

Division of Molecular Biology of Hematopoietic Stem Cells and Dendritic Cells

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Past Members

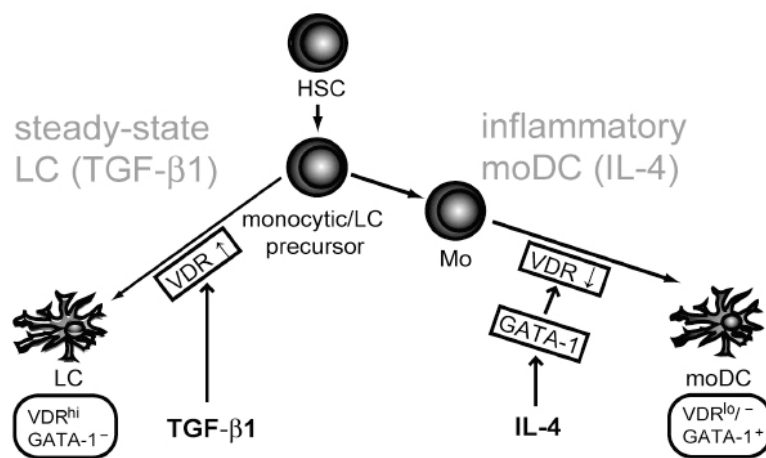
Sabine Taschner-Mandl, PhD, PostDoc, till
 3/2008
 Florian Göbel, PhD Student, PhD, till 4/2008
 Anastasia Meshcheryakova, Diploma
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Hematopoietic stem cells differentiate to lineage-committed progenitor cells, which in turn give rise to various leukocyte subtypes. These differentiation processes are regulated by a complex interplay between synergistic and antagonistic transcription factors in response to extra-cellular signals. In most of our projects, we use in vitro differentiation models of human CD34+ cord blood hematopoietic progenitor cells and monocytes to study molecular mechanisms underlying myelopoiesis and dendritic cell (DC) subset differentiation. We use retroviral and lentiviral gene delivery systems and flow cytometry to dissect transcriptional mechanisms underlying lineage commitment and differentiation. We are particularly interested in understanding how different subsets of human DCs develop from hematopoietic stem cells and monocytes. Epidermal/mucosal Langerhans cells (LCs) and inflammatorytype DCs represent two myeloid DC subsets, which differ in many phenotypic and functional characteristics. A better understanding of how these DC subsets develop and function is critical for both our basic understanding of immune system function and for the creation of novel immunotherapy procedures. In a second research area we analyzed molecular mechanisms underlying cell plasticity of myeloid cells. In one project we studied granulopoietic monocyte differentiation in response to inflammatory signals. In another project, we asked how epithelial genes are regulated during the life cycle of LCs.

1. Molecular mechanisms of human DC subset differentiation

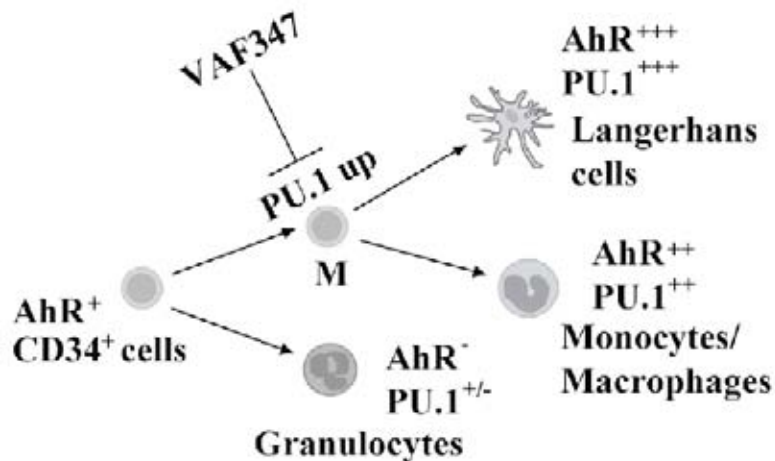
Two major pathways of human myeloid DC subset differentiation have been delineated in previous studies. LCs reside in epithelia at steady-state, whereas monocytes can provide DCs on demand in response to inflammatory signals. Both DC subset pathways arise from shared CD14⁺ monocyte precursors, which in turn develop from myeloid committed progenitor cells. The underlying hematopoietic mechanisms of myeloid DC subset differentiation still remain poorly defined. We identified a functional role of the transcription factors GATA-1, VDR and AhR in these processes.

