz Biotechnology) and anti-actin (Sigma-Alsrich) were diluted 1:1000 in 1xPBS-Tween/3% low-fat milk and added to the membrane over night at 4°C. Membranes were washed 3x with 1x PBS-Tween before adding the secondary antibodies which were conjugated to horse radish peroxidise (HPR); donkey anti-goat IgG HPR (for GKLF) or goat anti-rabbit IgG HPR (for actin; were used at a dilution of 1:2500 in 1xPBS-Tween/3% low-fat milk) for 1 to 2h at RT. Detection was performed with the chemiluminiscent substrate SuperSignal WestPico or WestDura (Pierce Biotechnology). Lumi-ImagerTM or the Fujifilm Las-4000 were used for data imaging.

2.5) Fluorescence Activated Cell Sorting (FACS)

For membrane staining cells were harvested and washed with 1x phosphate-buffered saline (PBS)/ bovine serum albumin (BSA)/azid. The supernatant got sucked off and the cells got remained in the needed volume with 1x PBS/BSA/azid [50µl cells were needed for one staining, transfer them into micronic tubes (Thermo Scientific)]. 1/10 Beriglobin was added to block unspecific binding and incubated for 10min on ice. Then 10µl of the needed mAbs were added and incubated for 20 to 30min at 4°C. After that the cells got washed again with 1x PBS/BSA/azid and, if needed, the Streptavidin (SA)-PerCP (10µl per staining) was added and incubated again for 20 to 30min. Next the cells were washed with 1x PBS before FACS analysis or in case of a intracellular staining fixed with 100µl Fixation Medium (An Der Grub) for 5 min at RT. Afterwards cells were washed and permeabilized by addition of 100 µl Permeabilization Medium (An Der Grub) followed by an

incubation with 10µl of the antibody against an intracellular marker for 20 to 30min at RT.

Murine monoclonal antibodies (mAbs) of the following specificity are used: phytocoerythrin (PE)-conjugated mAbs specific for Langerin, CD11b, CD14, CD15 (BD Biosciences) and Lactoferrin [LF; intracellular (An Der Grub)]; biotinylated mAb CD11b (BD Pharmingen) combined with SA-PerCP; and allophycyanin (APC)-conjugated anti-CD1a (BD Pharmingen) and anti-CD14 (Caltag Laboratories or BD Biosciences). All antibodies were pre-diluted 1:5 with 1xPBS/BSA/Azid in exception of PE-CD14 (1:10), PE-Langerin (1:10), APC-CD14 (1:30), LF (1:2), and SA-PerCP (1:100).

For analysis a FACS-Calibur flow cytometer and CellQuest software were used (Becton Dickinson). For cell sorting the BD FACS-Aria flow cytometer was used.

2.6) Statistical Analysis

Statistical analysis was performed using the paired, 2-tailed Student *t* test; *P* values less than <0.05 were considered significant.

3) Results

3.1) Up- and Down-regulated Factors via TGF-β1 – Microarray Approach

It has been shown that TGF-β1 is sufficient to induce LC commitment from $CD34^+$ HSC in vitro. However, also in vivo TGF-β1 seems to be absolutely required for LC development (Strobl et al, Blood 1997). In accordance with this, mice lacking TGF-β1 are devoid of LC (Borkowski et al, J Exp Med 1996). Although the importance of TGF-β1 in LC commitment was long ago established, the transcriptional mechanisms underlying this TGF-β1 dependent LC development still remain elusive.

We used a microarray approach to identify potential key molecules that act downstream of TGF-β1 to induce LC commitment. For this purpose $CD34^+/CD45RA^{high}$ cells, which are highly enriched in myeloid progenitor cells and can most efficiently give rise to both LC in the presence of TGF-β1 and monocytic precursors in the absence of TGF-β1, were FACS sorted and cultivated in LC-mix w/o TGF-β1 for two days. In contrast, the $CD45RA^{int}$ cells are believed to possess lymphoid potential and the erythroid lineages seem to arise from the $CD34^+/CD45RA^{low}$ cells. Afterwards, cells were stimulated with TGF-β1 or left untreated. RNA samples were taken after 6 and 24 hours. These RNA samples were then hybridized on an Affymetrix chip U133plus 2.0. (Jurkin J et al. unpublished data, Figure 12). By these means we wanted to identify novel transcriptional regulators that act downstream of TGF-β1 and might alone or in combination be sufficient to induce LC commitment.

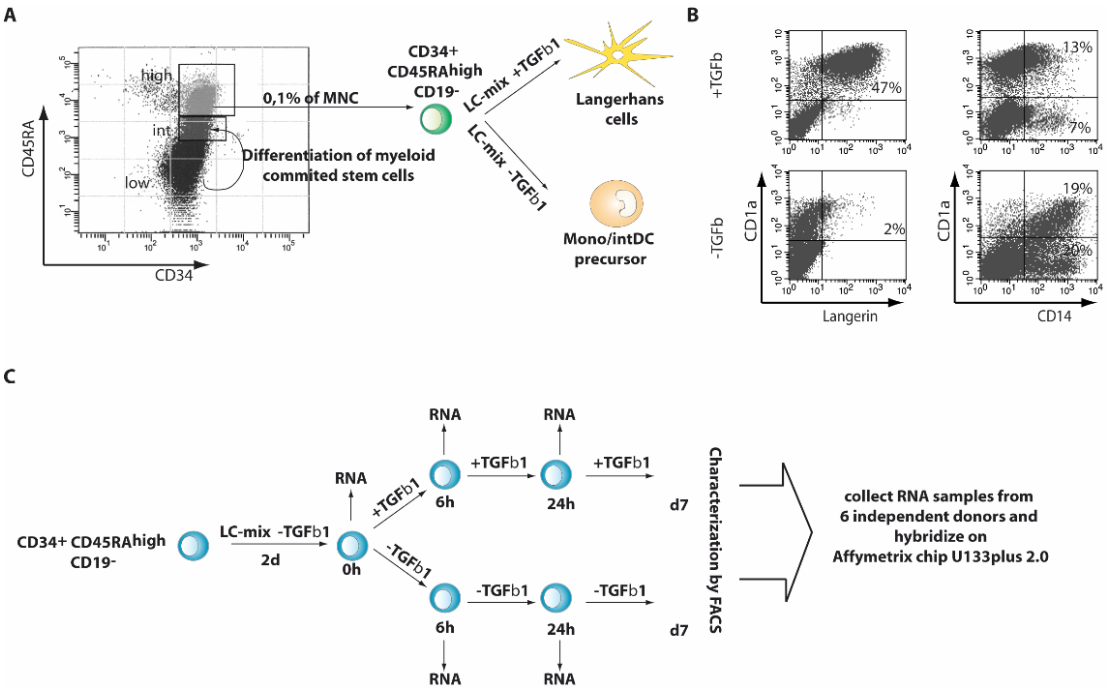


Figure 12: **Identification of factors which are regulated via TGF-β1 – experimental setup.**
A) $CD34^{+}/CD45RA^{high}$ cells were FACS sorted and cultivated plus TGF-β1 to acquire LC or without TGF-β1 for a Mo/DC precursor cell B) FACS blots from day 7. With TGF-β1 a LC population developed with CD207 and CD1a expression, whereas without TGF-β1 no CD207 expression is detectable but CD14. C) $CD34^{+}/CD45RA^{high}$ cells were cultivated in LC-Mix w/o TGF-β1 for two days. After that they get cultivated in presence or absence of TGF-β1 and RNA samples were taken after 6 and 24 hours. These RNA samples were hybridized on an Affymetrix chip U133plus 2.0. and used for further analysis (Jurkin J et al. unpublished data).

3.2) KLF4 is one of the Strongest Down-regulated Factors in Response to TGF-β1 Stimulation of Myeloid Progenitor Cells

In the above screen (Figure 12) we found several transcription factors to be significantly up- or down-regulated. We used Real Time- (RT-) PCR to validate several factors (Figure 13A). KLF4 was on of these factors of particular interest. Feinberg et al. previously showed that this factor is an active inducer of monopoiesis (Feinberg et al. EMBO J. 2007). In accordance with this, we found KLF4 specifically down-regulated by TGF-β1 during LC development, whereas its expression increases in the minus TGF-β1 condition, in which monocytic-like cells are generated (Figure 13B). In line

with this only Mo and intDC showed KLF4 protein expression, whereas LC and Gr lacked substantial levels of KLF4. Interestingly, we found the strongest KLF4 expression in intDC and not, as expected from literature, in Mo (Figure 13C).

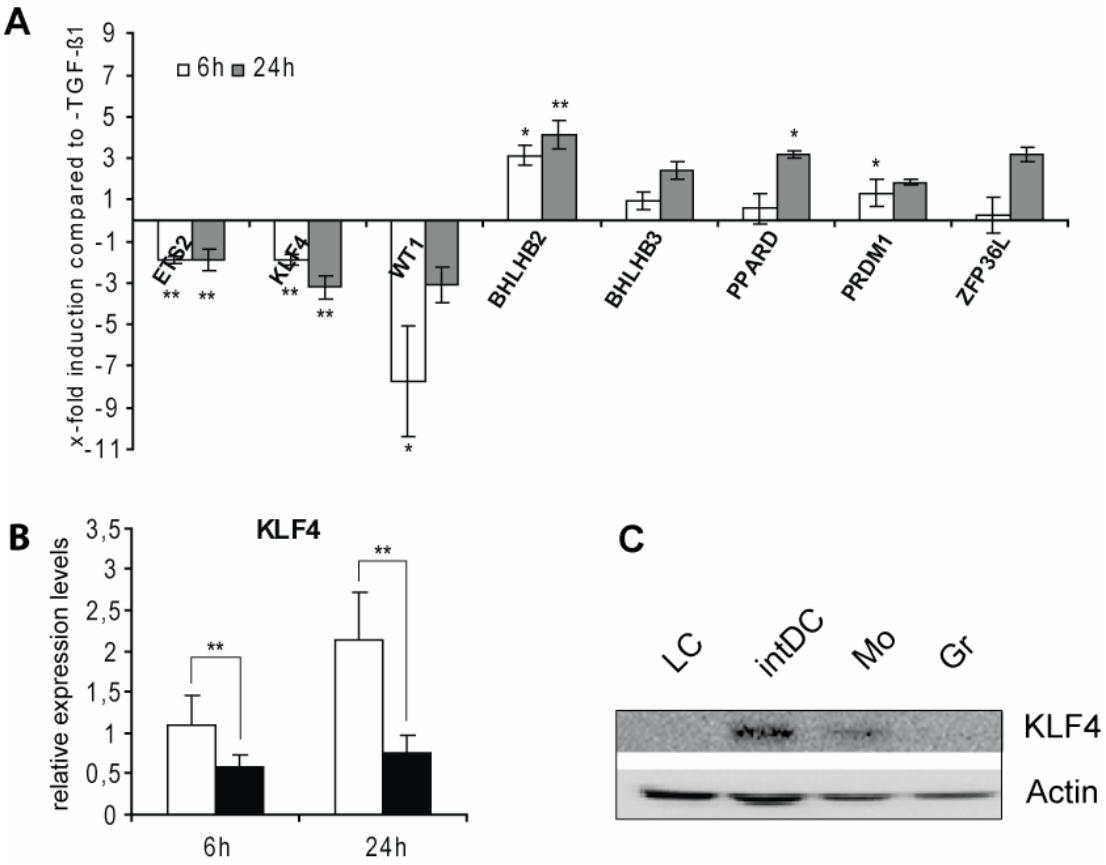


Figure 13: **Real time PCR analysis shows a significant down-regulation of KLF4 in the presence of TGF-β1.**
$CD34^+$ cells were cultivated in LC-mix in presence or absence of TGF-β1. RNA samples were taken at the indicated time points after TGF-β1 addition and RT-PCR analysis was performed. A) Expression levels were normalized to GAPDH and shown as x-fold induction or reduction compared to TGF-β1 unstimulated cells. Bar diagrams represent the mean value ± S.D. for 4-8 independent experiments. B) KLF4 expression levels were normalized to GAPDH and shown relative to unstimulated cells. ■ Black bars symbolize TGF-β1 treated cells, □ white bars the TGF-β1 untreated cells. Bar diagrams represent the mean value ± S.D. for four independent experiments. *p<0.05; **p<0.005, paired 2-tailed t test. C) Differentiation of $CD34^+$ cells into the different lineages in serum-free media. Used cytokine mixes were described in material and methods. LC ($CD1a^+$ $CD207^+$) were cluster purified at day 7, intDC ($CD1a^+$ $CD11b^+$) and Mo ($CD11b^+$ $CD14^+$) were FACS sorted at day 11, Gr (LF^+ $CD15^+$) at day 13. Cell lysates were analysed by western blot, actin served as a loading control. Data are representative of three independent experiments.

3.3) Ectopic Expression of KLF4 Results in a Significant Down-regulation of CD1a and Up-regulation of CD14

3.3.1) Retroviral Vectors and in vitro Differentiation Model

We ectopically expressed a retroviral KLF4 GFP construct (Figure 14) in $CD34^+$ cells to analyse the effect of this transcription factor on the myeloid lineage commitment. We were especially interested in the influence of KLF4 on Mo/DC subset differentiation.

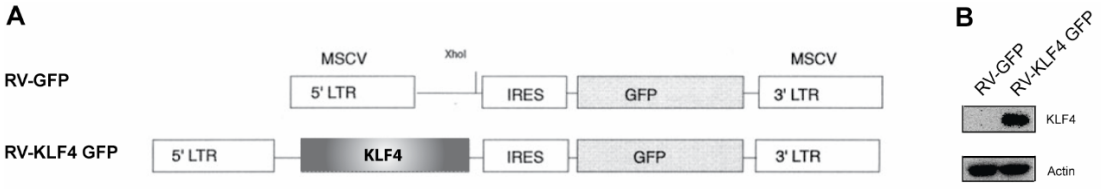


Figure 14: **The ectopic retroviral expression vector RV-GFP.**
A) Vector map of the RV-GFP control (CTRL) vector (Ranganath S and Murphy KM et al. J Immunol. 1998 and Mol Cell Biol. 2001) and RV-KLF4 GFP vector (Feinberg MW et al. J Biol Chem. 2005). B) U937Te cells were retroviral infected with 10µg RV-KLF GFP and CTRL vector DNA. After three days GFP^+ cells were FACS sorted and cell lysates got analysed by Western Blot. Actin served as a loading control.

For this purpose we used a two step in vitro generation model which was described previously (Chapter 2.2.5). In this model $CD34^+$ cells differentiated in serum-containing media with GM-CSF and TNFα into CD1a and CD14 single positive cells. $CD1a^+$ cells further give rise to LC whereas the $CD14^+$ population develops into CD14-derived DC (moDC) under the same culture conditions (Figure 15; Caux et al. J. Exp. Med. 1996).

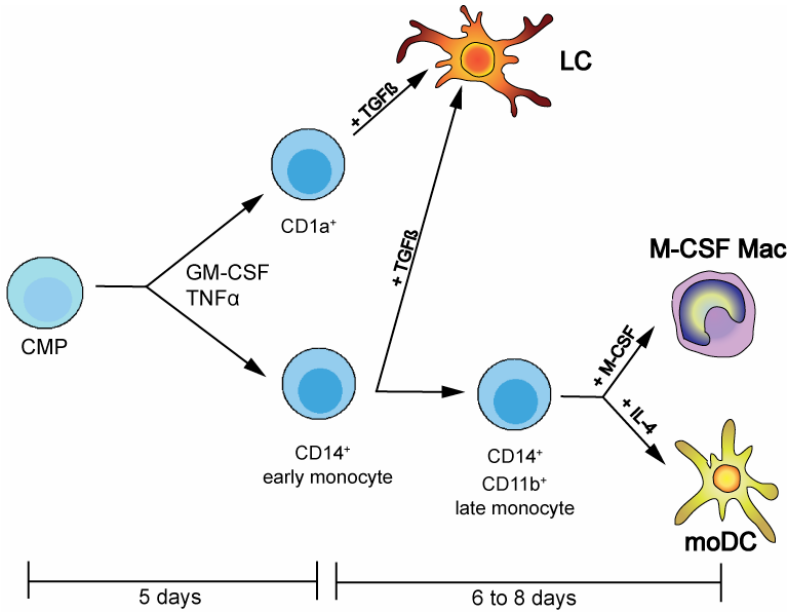


Figure 15: **Generation model in serum-containing media.**
The cultivation of isolated CD34$^+$ cells in presence of GM-CSF and TNFα results in two precursor populations: a CD1a$^+$ and a CD14$^+$ population (Caux et al. J. Exp. Med. 1996). Until day 11 to 13 the CD1a$^+$ population differentiates under TGF-β1 stimulation into a LC phenotype. Conversely, the CD14$^+$ population differentiates with IL-4 into a DC-like phenotype. Upon M-CSF stimulation CD14$^+$ cells differentiate into a macrophage-like phenotype (M-CSF Mac).

3.3.2) Ectopic KLF4 Promotes CD14$^+$ and CD11b$^+$ but Inhibits CD1a$^+$ Cell Differentiation

We cultivated RV-KLF4 GFP and empty control (CTRL) vector infected CD34$^+$ cells with GM-CSF and TNFα until day 13 to investigate the effect of KLF4 in this differentiation model. GFP$^+$ cells were gated for analysis of KLF4 and CTRL vector transduced cells. During culture, cells were analysed by FACS at day 5 and day 11 to 13 for different lineage informative markers (Figure 3). At day 5 we observed a significant decrease of the CD1a$^+$ cells in the KLF4 transduced population (Figure 16A, left panel). In addition, KLF4 transduced cells included high CD14 marker expression and reduced CD1a marker expression at day 13 (Figure 16, right panel). Furthermore, this CD14 induction and CD1a reduction went along with an increase of the CD11b marker which is a dendritic/monocytic marker. Therefore, ectopic KLF4 expression in CD34$^+$ cells seems to promote the development of CD14$^+$ monocytic cells at the expense of CD1a$^+$ dendritic cells in this culture system.

