Functional cooperation of Toll-like receptor signaling and the high-affinity receptor for IgE, FcεRI, on human Langerhans cells

Dissertation

zur

Erlangung des Doktorgrades (Dr. rer. nat.)

der

Mathematisch-Naturwissenschaftlichen Fakultät

der

Rheinischen Friedrich-Wilhelms-Universität Bonn

vorgelegt von

Nicole Leib

aus

Gießen

Bonn 2017

Die vorliegende Arbeit wurde mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Rheinischen Friedrich-Wilhelms-Universität Bonn angefertigt.

Erstgutachter: Prof. Dr. Dr. Thomas Bieber
Zweitgutachter: Prof. Dr. Waldemar Kolanus

Tag der Promotion: 10.01.2018
Erscheinungsjahr: 2018

Danksagung

Herrn Prof. Bieber danke ich für die Betreuung dieser Arbeit, seine wissenschaftliche Unterstützung und seine konstruktive Kritik, die zum entscheidenden Gelingen dieser Arbeit beigetragen haben. Darüber hinaus danke ich Prof. Bieber für die Überlassung des Themas und die Bereitstellung des Arbeitsplatzes.

Meinen Dank möchte ich ebenfalls Herrn Prof. Kolanus für die Übernahme des Koreferats aussprechen.

Herrn Prof. Hölzel und Herrn Prof. Bovier danke ich für die bereitwillige Mitwirkung im Promotionsausschuss.

Table of contents

Abbreviations

- APC	Allophycocyanin
7-AAD	7-amino-actinomycin D
Ab	Antibody
AB/AM	Antibiotic-antimycotic
Amp	Amplicon
APC	Antigen presenting cell
BSA	Bovine serum albumin
CD34LC	$CD34^+$ hematopoietic stem cell derived LC
cDNA	Complementary DNA
d	day
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DTT	1,4-dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
e.g.	*Exempli gratia*, for example
et al.	*Et alii*, and others
FACS	Fluorescence activated cell sorter
FCS	Fetal calf serum
FITC	Fluorescein-5-isothiocyanat
FLT3L	Fms like tyrosine kinase 3 ligand
FSL-1	Pam2CGDPKHPKSF, lipoprotein

gam	Goat anti-mouse
GM-CSF	Granulocyte-macrophage colony-stimulating factor
h	Hour
HCl	Hydrochloric acid
Ig	Immunoglobulin
kDa	kilo Dalton
L	Liter
LB	Luria broth (complex medium)
LC	Langerhans cell
LPS	Lipopolysaccharides
LTA	Lipoteichoic acid
M	Mol
mAb	Monoclonal antibody
MACS	Magnetic activated cell sorter
max	maximum
MFI	Mean fluorescence intensity
Milli-Q	Ultrapure water (type I)
min	Minute
miRNA	Micro-RNA
mRNA	Messenger RNA
n.s.	Not specified
N/A	Not applicable
P3C	$Pam_3Cys\text{-}Ser\text{-}(Lys)_4$ trihydrochloride
PCR	Polymerase chain reaction

PE	Phosphatidylethanolamine
qPCR	Quantitative polymerase chain reaction
RBP	RNA binding protein
rFI	Relative fluorescence index
RNA	Ribonucleic acid
RT	Room temperature
s	Second
S.a.	*Staphylococcus aureus*
S.p.	*Streptococcus pyogenes*
SCF	Stem cell factor
SD	Standard deviation
TE	Transfection efficiency
TGF-ß	Transforming growth factor beta
T_H	T helper cell
TNF-α	Tumor necrosis factor alpha
T_{reg}	regulatory T cell
Tris	Tris(hydroxymethyl-)aminomethan
URE	upstream regulatory element
UTR	Untranslated region

für Ildiko und Benno

1 Introduction

In the 19th century, Robert Koch identified the microorganisms causing anthrax, tuberculosis and cholera and deduced that each infectious disease is caused by a specific microorganism. In our daily live, we are surrounded by a multitude of different microorganisms – some of them are pathogenic, some are not. The immune system has to discriminate between harmful and harmless microorganisms and agents. Evolutionary, various mechanisms of the innate and the adaptive immunity have been evolved to recognize and to protect the organism against pathogens, but at the same time sustain immune homeostasis, thus preventing allergic reactions or auto-immunity against self-antigens.

The innate immunity comprises first line defense properties such as epithelial barriers, antimicrobial peptides and enzymes and special soluble plasma peptides that build the complement system. Once microorganisms overwhelm the first line barriers, they are recognized by tissue resident phagocytic cells like monocytes, macrophages, granulocytes and dendritic cells (DC). By presenting processed antigens to T cells, DC link innate to adaptive immunity. In contrast to the innate immune functions, adaptive immune responses develop over lifetime as specific reactions to certain pathogens (adaption). The major cell types of the adaptive immunity, the T and B lymphocytes, are endowed with an enormous repertoire of highly specialized receptors. Once a pathogen has been recognized, an immunological memory is generated which protects an individual from reinfections with the hence known agent.

1.1 Dendritic cells

“The cytoplasm of this large cell is arranged in pseudopods of varying length, width, form and number, resulting in a variety of cell shapes ranging from bipolar elongate cells to elaborate, stellate or dendritic ones. […] The term “dendritic” cell would thus be appropriate for this particular cell type.”, wrote Ralph Marvin Steinman in 1973 when he and his colleagues first described murine splenic DC (Steinman and Cohn 1973).

Since the DC discovery, substantial research concerning their ontogeny and development, their heterogeneity, their sources in the body and their function, especially in mice, was performed. Although human material is more difficult and less frequently available than the

murine counterpart, recently efforts were increased to investigate the human DC system. This elucidates human and mouse homologues as well as differences between the species.

1.1.1 DC ontogeny

During the last decades, it became apparent that human DC arise from myeloid and from lymphoid progenitors or even so called multi-lymphoid progenitors (Chicha *et al.* 2004, Doulatov *et al.* 2010), which may give rise to lymphoid as well as myeloid cell types like DC. Recent studies affirmed and complemented that human DC arise from $CD34^+$ hematopoietic stem cells through a series of lineage-determining progenitors (Lee *et al.* 2015). In the course of a sequential differentiation, granulocyte-monocyte-DC progenitors (hGMDP) develop into human monocyte-dendritic progenitors (hMDP), which have restricted potential to become monocytes or committed DC progenitors (CDP) (Lee, Breton *et al.* 2015). CDP give rise to plasmacytoid DC (pDC) and to circulating pre-DC precursors, which are released into the blood and differentiate into the myeloid $CD1c^+$ DC and $CD141^+$ DC subsets in the respective tissues (Breton *et al.* 2015). The first assumption was that those myeloid DC (mDC) subsets develop from one unique pre-CDC precursor, but current studies uncovered two distinct pre-CDC precursors for each mDC subtype (Breton *et al.* 2016).

DC development and differentiation is promoted by a set of transcription factors like runt-related transcription factor 1 (Runx1), PU.1 (Schotte *et al.* 2004), Spi-B (Schotte, Nagasawa *et al.* 2004), Batf3 (Poulin *et al.* 2012), Irf-4, Irf-8 (Hambleton *et al.* 2011, Salem *et al.* 2014, Schiavoni *et al.* 2002, Tsujimura *et al.* 2003), Id2, E2-2 (Nagasawa *et al.* 2008) as well as by the hematopoietins fms like tyrosine kinase 3 ligand (FLT3L) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Kingston *et al.* 2009, Maraskovsky *et al.* 2000) whose signaling is mediated by the signal transducer and activator of transcription 3 (STAT3) and STAT5, respectively (reviewed in (Chicha, Jarrossay *et al.* 2004, Collin *et al.* 2013, Haniffa *et al.* 2015)). In contrast, Langerhans cells (LC), a skin- and mucosal-restricted DC subtype, develop independently from FLT3L and require macrophage colony-stimulating factor (M-CSF), STAT5 and transforming growth factor beta 1 (TGF-β1).

In vitro, human DC can be generated from blood monocytes as well as from bone marrow or umbilical cord blood-derived $CD34^+$ hematopoietic stem cells by feeding a combination of GM-CSF and IL-4 (Bender *et al.* 1996, Sallusto and Lanzavecchia 1994) and TNF-α, respectively (Caux *et al.* 1992, Caux *et al.* 1996). For *in vitro* generation of LC from $CD34^+$

hematopoietic stem cells, TGF-ß1 is strictly required (Strobl *et al.* 1996). In this study, LC were generated *in vitro* from umbilical cord blood-derived $CD34^+$ hematopoietic stem cells as described by Herrmann *et al.* (Herrmann *et al.* 2013).

1.1.2 DC subsets

Different subsets of human DC are classified based on their ontogeny (myeloid, plasmacytoid), their phenotype (i.e. expression of blood dendritic cell antigens, BDCA (Dzionek *et al.* 2000)) or their spatial distribution (tissues, lymph nodes, blood). Most commonly, DC are divided into two main subgroups: the myeloid and the plasmacytoid DC (reviewed in (Collin, McGovern *et al.* 2013, Guilliams *et al.* 2014, Ziegler-Heitbrock *et al.* 2010)). The two main groups are accompanied by further subtypes such as monocyte-derived DC, migratory dermal $CD14^+$ DC, epidermal Langerhans cells (LC) as well as inflammatory dendritic epidermal cells (IDEC) and TNF-α and inducible nitric oxide synthase (INOS) -producing DC (Tip-DC).