Inverse role of GATA-1 and VDR in myeloid subset differentiation.



Using a microarray screen, we found that the vitamin D3 receptor (VDR) is induced by TGFβ1 during LC lineage commitment of hematopoietic progenitor cells. Moreover, we found that VDR exerts a positive role during LC generation. In contrast, VDR is repressed during IL-4-dependent monocyte-derived DC (moDC) differentiation. Using a functional retroviral cDNA library screen, we identified GATA-1 as a repressor of VDR in U937 monocytic cells. Studies of primary human DC subsets subsequently revealed that GATA-1 is induced by IL-4 in moDCs, but can not be detected in LCs. Moreover, we found that forced inducible expression of GATA-1 mimics IL-4 in re-directing moDC differentiation, and vice versa GATA-1 knockdown arrests moDC differentiation at the monocyte stage. Furthermore, ectopic GATA-1 expression stabilizes the moDC phenotype under monocyte promoting conditions in the presence of vitamin-D3 (VD3). In summary, human myeloid DC subset differentiation is inversely regulated by GATA-1 and VDR. GATA-1 mediates the repression of VDR and enables IL-4-dependent moDC differentiation. Conversely, VDR is induced downstream of TGF-β1 and is functionally involved in promoting LC differentiation.

Ligation of the aryl-hydrocarbon receptor (AhR) impairs in vitro generation of human monocytes and Langerhans-type DCs.

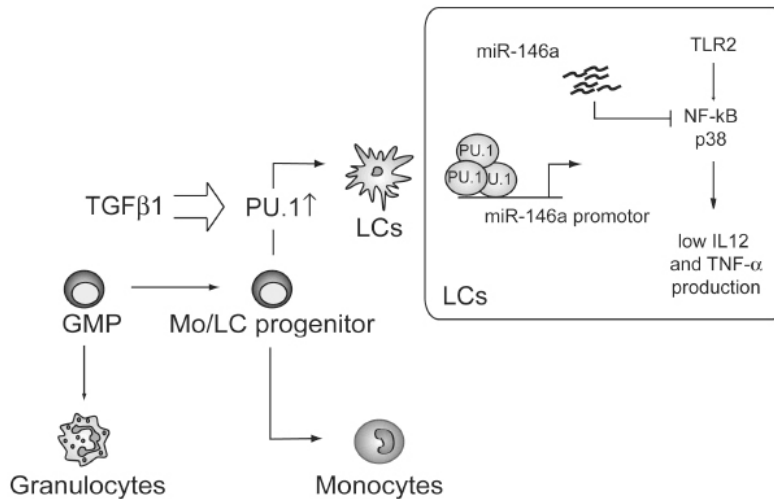


The transcription factor aryl hydrocarbon receptor (AhR) represents a promising therapeutic target in allergy and autoimmunity. AhR signalling induced by the newly described ligand VAF347 inhibits allergic lung inflammation as well as suppresses pancreatic islet allograft rejection. These effects are likely mediated via alterations in dendritic cell (DC) function. Moreover, VAF347 induces tolerogenic DCs. LCs are immediate targets of exogenous AhR ligands at epithelial surfaces; how they respond to AhR ligands remained undefined. We studied AhR expression and function in human LCs and myelopoietic cell subsets using a lineage differentiation and gene transduction model of human CD34⁺ hematopoietic progenitors. We found that AhR is highly regulated during myeloid subset differentiation. LCs expressed highest AhR levels followed by monocytes. Conversely, neutrophil granulocytes lacked AhR expression. We evaluated several AhR ligands including the newly identified compound VAF347, which we obtained through a collaboration with Novartis. We observed that various AhR ligands arrested the differentiation of monocytes and LCs at an early precursor cell stage, whereas progenitor cell expansion or granulopoiesis remained unimpaired AhR expression was co-regulated with the transcription factor PU.1 during myeloid subset differentiation. VAF347 inhibited PU.1 induction during initial monocytic differentiation, and ectopic PU.1 restored monocyte and LC generation in the presence of this compound. AhR ligands failed to interfere with cytokine receptor signalling during LC differentiation and failed to impair LC activation/maturation. VAF347-mediated antiproliferative effect on precursors undergoing LC lineage differentiation occurred in a clinically applicable serum-free culture model and was not accompanied by apoptosis induction. In conclusion, AhR agonist signalling interferes with transcriptional processes leading to monocyte/DC lineage commitment of human myeloid progenitor cells. These observations will now provide a basis for testing AhR ligand-modified DC subsets for their capacity to induce tolerogenic immune responses.