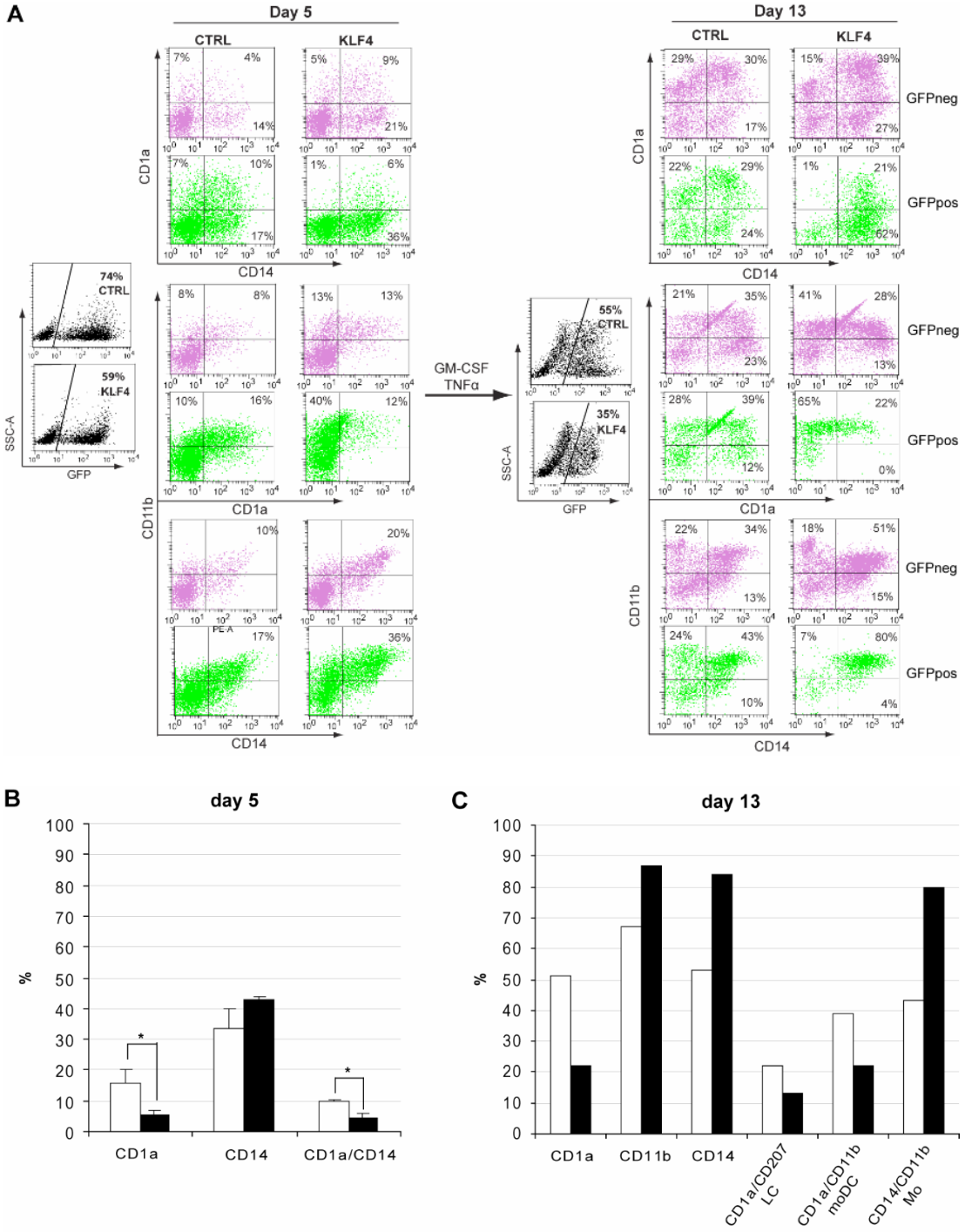


Figure 16: **Ectopic KLF4 promotes the generation of CD14$^+$ and CD11b$^+$ cells at expense of CD1a$^+$ cells.**
Isolated CD34$^+$ cells were infected with RV-KLF4 GFP and CTRL vector (three infection rounds) followed by a stimulation with GM-CSF and TNFα in serum-containing media until day 13. A) Expression of the different markers was determined by FACS analysis after day 5 and 13. Gates for the analysis were set for GFP$^+$ cells. The data shown represents one of three independent experiments with comparable results. B and C) Statistical analysis of day 5 and 13. ■ Black bars symbolize RV-KLF4 GFP transduced cells, ☐ white bars the empty CTRL vector transduced cells. Bar diagrams represent the mean value ± S.D. for four independent experiments (B) or one experiment (C). *p<0.05; paired 2-tailed *t* test.

3.3.3) KLF4 Promotes another DC-like Phenotype and Inhibits considerably the LC Pathway

It has been shown that moDC develop from $CD14^+$ precursor cells generated from $CD34^+$ cells after 5 days of culture with GM-CSF and TNFα. This precursor population can be further induced to develop into moDC or LC via addition of GM-CSF with either IL-4 or TGF-β1, respectively. The analysis of KLF4 expression levels in this culture system showed increased KLF4 expression after 5 days of culture with GM-CSF and TNFα relative to fresh $CD34^+$ cells. At day 13 KLF4 expression is up-regulated in IL-4 condition whereas reduced under TGF-β1 condition (Figure 17C).

moDC develop from $CD14^+$/$CD11b^+$ intermediate cells which share many features with blood Mo (Caux et al. Blood 1997). Furthermore, we found high KLF4 protein levels in intDC (Figure 13C). Therefore, we assessed the impact of KLF4 on the developmental pathway of the DC phenotype. $CD34^+$ cells were transduced with ectopic KLF4 or CTRL vectors and differentiated into moDC by applying the above described culture system. We found the percentage of $CD11b^+$ cells to be significantly increased in cells ectopically expressing KLF4. Interestingly, the CD14 marker expression remained constant. This increase in CD11b was furthermore accompanied by a significant loss of CD1a positive cells (Figure 17A). Therefore, we saw no inhibition of DC. This data, moreover, indicates that ectopic KLF4 expression promotes another DC-like phenotypes compared to the control.

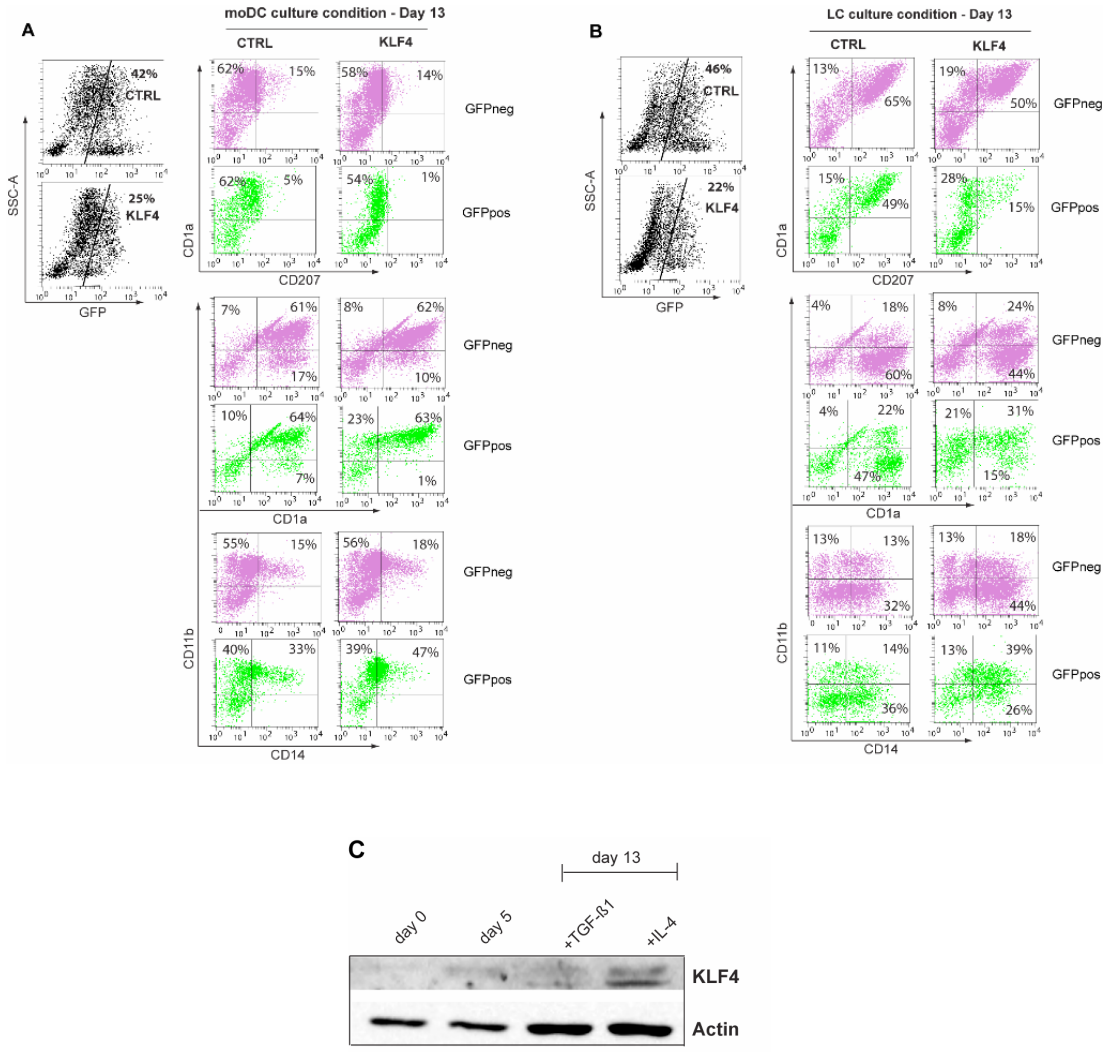


Figure 17: **KLF4 promotes moDC differentiation which shows another phenotype than CTRL cells.**
CD34^{+} cells were transduced with RV-KLF4 GFP or empty CTRL vector and then stimulated in serum-containing media with GM-CSF plus TNFα for five days. A) Cells were generated under moDC culture condition in serum-containing media until day 13. B) Cells were generated under LC culture condition in serum-containing media until day 13. Gated GFP^{+} cells were analysed by FACS at day 5 and day 13 for the indicated markers. The data shown represents one of three independent experiments with comparable results. C) Cell lysates were analysed by western blot, actin served as a loading control. Data are representative of two independent experiments.

We estimated an inhibition of the LC pathway with ectopic KLF4 because the previous experiments showed a significant inhibition of the CD1a marker expression (Figure 16 and 17). As expected, we detected a significant decline of the CD1a CD207 double positive population with ectopic KLF4 under TGF-β1 conditions. Furthermore, we observed an increase of CD11b and CD14 single as well as double positive cells in this population. This implicates that

KLF4 selectively impairs the LC-like DC phenotype, while promoting Mo and another DC-like phenotype compared to the control (Figure 17B). We noticed an increase of CD11b and an insignificant expand of monocytic cells in KLF4 transduced cells in moDC generation culture (Figure 18, left diagram); under LC culture condition this monocytic enhancement was even significantly advanced (Figure 18, right diagram).

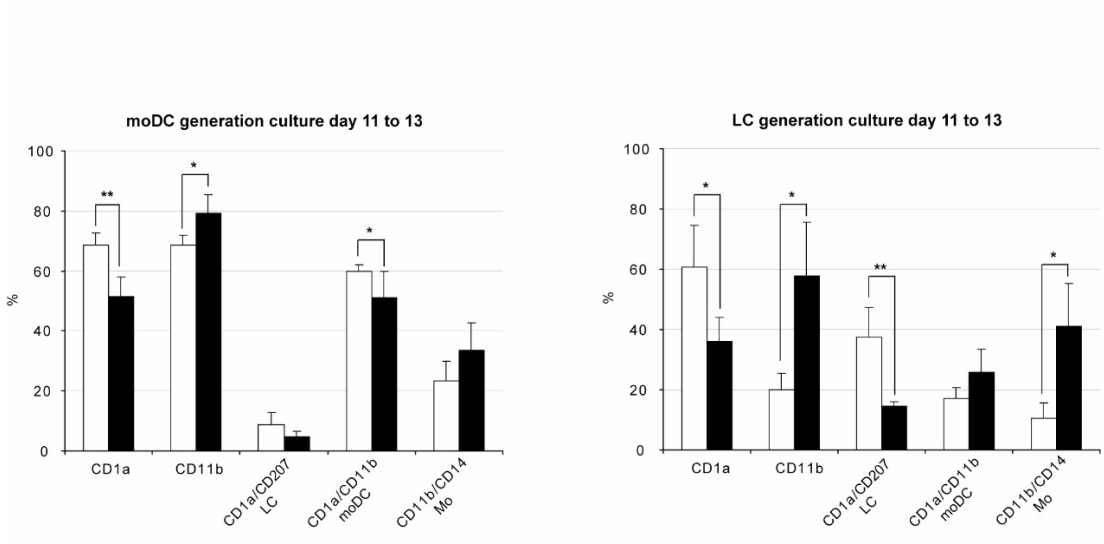


Figure 18: **Ectopic KLF4 inhibits significantly the LC generation and favours a dendritic/monocytic phenotype.**
$CD34^+$ cells were transduced with RV-KLF4 GFP and empty CTRL vector and generated in serum-containing media for five days with GM-CSF and TNFα followed by subculture in moDC or LC culture conditions until day 11 to 13. Gated GFP^+ cells were analysed by FACS at day 11 to 13 for the indicated markers. ■ Black bars symbolize ectopic KLF4 cells, □ white bars the CTRL cells. Bar diagrams represent the mean value ± S.D. for four independent experiments. *p<0.05; **p<0.005, paired 2-tailed *t* test.

3.4) Monocyte Development in the Absence of M-CSF is Facilitated by KLF4

KLF4 was found to be an active inducer of monopoiesis (Feinberg et al. EMBO J. 2007) by activating the CD14 as well as the M-CSFR promoter. We were interested in the analysis of the impact of ectopic KLF4 expression on cells cultivated in Mo conditions containing M-CSF.

We obtained an up-regulation of KLF4 protein levels shortly after M-CSF addition (two days after). However, it seemed as if KLF4 became slightly down-regulated from day 7 to day 13. We suppose a dependency on KLF4 at the beginning of Mo development, whereas the down-regulation of KLF4 is essential to finish this differentiation process (Figure 19C). Therefore, we analysed the impact of ectopic KLF4 on MO differentiation. All marker expressions remained constant in the assessed ectopically KLF4 infected cells, except a strong reduction of CD1a marker expression (Figure 19B). Many cells developed into CD11b/CD14 double positive cells even with the empty CTRL vector (Figure 19A, upper panel). Therefore, we tried a Mo generation culture system without M-CSF, which is an absolutely necessary cytokine for Mo generation. Interestingly, KLF4 compensated the absence of M-CSF. In conditions without M-CSF we observed a strong induction of the Mo population with ectopic KLF4 compared to the empty CTRL vector (Figure 19A, lower panel).

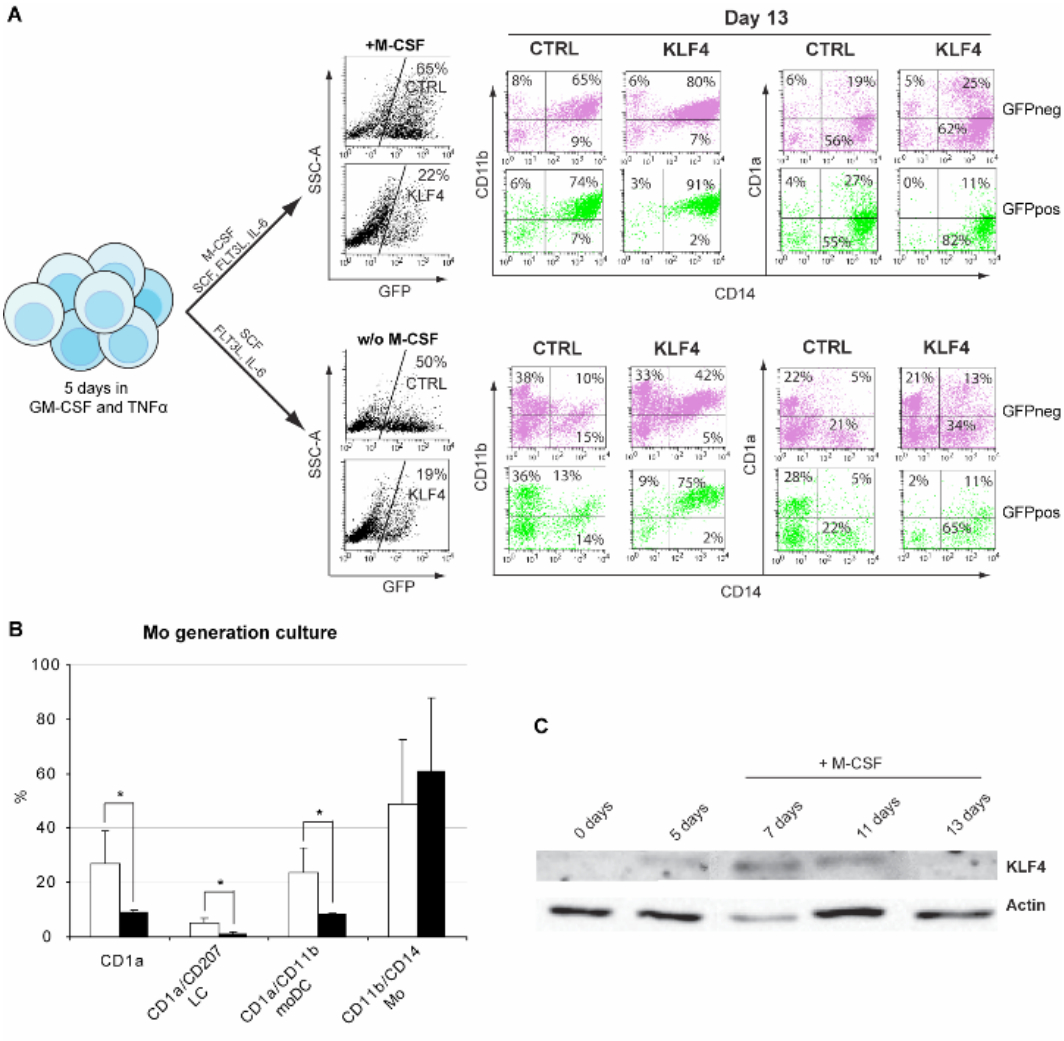


Figure 19: **CD11b is enhanced by ectopic KLF4 and results in another DC-like phenotype.**
RV-KLF4 GFP or empty CTRL vector transduced CD34$^+$ cells were cultivated in serum-containing media under Mo culture condition in serum-containing media until 13. A) Gated GFP$^+$ cells were analysed by FACS at day 5 and day 13 for the indicated markers. The data shown represents one of three independent experiments with comparable results for the condition containing M-CSF. B) Gated GFP$^+$ cells were analysed by FACS at day 11 to 13 for the indicated markers. ■ Black bars symbolize ectopic KLF4 cells, ☐ white bars the CTRL cells. Bar diagrams represent the mean value ± S.D. for four independent experiments. *p<0.05; paired 2-tailed *t* test. C) Cell lysates were analysed by western blot, actin serves as a loading control. Data are representative of two independent experiments.