Regardless of their subtype, DC are characterized by their high expression of major histocompatibility complex (MHC) class II molecules and lack the lymphoid lineage-specific markers CD3, (T cells), CD19/CD20 (B cells) and CD56 (natural killer cells, NK) (reviewed in (Collin, McGovern *et al.* 2013, Ziegler-Heitbrock, Ancuta *et al.* 2010)).

Below, human DC subtypes will be summarized in short by their most commonly accepted descriptive and functional classifications.

1.1.2.1 Myeloid DC

Myeloid DC correspond to the murine “classical” or “conventional” termed DC. They express myeloid lineage-associated antigens CD13 and CD33 (Siglec-3, sialic acid binding Ig-like lectin 3) as well as the integrins CD11b and CD11c, but no CD14 or CD16 (FcγRIII). Myeloid DC can be further split into $CD1c^+$ DC (BDCA-1^+) and $CD141^+$ DC (BDCA-3^+).

Myeloid $CD1c^+$ DC represent the major DC population and are found in the blood, in epithelial tissues and in lymphoid organs. They sense different microbial antigens via a set of pattern recognition receptors like TLR1, TLR2, TLT4, TLR5 and TLR8 (summarized in (Haniffa, Bigley *et al.* 2015). The expression of CD1c and CD1a (restricted to tissue DC) enables $CD1c^+$ DC to process glycolipid antigens of pathogens like mycobacteria (Hunger *et*

al. 2004, Van Rhijn *et al.* 2013). Further on, $CD1c^+$ DC are endowed with the C-type lectins Dectin-1 (CLEC7A) and Dectin-2 (CELC6A) promoting fungal recognition (Ariizumi *et al.* 2000, Drummond and Brown 2011, McGreal *et al.* 2006) as well as CD205 (DEC-205) and CD206, which allow them to recognize mannose residues of bacterial cell envelopes. Dependent on the stimuli, activated $CD1c^+$ DC secrete the interleukins IL-8, IL-10, IL-12p70 and in part IL-23 (Morelli *et al.* 2005). They are potent stimulators of naïve T cells and are able to promote T helper cells 1 (T_H1), T_H2 and T_H17 responses (reviewed in (Collin, McGovern *et al.* 2013, Haniffa, Bigley *et al.* 2015)).

A second myeloid DC type are the $CD141^+$ DC which find their murine homologue in $CD8^+$ DC and represent only 0.05 % of the human peripheral blood lymphocytes. Depending on certain conditions, the expression of CD141 (thrombomodulin) can also be found on migratory DC and $CD1a^+$ DC. In addition, myeloid $CD141^+$ DC can be distinguished from other $CD141^+$ DC by their lower expression of CD11b and CD11c. In comparison to other DC subtypes, $CD141^+$ DC are superior in the presentation of exogenous antigens via MHC class I molecules (cross-presentation) to naïve cytotoxic $CD8^+$ T cells. $CD141^+$ DC are able to take up dead or necrotic cells via CLEC9A, a recently discovered C-type lectin that is supposed to be only expressed on the $CD141^+$ subtype (Schreibelt *et al.* 2012). Through TLR3 and TLR8 expression, $CD141^+$ DC sense self or viral nucleic acids. Upon activation, $CD141^+$ DC secrete TNF-α, the C-X-C motif chemokine 10 (CXCL10) and the type III interferon IFN-λ. They polarize $CD4^+$ T cells to T_H2 (reviewed in (Collin, McGovern *et al.* 2013, Haniffa, Bigley *et al.* 2015)).

1.1.2.2 Plasmacytoid DC (pDC)

Initially, pDC were termed due to their plasma cell-like morphology and their inherent ability of immunoglobulin gene rearrangements (Colonna *et al.* 2004, Rissoan *et al.* 2002). They represent the most numerous DC circulating in the blood and can also be found in the lymph nodes, but are not abundant in healthy tissues (Ebner *et al.* 2004, Zaba *et al.* 2007). They can be distinguished from other DC subtypes by the low or even lacking expression of the myeloid lineage-associated markers CD11b, CD11c, CD13 and CD33 and the expression of CD123 (IL-3 receptor, IL-3R), CD303 (CLEC4C, BDCA-2) and CD304 (neutropilin, BDCA-4). In response to viral nucleic acids sensed by TLR7 and TLR9, pDC secret large amounts of type I interferons (Cella *et al.* 1999, Siegal *et al.* 1999). pDC may induce T_H1, T_H2 (Rissoan

et al. 1999) and regulatory T cell (T_{reg}) differentiation (Moseman *et al.* 2004) and are able to cross-present exogenous antigens to $CD8^+$ T cells (Hoeffel *et al.* 2007).

1.1.2.3 Skin DC

Human skin DC represent a first cellular defense for invading microorganisms which overcame the epithelial barrier of the skin. They are involved in bridging innate and adaptive immunity by presenting antigens to T cells of skin-draining lymph nodes, but also in keeping homeostasis by inducing tolerance (Novak and Bieber 2008, Novak *et al.* 2004). In non-inflamed skin, epidermal LC, mDC and $CD14^+$ dermal DC, recently discussed as monocyte-derived macrophages by virtue of their transcriptional profile (McGovern *et al.* 2014), can be found. During inflammation, the composition of DC subsets is changing and pDC, LC and IDEC are selectively recruited to the site of inflammation (Wollenberg *et al.* 2002).

1.1.2.4 Langerhans cells

In 1868, the medical student Paul Langerhans discovered a dendrite-shaped cell in the human epidermis. In *Virchows Archiv: The European Journal of Pathology*, he stated the question of a stromal or nerve cell origin of those special cells. Because of their alternating number of dendrites, he assumed that those cells were cells of the nervous system (Langerhans 1868). More than 100 years later, it was apparent that the young student unknowingly was the first one to describe a dendritic cell subset of the epidermis that was later named after his discoverer Paul Langerhans.

Up to date numerous research results shed light on the distribution, the development and the function of LC in mice and more recently in human, too. As described by Paul Langerhans, the morphology of LC is characterized by a various number of dendrites of variable length and shape, which are more pronounced in the mature LC phenotype. A hallmark of LC is the expression of Birbeck granules, discovered by Michael Birbeck in 1961 (Birbeck *et al.* 1961). Birbeck granules are tennis-racket-shaped cell organelles formed by the C-type lectin Langerin (CD207) (Valladeau *et al.* 2003, Valladeau *et al.* 2000). Today, Langerin is the most prevalent marker to identify LC populations and antibodies against Langerin replaced the formerly used Birbeck granule-detecting Lag antibody that most likely

detects a Langerin epitope within the Birbeck granule (Kashihara *et al.* 1986, Valladeau, Ravel *et al.* 2000).

LC are located in the supra-basal layer of the epidermis as well as in various mucosae of the body like nasal and oral mucosae (Allam *et al.* 2006) or those of the intestine (reviewed in (Romani *et al.* 2010)). In steady-state, LC represent 2-5 % of the epidermal cells in adults. Additionally, LC can be found in the paracortex of human skin-draining lymph nodes (Angel *et al.* 2009). More than a half of the LC found in the lymph nodes typically show a mature phenotype indicating a preceding inflammatory process.

As opposed to the case with other DC, LC homeostasis in undisturbed skin is sustained by the self-renewing capability and the longevity of the cells (Kanitakis *et al.* 1993). This was shown in a patient receiving a hand graft by the fact that LC from the donor could be found for more than four years in the graft's skin (Kanitakis *et al.* 2004). Moreover, mice experiments have shown that recruited monocytes can give rise to LC to repopulate the skin after inflammation-induced LC emigration (Ginhoux *et al.* 2006). In contrast to DC, LC can develop independently of FLT3L, but are strictly TGF-ß1-dependent as shown in TGF-ß1 knock-out mice that do not develop LC (Borkowski *et al.* 1996). Moreover, knock-out of the TGF-ß1 downstream transcription factors Id2 and Runx3 abolished LC development, too (summarized in (Romani, Clausen *et al.* 2010)). TGF-ß1 is secreted by the LC themselves supporting the self-renewal capacity in an autocrine way (Kaplan *et al.* 2007). *In vitro*, human LC can be generated from $CD34^+$ hematopoietic progenitors in the presence of FLT3L, stem cell factor (SCF), GM-CSF and TGF-ß1 (Strobl, Riedl *et al.* 1996).

In line with other DC, LC take up antigens by a numerous set of pattern recognition receptors (PRR) like Toll-like receptor 2 (TLR2) heterodimers and TLR4 (Novak *et al.* 2010). Activated LC migrate to skin draining lymph nodes and present the antigen/MHC complex to naïve T cells. Notably, LC are able to cross-present exogenous antigens and efficiently drive $CD8^+$ cells to become cytotoxic effector T cells (Klechevsky *et al.* 2008). Depending on the maturation state, the antigen and the given microenvironment, LC are described not only to induce immunity, but also tolerance, *i.e.* by negatively regulating T cell proliferation (Shklovskaya *et al.* 2011) or by the production of the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) (von Bubnoff *et al.* 2004) and the T_H1 anergy inducing IL-10 (Enk *et al.* 1993).

1.1.2.5 Inflammatory dendritic epidermal cells (IDEC)

In inflamed skin, a DC population was found which appears morphologically (dendrite-shaped), phenotypically ($CD1a^+$, $HLA\text{-}DR^+$,$Fc\varepsilon RI^+$) and locally (epidermal) similar to LC, but was strictly distinct from classical LC by the lack of Birbeck granules and the expression of the mannose receptor DC206 and the integrins CD11b and CD11c (Wollenberg *et al.* 1996, Wollenberg, Mommaas *et al.* 2002). Those DC were termed inflammatory dendritic epidermal cells (IDEC) and could be found in most inflammatory skin conditions like atopic dermatitis (AD) and psoriasis vulgaris. *In vitro*, IDEC can be generated under reducing conditions from monocytes of atopic donors (Novak *et al.* 2002).