2. Studies on Langerhans-type dendritic cell function

Our previous studies showed that the cytokine TGF- β 1 induces LC differentiation from CD34+ human hematopoietic progenitor cells. Based on these observations, large numbers of human LCs can now be generated. We performed gene array profiling of LC precursor cells to identify novel molecules induced during LC differentiation (TGF- β 1-induced genes). This screen led to the identification of several interesting cell surface molecules and transcription factors that we are currently analyzing. Furthermore, we compared LCs and monocytederived DCs for the expression of microRNAs and focused on one first interesting candidate.

miR-146a is differentially expressed by myeloid DC subsets and desensitizes cells to TLR2-dependent activation



Using microarray profiling we identified microRNA (miR)-146a to be constitutively expressed at higher levels in LCs compared to intDCs. Moreover, miR-146a levels were low in monocyte and non-detectable in neutrophil granulocytes. Constitutive high miR-146a expression in LCs is induced by the transcription factor PU.1 in response to TGF- β 1, a key micro-environmental signal for epidermal LC differentiation. We identified miR-146a as a regulator of monocyte and DC activation but not myeloid/DC subset differentiation. Specifically, ectopic miR-146a in monocytes and intDCs interfered with TLR2 downstream signaling and cytokine production, without affecting phenotypic DC maturation. Inversely, silencing of miR-146a in LCs enhanced TLR2-dependent NF- κ B signaling. We therefore conclude that high constitutive miR-146a levels in epithelial LCs are induced by micro-environmental signals and may render these cells less susceptible to commensal bacterial TLR2 triggers at body surfaces. (Jurkin, J. et al., submitted).

3. Molecular mechanisms of cell plasticity within the myelopoietic cell system

Usually, mature myeloid cells are considered end-stage differentiated cells. However, under certain conditions these cells are still capable of expressing genes affiliated to other developmental pathways. These processes might play important roles in autoimmune / inflammatory diseases and during steady-state immune homeostasis. In two projects we studied (1) granulopoietic monocyte differentiation in response to inflammatory signals and (2) the regulation of epithelial genes during the life cycle of Lcs.

Transcriptional control of “transdifferentiation” of granulopoietic cells to monocytes and osteoclasts.

How intracellular signalling cascades downstream of cytokine signals orchestrate transcription factors (TFs) to induce defined myeloid sub-lineage differentiation programs is so far poorly understood. We found that the direct upstream kinase of p38MAPK, MKK6, is more abundantly expressed by monocytes (Mo) than neutrophil granulocytes (G). Moreover, conditional induction of dominant-active (d.a.) MKK6 in G-CSF-dependent G reprograms them to Mo. D.a.MKK6-dependent G to Mo lineage conversion is mediated by p38-mediated proteasomal degradation of C/EBP α , and induction of c-Jun, followed by the upregulation of MafB/KLF4 and repression of Gfi-1. G to Mo differentiation required only low ectopic d.a.MKK6 expression levels as well as short-term (6 h) d.a.MKK6 expression. Moreover d.a.MKK6 rendered Mo or myeloid cell lines highly immunostimulatory. Since phospho-MKK6 expression marks Mo in rheumatoid arthritis (RA) joints we analyzed RA. Neutrophils from GCSF-mobilized but not normal blood differentiated to Mo in a murine RA model. Moreover, RA-associated pro-inflammatory cytokines enabled RANKL-dependent osteoclastogenesis from G-CSF mobilized human neutrophils via Mo intermediates in vitro. These data identified a critical role of MKK6/p38 as a trigger of emergency monocytopoiesis from left-shifted G under inflammatory situations.

The role of developmental concepts during epidermal Langerhans cell differentiation.

The expression of epithelial genes in LCs is a unique feature among hematopoietic cells. One crucial question is therefore, how hematopoietic LCs adopt these epithelial features. In a genome-wide analysis, we could identify the cytokine TGF- β 1 as an inducer of multiple epithelial genes during LC differentiation, including a restricted set of epithelia-associated transcription factors. We verified that LCs express a whole set of novel epithelial genes such as Cytokeratins (CK8,18). This indicates that LCs display substantial common features with epithelial cells, allowing them to integrate and interact within epithelial tissue. We therefore postulate that transcription factors involved in epithelial differentiation might also play a role in LCs. We currently address the involvement of candidate transcription factors identified by microarray in the induction of epithelial genes in LCs downstream of TGF- β 1. To perform functional studies, we use a state-of-art retroviral approach coupled with a Tet-inducible system, allowing to simultaneously express up to three genes in LC precursors and to induce gene-expression at specific time-points. This work will contribute to a better understanding of cellular mechanisms underlying LC function.