Additionally, we checked Mo development in serum-free culture conditions without M-CSF. In common with MO culture condition in serum-containing media, cells ectopically expressing KLF4 efficiently acquired the monocytic phenotype. The increase of monocytic markers was significant after 7 and 11 days in culture (Figure 20B).

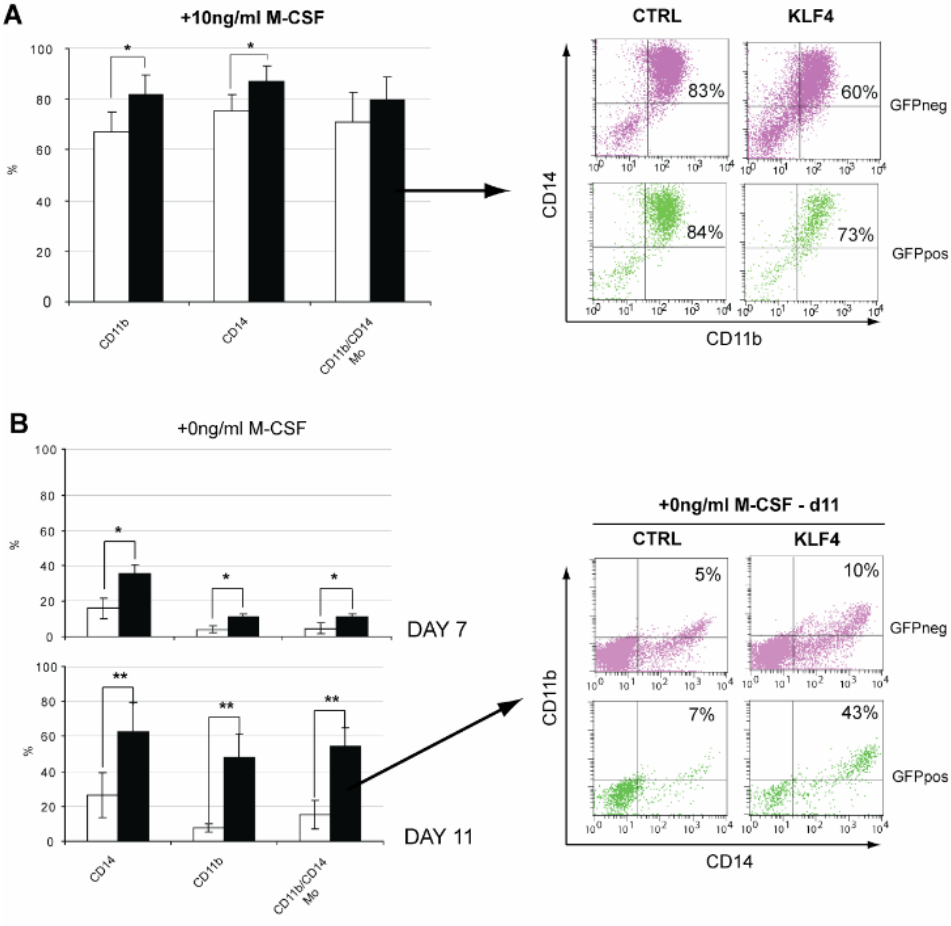


Figure 20: **KLF4 promotes the differentiation of Mo in the absence of M-CSF.**
RV-KLF4 GFP and empty CTRL vector transduced $CD34^+$ cells were cultivated with or w/o M-CSF under Mo culture condition in serum-free media. A) Gated GFP^+ and GFP^- cells were analysed by FACS for the indicated markers at day 11. 27% GFP^+ of CTRL vector and 11% GFP^+ of RV-KLF4 GFP transduced cells. B) FACS blots of gated GFP^+ and GFP^- cells at day 11 for the indicated markers. 34% GFP^+ of CTRL vector and 14% GFP^+ of RV-KLF4 GFP transduced cells. The data shown represents one of three independent experiments with comparable results. ☐ White bars symbolize empty CTRL vector, ■ black bars the ectopic KLF4. Bar diagrams represent the mean value ± S.D. for three independent experiments. *p<0.05; **p<0.005, paired 2-tailed t test.

3.5) The Granulocyte- as well as the LC Populations are Inhibited by Ectopic KLF4 Expression

KLF4 plays a role in inducing cell cycle arrest by inhibiting the Wnt/β-catenin signalling pathway and, therefore, consequently influences the proliferation of the cells (Zhang et al. 2006 Mol. Cell Biol.). Furthermore, it has been shown that KLF4 prevents apoptosis by up-regulating p21 (Ghaleb et al. Oncogene 2007).

We observed a significant reduction of the GFP$^+$ positive cells in all experiments compared to the control. We detected the strongest reduction in the Gr and LC lineages (Figure 11). This can be due to the fact that KLF4 plays a role in cell cycle arrest or because the cells are dying. We assessed this reduction in serum-free as well as in serum-containing differentiated KLF4 transduced cells. Interestingly, we realized a strong decrease of GFP$^+$ cells even in Mo generation cultures, whereas the moDC as well as the intDC showed the slightest decrease.

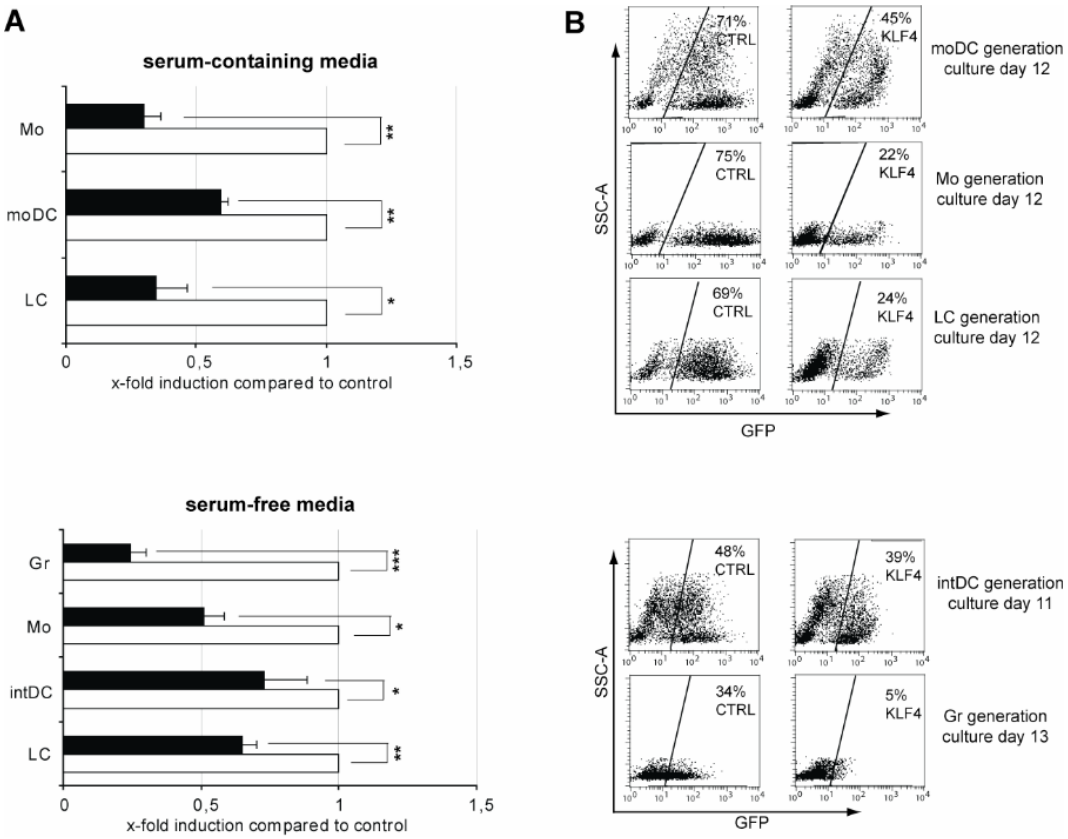


Figure 21: **Cell populations with ectopic KLF4 are inhibited over time.**
RV-KLF4 GFP and empty CTRL vector transduced CD34$^+$ cells were generated into the different lineages in serum-free or serum-containing media as described in material and methods. A) GFP$^+$ cells were counted and bar diagrams show x-fold induction compared to empty CTRL vector transduced cells. ☐ White bars symbolize empty CTRL vector, ■ black bars the RV-KLF4 GFP. Bar diagrams represent the mean value ± S.D. for three independent experiments. *p<0.05; **p<0.005 ***p<0.0005, paired 2-tailed t test. B) FACS blots of the GFP$^+$ cell populations in the different generation cultures. The data shown represents one of three independent experiments with comparable results.

3.6) Validation of Endogenous KLF4 Levels Shows an Inhibition in Monocyte-derived LC and an Increase in Monocyte-derived DC

Previous studies indicate that inflammatory Mo in mouse give rise to LC after LC depletion (Ginhoux et al. Nat Immunol. 2006). In human skin, special CD16 positive Mo are able to produce LC (Schaerli et al. Immunity 2005). Building up on these data we hypothesized that KLF4 down-regulated is essential to induce LC development from Mo. We used a special culture condition system to generate LC from cord blood- (CBM) or peripheral blood Mo (PBM). In this system the Notch ligand Delta-1 as well as TGF-β1 are required to obtain LC-type DC (Hoshino et al. J Leukoc Biol. 2005).

3.6.1) Down-regulation of KLF4 under Delta-1 plus TGF-β1 Conditions Favours a LC Phenotype which Shows LC Clusters

We suggested that down-regulation of KLF4 is necessary to generate CD207/CD1a positive LC. TGF-β1 as well as Delta-1 are inhibitors of KLF4 (Feinberg et al, J Biol Chem 2005; Ghaleb et al, Mol Cancer Res 2008). Apparently, it should also be possible to generate moLC without Delta-1 in conditions containing GM-CSF, IL-4 and TGF-β1 (Geißmann et al, J Exp Med. 1998).

However, when we tried to generate moLC with GM-CSF, IL-4 and TGF-β1 we observed a strong increase of the CD1a marker but only a little percentage of the cells developed into CD207 and CD1a double positive cells (Figure 22A, lower panel) without the typical LC cluster formations (Figure 22B, lower picture). Therefore, we came to the conclusion that cells acquired more a moDC than a moLC phenotype under this condition.

In our hands only CBM that were cultivated with Delta-1 and TGF-β1 developed into a $CD207^+$ and $CD1a^+$ cell population with typical LC cluster formations (Figure 22A and B, upper panel and picture). The cells retrained a monocytic phenotype when cultivated only in presence of TGF-β1. The cells showed no LC characteristics as well as no CD207/CD1a marker expression (Figure 22A and B, middle panel and middle picture).

In the case of LC differentiation from Mo, TGF-β1 alone is not sufficient to induce LC commitment. Both Factors, TGF-β1 and Delta-1, are required for efficient moLC generation. Recent papers showed that KLF4 expression is inhibited via Notch signalling through its ligand Delta-1 (Zheng et al. 2008), which would further strengthens our hypothesis that KLF4 down-regulation is an essential process to obtain moLC.

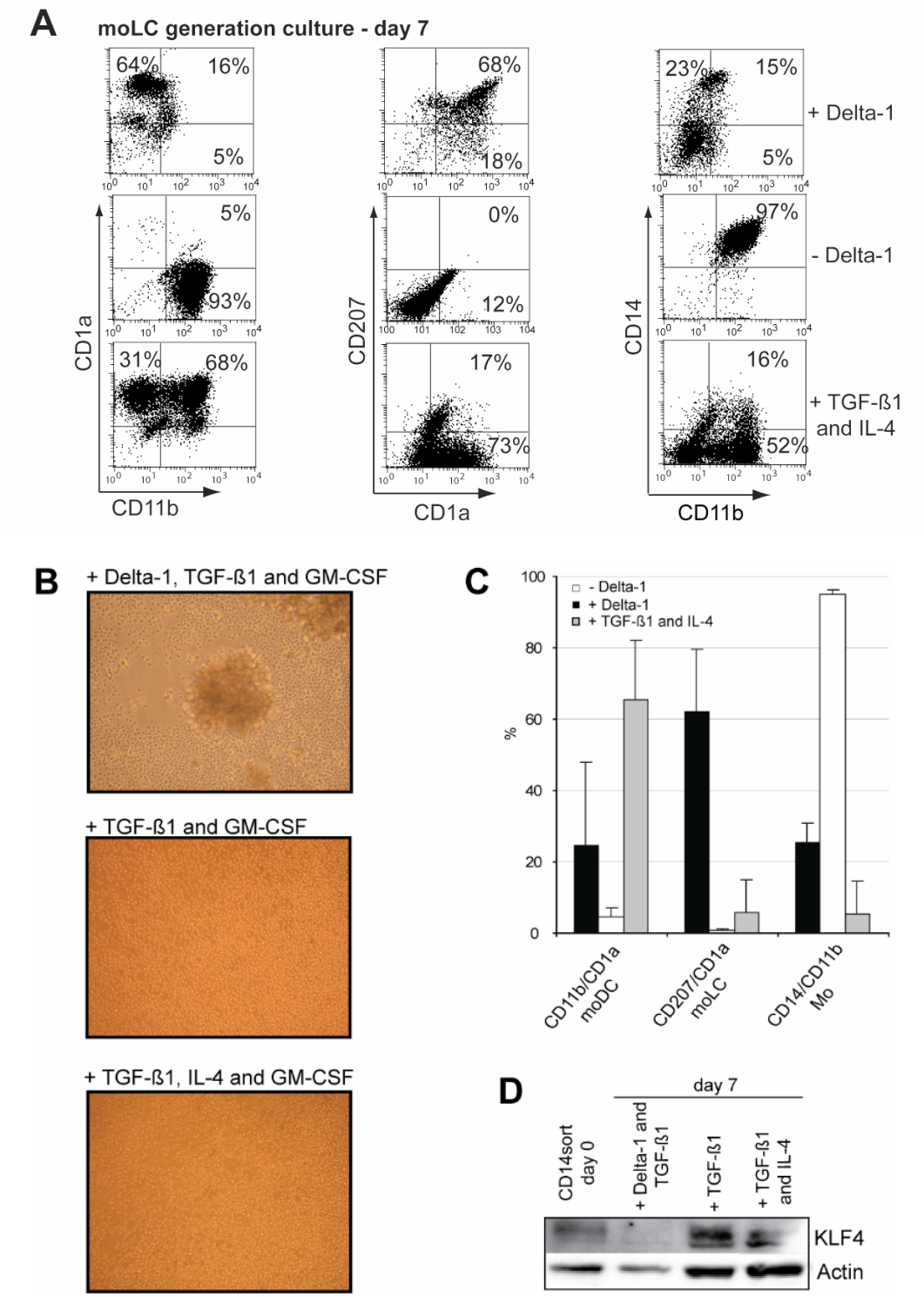


Figure 22: **LC development from Mo is absolutely depending on a combination of Delta-1 and TGF-β1.**
Isolated CBM were cultivated in serum containing media with GM-CSF, TGF-β1 and IL-4 or on dkαH-IgG coated plates with TGF-β1, GM-CSF and with and without Delta-1 until day 7. A) Cells are analysed at day 7 for the indicated markers by FACS. B) Microscope pictures were taken at 10fold magnification at day 7. C) Statistical analysis of day 7; ■ black bars symbolize the condition containing Delta-1; ☐ White bars the one without Delta-1; ▫ grey bars the TGF-β1, IL-4 and GM-CSF treated cells. Bar diagrams represent the mean value ± S.D. for three independent experiments. D) Cell lysates were analysed by western blot, actin served as a loading control. Data are representative of three independent experiments.

We checked endogenous KLF4 levels in the different conditions to assess whether Delta-1- and TGF-β1-dependent moLC development is accompanied by a down-regulation of KLF4 levels. As supposed, in conditions where we only added TGF-β1 we observed the presence of KLF4 levels. In contrast, combined addition of the Notch ligand Delta-1 and TGF-β1 showed absent KLF4 detection. This could be due to the fact that these two signalling pathways together strongly inhibit the expression of KLF4. In moDC or monocytic generation cultures we detected strong endogenous KLF4 levels (Figure 22D).