1.1.2.6 TNF-α and INOS-producing DC (Tip-DC)

In contrast, TNF-α and INOS-producing DC (Tip-DC) can be found in the context of psoriasis vulgaris and are located in the dermis as well as the epidermis. Tip-DC belong to the myeloid $CD11c^+$ DC population and express typical DC markers such as HLA-DR, CD40, CD83 and CD86, but lack Langerin, CD1a and CD14 antigens. As indicated by their name, Tip-DC are potential mediators of inflammation by the release of TNF-α and INOS (Lowes *et al.* 2005, Serbina *et al.* 2003, Wilsmann-Theis *et al.* 2013).

1.1.3 DC function

Immature DC sense and take up antigens by different receptor-dependent mechanisms including mannose receptor-mediated endocytosis of mannosylated microbial protein antigens (Wollenberg, Mommaas *et al.* 2002) or FcγRII-mediated internalization of antigen-receptor complexes (reviewed in (Nimmerjahn and Ravetch 2007)). Antigen-uptake and internalization is often accompanied by DC maturation. During this process, DC undergo a series of phenotypical and functional alterations such as the upregulation of surface MHC class II molecules and the co-stimulatory molecules CD40, CD80, CD86 and CD83. Additionally, migratory capacities increase due to the upregulation of the CCRX chemokine 7 receptor (CCR7). During their maturation, DC almost lose their phagocytic properties, but gain their T cell priming capability.

In general there are two ways of antigen presenting via MHC molecules depending on the nature of the antigens. Intracellular antigens including self-proteins are normally degraded proteasomally and assemble with MCH class I molecules in the endoplasmic reticulum before the complex is transported to the DC's surface for recognition by $CD8^+$ T cells. In contrast, extracellular antigens are processed and loaded on MHC class II molecules in late endosomal compartments. Antigen/MHC class II complexes are recognized by $CD4^+$ T cells (reviewed in (Blum *et al.* 2013)). In some DC subsets including LC, exogenous antigens might be presented by MHC class I complexes. This so called cross-presentation enables antigen presenting cells (APC) to present exogenous antigens to $CD8^+$ T cells and induce either immunity (i.e. in the case of pathogens) or tolerance against self-antigens by peripheral deletion of autoreactive $CD8^+$ T cells (Kurts *et al.* 2001).

The kind of antigen, its dose and the duration of contact in a given environment may induce the processing pathway and select the MHC, but the T cell response leading to either immunity or tolerance is dictated by a series of co-stimulatory and accessory molecules and signals. The first of three signals determining the T cell fate is the binding of the T cell receptor (TCR) to the respective antigen/MHC complex. Standing alone, signal 1 promotes tolerance by provoking anergy or deletion of the respective naïve T cell (Wang *et al.* 2002). The second signal encompasses co-stimulatory molecules consisting of receptors of the APC and its respective ligand on the T cell. DC express CD80/CD86 (B7.1/B7.2) for CD28 binding that results in IL-2 production and subsequent T cell proliferation, while the binding of the anti-proliferative cytotoxic T-lymphocyte antigen 4 (CTLA-4) leads to peripheral tolerance (Probst *et al.* 2005). Moreover, the absence of CD80/CD86 on the DC leads to anergy and deletion of the respective T cell. Third, the fate of a T cell to develop into T_H1, T_H2 or into cytotoxic T cells (CTL) is dictated by the expression of T_H cell-polarizing molecules such as IL-12 family members, type I interferons or TGF-ß for T_H1 priming or the C-C motif ligand 2 (CCL2; also known as monocytic chemotactic protein 1 (MCP1)) and OX40 ligand for T_H2 development (reviewed in (Kapsenberg 2003)).

1.1.4 The role of DC in atopic dermatitis

Atopic dermatitis (AD) is a multifactorial chronic relapsing skin disease with increasing incidence in western industrialized countries. The disease usually starts in the early childhood and is often the beginning of the so called atopic march that is followed

characteristically by allergic rhinitis or allergic asthma (reviewed in (Bieber 2008)). One hallmark of the disease is a genetically predisposed impairment of epidermal barrier functions which facilitate the entrance of foreign antigens into skin compartments, where they can activate resident DC (reviewed in (Novak 2012)).

Unlike LC, which are present in healthy and infected skin, IDEC are restricted to inflamed skin only. In contrast to other skin diseases like psoriasis vulgaris or contact dermatitis, the amount of pDC in dermal AD skin is controversially discussed varying from absent (Wollenberg *et al.* 2002) to substantial numbers (Guttman-Yassky *et al.* 2007). However, diminished pDC expression in AD may explain the enhanced susceptibility for viral infections like the herpes simplex -induced eczema herpeticum (Wollenberg, Wagner *et al.* 2002).

Both IDEC and LC, but not pDC, express CD1a which mediates the presentation of lipid antigens to T cells (reviewed in (Mizumoto and Takashima 2004)). Additionally, skin DC in AD are equipped with a particular set of intra- and extracellular TLR. TLR2 is expressed by epidermal LC as well as dermal DC (reviewed in (Novak, Koch *et al.* 2010)). For ligand binding, TLR2 forms heterodimers with TLR1 and TLR6 (Biedermann 2006). TLR1/2 heterodimers recognize triacylated lipopeptides like Pam_3Cys-Ser-$(Lys)_4$ (P3C) (Buwitt-Beckmann *et al.* 2006) while TLR2/6 heterodimers interact with diacylated lipopeptides such as Pam_2CGDPKHPKSF (FSL-1) (Skabytska *et al.* 2014). *In vivo*, TLR2 heterodimers are described to bind bacterial peptidoglycans like from the Gram-positive bacterium *Staphylococcus aureus* (*S.a.*) , which predominantly colonizes the skin of AD patients (Boguniewicz and Leung 2010). In contrast, TLR4 represents the main structure in recognizing lipopolysaccharides (LPS) of Gram-negative bacteria. Human skin LC, but not oral and mucosal LC, are described to lack TLR4 expression and do not respond to LPS (Allam *et al.* 2008, Flacher *et al.* 2006, van der Aar *et al.* 2007). Controversially, unpublished results of our group showed that *ex vivo* skin LC of healthy individuals are responding to LPS (personal communication Tim Stroisch). However, TLR-mediated activation of DC usually drives them to leave the skin towards skin-draining lymph nodes for antigen presentation and T cell priming. Thereby, the particular microenvironment, the dose, the exposure duration and the type of invading pathogens dictate the T cell developmental direction towards T_H1, T_H2 or T_{reg} (Fujita *et al.* 2011).

1.2 The high-affinity receptor for IgE, FcεRI

A hallmark of skin DC of atopic individuals is the expression of the high-affinity receptor for IgE, FcεRI ((Bieber *et al.* 1992) and reviewed in (Bieber 2008)). FcεRI is a multimeric immune receptor existing in a tetrameric and a trimeric form. The tetrameric receptor consists of one α-chain for IgE binding, one ß-chain with signal amplifying and stabilizing functions (Lin *et al.* 1996) and two γ-chains for signal transduction. The ß-chain and the γ-chains contain intracellular immunoreceptor tyrosine-based activation motifs (ITAM) which are phosphorylated after cross-linking of the receptor and induce the downstream signaling. The γ-chain is shared by other Fc receptors like FcγRI and FcγRII. In mice and humans, the tetrameric form is expressed on mast cells and basophiles. In contrast, the ß-chain lacking trimeric form is restricted to human APC like DC and LC (Bieber, de la Salle *et al.* 1992, Kinet 1999). Rodents do not express FcεRI on DC. In AD, the trimeric FcεRI ($\alpha\gamma_2$) shows a heterogeneous and higher expression on LC and especially on IDEC compared to skin DC from healthy individuals (Herrmann, Koch *et al.* 2013, Jurgens *et al.* 1995, Wollenberg, Kraft *et al.* 1996). The high expression of the receptor correlates with serum IgE levels and the severity of the disease (Potaczek *et al.* 2014, Weidinger *et al.* 2008). Binding of monomeric, allergen-independent IgE stabilizes the receptor on the cell surface, while cross-linking of the receptor by specific allergen-bound IgE leads to internalization and subsequent processing of the allergen-IgE-FcεRI complexes by MHC class II compartments (Maurer *et al.* 1998). Allergen-derived antigens are presented to naïve T cells of skin-draining lymph nodes or locally to transiting antigen-specific T cells in the context of secondary immune responses. Thereby, T_H2 responses are mainly induced by LC, while IDEC enhance pro-inflammatory T_H1-mediated INF-γ release (reviewed in (Novak and Bieber 2005)). This phenomenon explains why the initial phase of inflammation in AD skin is characterized by T_H2 responses by skin resident LC, while the later phase switches to T_H1 prevalence due to invading IDEC that occurs within 72 hours and is maintained in chronic AD (Kerschenlohr *et al.* 2003). Moreover, cross-linking of FcεRI on DC can induce the production of pro-inflammatory cytokines such as TNF-α, IL-1ß or IL-8 and chemokines like CCL2 via the activation of nuclear factor-κB (NF-κB) (Kraft *et al.* 2002). More recently, FcεRI-mediated tolerogenic properties such as the release of the anti-inflammatory interleukin IL-10 (Novak *et al.* 2001) and the production of the immunoregulatory enzyme indoleamine, 2,3 dioxygenase (IDO) (von Bubnoff, Bausinger *et al.* 2004, von Bubnoff *et al.* 2004) have been reported. Our former studies revealed that TLR-mediated activation of *in vitro* generated LC

results in a downregulation of FcεRI expression (Herrmann, Koch *et al.* 2013), thus influencing the course of AD by cross-talking of those receptors.