Grants

- FWF (Austrian Science Fund) SFB-2304 „TGF- β 1-polarized epithelial Langerhans cells - a candidate tolerogenic DC subset.“ 5.10.2004-30.8.2009
- FWF (Austrian Science Fund) P19425 „Transcriptional networks in human dendritic cell subset differentiation“ 1.2.2007-30.1.2010
- OeNB (Österreichische Nationalbank) Jubiläumsfonds #12111. “Signal integration by epithelial Langerhans cells” 1.7.2006-30.6.2008
- FWF (Austrian Science Fund) PhD Program Immunity and Inflammation. International PhD program, Deputy Director. 1.1.2007-31.12.2009
- FWF (Austrian Science Fund) Lise-Meitner Stipendium M1096-B13 to Dr. Sabine Witzel (now Konradi). Approved: 6.10.2008 (2 years)

Thesis

PhD Theses

- Florian Göbel: Molecular analysis of differentiation and life cycle of dendritic cells, completed 2008
- Susanne Richter: Regulation of myeloid subset differentiation: - Signals directing pDC development - Role of AhR ligation during myeloid cell differentiation and maturation. Started: August 2005
- Jennifer Jurkin: Transcriptional control of LC differentiation and activation. Started: February 2007
- Thomas Bauer: Novel surface receptors and their role in Langerhans cell differentiation and activation. Started: August 2007
- Nighat Yasmin: Regulation of epithelial gene expression during Langerhans cell differentiation. Started: February 2008
- Gregor Eisenwort: TACSTD2 – a novel molecule in Langerhans cell biology. Started: Sept. 2008

Diploma Thesis

- Anastasia Meshcheryakova: Molecular Analysis of p38MAPK Signal Integration in Granulocytes. Started: Nov. 2007
- Christina Mühlbacher: Transcription Factors in Myeloid Development – the Role of Krüppel-like factor 4 (KLF4). Started: January 2008
- Doris Kneidinger: Functional and molecular analysis of different transcription factors (Ahr, VDR and GATA-1) in dendritic cell development. Started: February, 2008

Awards

- Sabine Witzel: Lise-Meitner Scholarship from the Austrian Science Fund for her project „Regulation of epithelial properties in Langerhans cells“

Publications 2007-2009

- Jörgl, A., B.Platzer, S.Taschner, L.X.Heinz, B.Höcher, P.M.Reisner, F.Göbel, H.Strobl. Human Langerhans cell activation triggered in vitro by conditionally expressed MKK6 is counter-regulated by the downstream effector RelB. *Blood* 109:185-193 (2007)
- Taschner S., C.Koesters, B.Platzer, A.Jorgl, W.Ellmeier, T.Benesch, H.Strobl Downregulation of RXRa expression is essential for neutrophil development from granulocyte/monocyte progenitors. *Blood* 109:971-979 (2007)
- Strehl, S., K.Nebral, M.König, J.Harbott, H.Strobl, R.Ratei, S.Struski, B.Bieloraj, M.Lessard, M.Zimmermann, O.A.Haas, S.Izraeli. ETV6-NCOA2: a novel fusion gene in acute leukemia associated with coexpression of T-lymphoid and myeloid markers and frequent NOTCH1 mutations. *Clinical Cancer Research* 14:977-983 (2008)
- Göbel, F., S.Taschner, J.Jurkin, S.Witzel, C.Vaculik, S.Richter, D.Kneidinger, C.Mühlbacher, C.Bieglmayer, A.Bürger-Elbe, H.Strobl. Reciprocal role of GATA-1 and vitamin D receptor in human myeloid dendritic cell differentiation. Pre published *Blood*, Aug.31 DOI 10.1182/blood-2009-03-210484 (2009)
- Platzer, B., S.Richter, S., D.Kneidinger, D.Waltenbegger, M.Woitschläger, H.Strobl. Aryl hydrocarbon receptor activation inhibits in vitro differentiation of human monocytes and Langerhans dendritic cells. *J.Immunol.*, 183 (1): 66-74 (2009)