3.6.2) IL-4 Up-regulates KLF4 Levels and thus Leads to the Generation of moDC

We examined an inhibition of the LC pathway and a facilitation of Mo/moDC-like phenotypes with ectopic KLF4. Hence, we proposed higher KLF4 protein levels in moDC compared to moLC. IL-4 was found to be absolutely necessary to obtain moDC from CBM (Chapuis et al, Eur J Immunol. 1997). Interestingly, we generated moDC instead of moLC in presence of IL-4 and TGF-β1. These cells differentiated with a high percentage to CD11b and CD1a double positive cells. Furthermore, nearly all cells developed were CD1a positive (Figure 22A, lower panel). In contrast, nearly all cells cultured with IL-4 alone turned CD11b positive (Figure 23A and D). Independently of this different CD1a and CD11b expressions, the endogenous KLF4 levels remained the same under both conditions (Figure 23C) although we expected a decrease in the condition containing TGF-β.

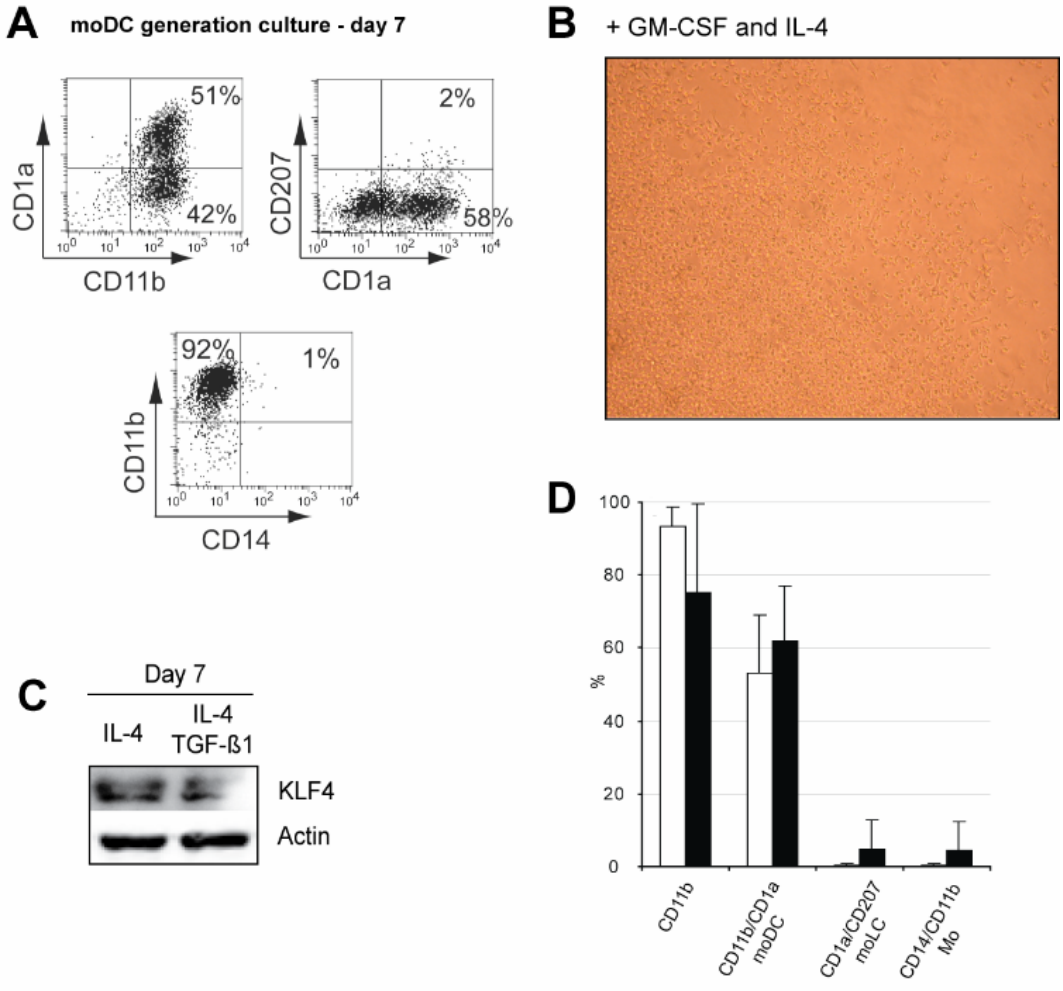


Figure 23: **Endogenous KLF4 levels are up-regulated in moDC under IL-4 as well as under Il-4 plus TGF-β1 conditions.**
Isolated CBM were cultivated in serum containing media with GM-CSF and IL-4 or with GM-CSF, IL-4 and TGF-β1. A) FACS analysis of the cells at day 7 for the indicated markers in serum containing media with GM-CSF and IL-4. B) Microscope pictures were taken at 10fold magnification at day 7. C) Cell lysates were analysed by western blot, actin served as a loading control. Data are representative of three independent experiments. D) Statistical analysis of day 7; ☐ White bars the one with IL-4; ■ black bars symbolize the condition containing IL-4 plus TGF-β1. Bar diagrams represent the mean value ± S.D. for three independent experiments.

The generation of moDC from Mo also occurs in other conditions, involving TNFα, IL15 or IFNα. TNFα gives rise to a special moDC-like phenotype which has monocytic characteristics but also shows expression of CD1a (Iwamoto et al, J Immunol. 2007). IL-15 induces a CD1a positive cell population from Mo. Those cells loose CD14 but only half of the population gains CD11b. Interestingly, those cells are also reported to become CD207 positive

(Mohamadzadeh et al, J Exp Med. 2001). In addition, it is reported that IFNα can also induce the development of CD1a positive cells from Mo (Santini et al, J Exp Med. 2000).

We analyzed KLF4 expression under these conditions in PBM. We treated Mo with either IL15 or IFNα or TNFα and analyzed the generated cells for informative lineage markers as well as KLF4 protein expression levels. Under these different conditions we generated only a small CD1a/CD11b double positive cell population. Additionally, all cells developed very high CD11b expression levels which is characterizing for a monocytic/dendritic phenotype. Interestingly, we found a higher percentage of monocytic-like cells with IFNα than in the other conditions. Additionally, these cells revealed the existence of the monocytic marker CD14 (Figure 24A, lower panel). However, the cells failed to form clusters (Figure 24B, pictures on the right side), to acquire a LC phenotype or a CD11b/CD1a positive moDC-like population under these three different generation culture systems. In contrast, the cells acquired LC characteristics when generated with Delta-1 and TGF-β1 (Figure 24A, left side), formed LC clusters (Figure 24B, left side) and showed absent KLF4 protein levels (Figure 24C). Additionally, the generation of moDC occurred under IL-4 influence (Figure 24A and B, left side).

Assessment of endogenous KLF4 revealed less KLF4 protein levels in the IFNα condition, although these cells only acquired monocytic-like features. However, cells treated with TNFα or IL-15 revealed equalled KLF4 levels as in IL-4 situation (Figure 24C) although the generation of moDC failed under these conditions. KLF4 down-regulation occurred only in conditions with Delta-1 and TGF-β1.

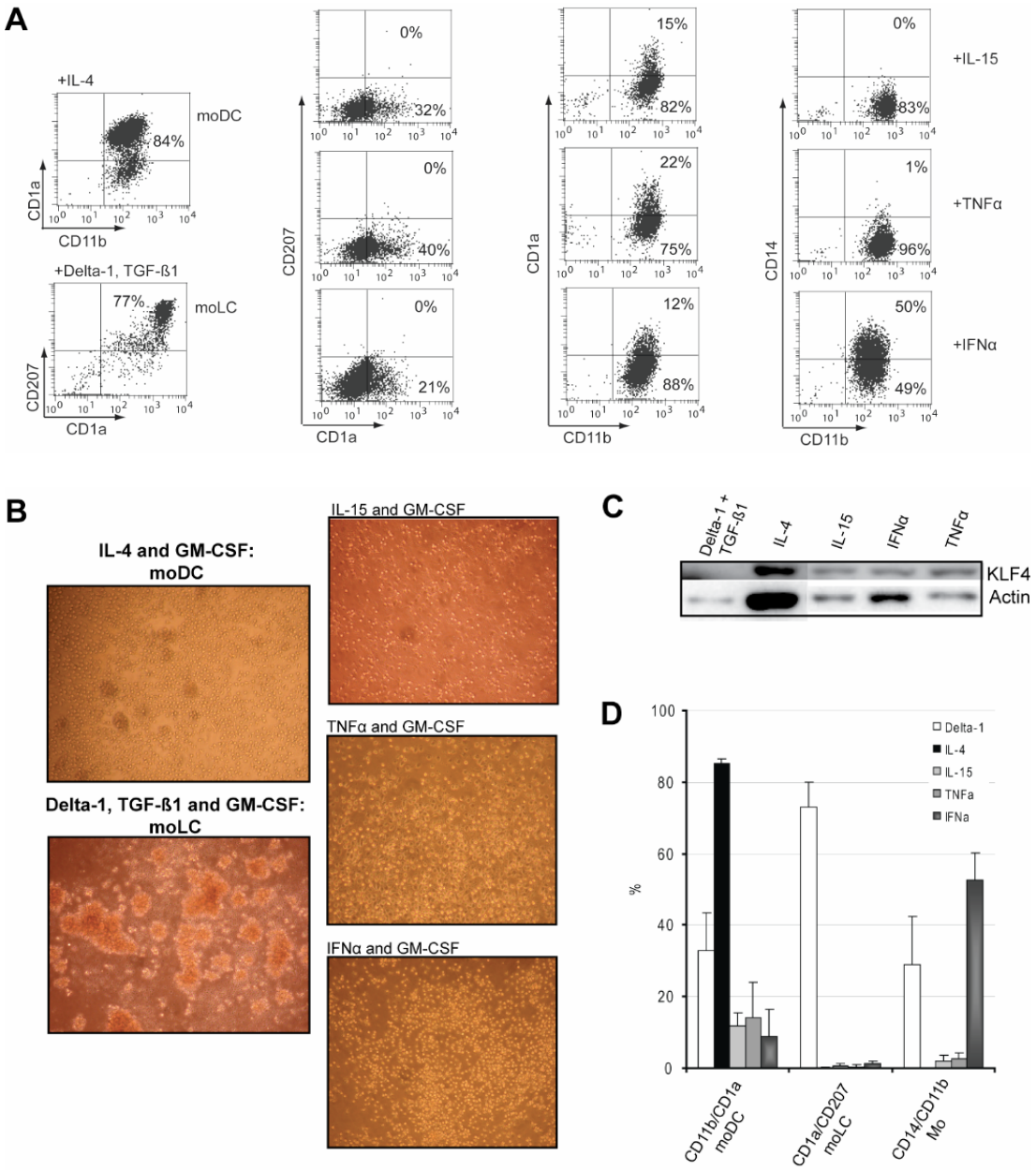
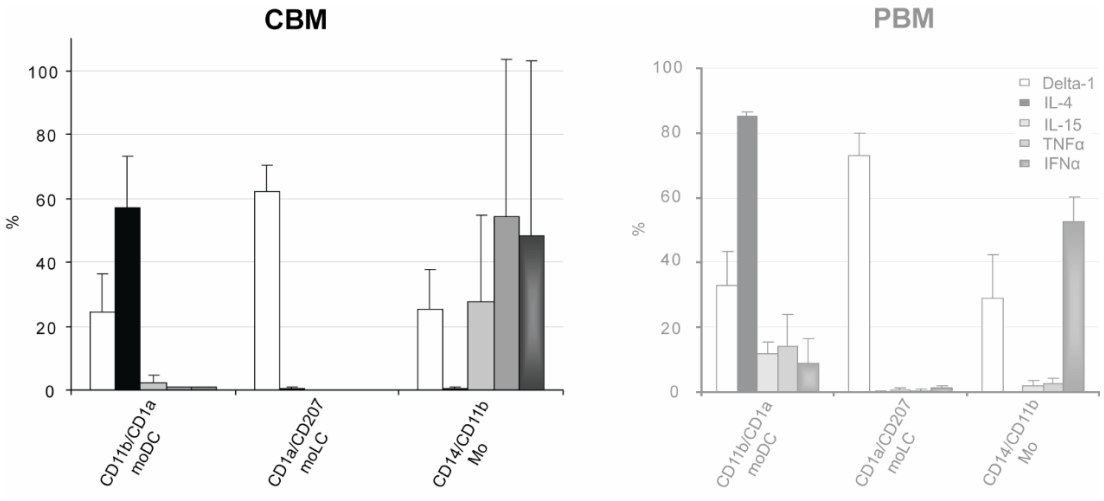


Figure 24: **IL-4 is absolutely necessary to obtain moDC as well as Delta-1 plus TGF-β1 is required to generate moLC.**
Isolated CD14^{+} cells from the peripheral blood (PBM) of three different donors were cultivated in serum containing media with GM-CSF plus IL-4 or IL-15 or IFNα or TNFα or on dkαHuIgG (donkey-anti-human IgG) coated plates with GM-CSF, Delta-1 and TGF-β1 until day 7. A) FACS analysis at day 7, on the left site control FACS blots for moLC and moDC are shown. B) Microscope pictures were taken at 10fold magnification at day 7, on the left site control microscope pictures for moLC and moDC are shown. C) Cell lysates were analysed by western blot, actin served as a loading control. Data are representative for three individual donors. D) Statistical analysis of day 7; ☐ White bars the one with Delta-1 plus TGF-β1, a moLC control; ■ black bars symbolize the condition containing IL-4, the moDC control. Bar diagrams represent the mean value ± S.D. from three different donors.

We recognized different expression profiles in the differentiation of cord blood Mo (CBM) compared to PBM in these conditions. We detected more monocytic-like cells with either TNFα or IFNα or IL-15. Additionally, the cells failed to develop a CD11b and CD1a double positive cell population (Figure 25).



.

Figure 25: **After different cytokine stimulation CBM develop another phenotype than PBM.**
CBM were cultivated in serum-containing media with GM-CSF plus IL-4 or IL-15 or IFNα or TNFα or on dkαHuIgG coated plates with GM-CSF, Delta-1 and TGF-β1 until day 7. The cells were analysed by FACS at day 7 and statistical analysis are shown in the bar diagrams; ☐ White bars represent the condition with Delta-1 plus TGF-β1, a moLC control; ■ black bars symbolize the condition containing IL-4, the moDC control. Bar diagrams represent the mean value ± S.D. from two different donors. On the right side the results from PBM were shown as before (Figure 24D).

4) Discussion

Hematopoiesis is a well coordinated process which requires expression of distinct transcription factors. The myeloid lineages differentiate from HSC under the control of factors which activate or repress lineages specific target genes and thus drive lineage commitment. Ordered myelopoiesis relies on the correct expression pattern, the combination of specific factors and their coordinated interplay (Tenen et al, Blood 1997; Nerlov, Tenen and Graf, University Press 2001; Orkin SH, Nat Rev Genet. 2000). There are many factors known which seem to have an influence on myeloid sublineage expression, such as Id2 and RelB for DC, VDR and MafB for Mo, Gfi-1 and C/EBPε and α for Gr or Id2 and IRF8 genes for LC differentiation. However, there is no single factor known which is only expressed in the myeloid lineages and acts as a master transcription factor for myeloid cell fate and choice. Representing a sublineage of myeloid DC, LC differentiation similarly relies on a complex interplay of several transcription factors. The identity of these factors and their exact role in LC differentiation is currently only poorly understood. Our laboratory previously demonstrated that LC differentiation from hematopoietic progenitor cells is critically dependent on the cytokine TGF-β1. For in vitro LC differentiation, TGF-β1 has to cooperate with other cytokines and ligands such as GM-CSF and Delta-1. GM-CSF may be replaced by M-CSF, and TNFα plus FLT3L enhance LC differentiation. However, TGF-β downstream (co-)signalling is absolutely essential for LC differentiation. This notion is supported by recent observations that Activin A, a TGF-β/BMP family member can replace TGF-β1 for LC differentiation. The goal of this project was to identify the critical transcription factor combinations that induce LC differentiation downstream of TGF-β1 signalling.

Previous studies indicated KLF4 as an important factor for Mo development. It regulates the monocytic CD14 promoter as well as iNOS production in activated Mac (Feinberg et al, J Biol Chem 2005 and EMBO J 2007). It has also been shown that KLF4 acts either as tumor suppressor or oncogene (McConnell et al, Bioessays 2007) and regulates proliferation and apoptosis

(Zhang et al, Mol Cell Biol 2006; Ghaleb et al, Oncogene 2007). KLF4 belongs to the Kruppel-like transcription factor family which contains three zinc fingers for DNA-binding and is implicated in a variety of physiological processes (Dang et al, Int J Biochem Cell Biol 2000).

We used culture systems which allow us to generate different myeloid lineages from $CD34^+$ cord blood progenitor cells. Additionally, certain DC subsets can also be generated in vitro from peripheral blood $CD14^+$ Mo. The addition of specific cytokines drives the commitment into a distinct cell type which is identified by FACS for its lineage specific marker expression shown in Figure 3. We also infected the cells by using a retrovirus-mediated gene transfer system. Specifically, we studied the role of KLF4 in myeloid sublineage development.