FcεRI is transcriptionally regulated by two promotor elements. The proximal promotor is found in humans and rodents, while the distal promotor element is specific only for humans. The proximal promotor contains binding sites for two E26 transformation-specific (ETS) transcription factors, namely PU.1 (encoded by the *SPI1* gene) and E74-like factor 1 (Elf-1), and for the GATA-binding factor 1 (Gata-1). Furthermore, transcription factor Yin-Yang-1 (Yy1) shares an overlapping binding site with PU.1 and Elf-1 (see Figure 1) (Nishiyama, Hasegawa *et al.* 2002). Binding of PU.1 and Yy1 promotes FcεRIα transcription, while Elf-1 acts inhibitory on the FcεRI promotor element by competing with PU.1 for the respective binding site (Wang *et al.* 2008). Gata-1 is required for FcεRI expression on mast cells, but is rather weakly expressed in LC (Herrmann, Koch *et al.* 2013, Nishiyama *et al.* 2005) compared to PU.1. Additionally, upstream of the PU.1/Elf-1/Yy1 binding region high mobility group box 1 and/or box 2 proteins (Hmgb1/Hmgb2) and the transcription factor Sp1 share a binding site in the promotor (see Figure 1). Which of those factors bind to the promotor sequence depends on the given single nucleotide polymorphism (SNP) in the binding region. Alleles promoting Hmgb1/2 binding reveal a stronger promotor activity compared to those that bind Sp1 (Kanada, Nakano *et al.* 2008).

The distal promotor is IL-4-dependent. In contrast to the proximal promotor, the activity of the distal one is negatively regulated not only by Elf-1, but also by PU.1 and Yy1 (Hasegawa *et al.* 2003).

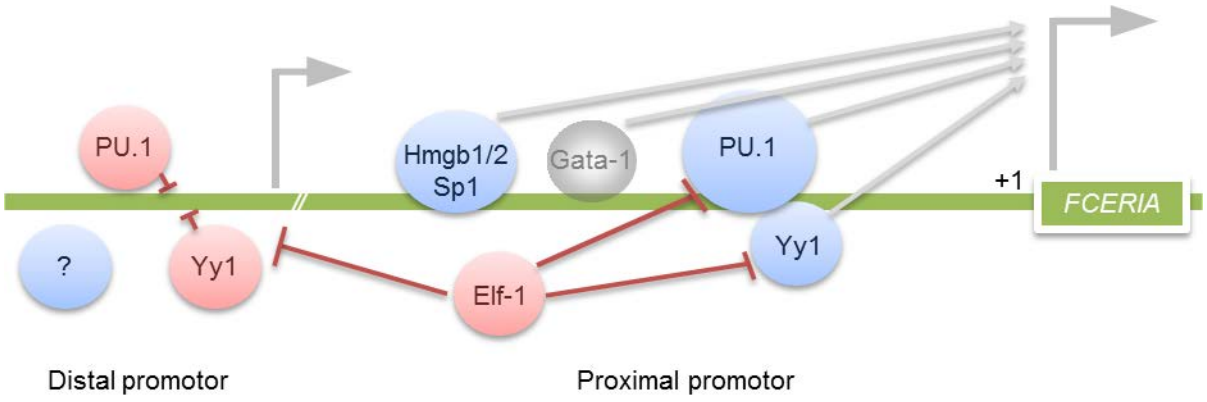

Figure 1 ***Schematic representation of the FCER1A promotor.*** Positive regulators are depicted in blue; negative ones in red. Gata-1 (grey) is not expressed in CD34LC. (Adapted from (Kanada *et al.* 2008, Nishiyama *et al.* 2002))

Among the FcεRI-associated transcription factors, PU.1 is stronger expressed in LC compared to Elf-1 and Yy1 and is regulated upon activation of the cells (Herrmann, Koch *et al.* 2013). In general, PU.1 is highly expressed in myeloid DC subsets and controls DC development *inter alia* by the regulation of the hematopoietic factor FLT3 (Carotta *et al.* 2010). Similarly, PU.1 enhances LC development in concert with the TGF-ß1 responsive transcription factor Runx3 (Chopin *et al.* 2013, Heinz *et al.* 2006). To date, more than 100 PU.1 target genes have been identified. Those are predominantly genes coding for extracellular or membrane-associated proteins such as antibodies and antibody receptors like *FCER1A*, *FCER2* or genes for Fc gamma receptors (*FCGR1A*, *FCGR2B*, *FCGR3A*) (summarized in (Turkistany and DeKoter 2011)).

Since a precisely regulated expression of PU.1 is crucial for normal hematopoiesis, several mechanisms exist for the control of PU.1 activity and expression. First, the PU.1 expression is transcriptionally regulated by the binding of tissue-specific transcription factors to the PU.1 promotor. In myeloid cells, the transcription factors Sp1 and mainly PU.1 itself promote *SPI1* gene expression (Chen *et al.* 1995, Hoogenkamp *et al.* 2007). Additionally, distal regulatory elements upstream of the *SPI1* gene promotor cooperate to induce PU.1 expression. Thereby, CCAAT/enhancer binding protein alpha (C/EBPα) opens the chromatin structure of the upstream regulatory element for autoregulatory PU.1 binding (Leddin *et al.* 2011). Secondly, protein interactions can influence the binding of PU.1 to a given promotor. C/EBPα plays a reciprocal role in PU.1 regulation. It physically inhibits the function of PU.1 by displacing its co-activator c-Jun and thereby abolishes PU.1-induced DC development (Reddy *et al.* 2002). Furthermore, PU.1 function can be blocked by the interaction of the Gata-1 zinc finger domain with PU.1's DNA-binding domain (Nerlov *et al.* 2000). Thirdly, post-transcriptional mechanisms can modulate PU.1 protein levels. Non-coding antisense RNA have been described to negatively regulate PU.1 gene expression through translational interference (Ebralidze *et al.* 2008). Another way to prevent the translation of mRNA transcripts into a functional protein is the binding of micro-RNA (miRNA). This will be the topic of the next section.

1.3 micro-RNA in the immune system

In the 1990, it was found that in *Caenorhabditis elegans* (*C. elegans*) a small RNA encoded by the lin-4 gene was able to suppress lin-14 protein expression by antisense complementarity to the gene's 3' untranslated region (3' UTR) (Lee *et al.* 1993, Wightman *et*

al. 1993). A couple of years later, another small regulatory RNA, let-7, was discovered (Reinhart *et al.* 2000) and homologues of this small RNA were found in several other metazoan lineages including vertebrates, molluscs, annelids and arthropods (Pasquinelli *et al.* 2000). Nowadays, the term micro-RNA has been assigned to these special small (~ 22 nucleotides, nt) regulatory RNA molecules.

1.3.1 Origin of miRNA

Primary miRNA precursor molecules (pri-miRNA) originate from independent genes or from intronic regions of protein-coding genes (Rodriguez *et al.* 2004, Saini *et al.* 2007). In the canonical pathway, the RNA polymerase II processes hairpin-shaped gene transcripts, which are further cleaved into ~70 nt pre-miRNA by the RNase III family member enzyme Drosha. Some miRNA orginate from short introns and are processed into pre-miRNA in a spliceosome complex independently of Drosha (non-canonical pathway). The pre-miRNA are exported into the cytoplasm, where Dicer, another RNase III family member enzyme, cleaves the pre-miRNA products into a double-stranded (ds) miRNA molecule. In the miRNA biogenesis, Drosha and Dicer operate in concert with accessory proteins and enzymes like ds-RNA-binding-proteins (RBP) or transactivation-responsive RBP. Typically, the ds-miRNA possesses one guide strand and one passenger strand. The guide strand is preferentially loaded into a miRNA-silencing complex (miRISC), where the mature miRNA base pairs with its respective mRNA. In opposition to former assumptions, where the passenger strand was determined to degradation, it is now accepted, that this strand can also be incorporated into the miRISC. Besides a number of cofactors, Argonaute (AGO) proteins and glycine-tryptophan protein of 182 kDa (GW182) represent the core components of the miRISC. In mammals, AGO2 proteins interact with the miRNA and promote mRNA cleavage and subsequently degradation, while GW182 proteins contain functional regions which repress the translation or facilitate 3‘ deadenylation of the mRNA (Krol *et al.* 2010).

Since the spatiotemporal expression of miRNA is indispensable for adequate protein expression from very early developmental processes on, their expression, processing and function have to be strictly controlled. Similar to protein-coding genes, miRNA encoding genes can be activated by transcription factors, enhancers and transcriptional co-factors. miRNA often act in autoregulatory feedback circuits by targeting factors involved in their biogenesis or their function. Additionally, several factors regulate miRNA processing through

protein-protein interaction as described for the proteins Drosha and Dicer, whose function is dependent on sufficient RBP levels. Modifications of the 3' end through adenylation, uridylation or methylation can further stabilize or destabilize the miRNA. Moreover, miRNA function can be altered by the respective AGO protein or the AGO protein level as shown for AGO2, where low levels are limiting the miRISC loading.