PU.1 expression is necessary for the development of the different DC subsets. Ectopic PU.1 expression promotes DC differentiation from hematopoietic progenitor cells; however, only in the presence of defined co-signals. Importantly, TGF-β1 is required for LC differentiation from PU.1 transduced progenitors. PU.1 is also able to activate KLF4 expression which is an important factor for Mo development (Feinberg et al, EMBO J 2007). It is very interesting that one single factor such as PU.1 is essential for several different myeloid subsets and it is, therefore, unlikely that this factor alone is able to make a choice of cell fate. Other factors seem to be implicated in this selection process. We observed that KLF4 is expressed at much lower levels in LC compared to Mo/Mac and intDC, and that ectopic KLF4 abrogates LC differentiation, while promotes DC differentiation. Therefore, the repression of KLF4 seems to be functionally required for LC differentiation from monocytic cells, while it plays a positive role in DC differentiation. Our observations point to a model of LC lineage commitment, whereby TGF-β1 signalling represses KLF4. In vivo TGF-β1 within the epidermis provides the critical signal for LC differentiation from Mo. Therefore, KLF4 repression seems to enable Mo to differentiate to LC in the epidermis. During initial Mo differentiation PU.1 seems to up-regulate KLF4 expression. Therefore, subsequent differentiation processes of monocytic cells into LC versus DC seem to be critically

regulated by KLF4 expression levels. Interestingly, PU.1-mediated KLF4 induction during Mo differentiation might be the reason why PU.1 alone is not able to induce LC development as observed in previous studies. Although PU.1 is one of the necessary transcription factors for LC differentiation, PU.1 requires TGF-β1 co-signalling for LC differentiation. According to our model, TGF-β1-mediated repression of KLF4 may cooperate with PU.1 for inducting lineage specification of LC within the epidermis.

Previous studies indicate that TGF-β1 antagonizes KLF4 and vice versa (Feinberg et al, J Biol Chem 2005) and we detected a strong reduction of KLF4 in $CD34^+$ cells cultivated with TGF-β1 on RNA as well as on protein level (Figure 13B and C). LC and Gr showed little to no KLF4 protein expression whereas intDC and Mo clearly expressed KLF4. intDC showed among all myeloid cell subsets tested the highest KLF4 protein expression level (Figure 13C). They were generated from Mo in response to the cytokine signals GM-CSF, IL-4 and TNFα; GM-CSF is an important cytokine in inflammation and it seems as if it is involved in the development of "inflammatory" DC (Hamilton JA, Nat Rev Immunol 2008; Shortman et al, Nat Rev Immunol 2007). A special "inflammatory" DC subset is the Tip-DC which is characterised by its TNFα and iNOS production (Lowes et al, Proc Natl Acad Sci USA 2005). KLF4 is able to induce iNOS; considering these previous studies, DC generated in our system might better be termed "inflammatory" DC, given that these cells express higher KLF4 protein levels than other myeloid cell subsets. In comparison, in vitro generated M-CSF-dependent Mo revealed lower KLF4 protein levels (Feinberg et al, EMBO J 2007). It is therefore interesting to speculate that inflammatory stimuli induce KLF4, which in turn promotes inflammatory-type DC differentiation. Furthermore, we suggest that KLF4 is very important for the initiation of Mo differentiation but is not required any more after completing this process (Figure 19C). Therefore, further studies of KLF4 expression during Mo development are of particular interest. KLF4 might regulate the differentiation of inflammatory DC and Mo which are characterized by high expression levels of iNOS.

Ectopic KLF4 expression experiments in $CD34^+$ cells indicated an involvement of KLF4 in the regulation of CD1a expression. Interestingly, ectopic KLF4 significantly drops CD1a expression density in cells cultivated with GM-CSF and TNFα. However, KLF4 did not seem to alternatively promote Mo differentiation from these cells, given that KLF4 did not significantly induce CD14 in these cells at day 5. Only at later culture time points we observed an increase in CD14 as well as CD11b indicative of Mo differentiation by KLF4 transduced cells (Figure 16). Under moDC generation conditions (GM-CSF plus IL-4), ectopic KLF4 promoted the differentiation into a monocytic-like DC subset which was different in marker expression levels than the CTRL DC subset. The expression pattern of the CD11b marker increased significantly with ectopic KLF4 whereas CD1a decreased (Figure 17A). In sharp contrast in LC generation conditions, ectopic KLF4 prevented LC development as revealed from a significant loss of CD1a and CD207 expression in favour of a monocytic phenotype (as indicated by enhanced CD14 as well as CD11b expression; Figure 17B). These results suggest that under LC conditions, when TGF-β1 is not able to repress ectopic KLF4 anymore, the differentiation into a monocytic cell is favoured whereas under moDC generation culture another monocytic-like DC cell type is promoted. The availability of IL-4 or the proinflammatory cytokine TNFα in combination with high KLF4 protein levels seems to be a reason for that; TNFα and KLF4 facilitate monocytic cells (Figure 26A) whereas IL-4 and KLF4 stimulate a type of “inflammatory” monocytic-like DC type (Figure 26B). The previous description that GM-CSF/IL-4-dependent moDC phenotypically resemble “inflammatory dendritic epidermal cells” (IDECs) (Wollenberg et al, J Invest Dermatol. 2002) is in line with this model.

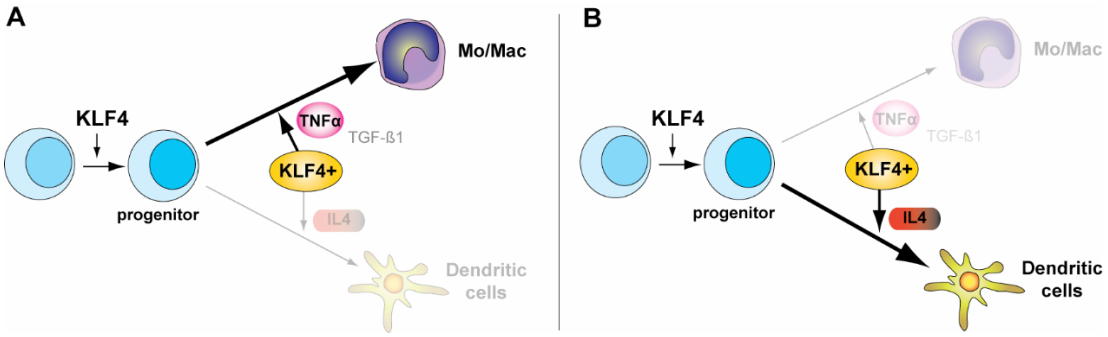


Figure 26: **KLF4 and its implication in Mo or DC differentiation.**
A) KLF4 transduced cells cultured under LC differentiation condition in the presence of TNFα and TGF-β1 fail to give rise to LC, but instead acquire a monocytic phenotype. B) Under moDC culture condition with IL-4 KLF4 transduced cells fail to develop into monocytic cells as might be expected from observations by Feinberg et al. Instead these cells give rise to moDC, as evidenced by the observed increase in CD11b and reduced CD1a expression. The interaction of KLF4 with either TNFα and TGF-β1 or IL-4 seems to be critical for this differentiation process.

The high expression level of KLF4 and the presence of the proinflammatory cytokine TNFα might be a reason for some inflammatory diseases like RA. In this disease, TNFα occurs at high levels in the inflamed joints (Feldmann et al, Springer Semin Immunopathol. 1998). High levels of KLF4 can promote some sorts of over-reactive Mac and DC. It would be further interesting if high KLF4 expression influences the expression of other surface markers which are important for migration or T-cell stimulation or some other immune system function. Additionally, the examination if these types of Mac or DC are implicated in e.g. RA, atherosclerosis or psoriasis is also of particular interest. Therefore, there might be an awakening interest to examine if there is any change in the KLF4 expression in such diseases.

The transcription factor PU.1 plays an important role in the development of myeloid subsets since high expression levels favour Mo and DC development whereas low levels facilitate Gr differentiation. PU.1 null mice lack all peripheral blood leukocytes and died of acute septicaemia within 48h (McKercher et al, EMBO J. 1996 and J Leukoc Biol. 1999). Feinberg et al. demonstrated that in fetal liver cells from PU.1$^{-/-}$ mice KLF4 expression is absent and that there are PU.1 binding sites on the KLF4 promoter. Interestingly when KLF4 is ectopically expressed in those PU.1-deficient cells, only the expression of the M-CSFR can be restored indicating its important

role in Mo development whereas when PU.1 is over expressed the M-CSFR as well as the G-CSFR are re-detectable (Feinberg et al, EMBO J 2007).

We examined that the differentiation process of Mo remained constant when KLF4 was ectopically expressed. This might be due to the fact that even under normal culture conditions over 75% of generated cells exhibited a monocytic phenotype. Therefore, we analyzed KLF4 under sub-optimal Mo culture conditions. Hence, we omitted one of the essential cytokines for Mo differentiation, i.e. M-CSF. When M-CSF is absent we only detected a very low number of CD14 and CD11b double positive cells (~15%) compared to control cultures in presence of M-CSF (Figure 20). Ectopic KLF4 restored this failure of Mo generation in culture conditions missing M-CSF (Figure 20B) thus indicating that KLF4 acts downstream of M-CSF as strong inducer of Mo development. KLF4 restores the impact of M-CSF and therefore, reconstitutes Mo differentiation process when M-CSF is absent. It might be interesting if KLF4 is able to affect not only the expression of the M-CSFR and CD14 but also of other important Mo surface expression molecules, like CCR2 which is expressed on “inflammatory” but not on “resident” Mo. “Inflammatory” Mo reveal a stronger dependence on KLF4 than “resident” Mo. Interestingly, previous studies demonstrated that these “inflammatory” Mo replenishing the LC pool in the skin after LC depletion (Alder et al, J Immunol 2008; Ginhoux et al, Nat Immunol 2006). CCR2 as well as M-CSFR play an essential role for this migration process into the skin and the repopulation of the LC (Merad et al, Nat Immunol 2002; Ginhoux et al, Nat Immunol. 2006). Another important fact in this field is that Notch signalling is essential for LC differentiation from $CD14^+$ progenitors. The Notch ligand Delta-1 in combination with TGF-β1 triggers isolated $CD14^+$ Mo to differentiate into LC (Hoshino et al, J Leukoc Biol 2005). It is demonstrated that Notch signalling interferes with KLF4 expression (Ghaleb et al, Mol Cancer Res 2008). Therefore, it is surprising that Mo reveal a strong expression of Notch signalling receptors on their surface. When these receptors come into contact with their ligands the survival of Mo is inhibited under M-CSF influence whereas under GM-CSF stimulation they develop into DC-like phenotypes (Ohishi et al, Blood 2000 and 2001). If additionally TGF-β1 is present, the cells develop LC

characteristics (Figure 7). The Notch ligand Delta-1 as well as TGF-β1 and GM-CSF are factors present in the skin environment in vivo (Hoshino et al, J Leukoc Biol 2005; Lowell et al, Curr Biol 2000). Figure 27 shows the interplay of the different cytokines and the transcription factor KLF4 in the various cell lineage differentiation processes.

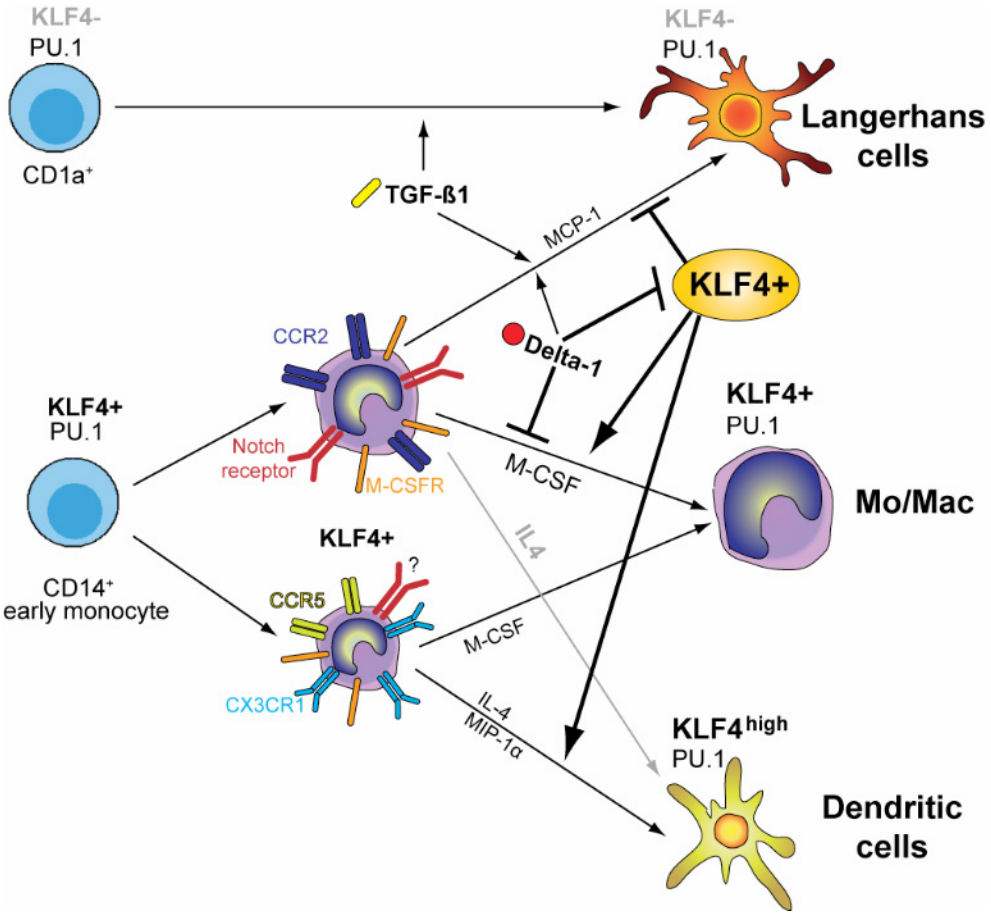


Figure 27: **The involvement of KLF4 in the complex process of dendritic-like or Langerhans-like cell differentiation.**
It is already known that TGF-β1 is an essential factor for LC development. However, during Mo to a LC differentiation TGF-β1 alone is not sufficient to induce LC development. An additional factor is required; Delta-1 which is a ligand for Notch signalling (Hoshino et al, J Leukoc Biol 2005). KLF4 is repressed by TGF-β1 as well as Delta-1, whereas KLF4 is able to interact with TGF-β1 signalling and inhibit it (Feinberg et al, J Biol Chem 2005; Ghaleb et al, Mol Cancer Res 2008). Our study indicates that KLF4 represses the differentiation process of LC whereas a special type of DC is favoured. Previous studies delineated at least two different Mo subsets in human as well as in mice. The first one is characterised by its expression of CCR2 while the second expresses CCR5 as well as CX_3CR1. The $CCR2^+$ subset is known as “classical” (human) or “inflammatory” (mouse) subset and is shown to give rise to LCs whereas the $CCR2^-$ subset is the “proinflammatory” (human) or “resident” (mouse) subset which has a higher predisposition to develop into moDC (Grage-Griebenow et al., Eur. J. Immunol. 2001; Randolph et al., J. Exp. Med. 2002; Ginhoux et al, Nat Immunol. 2006). MCP-1 is a ligand for CCR2; MIP-1α is the ligand for CCR5.

We revealed an absence of KLF4 in moLC (Figure 22D). $CD14^+$ cells cultivated only with TGF-β1 and GM-CSF showed a monocytic phenotype ($CD14^+CD11b^+$) associated with high KLF4 expression, whereas cells generated with TGF-β1, Delta-1 and GM-CSF expressed CD207 and CD1a, formed LC clusters and lacked detectable KLF4 protein expression (Figure 22). Interestingly, in all other conditions which were used to obtain a moDC-like phenotype, KLF4 expression was present and only under Delta-1 plus TGF-β1 condition, cells showed LC characteristics and absent KLF4 protein expression (Figure 22 and 24). The interplay between Notch signalling and the TGF-β1 pathway is absolutely required for LC differentiation from a $CD14^+$ precursors. Furthermore, exogenous Delta-1 is not required for LC differentiation from $CD34^+$ precursors. It is attractive to speculate that these two factors have to act synergistically to allow the development into LC from fully differentiated Mo because several reports show a regulation of common target genes by Notch signalling and TGF-β1.

A possible target gene might be KLF4 because it is demonstrated that both factors, TGF-β1 as well as the Notch signalling pathway, repress KLF4 expression. In line with this, we failed to detect KLF4 in moLC (Figure 22). It is interesting to speculate that in $CD34^+$ cells, where only low levels of KLF4 are present, TGF-β1 alone is sufficient to suppress KLF4 expression and allow LC differentiation, while in fully differentiated Mo, which express higher levels of KLF4, the synergism between both Notch and TGF-β1 signalling is required to sufficiently repress KLF4 and allow LC development. In line with this, the addition of TGF-β1 alone to Mo is not able to suppress KLF4 protein expression or induce the development of LC-like cells. There seems to be a complicated interplay of transcription factors, signalling pathways and the expression of distinct surface receptors during Mo/DC subset development. We suppose that after Mo are infiltrating the skin KLF4 down-regulation is absolutely necessary to obtain LC. Notch- and TGF-β1 co-signalling mediates this KLF4 down-regulation.

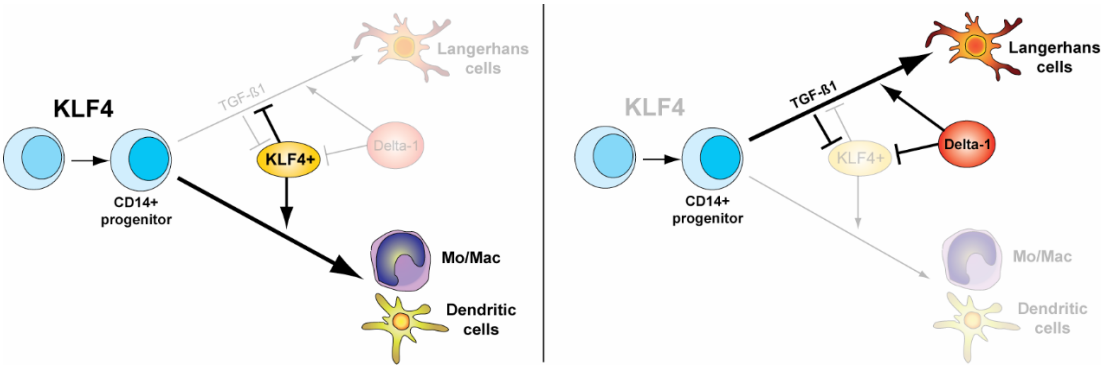


Figure 28: **KLF4 and its implication in LC development.**
LC can be generated from CD14$^+$ Mo progenitor cells under the influence of Delta-1 and TGF-β1. Both stimuli together repress KLF4. When KLF4 levels are very high a distinct pathway of monocytic cells develops. Depending on the cytokine influence these KLF4$^+$ cells acquire moDC or Mac phenotype.