1.3.2 miRNA function in the immune system

The role of miRNA in immune dysfunctions such as allergic diseases became more evident during the last years. Several miRNA were discovered to be involved in the regulation of inflammation, in immune receptor expression and signal transduction processes. Among them, miRNA-146a-5p (abbreviated to miRNA-146a) and miRNA-155-5p (abbreviated to miRNA-155) may be the most intensively studied miRNA in pro- and in anti-inflammatory immune responses (reviewed in (Rebane and Akdis 2014)). Both miRNA have been described to be involved in different TLR signaling processes.

In 1997, Tam *et al.* became aware that the B cell integration cluster (*BIC*) gene is activated by proviral integration of the avian leucosis virus in B cell lymphomas. Interestingly, the *BIC* gene was found to lack an open reading frame, but formed an imperfect RNA duplex probably functioning as a non-coding RNA (Tam 2001, Tam *et al.* 1997). Further studies revealed that miRNA-155 is processed by the *BIC* gene and that both, *BIC* as well as miRNA-155, accumulate in human lymphoma cells (Eis *et al.* 2005). Due to the fact that the *BIC* gene harbors the primary miR-155 (pri-miR-155) in a phylogenetically conserved region of the *BIC* gene, the gene's name was designated MIR155 host gene (*MIR155HG*). In the following years, miRNA-155 was found to be involved in many physiological and pathological processes like hematopoiesis, immunity, inflammation or cancer (reviewed in (Elton *et al.* 2013)). For example, miRNA-155 is indispensable for normal immune function as shown by miRNA-155 deficient mice, which produce less IgM, less antigen-specific antibodies and show an impaired production of IL-2 and IFN-γ cytokines by splenocytes upon activation (Rodriguez *et al.* 2007). During monocyte differentiation into DC (mDC), miRNA-155 is upregulated and is involved in the regulation of DC development as well as in apoptotic processes (Lu *et al.* 2011). DC lacking miRNA-155 are incapable of presenting antigens to naïve T cells (Rodriguez, Vigorito *et al.* 2007). Compared to DC, the expression of miRNA-155 is low in LC (Jurkin *et al.* 2010). In different myeloid cells, miRNA-155 is

highly induced upon activation through different TLR ligands such as Pam_3Cys-Ser-$(Lys)_4$ (P3C) for TLR2, polyriboinosinic:polyribocytidylic acid (poly(I:C)) for TLR3, lipopolysaccharides (LPS) for TLR4 and hypomethylated DNA for TLR9 (O'Connell *et al.* 2007).

For miRNA-155, a huge number of validated and predicted target mRNA (http://www.mirbase.org/) is indicative for the multi-functionality of this special miRNA that is involved in many biological and pathological processes like haematopoiesis, cancer and inflammation (reviewed in (Faraoni *et al.* 2009)). It directly targets many key regulators such as *PU.1* (Martinez-Nunez *et al.* 2009) or *C/EBPß* (Costinean *et al.* 2009). In immunity, miRNA-155 exhibits pro- and anti-inflammatory functions. The pro-inflammatory ones are based on targeting the suppressor of cytokine signaling 1 (*SOCS-1*), which attenuates the production of inflammatory cytokine production (Wang *et al.* 2010), or the inositol polyphosphate-5-phosphatase D (SHIP-1), a negative regulator of myeloid cell proliferation (Cremer *et al.* 2009, O'Connell *et al.* 2009). Moreover, miRNA-155 overexpression in activated $CD4^+$ T cells is expected to promote T_H1 proliferation by suppressing IFN-γ (Banerjee *et al.* 2010). Additionally, miRNA-155 targets *CTLA-4*, an anti-proliferative molecule in T cell responses (Sonkoly *et al.* 2010). On the other side, the termination of inflammation is supported by targeting TLR/IL-1 signaling downstream factors like the myeloid differentiation primary response gene 88 (*MYD88*) or the TGF-ß activated kinase 1/MAP3K7 binding protein 2 (*TAB2*) (Ceppi *et al.* 2009).

By targeting mRNA of genes mentioned above, miRNA-155 is suggested to influence cytokine release, T cell proliferation and inflammation in allergic disease such as AD, where miRNA-155 is elevated in lesional skin compared to skin of healthy donors (Sonkoly, Janson *et al.* 2010). Major contributors to the increased miRNA-155 levels in inflamed skin are T cells and DC.

While miRNA-155 has pro- and anti-inflammatory properties, miRNA-146a has been described to act predominantly as a negative regulator of innate immune responses (reviewed in (Saba *et al.* 2014)). In human LC, miRNA-146a shows a high basal expression level which is maintained by a TGF-ß1-dependent PU.1 expression, a positive regulator for this miRNA (Jurkin, Schichl *et al.* 2010). Upon TLR2 engagement, miRNA-146a expression was shown to be further elevated in the monocytic cell line THP-1 but not in LC (Taganov *et al.* 2006). miRNA-146a is not only regulated through PU.1, but also through NF-κB which in turn is increased upon TLR activation. In an auto-regulatory loop, miRNA-146a controls its

own expression by reducing NF-κB expression through targeting TLR signaling upstream molecules such as TNF receptor-associated factor 6 (*TRAF6*) or IL-1 receptor-associated kinase 1 (*IRAK1*) (Taganov, Boldin *et al.* 2006). In monocytes, miRNA-146a plays a pivotal role in endotoxin tolerance against LPS-induced TLR4 activation. Cells subsequently primed with low doses of LPS show a hyporesponsiveness to LPS as a result of miRNA-146a-dependent TRAF6 and IRAK1 reduction (Nahid *et al.* 2009). Moreover, challenging monocytes with LPS induces cross-tolerance to other TLR ligands like peptidoglycan (PGN), P3C or even inactivated bacteria (Nahid *et al.* 2011). In AD, miRNA-146a levels are upregulated in cultured primary keratinocytes and lesional as well as non-lesional skin samples. Here, miRNA-146a inhibits the expression of several pro-inflammatory factors such as IL-6, IL-8 or the chemokine C-C motif ligand 8 (CCL8) via IRAK1 and caspase recruitment domain-containing protein 10 (CARD10) -dependent NF-κB reduction. Moreover, miRNA-146a directly targets the inducible chemokine C-C motif ligand 5 (*CCL5*, also known as *RANTES*), a chemokine for the recruitment of T cells, macrophages and eosinophils to the site of inflammation (Rebane *et al.* 2014). Thus, in AD miRNA-146a is an important player in controlling NF-κB- and CCL5-dependent skin inflammation.

1.4 Objectives of this work

Allergic diseases show an increasing prevalence mostly in industrialized countries. Among them, AD is considered to be the most common inflammatory skin disease that appears in 10 to 20 % during the childhood and persists throughout adulthood in 1 to 3 %. People suffering from AD are often restricted in their life quality. Although considerable research shed light on the genetics, the epidemiology and the pathophysiology of the disease, there is further need to understand the complexity of AD and to evolve new therapeutic strategies. Previous work has demonstrated that skin DC expressing FcεRI play a pivotal role in the course and the severity of TLR-induced skin inflammation (Herrmann, Koch *et al.* 2013).

This study was designed to gain further insights into the molecular regulation of FcεRI on human DC. For this purpose, a model of human $CD34^+$-derived LC expressing FcεRI was used:

- first to analyze the regulation of FcεRI upon stimulation of the cells with different TLR ligands as well as with heat-killed bacteria suspensions to mimic AD skin conditions;

- secondly to elucidate how FcεRI is regulated at the transcriptional level and

- thirdly to find out how the factors promoting FcεRI expression are regulated in steady-state cells compared to inflammatory conditions.

2 Material

2.1 Chemicals

Table 1 Chemicals

Chemical	Manufacturer
2-Mercaptoethanol	Sigma-Aldrich Chemie GmbH; Taufkirchen, Germany
7-AAD	Sigma-Aldrich Chemie GmbH; Taufkirchen, Germany
Agarose	Sigma-Aldrich Chemie GmbH; Taufkirchen, Germany
Antibiotic-antimycotic (100x)	Gibco®; Karlsruhe, Germany
BSA	Sigma-Aldrich Chemie GmbH; Taufkirchen, Germany
Chloroform	AppliChem GmbH; Darmstadt, Germany
Dextran	Sigma-Aldrich Chemie GmbH; Taufkirchen, Germany
DMSO	Sigma-Aldrich Chemie GmbH; Taufkirchen, Germany
dNTP	Life Technologies GmbH; Darmstadt, Germany
DTT	Carl Roth GmbH & Co. KG; Essen, Germany
EDTA	Merck KGaA, Darmstadt, Germany
Ethanol	AppliChem GmbH; Darmstadt, Germany
Ethidium bromid solution	Sigma-Aldrich Chemie GmbH; Taufkirchen, Germany
Fetal calf serum (Gibco®)	Life Technologies GmbH; Darmstadt, Germany
FLT3L (rhFlt3/Flk2 ligand)	R&D Systems Inc.; Minneapolis, USA
Gel Loading Dye Blue, (6x)	New England Biolabs; Frankfurt am Main, Germany
Glycin	AppliChem GmbH; Darmstadt, Germany

Table 1 Chemicals (continued)