Another interesting point is that the generation of DC-like cells in presence of GM-CSF with IL15, TNFα or IFNα failed, as demonstrated from other groups (Mohamadzadeh et al, J Exp Med 2001; Iwamoto et al, J Immunol 2007; Santini et al, J Exp Med 2000). IL15 should also be able to induce CD207 expression but we were never able to demonstrate this (Figure 24). Why our peripheral blood isolated CD14$^+$ cells did not develop such a DC-like phenotype remains elusive. We detected only low percentages of CD11b and CD1a double positive cells, whereas with IFNα more monocytic cells developed (Figure 24). Interestingly, when we tried the same with cord blood isolated CD14$^+$ cells, nearly all cells developed monocytic-like features and did not gain CD1a (Figure 25). Maybe culture conditions still need to be further improved to develop these cell types with the described cytokine combinations.

We found that Mo up-regulate KLF4 concomitant with moDC differentiation in response to G-CSF and IL-4 stimulation. Conversely, they down-regulate KLF4 during moLC differentiation induced by TGF-β1, Delta-1 and GM-CSF. It would be interesting to transfect Mo with ectopic KLF4 to provide further evidence that KLF4 repression is required for LC differentiation. We would expect an abolishment of moLC differentiation. Inversely, ectopic KLF4 in CD14$^+$ cells may favour a type of “inflammatory” moDC, or alternatively Mo/Mac differentiation might be promoted. We would assume an increase in

some type of “inflammatory” moDC because of the high KLF4 protein levels in DC.

Another interesting part is silencing studies for the assessment of the effect of KLF4 on the myeloid lineages. KLF4 silencing in Mo might promote the differentiation process into moLC without the presence of Delta-1 or TGF-β1. Another question would be the differentiation process of moDC and whether it still takes place or switches into a moLC phenotype with KLF4 silencing. It is interesting if it might be possible to generate LC in absence of TGF-β1, when KLF4 is silenced in $CD34^+$, or if TGF-β1 remains still essential. Furthermore, we would expect an increase of Gr and LC because of their absent KLF4 expression levels (Figure 13C). It seems as if KLF4 down-regulation is necessary for their differentiation. Our generated DC showed high protein levels and we termed them “inflammatory” DC but maybe without KLF4 some sort of immature or anti-inflammatory DC subset can be generated. Another interesting experiment would be to silence KLF4 during DC subset differentiation of Mo. KLF4 silencing in Mo may promote moLC differentiation under sub-optimal stimulation conditions (e.g. without Delta-1 or TGF-β1). Furthermore, it would be interesting to analyze whether KLF4 silencing impairs moDC differentiation. Since KLF4 is repressed by TGF-β1, KLF4 silencing may allow LC differentiation in the absence of TGF-β1. Furthermore, granulopoiesis and LC differentiation might be facilitated as a consequence of diminished KLF4 expression levels (Figure 13C). moDCs generated in response to GM-CSF plus IL-4 showed high KLF4 protein levels and we termed them “inflammatory” DC. It will be interesting whether KLF4 silencing may convert these cells to a more immature or tolerogenic DC subset.

An attractive point of future research will be to address whether Mac and DCs from inflammatory lesions express high levels of KLF4 and if the inhibition of elevated KLF4 expression has an anti-inflammatory effect. Maybe with low to absent KLF4 levels the cells switch from an inflammatory to a resident or tolerogenic state. Therefore KLF4 might represent an interesting therapeutic target in autoimmune-inflammatory diseases.

KLF4 seems to represent a key player in the transcription factor network that controls development of the mononuclear phagocytic and DC system (Figure 29). KLF4 function seems to be important for DC as well as Mo/Mac differentiation whereas its down-regulation seems to be absolutely essential for Gr and LC differentiation. Interestingly, PU.1 was previously shown to directly induce KLF4 expression, and to promote LC differentiation when ectopically expressed in myeloid progenitor cells. However, we found that KLF4 is down-regulated during LC differentiation. Down-regulation of this important factor is mediated by TGF-β1 plus Delta-1, both indispensable signals for LC differentiation. Future studies will show whether therapeutic targeting of key transcription factors within this network may alter DC function for the control of immunity and tolerance.

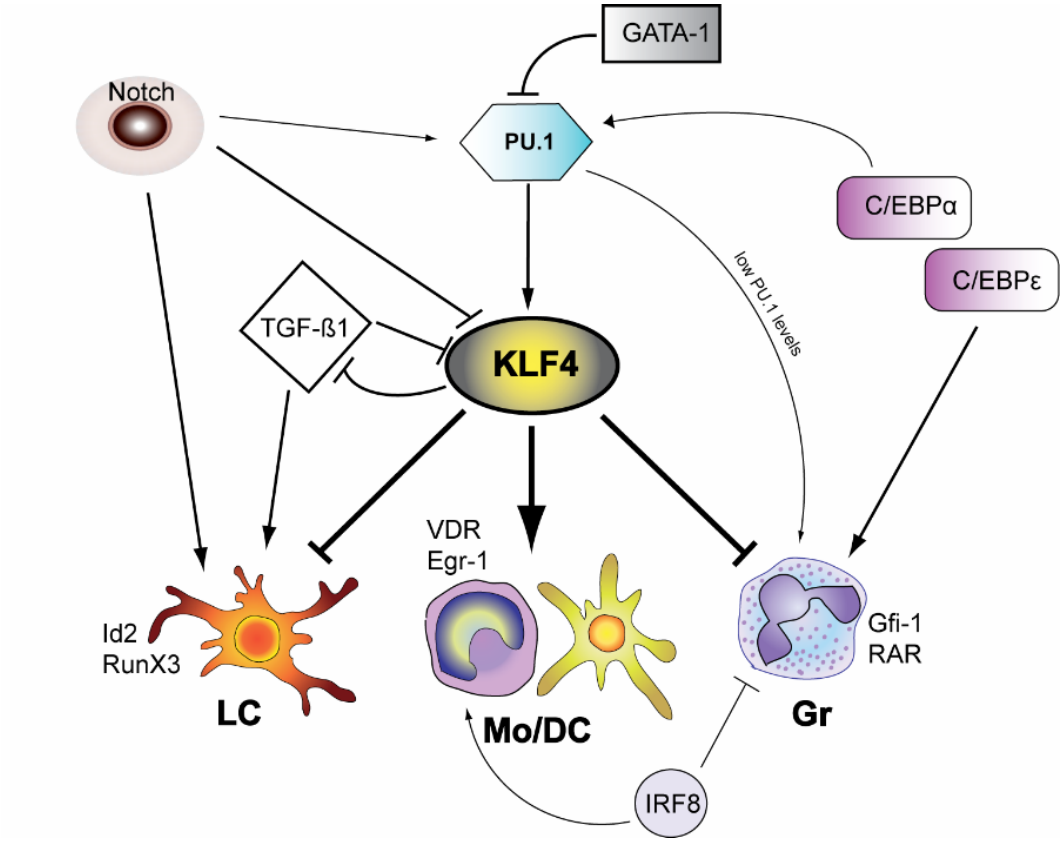


Figure 29: **KLF4 and its implication in myeloid cell development.**
KLF4 is necessary for DC and Mo whereas its down-regulation is essential for LC and Gr. Whereas PU.1 is an inducer, TGF-β1 and Delta-1 are inhibitors of KLF4. It might be interesting if KLF4 is able to affect other transcription factors or if itself is influenced.

5) References

Akashi, K., Traver, D., Miyamoto, T., and Weissman, I.L. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature 2000; 404:193–197.

Alder JK, Georgantas RW 3rd, Hildreth RL, Kaplan IM, Morisot S, Yu X, McDevitt M, Civin CI. Kruppel-like factor 4 is essential for inflammatory monocyte differentiation in vivo. J Immunol. 2008 Apr 15;180(8):5645-52

Angel CE, George E, Brooks AE, Ostrovsky LL, Brown TL, Dunbar PR. Cutting edge: CD1a+ antigen-presenting cells in human dermis respond rapidly to CCR7 ligands. J Immunol. 2006 May 15;176(10):5730-4

Artavanis-Tsakonas, S., Rand, M. D., Lake, R. J. Notch signaling: cell fate control and signal integration in development. Science 1999; 284, 770–776.

Barral DC, Brenner MB. CD1 antigen presentation: how it works. Nat Rev Immunol. 2007 Dec;7(12):929-41

Bastie JN, Balitrand N, Guidez F, Guillemot I, Larghero J, Calabresse C, Chomienne C, Delva L. 1 alpha,25-dihydroxyvitamin D3 transrepresses retinoic acid transcriptional activity via vitamin D receptor in myeloid cells. Mol Endocrinol 2004 Nov;18(11):2685-99

Bieker JJ. Krüppel-like factors: three fingers in many pies. J Biol Chem. 2001 Sep 14;276(37):34355-8.

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 1996;184:2417-2422

Calzada-Wack JC, Frankenberger M, Ziegler-Heitbrock HW. Interleukin-10 drives human monocytes to CD16 positive macrophages. J Inflamm 1996;46(2):78-85

Carlson CM, Endrizzi BT, Wu J, Ding X, Weinreich MA, Walsh ER, Wani MA, Lingrel JB, Hogquist KA, Jameson SC. Kruppel-like factor 2 regulates thymocyte and T-cell migration. Nature 2006 Jul 20;442(7100):299-302

Caux C, Dezutter-Dambuyant C, Schmitt D, Banchereau J. GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells. Nature 1992; 360:258-261.

Caux C, Vanbervliet B, Massacrier C, Dezutter-Dambuyant C, de Saint-Vis B, Jacquet C, Yoneda K, Imamura S, Schmitt D, Banchereau J. CD34+ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF+TNF alpha. J Exp Med. 1996 Aug 1; 184(2):695-706.

Caux C, Massacrier C, Vanbervliet B, 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. 1997; 90:1458-1470.

Chapuis F, Rosenzwajg M, Yagello M, Ekman M, Biberfeld P, Gluckman JC. Differentiation of human dendritic cells from monocytes in vitro. Eur J Immunol. 1997 Feb; 27(2):431-41

Charbonnier AS, Kohrgruber N, Kriehuber E, Stingl G, Rot A, Maurer D. Macrophage inflammatory protein 3alpha is involved in the constitutive trafficking of epidermal langerhans cells.. J Exp Med. 1999 Dec 20; 190(12):1755-68

Chen X, Whitney EM, Gao SY, Yang VW. Transcriptional profiling of Krüppel-like factor 4 reveals a function in cell cycle regulation and epithelial differentiation. J Mol Biol. 2003 Feb 21; 326(3):665-77

Dahl R, Walsh JC, Lancki D, Laslo P, Iyer SR, Singh H, Simon MC. Regulation of macrophage and neutrophil cell fates by the PU.1:C/EBPalpha ratio and granulocyte colony-stimulating factor. Nat Immunol. 2003 Oct; 4(10):1029-36

Dai XM, Ryan GR, Hapel AJ, et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. Blood 2002;99:111-120

Dang DT, Pevsner J, Yang VW. The biology of the mammalian Krüppel-like family of transcription factors. Int J Biochem Cell Biol. 2000 Nov-Dec; 32(11-12):1103-21.

Dang DT, Chen X, Feng J, Torbenson M, Dang LH, Yang VW. Overexpression of Krüppel-like factor 4 in the human colon cancer cell line RKO leads to reduced tumorigenecity. Oncogene. 2003 May 29; 22(22):3424-30

Dieu-Nosjean MC, Massacrier C, Vanbervliet B, Fridman WH, Caux C. IL-10 induces CCR6 expression during Langerhans cell development while IL-4 and IFN-gamma suppress it. J Immunol. 2001 Nov 15;167(10):5594-602

Emery DW, Gavriilidis G, Asano H, Stamatoyannopoulos G. The transcription factor KLF11 can induce gamma-globin gene expression in the setting of in vivo adult erythropoiesis. J Cell Biochem. 2007 Mar 1;100(4):1045-55

Esashi E, Wang YH, Perng O, Qin XF, Liu YJ, Watowich SS. The signal transducer STAT5 inhibits plasmacytoid dendritic cell development by suppressing transcription factor IRF8. Immunity 2008;28:509-520

Fainaru O, Woolf E, Lotem J, Yarmus M, Brenner O, Goldenberg D, Negreanu V, Bernstein Y, Levanon D, Jung S, Groner Y. Runx3 regulates mouse TGF-beta-mediated dendritic cell function and its absence results in airway inflammation. EMBO J. 2004 Feb 25;23(4):969-79

Feinberg MW, Shimizu K, Lebedeva M, Haspel R, Takayama K, Chen Z, Frederick JP, Wang XF, Simon DI, Libby P, Mitchell RN, Jain MK. Essential role for Smad3 in regulating MCP-1 expression and vascular inflammation. Circ Res. 2004 Mar 19;94(5):601-8

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: 38247–38258

Feinberg MW, Wara AK, Cao Z, Lebedeva MA, Rosenbauer F, Iwasaki H, Hirai H, Katz JP, Haspel RL, Gray S, Akashi K, Segre J, Kaestner KH, Tenen DG, Jain MK. The Kruppel-like factor KLF4 is a critical regulator of monocyte differentiation. EMBO J. 2007 Sep 19; 26(18):4138-48. Epub 2007 Aug 30.

Feldmann M, Charles P, Taylor P, Maini RN. Biological insights from clinical trials with anti-TNF therapy. Springer Semin Immunopathol. 1998;20(1-2):211-28

Fingerle, G., Pforte, A., Passlick, B., Blumenstein, M., Ströbel, M., Ziegler-Heitbrock, H. W. L. The novel subset of $CD14^+/CD16^+$ blood monocytes is expanded in sepsis patients. Blood 1993; 82, 3170–3176.

Fisch S, Gray S, Heymans S, Haldar SM, Wang B, Pfister O, Cui L, Kumar A, Lin Z, Sen-Banerjee S, Das H, Petersen CA, Mende U, Burleigh BA, Zhu Y, Pinto YM, Liao R, Jain MK. Kruppel-like factor 15 is a regulator of cardiomyocyte hypertrophy. Proc Natl Acad Sci U S A. 2007 Apr 24;104(17):7074-9

Foster KW, Ren S, Louro ID, Lobo-Ruppert SM, McKie-Bell P, Grizzle W, Hayes MR, Broker TR, Chow LT, Ruppert JM. Oncogene expression cloning by retroviral transduction of adenovirus E1A-immortalized rat kidney RK3E cells: transformation of a host with epithelial features by c-MYC and the zinc finger protein GKLF. Cell Growth Differ. 1999 Jun;10(6):423-34

Foster KW, Frost AR, McKie-Bell P, Lin CY, Engler JA, Grizzle WE, Ruppert JM. Increase of GKLF messenger RNA and protein expression during progression of breast cancer. Cancer Res. 2000 Nov 15;60(22):6488-95

Frankenberger M, Sternsdorf T, Pechumer H, Pforte A, Ziegler-Heitbrock HW. Differential cytokine expression in human blood monocyte subpopulations: a polymerase chain reaction analysis.. Blood. 1996 Jan 1; 87(1):373-7

Ghaleb AM, Katz JP, Kaestner KH, Du JX, Yang VW. Krüppel-like factor 4 exhibits antiapoptotic activity following gamma-radiation-induced DNA damage. Oncogene. 2007 Apr 5;26(16):2365-73

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 Dec;6(12):1920-7

Garrett-Sinha LA, Eberspaecher H, Seldin MF, de Crombrugghe B.A gene for a novel zinc-finger protein expressed in differentiated epithelial cells and transiently in certain mesenchymal cells. J Biol Chem. 1996 Dec 6;271(49):31384-90

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. 1998 Mar 16; 187(6):961-6

Geissmann F, Jung S, Littman DR. Blood monocytes consist of two principal subsets with distinct migratory properties. Immunity. 2003 Jul; 19(1):71-82

Ghaleb, A. M., J. P. Katz, K. H. Kaestner, J. X. Du, and V. W. Yang. Kruppel-like factor 4 exhibits antiapoptotic activity following gamma-radiationinduced DNA damage. Oncogene 2007; 26: 2365–2373

Ginhoux F, Tacke F, Angeli V, Bogunovic M, Loubeau M, Dai XM, Stanley ER, Randolph GJ, Merad M. Langerhans cells arise from monocytes in vivo. Nat Immunol. 2006 Mar; 7(3):223-4.

Gordon, S., Taylor, P.R. Monocyte and macrophage heterogeneity. Nat. Rev. Immunol. 2005; 5, 953–964.

Grage-Griebenow, E., Zawatzky, R., Kahlert, H., Brade, L., Flad, H.D., Ernst, M. Identification of a novel dendritic cell-like subset of CD64(+)/CD16(+) blood monocytes. Eur. J. Immunol. 2001; 31, 48–56.