Chemical	Manufacturer
GM-CSF	Bayer AG; Leverkusen, Germany
Human IgG, Myeloma	Calbiochem® by Merck Chemicals GmbH; Darmstadt, Germany
Isopropanol	AppliChem GmbH; Darmstadt, Germany
LB Broth (Lennox)	Carl Roth GmbH + Co. KG; Karlsruhe, Germany
Lipofectamine® RNAiMAX Transfection Reagent (Ambion™)	Life Technologies GmbH; Darmstadt, Germany
Lymphoprep™	Progen Biotechnik GmbH; Heidelberg, Germany
Normal mouse serum	Jackson ImmunoResearch Labaratories, Inc; West Grove, USA
Opti-MEM® I Reduced Serum Medium (Gibco™)	Life Technologies GmbH; Darmstadt, Germany
Pam3Cys-Ser-(Lys)4 trihydrochloride	EMC microcollections GmbH; Tübingen, Germany
Quick-Load® 2-Log DNA Ladder (0.1-10.0 kb)	New England Biolabs; Frankfurt am Main, Germany
Quick-Load® 50 bp DNA Ladder	New England Biolabs; Frankfurt am Main, Germany
RPMI Medium 1640 (1x) + GlutaMAX™ -I (Gibco®)	Life Technologies GmbH; Darmstadt, Germany
SCF	R&D Systems Inc.; Minneapolis, USA
SYBR® Green Supermix with ROX™	Bio-Rad Laboratories Inc.; Hercules, USA
TGF-ß	R&D Systems Inc.; Minneapolis, USA
TNF-α	R&D Systems Inc.; Minneapolis, USA
TRIzol®	Life Technologies GmbH; Darmstadt, Germany

2.2 Buffers

Table 2 Composition of buffers

Buffers (1x)	Composition
FACS buffer	137 mM NaCl; 75 mM Na_2HPO_4; 32.5 mM NaH_2PO_4; 10 % (v/v) FCS; 155 mM NaN_3
MACS buffer	137 mM NaCl; 2.7 mM KCl; 8.5 mM Na_2HPO_4; 1.47 mM KH_2PO_4; 0.5 % (w/v) BSA; 1 % (v/v) 0.5 M EDTA
PBS	137 mM NaCl; 2.7 mM KCl; 8.5 mM Na_2HPO_4; 1.47 mM KH_2PO_4
TAE buffer	40 mM Tris/acetic acid, pH 7.8; 10 mM NaAc; 1 mM EDTA, pH 8.0

2.3 Cell culture media

Table 3 Composition of cell culture media

Name	Composition
$CD34^+$ medium	RPMI Medium 1640 (1x) + GlutaMAX™ -I; 10 % (v/v) FCS; 1 % (v/v) AB/AM; 50 µM 2-mercaptoethanol
$CD34^+$ transfection medium	RPMI Medium 1640 (1x) + GlutaMAX™ -I; 10 % (v/v) FCS; 50 µM 2-mercaptoethanol
freezing medium	FCS; 10 % DMSO
LB medium	Tryptone 10 g/L; Yeast extract 5 g/L; NaCl 5 g/L; ad 1 L distilled water

2.4 Kits

Table 4 Kits

Kit	Manufacturer
BD Cytofix/Cytoperm™	Beckton Dickinson GmbH; Heidelberg, Germany
CD1a MicroBeads, human	Miltenyi Biotec GmbH; Bergisch Gladbach, Germany
DNA-free™ DNA Removal Kit (Ambion®)	Life Technologies GmbH; Darmstadt, Germany
Indirect CD34 MicroBead Kit, human	Miltenyi Biotec GmbH; Bergisch Gladbach, Germany
Invisorb® Spin Plasmid Mini Two	STRATEC Biomedical AG; Birkenfeld, Germany
TaqMan® MicroRNA Assays - hsa-miR-155-5p (ID 002623) - hsa-miR-146a-5p (ID 000468) - RNU48 (ID 001006)	Life Technologies GmbH; Darmstadt, Germany

2.5 Antibodies

Table 5 Antibodies used for flow cytometry analysis

Antibody / conjugation	Immunogen / specificity	Clone	Species	Isotype	Manufacturer
CD14-APC	human CD14	TÜK4	mouse	IgG2a	Miltenyi Biotec GmbH; Bergisch Gladbach, Germany
CD34-PE	human CD34	581	mouse	IgG1, κ	Beckton Dickinson GmbH; Heidelberg, Germany
CD83	human CD83	HB15a	mouse	IgG2b	Beckman Coulter Inc.; Krefeld, Germany
FcεRIα	human FcεRIα	AER-37 (CRA1)	mouse	IgG2b, κ	eBioscience; San Diego, USA
Goat anti-mouse IgG, Fc_γ-FITC	mouse IgG Fcγ	polyclonal	goat	IgG	Jackson ImmunoResearch Europe Ltd.; Suffolk, UK
Langerin (CD207)	CD34-derived dendritic cells	DCGM4	mouse	IgG1	Beckman Coulter Inc.; Krefeld, Germany
PU.1	human PU.1	A-7	mouse	IgG1	Santa Cruz Biotechnology, Inc.; Heidelberg, Germany
CD1a-RD1 (T6-RD1)	human CD1a	SFCI19Thy1 A8	mouse	IgG1	Beckman Coulter Inc.; Krefeld, Germany
TLR2	human TLR2	1030A5.138	mouse	IgG1	Immgenex; San Diego, USA

Table 6 Isotype control antibodies used for flow cytometry analysis

Antibody / conjugation	Clone	Isotype control for	Manufacturer
IgG1, κ	MOPC-21	anti-PU.1	Sigma-Aldrich Chemie GmbH; Taufkirchen, Germany
IgG2b, κ	MOPC-141	anti-FcεRI, anti-CD83, anti-Langerin	Sigma-Aldrich Chemie GmbH; Taufkirchen, Germany
IgG1-PE	X40	anti-CD34-PE	Beckton Dickinson GmbH; Heidelberg, Germany
IgG1-RD1	2T8-2F5	anti-CD1a-RD1	Beckman Coulter Inc.; Krefeld, Germany
IgG2a-APC	S43.10	anti-CD14-APC	Miltenyi Biotec GmbH; Bergisch Gladbach, Germany

2.6 Oligonucleotides

Table 7 Amplicons, Oligonucleotides and cDNA clone used for qPCR. Matching sequences for oligonucleotides in amplicons are underlined. Amplicons and oligonucleotides were manufactured by Life Technologies GmbH; Darmstadt, Germany.

Gene	**Forward sequence (5' → 3')**	**Reverse sequence (5' → 3')**
Amplicon	**Amplicon sequence (5' → 3')**	
hACTB	AGCGCGGCTACAGCTTCA	TCCTTAATGTCACGCACGATTT
AmphACTB	TCCTCACCGAGCGCGGCTACAGCTTCACCACCACGGCCGAGCGGGAAATCGTGCGTGACATTAAGGAGAAGCTGT	
FCER1A	GGCAGCTGGACTATGAGTCTGA	CTTCTCACGCGGAGCTTTTATT
pDNR -LIB FCER1A	For further information and *FCER1A* sequence in this clone see: http://www.ncbi.nlm.nih.gov/nuccore/BC005912 and Table 8.	
FCER1G	GATGCCATCCTGTTTCTGTATGG	CACTTGGATCTTCAGTCGACAGTAG
AmpFCER1G	CTATATCCTGGATGCCATCCTGTTTCTGTATGGAATTGTCCTCACCCTCCTCTACTGTCGACTGAAGATCCAAGTGCGAAAGGCAG	
ELF1	TGCCCCAGTCACCCATGT	ACCCGGTGAGTCTGCATATT
AmpELF1_IsoA	TGTTGCCCCAGTCACCCATGTGTCCGTCACATTAGATGGGATTCCTGAAGTGATGGAAACACAGCAGGTGCAAGAAAAATATGCAGACTCACCGGGAGCC	
PU.1	GGAGAGCCATAGCGACCATT	GGAGCTCCGTGAAGTTGTTC
AmpPU.1	ATGGGGAGAGCCATAGCGACCATTACTGGGACTTCCACCCCCACCACGTGCACAGCGAGTTCGAGAGCTTCGCCGAGAACAACTTCACGGAGCTCCAGAG	
YY1	GTTCAGGGATAACTCGGCCA	TTCTGCACAGACGTGGACTC
AmpYY1	GCACAAAGATGTTCAGGGATAACTCGGCCATGAGAAAACATCTGCACACCCACGGTCCCAGAGTCCACGTCTGTGCAGAATGTGGCAAAG	
HMGB1	GATCCTAAGAAGCCGAGAGGC	CTTATGCTCCTCCCGACAAGT
AmpHMGB1	GGGCAAAGGAGATCCTAAGAAGCCGAGAGGCAAAATGTCATCATATGCATTTTTTGTGCAAACTTGTCGGGAGGAGCATAAGAAGAAGCACC	
HMGB2	CCCGGACTCTTCCGTCAATT	TCTTCCATCTCTCCGAACACTTC
AmpHMGB2	AGAAGAAACACCCGGACTCTTCCGTCAATTTCGCGGAATTCTCCAAGAAGTGTTCGGAGAGATGGAAGACCATGTCTGC	

Table 7 Amplicons, Oligonucleotides and cDNA clone used for qPCR (continued I)