Gray S, Feinberg MW, Hull S, Kuo CT, Watanabe M, Sen-Banerjee S, DePina A, Haspel R, Jain MK. The Krüppel-like factor KLF15 regulates the insulin-sensitive glucose transporter GLUT4. J Biol Chem. 2002 Sep 13;277(37):34322-8

Gray S, Wang B, Orihuela Y, Hong EG, Fisch S, Haldar S, Cline GW, Kim JK, Peroni OD, Kahn BB, Jain MK. Regulation of gluconeogenesis by Krüppel-like factor 15. Cell Metab. 2007 Apr;5(4):305-12

Guerriero A, Langmuir PB, Spain LM, Scott EW. PU.1 is required for myeloid-derived but not lymphoid-derived dendritic cells. Blood. 2000 Feb 1; 95(3):879-85

Hacker C, Kirsch RD, Ju XS, Hieronymus T, Gust TC, Kuhl C, Jorgas T, Kurz SM, Rose-John S, Yokota Y, Zenke M. Transcriptional profiling identifies Id2 function in dendritic cell development. Nat Immunol. 2003 Apr;4(4):380-6

Hakkinen, T., Karkola, K., Yla-Herttuala, S. Macrophages, smooth muscle cells, endothelial cells, and T-cells express CD40 and CD40L in fatty streaks and more advanced human atherosclerotic lesions. Colocalization with epitopes of oxidized low-density lipoprotein, scavenger receptor, and CD16 (Fc γRIII). Virchows Arch. 2000; 437, 396–405.

Hamilton JA. Colony-stimulating factors in inflammation and autoimmunity. Nat Rev Immunol 2008;8:533-544

Hamon G, Mulloy RH, Chen G, Chow R, Birkenmaier C, Horn JK. Transforming growth factor-beta 1 lowers the CD14 content of monocytes. J Surg Res. 1994 Nov; 57(5):574-8

Hock H, Hamblen MJ, Rooke HM, Traver D, Bronson RT, Cameron S, Orkin SH. Intrinsic requirement for zinc finger transcription factor Gfi-1 in neutrophil differentiation. Immunity. 2003 Jan; 18(1):109-20

Hoshino N, Katayama N, Shibasaki T, Ohishi K, Nishioka J, Masuya M, Miyahara Y, Hayashida M, Shimomura D, Kato T, Nakatani K, Nishii K, Kuribayashi K, Nobori T, Shiku H. A novel role for Notch ligand Delta-1 as a regulator of human Langerhans cell development from blood monocytes. J Leukoc Biol. 2005 Oct; 78(4):921-9

Hwang CK, D'Souza UM, Eisch AJ, Yajima S, Lammers CH, Yang Y, Lee SH, Kim YM, Nestler EJ, Mouradian MM. Dopamine receptor regulating factor, DRRF: a zinc finger transcription factor. Proc Natl Acad Sci U S A. 2001 Jun 19;98(13):7558-63

Imai T, Hieshima K, Haskell C, Baba M, Nagira M, Nishimura M, Kakizaki M, Takagi S, Nomiyama H, Schall TJ, Yoshie O. Identification and molecular characterization of fractalkine receptor CX3CR1, which mediates both leukocyte migration and adhesion.. Cell. 1997 Nov 14; 91(4):521-30

Imaizumi T, Yoshida H, Satoh K. Regulation of CX3CL1/fractalkine expression in endothelial cells.; 11(1):15-21

Imhof A, Schuierer M, Werner O, Moser M, Roth C, Bauer R, Buettner R. Transcriptional regulation of the AP-2alpha promoter by BTEB-1 and AP-2rep, a novel wt-1/egr-related zinc finger repressor. Mol Cell Biol. 1999 Jan;19(1):194-204

Iwamoto S, Iwai S, Tsujiyama K, Kurahashi C, Takeshita K, Naoe M, Masunaga A, Ogawa Y, Oguchi K, Miyazaki A. TNF-alpha drives human CD14+ monocytes to differentiate into CD70+ dendritic cells evoking Th1 and Th17 responses. J Immunol. 2007 Aug 1; 179(3):1449-57.

Katz JP, Perreault N, Goldstein BG, Lee CS, Labosky PA, Yang VW, Kaestner KH. The zinc-finger transcription factor Klf4 is required for terminal differentiation of goblet cells in the colon. Development. 2002 Jun;129(11):2619-28

Katz JP, Perreault N, Goldstein BG, Actman L, McNally SR, Silberg DG, Furth EE, Kaestner KH. Loss of Klf4 in mice causes altered proliferation and differentiation and precancerous changes in the adult stomach. Gastroenterology 2005 Apr;128(4):935-45

Kawanaka, N., Yamamura, M., Aita, T., Morita, Y., Okamoto, A., Kawashima, M., Iwahashi, M., Ueno, A., Ohmoto, Y., Makino, H. $CD14^+$,$CD16^+$ blood monocytes and joint inflammation in rheumatoid arthritis. Arthritis Rheum. 2002; 46, 2578–2586.

Kissenpfennig A, Henri S, Dubois B, Laplace-Builhé C, Perrin P, Romani N, Tripp CH, Douillard P, Leserman L, Kaiserlian D, Saeland S, Davoust J, Malissen B. Dynamics and function of Langerhans cells in vivo: dermal dendritic cells colonize lymph node areas distinct from slower migrating Langerhans cells. Immunity. 2005 May;22(5):643-54

Klechevsky E, Morita R, Liu M, Cao Y, Coquery S, Thompson-Snipes L, Briere F, Chaussabel D, Zurawski G, Palucka AK, Reiter Y, Banchereau J, Ueno H. Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells. Immunity. 2008 Sep; 29(3):497-510

Kondo, M., Weissman, I.L., and Akashi, K. Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell 1997; 91:661–672.

Lande R, Gregorio J, Facchinetti V, Chatterjee B, Wang YH, Homey B, Cao W, Wang YH, Su B, Nestle FO, Zal T, Mellman I, Schröder JM, Liu YJ, Gilliet M. Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. Nature. 2007 Oct 4;449(7162):564-9

Laouar Y, Welte T, Fu XY, Flavell RA. STAT3 is required for Flt3L-dependent dendritic cell differentiation. Immunity 2003;19:903-912

Larregina AT, Morelli AE, Kolkowski E, Sanjuan N, Barboza ME, Fainboim L. Pattern of cytokine receptors expressed by human dendritic cells migrated from dermal explants. Immunology. 1997 Jun; 91(2):303-13

Larregina AT, Morelli AE, Spencer LA, Logar AJ, Watkins SC, Thomson AW, Falo LD Jr. Dermal-resident CD14+ cells differentiate into Langerhans cells. Nat Immunol. 2001 Dec;2(12):1151-8

Laslo P, Spooner CJ, Warmflash A, Lancki DW, Lee HJ, Sciammas R, Gantner BN, Dinner AR, Singh H. Multilineage transcriptional priming and determination of alternate hematopoietic cell fates. Cell 2006 Aug 25;126(4):755-66

Laub F, Lei L, Sumiyoshi H, Kajimura D, Dragomir C, Smaldone S, Puche AC, Petros TJ, Mason C, Parada LF, Ramirez F. Transcription factor KLF7 is important for neuronal morphogenesis in selected regions of the nervous system. Mol Cell Biol. 2005 Jul;25(13):5699-711

Lavallée G, Andelfinger G, Nadeau M, Lefebvre C, Nemer G, Horb ME, Nemer M. The Kruppel-like transcription factor KLF13 is a novel regulator of heart development. EMBO J. 2006 Nov 1;25(21):5201-13

Liu J, Zhang H, Liu Y, Wang K, Feng Y, Liu M, Xiao X. KLF4 regulates the expression of interleukin-10 in RAW264.7 macrophages. Biochem Biophys Res Commun 2007 Oct 26;362(3):575-81

Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol. 2005;23:275-306

Loh YH, Agarwal S, Park IH, Urbach A, Huo H, Heffner GC, Kim K, Miller JD, Ng K, Daley GQ. Generation of induced pluripotent stem cells from human blood. Blood 2009 May 28;113(22):5476-9

Lowell, S., Jones, P., Le Roux, I., Dunne, J., Watt, F. M. Stimulation of human epidermal differentiation by delta-notch signaling at the boundaries of stem-cell clusters. Curr. Biol. 2000; 10, 491–500.

Lowes MA, Chamian F, Abello MV, Fuentes-Duculan J, Lin SL, Nussbaum R, Novitskaya I, Carbonaro H, Cardinale I, Kikuchi T, Gilleaudeau P, Sullivan-Whalen M, Wittkowski KM, Papp K, Garovoy M, Dummer W, Steinman RM, Krueger JG. Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). Proc Natl Acad Sci USA 2005 Dec 27;102(52):19057-62

Manz, M.G., Traver, D., Miyamoto, T., Weissman, I.L., and Akashi, K. Dendritic cell potentials of early lymphoid and myeloid progenitors. Blood 2001; 97:3333–3341.

Masuda S, Kumano K, Shimizu K, et al. Notch1 oncoprotein antagonizes TGF-β/Smad-mediated cell growth suppression via sequestration of coactivator p300. Cancer Sci 2005; 96:274-282.

Matsumoto N, Kubo A, Liu H, Akita K, Laub F, Ramirez F, Keller G, Friedman SL. Developmental regulation of yolk sac hematopoiesis by Kruppel-like factor 6. Blood. 2006 Feb 15;107(4):1357-65

McConnell BB, Ghaleb AM, Nandan MO, Yang VW. The diverse functions of Krüppel-like factors 4 and 5 in epithelial biology and pathobiology. Bioessays 2007 Jun;29(6):549-57

McKenna HJ, Stocking KL, Miller RE, et al. Mice lacking flt3 ligand have deficient hematopoiesis affecting hematopoietic progenitor cells, dendritic cells, and natural killer cells. Blood 2000;95:3489-3497

McKercher SR, Torbett BE, Anderson KL, Henkel GW, Vestal DJ, Baribault H, Klemsz M, Feeney AJ, Wu GE, Paige CJ, Maki RA. Targeted disruption of the PU.1 gene results in multiple hematopoietic abnormalities. EMBO J. 1996 Oct 15; 15(20):5647-58

McKercher SR, Henkel GW, Maki RA. The transcription factor PU.1 does not regulate lineage commitment but has lineage-specific effects. J Leukoc Biol. 1999 Nov;66(5):727-32

Merad M, Manz MG, Karsunky H, Wagers A, Peters W, Charo I, Weissman IL, Cyster JG, Engleman EG. Langerhans cells renew in the skin throughout life under steady-state conditions. Nat Immunol. 2002 Dec; 3(12):1135-41

Meuter S, Schaerli P, Roos RS, Brandau O, Bösl MR, von Andrian UH, Moser B. Murine CXCL14 is dispensable for dendritic cell function and localization within peripheral tissues. Mol Cell Biol. 2007 Feb; 27(3):983-92.

Mohamadzadeh M, Berard F, Essert G, Chalouni C, Pulendran B, Davoust J, Bridges G, Palucka AK, Banchereau J. Interleukin 15 skews monocyte differentiation into dendritic cells with features of Langerhans cells. J Exp Med. 2001 Oct 1; 194(7):1013-20.

Mori T, Sakaue H, Iguchi H, Gomi H, Okada Y, Takashima Y, Nakamura K, Nakamura T, Yamauchi T, Kubota N, Kadowaki T, Matsuki Y, Ogawa W, Hiramatsu R, Kasuga M. Role of Krüppel-like factor 15 (KLF15) in transcriptional regulation of adipogenesis. J Biol Chem. 2005 Apr 1;280(13):12867-75

Morita M, Kobayashi A, Yamashita T, Shimanuki T, Nakajima O, Takahashi S, Ikegami S, Inokuchi K, Yamashita K, Yamamoto M, Fujii-Kuriyama Y Functional analysis of basic transcription element binding protein by gene targeting technology.. Mol Cell Biol. 2003 Apr;23(7):2489-500.

Moser, B., Wolf, M., Walz, A., and Loetscher, P. Chemokines: multiple levels of leukocyte migration control. Trends Immunol. 2004; 25, 75–84.

Nagasawa T., Kobayashi H., Aramaki M., Kiji M., Oda S., Izumi Y. Expression of CD14, CD16 and CD45RA on monocytes from periodontitis patients. J. Periodontal Res. 2004; 39**,** 72–78.

Nerlov C, Tenen DG, Graf T, Hematpoiesis: A developmental approach, LI Zon, ed. Oxford University Press 2001, New York, pp. 363-367

Nestle FO, Filgueira L, Nickoloff BJ, Burg G. Human dermal dendritic cells process and present soluble protein antigens. J Invest Dermatol. 1998 May;110(5):762-6

Nestle FO, Conrad C, Tun-Kyi A, Homey B, Gombert M, Boyman O, Burg G, Liu YJ, Gilliet M. Plasmacytoid predendritic cells initiate psoriasis through interferon-alpha production. J Exp Med. 2005 Jul 4;202(1):135-43

Ohishi K, Varnum-Finney B, Flowers D, Anasetti C, Myerson D, Bernstein ID. Monocytes express high amounts of Notch and undergo cytokine specific apoptosis following interaction with the Notch ligand, Delta-1. Blood. 2000 May 1;95(9):2847-54

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 Sep 1;98(5):1402-7

Ohishi, K., Varnum-Finney, B., Bernstein, I. D. Delta-1 enhances marrow and thymus repopulating ability of human CD34+CD38– cord blood cells. J. Clin. Invest. 2002; 110, 1165–1174.

Oishi Y, Manabe I, Tobe K, Tsushima K, Shindo T, Fujiu K, Nishimura G, Maemura K, Yamauchi T, Kubota N, Suzuki R, Kitamura T, Akira S, Kadowaki T, Nagai R. Krüppel-like transcription factor KLF5 is a key regulator of adipocyte differentiation. Cell Metab. 2005 Jan;1(1):27-39

Orkin SH. Diversification of haematopoietic stem cells to specific lineages. Nat Rev Genet. 2000 Oct;1(1):57-64

Palframan RT, Jung S, Cheng G, Weninger W, Luo Y, Dorf M, Littman DR, Rollins BJ, Zweerink H, Rot A, von Andrian UH. Inflammatory chemokine transport and presentation in HEV: a remote control mechanism for monocyte recruitment to lymph nodes in inflamed tissues. J Exp Med. 2001 Nov 5; 194(9):1361-73

Passlick B, Flieger D, Ziegler-Heitbrock HW. Identification and characterization of a novel monocyte subpopulation in human peripheral blood. Blood. 1989 Nov 15; 74(7):2527-34

Pastore, S., Fanales-Belasio, E., Albanesi, C., Chinni, L. M., Giannetti, A., Girolomoni, G. Granulocyte macrophage colony-stimulating factor is overproduced by keratinocytes in atopic dermatitis: implications for sustained dendritic cell activation in the skin. J. Clin. Invest.1997; 99, 3009–3017.

Pearson R, Fleetwood J, Eaton S, Crossley M, Bao S Krüppel-like transcription factors: a functional family. Int J Biochem Cell Biol. 2008; 40(10):1996-2001.

Perkins AC, Sharpe AH, Orkin SH. Lethal beta-thalassaemia in mice lacking the erythroid CACCC-transcription factor EKLF. Nature 1995 May 25;375(6529):318-22

Platzer B, Jörgl A, Taschner S, Höcher B, Strobl H. RelB regulates human dendritic cell subset development by promoting monocyte intermediates. Blood. 2004 Dec 1;104(12):3655-63. Epub 2004 Aug 17

Pulliam, L., Gascon, R., Stubblebine, M., McGuire, D., McGrath, M. S. Unique monocyte subset in patients with AIDS dementia. Lancet 1997; 349, 692–695.

Radomska HS, Huettner CS, Zhang P, Cheng T, Scadden DT, Tenen DG. CCAAT/enhancer binding protein alpha is a regulatory switch sufficient for induction of granulocytic development from bipotential myeloid progenitors. Mol Cell Biol. 1998 Jul; 18(7):4301-14

Randolph, G.J., Sanchez-Schmitz, G., Liebman, R.M., Schakel, K. The CD16(+) (FcgammaRIII(+)) subset of human monocytes preferentially becomes migratory dendritic cells in a model tissue setting. J. Exp. Med. 2002; 196, 517–527.

Ranganath S, Ouyang W, Bhattarcharya D, Sha WC, Grupe A, Peltz G, Murphy KM. GATA-3-dependent enhancer activity in IL-4 gene regulation. J Immunol. 1998 Oct 15; 161(8):3822-6

Ranganath S, Murphy KM. Structure and specificity of GATA proteins in Th2 development. Mol Cell Biol. 2001 Apr; 21(8):2716-25

Rathinam C, Geffers R, Yücel R, Buer J, Welte K, Möröy T, Klein C. The transcriptional repressor Gfi1 controls STAT3-dependent dendritic cell development and function. Immunity. 2005 Jun;22(6):717-28

Rivier, A., Pene, J., Rabesandratana, H., Chanez, P., Bousquet, J., Campbell, A. M. Blood monocytes of untreated asthmatics exhibit some features of tissue macrophages. Clin. Exp. Immunol. 1995; 100, 314–318.

Romani N, Holzmann S, Tripp CH, Koch F, Stoitzner P. Langerhans cells - dendritic cells of the epidermis. APMIS. 2003 Jul-Aug;111(7-8):725-40

Saleh, M.N., Goldman, S.J., LoBuglio, A.F., Beall, A.C., Sabio, H., McCord, M.C., Minasian, L., Alpaugh, R.K., Weiner, L.M., Munn, D.H. CD16+ monocytes in patients with cancer: spontaneous elevation and pharmacologic induction by recombinant human macrophage colony-stimulating factor. Blood 1995; 85, 2910–2917.

Sallusto F, 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 alpha. J Exp Med 1994;179:1109-1118.

Santini SM, Lapenta C, Logozzi M, Parlato S, Spada M, Di Pucchio T, Belardelli F. Type I interferon as a powerful adjuvant for monocyte-derived dendritic cell development and activity in vitro and in Hu-PBL-SCID mice. J Exp Med. 2000 May 15; 191(10):1777-88.