Gene	Forward sequence (5' → 3')	Reverse sequence (5' → 3')
Amplicon	**Amplicon sequence (5' → 3')**	
SP1	GGACTACCTGGAGTGATGCCTAA	CCCATCAACGGTCTGGAACT
AmpSP1	AGTTCTGACAGGACTACCTGGAGTGATGCCTAATATTCAGTATCAAGTAATC CCACAGTTCCAGACCGTTGATGGGCAACAGCTGC	
GATA1	TGTGAACTGCGGAGCAACAG	GCAGGCGTTGCATAGGTAGTG
AmpGATA1	CCAGGGAGTGTGTGAACTGCGGAGCAACAGCCACTCCACTGTGGCGGAGG GACAGGACAGGCCACTACCTATGCAACGCCTGCGGCCTCTATC	
GATA2	AAGGACGGCGTCAAGTACCA	GGGACTGCCACTTTCCATCTT
AmpGATA2	AGGAGAGGACAAGGACGGCGTCAAGTACCAGGTGTCACTGACGGAGAGCA TGAAGATGGAAAGTGGCAGTCCCCTGCGCCCAG	
TLR1	TGTGCTGCCAATTGCTCATT	TTTTCCCCATAAGTCTCTCCTAAGAC
AmpTLR1	CACTTAGAAAAATCTAGTGTGCTGCCAATTGCTCATTTGAATATCAGCAAGG TCTTGCTGGTCTTAGGAGAGACTTATGGGGAAAAAGAAGACCCT	
TLR2	CCAAGGAAGAATCCTCCAATCA	GCTGCCCTTGCAGATACCA
AmpTLR2	ATCAGCCTCTCCAAGGAAGAATCCTCCAATCAGGCTTCTCTGTCTTGTGACC GCAATGGTATCTGCAAGGGCAGCTCAGGATCTT	
TLR6	GGGACTCAGCATGGTAGAAGGTA	CTCCTGTTACTCTGCAAGCTTTCA
AmpTLR6	TGCAGACAAGTGTGAGGGACTCAGCATGGTAGAAGGTAGCCTGGCATCCCA CTCCAAGACTGAAAGCTTGCAGAGTAACAGGAGCACACAGGTTCAGTG	
MYD88	TCACTGTCTGCGACTACACCAA	GGCAAGGCGAGTCCAGAAC
AmpMYD88	CCTGAGGTTCATCACTGTCTGCGACTACACCAACCCCTGCACCAAATCTTGG TTCTGGACTCGCCTTGCCAAGGCCTTGT	
CCR6	CCATTCTGGGCAGTGAGTCA	GCACGTGGCATTGCTGAA
AmpCCR6	TCTTACTCTCCCATTCTGGGCAGTGAGTCATGCCACCGGTGCGTGGGTTTT CAGCAATGCCACGTGCAAGTTGCTAA	
CCR7	GCTGCGTCAACCCTTTCTTG	AAGAGATCGTTGCGGAACTTG
AmpCCR7	TGCGTCCGCTGCTGCGTCAACCCTTTCTTGTACGCCTTCATCGGCGTCAAG TTCCGCAACGATCTCTTCAAGCTCTTC	

Table 7 Amplicons, Oligonucleotides and cDNA clone used for qPCR (continued II)

Gene	**Forward sequence (5' → 3')**	**Reverse sequence (5' → 3')**
Amplicon	**Amplicon sequence (5' → 3')**	
CD83	GCCTCGAAAACCATCACATGA	GGTGGCCATGGAGAAGCA
AmpCD83	CTGTGGGCAGGCCTCGAAAACCATCACATGACCACATAGCATGAGGCCA CTGCTGCTTCTCCATGGCCACCTTTTCAGCGA	
hGABPA	ACGCCTTGGGATACCCTATGA	ACCACCCAATGCAGGACTTG
AmpGABPA	AAGAACAAGAACGCCTTGGGATACCCTATGATCCCATACAGTGGTCCACA GACCAAGTCCTGCATTGGGTGGTTTGGGTAAT	
hCEBPA	CGAGCACGAGACGTCCATC	AGGAACTCGTCGTTGAAGGC
AmpCEBPA	CCGCTGGGCGGCATCTGCGAGCACGAGACGTCCATCGACATCAGCGCCT ACATCGACCCGGCCGCCTTCAACGACGAGTTCCTGGCCGACCTG	

2.7 cDNA clone

Table 8 Specifications of the cDNA clone used for human *FCER1A* cDNA. For *FCER1A* sequence in this clone see: http://www.ncbi.nlm.nih.gov/nuccore/BC005912.

Clone ID	**Gene bank accession**	**Gene**	**Species**	**Vector**	**Selection**
4294467	BC005912	*FCER1A*	Homo sapiens	pDNR-LIB	chloramphenicol

2.8 Restriction enzymes

Table 9 Restriction enzymes

Name	**Recognition site**	**Manufacturer**
EcoRI	5'…G'AATTC…3' 3'…CTTAA'G…5'	New England Biolabs; Frankfurt am Main, Germany
XhoI	5'…C'TCGAG…3' 3'…GAGCZ'C…5'	New England Biolabs; Frankfurt am Main, Germany

2.9 Micro-RNA molecules

Table 10 Micro-RNA (miRNA) molecules used for transfection experiments. miRNA precursor and inhibitor molecules were purchased from Life Technologies GmbH; Darmstadt, Germany.

miRNA Name	miRBase Accession #	Species
Ambion® Pre-miR™ Precursor for hsa-miR-155-5p	MIMAT0000646	Homo sapiens
Ambion® Pre-miR™ Precursor for hsa-miR-146a-5p	MIMAT0000449	Homo sapiens
Ambion® Pre-miR™ miRNA Precursor Molecules - Negative Control # 1	synthetically derived miRNA	-
BLOCK-iT™ Alexa Fluor® Red Fluorescent Control (Ambion™)	synthetically derived miRNA	-

2.10 Devices and consumables

Table 11 Devices and consumables

Name	Manufacturer
Nunc™ Biobanking and Cell Culture Cryogenic Tubes, 1.8 mL	Thermo Fisher Scientific Inc.; Waltham, USA
Mr. Frosty™ Freezing Container	Thermo Fisher Scientific Inc.; Waltham, USA
Safe-Lock Tubes, 0.5 mL / 1.5 mL, PCR clean	Eppendorf AG; Hamburg, Germany
15 mL / 50 mL CELLSTAR® Polypropylene Tube	Greiner Bio-One GmbH; Frickenhausen, Germany
Pipettors	Gilson Inc.; Middleton, USA Eppendorf AG; Hamburg, Germany
CELLSTAR® Serological Pipet, 10 mL, sterile	Greiner Bio-One GmbH; Frickenhausen, Germany
Costar® Stripette® Serological Pipets, Polystyrene, 5 mL / 10 mL, sterile	Corning GmbH HQ; Wiesbaden, Germany
Costar® 24 Well Clear TC-Treated Multiple Well Plates, Individually Wrapped, sterile	Corning GmbH HQ; Wiesbaden, Germany
Polypropylene Tubes - Two-Position Vent Stopper Tube, 5 mL	Greiner Bio-One GmbH; Frickenhausen, Germany
Falcon® 14 mL Round Bottom High Clarity PP Test Tube, Graduated, with Snap Cap, Sterile,	Corning GmbH HQ; Wiesbaden, Germany
Whatman® Puradisc 30 syringe filters, pore size 0.2 µm	GE Healthcare; Chalfont St. Giles, Great Britain
Pre-Separation Filters (30 µm)	Miltenyi Biotec GmbH; Bergisch Gladbach, Germany
MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	Applied Biosystems® by Life Technologies GmbH; Darmstadt, Germany
Opti-Seal Optical Disposable Adhesive	BIOplastics; Landgraaf, Netherlands

2.11 Instruments

Table 12 Instruments

Name	Manufacturer
Autoclave Varioclav 500 H+P	Labortechnik AG; Oberschleißheim, Germany
AutoMACS Pro® Separator	Miltenyi Biotec GmbH; Bergisch Gladbach, Germany
Centrifuge 5417R	Eppendorf AG; Hamburg, Germany
Centrifuge Allegra® X-15R	Beckman Coulter Inc.; Brea, USA
Centrifuge Rotixa 120 RS	Hettich GmbH & Co.KG; Tuttlingen, Germany
Flow Cytometer FACSCanto™	Beckton Dickinson GmbH; Heidelberg, Germany
Horizontal Electrophoresis System Wide Mini-Sub Cell GT Cell	Bio-Rad Laboratories GmbH; München; Germany
ImageQuant™ LAS 4000	GE Healthcare; Chalfont St. Giles, UK
Incubator Heracell™ 150 and 150i	Thermo Fisher Scientific Inc.; Waltham, USA
Incubator Heraeus Function Line	Hereaus Material Technology GmbH & Co. KG; Hanau, Germany
Incubator Shaker Innova 4000	New Brunswick Scientific; Enfield, USA
Laboclav 135 MSLV	SHP Steriltechnik AG; Detzel Schloss, Germany
Mastercycler nexus gradient	Eppendorf AG; Hamburg, Germany
Microscope Leica DM IRB	Leica Camera AG; Bensheim, Germany
Microscope Nikon Eclipse TS100	Nikon GmbH; Düsseldorf, Germany
Milli-Q® Reference Water Purification System	Merck KGaA, Darmstadt, Germany
Neubauer improved hemocytometer	Brand GmbH & Co. KG; Wertheim, Germany
Power supply PowerPac™ 3000	Bio-Rad Laboratories GmbH; München; Germany
qPCR machine StepOne™ plus	Applied Biosystems® by Life Technologies GmbH; Darmstadt, Germany
Synergy™ HT Multi-Mode Microplate Reader	BioTek Germany; Bad Friedrichshall, Germany
Thermomixer 5436	Eppendorf AG; Hamburg, Germany