Schaerli P, Willimann K, Ebert LM, Walz A, Moser B. Cutaneous CXCL14 targets blood precursors to epidermal niches for Langerhans cell differentiation. Immunity 2005 Sep; 23(3):331-42

Schiavoni G, Mattei F, Borghi P, Sestili P, Venditti M, Morse HC 3rd, Belardelli F, Gabriele L. ICSBP is critically involved in the normal development and trafficking of Langerhans cells and dermal dendritic cells. Blood 2004 Mar 15; 103(6):2221-8

Schotte R, Nagasawa M, Weijer K, Spits H, Blom B. The ETS transcription factor Spi-B is required for human plasmacytoid dendritic cell development. J Exp Med. 2004 Dec 6;200(11):1503-9

Segre JA, Bauer C, Fuchs E. Klf4 is a transcription factor required for establishing the barrier function of the skin. Nat Genet. 1999 Aug; 22(4):356-60

Serbina NV, Pamer EG. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. Nat Immunol. 2006 Mar;7(3):311-7

Shindo T, Manabe I, Fukushima Y, Tobe K, Aizawa K, Miyamoto S, Kawai-Kowase K, Moriyama N, Imai Y, Kawakami H, Nishimatsu H, Ishikawa T, Suzuki T, Morita H, Maemura K, Sata M, Hirata Y, Komukai M, Kagechika H, Kadowaki T, Kurabayashi M, Nagai R. Krüppel-like zinc-finger transcription factor KLF5/BTEB2 is a target for angiotensin II signaling and an essential regulator of cardiovascular remodeling. Nat Med. 2002 Aug;8(8):856-63

Shortman K, Liu YJ. Mouse and human dendritic cell subtypes. Nat Rev Immunol. 2002 Mar;2(3):151-61

Shortman K, Naik SH. Steady-state and inflammatory dendritic-cell development. Nat Rev Immunol 2007 Jan;7(1):19-30

Simmen RC, Eason RR, McQuown JR, Linz AL, Kang TJ, Chatman L Jr, Till SR, Fujii-Kuriyama Y, Simmen FA, Oh SP. Subfertility, uterine hypoplasia, and partial progesterone resistance in mice lacking the Kruppel-like factor 9/basic transcription element-binding protein-1 (Bteb1) gene. J Biol Chem. 2004 Jul 9;279(28):29286-94

Skrzeczyńska-Moncznik J, Bzowska M, Loseke S, Grage-Griebenow E, Zembala M, Pryjma J. Peripheral blood CD14high CD16+ monocytes are main producers of IL-10. Scand J Immunol. 2008 Feb; 67(2):152-9.

Song CZ, Gavriilidis G, Asano H, Stamatoyannopoulos G. Functional study of transcription factor KLF11 by targeted gene inactivation. Blood Cells Mol Dis. 2005 Jan-Feb;34(1):53-9

Sporn, M. B., Roberts, A. B. Transforming growth factor-β: recentprogress and new challenges. J. Cell Biol. 1992; 119, 1017–1021.

Strauss-Ayali D, Conrad SM, Mosser DM. Monocyte subpopulations and their differentiation patterns during infection. J Leukoc Biol. 2007 Aug;82(2):244-52. Epub 2007 May 2

Strobl H, Riedl E, Scheinecker C, Bello-Fernandez C, Pickl WF, Rappersberger K, Majdic O, Knapp W. TGF-beta 1 promotes in vitro development of dendritic cells from CD34+ hemopoietic progenitors. J Immunol. 1996 Aug 15;157(4):1499-507

Strobl H, Bello-Fernandez C, Riedl E, 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. 1997; 90:1425-1434

Subramaniam M, Gorny G, Johnsen SA, Monroe DG, Evans GL, Fraser DG, Rickard DJ, Rasmussen K, van Deursen JM, Turner RT, Oursler MJ, Spelsberg TC. TIEG1 null mouse-derived osteoblasts are defective in mineralization and in support of osteoclast differentiation in vitro. Mol Cell Biol. 2005 Feb;25(3):1191-9

Suda S, Rai T, Sohara E, Sasaki S, Uchida S. Postnatal expression of KLF12 in the inner medullary collecting ducts of kidney and its trans-activation of UT-A1 urea transporter promoter. Biochem Biophys Res Commun. 2006 May 26;344(1):246-52

Sue N, Jack BH, Eaton SA, Pearson RC, Funnell AP, Turner J, Czolij R, Denyer G, Bao S, Molero-Navajas JC, Perkins A, Fujiwara Y, Orkin SH, Bell-Anderson K, Crossley M. Targeted disruption of the basic Krüppel-like factor gene (Klf3) reveals a role in adipogenesis. Mol Cell Biol. 2008 Jun;28(12):3967-78

Sunderkotter, C., Nikolic, T., Dillon, M.J., van Rooijen, N., Stehling, M., Drevets, D.A., Leenen, P.J.M. Subpopulations of mouse blood monocytes differ in maturation stage and inflammatory response. J. Immunol. 2004; 172, 4410–4417.

Suske G, Bruford E, Philipsen S. Mammalian SP/KLF transcription factors: bring in the family. Genomics. 2005 May; 85(5):551-6.

Tacke F, Randolph GJ. Migratory fate and differentiation of blood monocyte subsets. Immunobiology. 2006; 211(6-8):609-18.

Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25;126(4):663-76

Tamura T, Nagamura-Inoue T, Shmeltzer Z, Kuwata T, Ozato K. ICSBP directs bipotential myeloid progenitor cells to differentiate into mature macrophages. Immunity 2000 Aug;13(2):155-65

Tamura T, Tailor P, Yamaoka K, Kong HJ, Tsujimura H, O'Shea JJ, Singh H, Ozato K. IFN regulatory factor-4 and -8 govern dendritic cell subset development and their functional diversity. J Immunol 2005 Mar 1;174(5):2573-81

Tenen DG, Hromas R, Licht JD, Zhang DE. Transcription factors, normal myeloid development, and leukemia. Blood 1997 Jul 15;90(2):489-519

Tezuka H, Abe Y, Iwata M, Takeuchi H, Ishikawa H, Matsushita M, Shiohara T, Akira S, Ohteki T. Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. Nature 2007 Aug 23;448(7156):929-33

Truty MJ, Lomberk G, Fernandez-Zapico ME, Urrutia R. Silencing of the transforming growth factor-beta (TGFbeta) receptor II by Kruppel-like factor 14 underscores the importance of a negative feedback mechanism in TGFbeta signaling. J Biol Chem. 2009 Mar 6; 284(10):6291-300

Tsai S, Collins SJ. A dominant negative retinoic acid receptor blocks neutrophil differentiation at the promyelocyte stage. Proc Natl Acad Sci USA 1993 Aug 1;90(15):7153-7

Tohyama M, Shirakara Y, Yamasaki K, Sayama K, Hashimoto K. Differentiated keratinocytes are responsible for TNF-alpha regulated production of macrophage inflammatory protein 3alpha/CCL20, a potent chemokine for Langerhans cells. J. Dermatol Sci 2001; 27 (2): 130–9.

Valladeau J, Ravel O, Dezutter-Dambuyant C, Moore K, Kleijmeer M, Liu Y, Duvert-Frances V, Vincent C, Schmitt D, Davoust J, Caux C, Lebecque S, Saeland S. Langerin, a novel C-type lectin specific to Langerhans cells, is an

endocytic receptor that induces the formation of Birbeck granules. Immunity 2000 Jan;12(1):71-81

van Vliet J, Crofts LA, Quinlan KG, Czolij R, Perkins AC, Crossley M. Human KLF17 is a new member of the Sp/KLF family of transcription factors. Genomics 2006 Apr;87(4):474-82

Vremec D, Lieschke GJ, Dunn AR, Robb L, Metcalf D, Shortman K. The influence of granulocyte/macrophage colony-stimulating factor on dendritic cell levels in mouse lymphoid organs. Eur J Immunol 1997;27:40-44.

Wahl SM, Allen JB, Welch GR, Wong HL. Transforming growth factor-beta in synovial fluids modulates Fc gamma RII (CD16) expression on mononuclear phagocytes. J Immunol 1992 Jan 15;148(2):485-90

Wang X, Zhao J. KLF8 transcription factor participates in oncogenic transformation. Oncogene 2007 Jan 18;26(3):456-61

Wang X, Zheng M, Liu G, Xia W, McKeown-Longo PJ, Hung MC, Zhao J Krüppel-like factor 8 induces epithelial to mesenchymal transition and epithelial cell invasion.. Cancer Res 2007 Aug 1;67(15):7184-93

Wang X, Urvalek AM, Liu J, Zhao J. Activation of KLF8 transcription by focal adhesion kinase in human ovarian epithelial and cancer cells. J Biol Chem 2008 May 16;283(20):13934-42

Wani MA, Means RT Jr, Lingrel JB. Loss of LKLF function results in embryonic lethality in mice. Transgenic Res 1998 Jul;7(4):229-38

Weber, C., Belge, K.U., von Hundelshausen, P., Draude, G., Steppich, B., Mack, M., Frankenberger, M., Weber, K.S.C., Ziegler-Heitbrock, H.W.L. Differential chemokine receptor expression and function in human monocyte subpopulations. J Leukoc Biol 2000; 67, 699–704.

Wei H, Wang X, Gan B, Urvalek AM, Melkoumian ZK, Guan JL, Zhao J. J Sumoylation delimits KLF8 transcriptional activity associated with the cell cycle regulation. Biol Chem 2006 Jun 16;281(24):16664-71

Werner F, Jain MK, Feinberg MW, Sibinga NE, Pellacani A, Wiesel P, Chin MT, Topper JN, Perrella MA, Lee ME. Transforming growth factor-beta 1 inhibition of macrophage activation is mediated via Smad3. J Biol Chem 2000 Nov 24;275(47):36653-8

Wollenberg A, Wagner M, Günther S, Towarowski A, Tuma E, Moderer M, Rothenfusser S, Wetzel S, Endres S, Hartmann G. Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases. J Invest Dermatol. 2002 Nov;119(5):1096-102

Wu L, Nichogiannopoulou A, Shortman K, Georgopoulos K. Cell-autonomous defects in dendritic cell populations of Ikaros mutant mice point to a developmental relationship with the lymphoid lineage. Immunity 1997 Oct; 7(4):483-92

Yamanaka R, Barlow C, Lekstrom-Himes J, Castilla LH, Liu PP, Eckhaus M, Decker T, Wynshaw-Boris A, Xanthopoulos KG. Impaired granulopoiesis, myelodysplasia, and early lethality in CCAAT/enhancer binding protein

epsilon-deficient mice. Proc Natl Acad Sci USA 1997 Nov 25; 94(24):13187-92

Yano R, Yamamura M, Sunahori K, Takasugi K, Yamana J, Kawashima M, Makino H. Recruitment of CD16+ monocytes into synovial tissues is mediated by fractalkine and CX3CR1 in rheumatoid arthritis patients. Acta Med Okayama 2007 Apr; 61(2):89-98

Yoon HS, Yang VW. Requirement of Krüppel-like factor 4 in preventing entry into mitosis following DNA damage. J Biol Chem 2004 Feb 6;279(6):5035-41

Zaba LC, Fuentes-Duculan J, Steinman RM, Krueger JG, Lowes MA. Normal human dermis contains distinct populations of CD11c+BDCA-1+ dendritic cells and CD163+FXIIIA+ macrophages. J Clin Invest. 2007 Sep;117(9):2517-25

Zaba LC, Fuentes-Duculan J, Eungdamrong NJ, Abello MV, Novitskaya I, Pierson KC, Gonzalez J, Krueger JG, Lowes MA. Psoriasis is characterized by accumulation of immunostimulatory and Th1/Th17 cell-polarizing myeloid dendritic cells. J Invest Dermatol. 2009 Jan;129(1):79-88

Zhang W, Chen X, Kato Y, Evans PM, Yuan S, Yang J, Rychahou PG, Yang VW, He X, Evers BM, Liu C. Novel cross talk of Kruppel-like factor 4 and beta-catenin regulates normal intestinal homeostasis and tumor repression. Mol Cell Biol 2006 Mar; 26(6):2055-64

Zheng H, Pritchard DM, Yang X, Bennett E, Liu G, Liu C, Ai W. 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 2009 Mar; 296(3):G490-8. Epub 2008 Dec 24.

Zhou M, McPherson L, Feng D, Song A, Dong C, Lyu SC, Zhou L, Shi X, Ahn YT, Wang D, Clayberger C, Krensky AM. Kruppel-like transcription factor 13 regulates T lymphocyte survival in vivo. J Immunol 2007 May 1;178(9):5496-504

Ziegler-Heitbrock HW, Fingerle G, Ströbel M, Schraut W, Stelter F, Schütt C, Passlick B, Pforte A. The novel subset of CD14+/CD16+ blood monocytes exhibits features of tissue macrophages. Eur J Immunol 1993 Sep; 23(9):2053-8

Ziegler-Heitbrock HW. Heterogeneity of human blood monocytes: the CD14+ CD16+ subpopulation. Immunol Today 1996 Sep;17(9):424-8

Ziegler-Heitbrock, H.W. Definition of human blood monocytes. J Leukoc Biol 2000; 67, 603–606.

Ziegler-Heitbrock L. The CD14+ CD16+ blood monocytes: their role in infection and inflammation. J Leukoc Biol 2007 Mar;81(3):584-92

6) Acknowledgements

Also erstes möchte ich vor allem meiner Betreuerin, Jennifer Jurkin, danken. Sie führte mich in die Welt des Labor-Lebens ein, mit all seinen Höhen und Tiefen, half mir mein grundlegendes praktisches und theoretisches Wissen zu erlangen und gab mir hilfreiche Tipps wenn Dinge sich nicht so entwickelten wie sie sollten. Ebenso bedanke ich mich für ihre Geduld mich in die FACS Auswertungen, Western Blots und Zellkultur Arbeit einzuschulen. Ein großes Dankeschön auch meinem Gruppenleiter Herbert Strobl, der mir es überhaupt erst ermöglichte in einem so tollen Labor mitzuarbeiten, der meine Arbeit immer mit Enthusiasmus verfolgte, interessante und kritische Beurteilungen meiner Resultate parat hatte und mir bei der Lösung von Problemen ebenso zur Seite stand.

Ebenso gilt ein Dankeschön allen restlichen Labor Mitgliedern, insbesonders Doris Kneidinger, Renè Köffel, Sabine Witzel, Susanne Richter und Thomas Bauer. Sie beteiligten sich immer bei Diskussionen zu meiner Arbeit und gaben manch hilfreiche Tipps. Thomas Bauer möchte ich für seine Hilfe im Bezug auf Klonierungen und Ligationen danken. Ebenso möchte ich Bernhard Gesselbauer für die außerordentliche Hilfe bei den Isolierungen der $CD34^+$ und $CD14^+$ Zellen danken und Dieter Prinz vom St. Anna Kinderspital für die Hilfe beim FACS Sorten von Zellen.

Zu guter Letzt gilt mein größtes Dankeschön meiner Familie, meinen Freunden und meinem Freund, die mich immer unterstützten wenn ich mal meine Motivation verlor und eine Ablenkung oder ein nettes Wort benötigte. Vor allem meiner Mama und meinem Papa, die es mir überhaupt ermöglichten soweit zu kommen, mir immer Ratschläge und gute Zuversicht gaben, und jederzeit ein offenes Ohr für mich hatten, gilt mein größter Dank. Danke für eure Liebe und Unterstützung während dieser langen Zeit und danke für euren Glauben, dass ich das alles bewältige, das ihr nie aufgegeben habt und mir immer unterstützend zur Seite gestanden seit.

DANKE!

7) Curriculum Vitae

Personal data

Name:	Mühlbacher Christina
Date of birth:	September 30th, 1982 Klagenfurt, Austria
Nationality:	Austria
Home Address:	Forsthausgasse 2-8/3115, A-1200 Wien
E-mail:	christina.muehlbacher@meduniwien.ac.at
Mobile:	+43650/ 33 200 10

Education

1989 – 1993:	Volksschule (primary school) Velden, Austria
1993 – 2001:	Gymnasium (High school) Viktring (Schwerpunkt Bildnerische Erziehung), Austria

Studies

2001 – 2002:	Study of architecture, University of Graz, Austria
2002 – 2009:	Study of Biology, University of Vienna, Austria
2006:	Finish the first part of the studies in basic Biology
2006 – 2009:	Major Field of studies: Immunology Minor Field of studies: Microbiology and Molecular Medicine
2007 – 2009:	Medical University of Vienna, Austria Institute of Immunology
	Diploma Thesis: ***Transcription Factors in myeloid development – the role of Kruppel-like factor 4 (KLF4)***

Research and practical experience

09. 2007 – 10. 2007:

Univ.-Prof. Dr. Udo Bläsi Group, Max F. Perutz Laboratories, Department of RNA biology, University of Vienna, "Translational Regulation by non coding RNAs and Hfq in Eubacteria and by Initiation Factors in Archaea"

10. 2007 – 11. 2007:

Dr. Joachim Seipelt, Max F. Perutz Laboratories, Department of Infection Biology, University of Vienna and Department of Medical Biochemistry, Medical University of Vienna, "PDTC inhibits picornavirus polyprotein processing and RNA replication by transporting zinc ions into cells"

11. 2007 – 02. 2009:

Ao. Univ. Prof. Dr. Herbert Strobl Group, Institute of Immunology, Medical University of Vienna, Diploma Thesis: "Transcription Factors in myeloid development – the role of Kruppel-like factor 4 (KLF4)"

Publications

Reciprocal role of GATA-1 and vitamin D receptor in human myeloid dendritic cell differentiation.

Goebel F, Taschner S, Jurkin J, Vaculik C, Richter S, Kneidinger D, Mühlbacher C, Bieglmayer C, Elbe-Bürger A, Strobl H. Blood 2009

Skills and qualifications

Languages: German native, English fluent

Talks: power-point presentations and oral talks during lab- and group meetings; journal clubs

Computer: Operating System Microsoft Windows & Apple Macintosh: Microsoft Office, Genetic Databases, Bioinformatics Software, Adobe CS