2.12 Software

Table 13 Software

Software	Manufacturer
FlowJo 7.6.1	FlowJo, LLC Data Analysis Software; Ashland, USA
BD FACS Diva Software	Beckton Dickinson GmbH; Heidelberg, Germany
StepOne™ Software v2.2.2	Applied Biosystems® by Life Technologies GmbH; Darmstadt, Germany
Microsoft Office 2010	Microsoft Corporation; Redmond, USA
IBM SPSS Statistics 22	IBM Deutschland GmbH; Ehningen, Germany
Primer Express 3.0.1	Applied Biosystems® by Life Technologies GmbH; Darmstadt, Germany
NCBI/ Primer-BLAST	www.ncbi.nlm.nih.gov/tools/primer-blast/

3 Methods

3.1 Cell biological methods

3.1.1 Isolation of peripheral blood mononuclear cells (PBMC) from human cord blood

Human cord blood was provided by the Johanniter-Hospital Bonn and the St. Marien-Hospital Bonn, Germany, respectively. The use of human cord blood was approved by the local ethics committee of the University of Bonn in accordance with the declaration of the Helsinki principles. Samples were collected in 50 mL tubes prepared with 250 µL Heparin and stored at 4 °C until processing. PBMC were isolated using Lymphoprep™ density gradient medium. Cord blood was mixed 1:2 with PBS, layered on Lymhpoprep™ and centrifuged (800 x g, 20 °C, 28 min, break off). The PBMC containing interphase was collected and subject to magnetic-activated cell sorting (MACS®) for enrichment of $CD34^+$ hematopoietic stem cells (see 3.1.2).

3.1.2 Magnetic-activated cell sorting (MACS®)

The MACS® Technology (Miltenyi Biotec GmbH; Bergisch Gladbach, Germany) is used to enrich different cell types of a single-cell solution by magnetic labeling of the cell surface molecules. Cells are separated by a magnetic field within an AutoMACS Pro® Separator (Miltenyi Biotec GmbH; Bergisch Gladbach, Germany).

PMBC (see 3.1.1) were washed first with 15 mL PBS (400 x g, 20 °C, 10 min) and then with 25 mL 4 °C MACS buffer (230 x g, 4 °C, 10 min). Cells were resuspended in 1 mL 4 °C MACS buffer and were labeled magnetically according to the manufacturers' instructions of the CD34 MicroBead Kit (Miltenyi Biotec GmbH; Bergisch Gladbach, Germany). $CD34^+$ hematopoietic stem cells were prepared for cryopreservation in liquid nitrogen (see 3.1.3) or were differentiated into $CD34^+$ hematopoietic stem cell derived LC (CD34LC) (see 3.1.4).

$CD1a^+$ cells were enriched from CD34LC following the manufacturer's protocol of the CD1a MicroBeads kit from Miltenyi Biotec GmbH; Bergisch Gladbach, Germany. $CD1a^+$ enriched cells were resuspended in 1 mL TRIzol® reagent for RNA isolation (see 3.2.2).

The purity of enriched cell fractions was confirmed by flow cytometry analysis (see 3.1.8).

3.1.3 Cryopreservation of CD34$^+$ hematopoietic stem cells

Freshly isolated CD34$^+$ stem cells can be stored in liquid nitrogen for extended times in order to use them for further experiments at later time points.

I Freezing of CD34$^+$ hematopoietic stem cells

CD34$^+$ hematopoietic stem cells were counted (Neubauer improved hemocytometer), centrifuged (300 x g, 4 °C, 5 min), resuspended in 1 mL freezing medium and transferred into a 1.8 mL cryogenic tube. For slow freezing conditions, cells were placed into an isopropanol freezing container (cooling speed 1 °C/min) and were stored at -70 °C for one day before they were transferred into liquid nitrogen for long term storage.

II Thawing of CD34$^+$ hematopoietic stem cells

CD34$^+$ hematopoietic stem cells were thawed in a 37 °C water bath directly after removing them from liquid nitrogen. Cells were washed in 37 °C RPMI Medium 1640 (1x) + GlutaMAX™ -I (Gibco®) (300 x g, 20 °C, 5 min) and adjusted to 0.6 to 0.8 x 10^6 cells/mL in CD34$^+$ medium. CD34$^+$ hematopoietic stem cells were processed to *in vitro* generated CD34LC (see 3.1.4).

3.1.4 *In vitro* generation of CD34$^+$ hematopoietic stem cell derived LC

Freshly isolated or thawed CD34$^+$ hematopoietic stem cells were adjusted to 0.6 to 0.8 x 10^6 cells/mL CD34$^+$ medium and cultured in a 24-well plate at 37 °C and 5 % CO_2 for 8 to 12 days. Culture conditions are summarized in Table 13. Cells were harvested between d8 and d12. The LC phenotype was analyzed by flow cytometry (see 3.1.9). CD34LC were used for stimulation or transfection experiments (see 3.1.5 and 3.1.6).

Table 14 Culture conditions for CD34$^+$ hematopoietic stem cell derived LC. Final concentrations are listed.

Reagent	d0	d2	d4	d6	d8
GM-CSF	300 U/mL		200 U/mL		300 U/mL
mIgE	10^3 ng/mL	10^3 ng/mL	10^3 ng/mL	10^3 ng/mL	10^3 ng/mL
FLT3L	10 ng/mL				
SCF	10 ng/mL				
TGF-ß	0.5 ng/mL		0.5 ng/mL		0.5 ng/mL
TNF-α	20 U/mL				
Culture volume / well	0.50 mL	0.50 mL	0.75 mL	0.75 mL	1.00 mL

3.1.5 TLR-mediated stimulation of CD34LC

CD34LC were adjusted to 10^6 cells/mL in freshly prepared CD34$^+$ medium including 300 U/mL GM-CSF and were distributed in 24-well plates with 1 mL per well. For time course experiments a maximum of 2 mL cell suspension was used per 14 mL stimulation tube. Cells were left untreated or treated with specific stimulants (see Table 14) and were incubated at 37 °C and 5 % CO_2 for 24 h or for the indicated time periods. Stimulation effects were analyzed by flow cytometry of all CD34LC (see 3.1.7). CD1a$^+$ enriched cell fractions (see 3.1.2) were examined by quantitative polymerase chain reaction (qPCR) (see 3.2.8) and TaqMan® MicroRNA Assays (see 3.2.9).

Table 15 Ligands for TLR-mediated stimulation of CD34LC

Stimulant / treatment	Derived from	Final concentration	Used as ligand for	Achieved from
FSL-1	Synthetic diacylated lipoprotein derived from *Mycoplasma salivarium*	16.6 x 10^{-3} µg/mL	TLR2 / TLR6	EMC microcollections GmbH; Tübingen, Germany
LTA	*Staphylococcus aureus* (*S.a.*)	1.0 µg/mL	TLR2 / TLR6	InvivoGen; San Diego, USA
LPS	*E. coli* 0111:B4	0.1 µg/mL	TLR4	InvivoGen; San Diego, USA
S.p.	Heat-killed *Streptococcus pyogenes* (*S.p.*) serotype M49	6.5 x 10^{9} cells/mL	TLR2	kind gift from R. Lütticken (University of Aachen, Germany)
S.a.	Heat-killed, formalin-fixed *S.a.* Cowan 1	10 µL suspension/mL	TLR2	Merck KGaA; Darmstadt, Germany
P3C	Synthetic triacylated lipopeptide	1.0 µg/mL	TLR1 / TLR2	EMC microcollections GmbH; Tuebingen, Germany
"untreated"	-	equal volume of medium	-	

3.1.6 Transfection of CD34LC with miRNA precursor molecules

miRNA precursor molecules are synthetically produced double-stranded small RNA molecules. They mimic endogenous pre-miRNA molecules and are processed to functionally mature miRNAs in the cell. Transfection of CD34LC with miRNA precursor molecules was achieved by using Lipofectamine® RNAiMAX Reagent (Life Technologies GmbH; Darmstadt, Germany). In aqueous environment, Lipofectamine® RNAiMAX forms cationic liposomes that incorporate RNA molecules. These liposomes can fuse with the negatively charged cell membrane and the RNA is released into the cytoplasm.

For transfection experiments, CD34 medium without AB/AM was used. This avoided cell irritations due to a possible transferring of AB/AM into the cells via Lipofectamine vesicles. miRNA were adjusted to a working concentration of 10 µM. Target miRNA pre-miRNA-155-5p, pre-miRNA-146a-5p and negative control miRNA Pre-miR™ miRNA Precursor Molecules - Negative Control # 1 (Ambion®, Life Technologies GmbH; Darmstadt, Germany) were resuspended in nuclease-free water. Control miRNA BLOCK-iT™ Alexa Fluor® Red Fluorescent Control (Ambion™, Life Technologies GmbH; Darmstadt, Germany) was diluted in Opti-MEM® I Reduced Serum Medium (Gibco™, Life Technologies GmbH; Darmstadt, Germany). Control miRNA are unspecific and non-functional miRNA duplexes that mimic classical endogenous miRNA molecules. BLOCK-iT™ Alexa Fluor® Red Fluorescent Control coupled to Alexa Fluor® 555 was used to evaluate transfection efficiency via flow cytometry analysis (see 3.1.7). miRNA were stored at -20 °C until use.

CD34LC were harvested between d8 and d12. Prior to transfection, cells were distributed into 24-well plates at 0.5 x 10^6 cells/0.5 mL per well. Cells were allowed to sediment for 1 h at 37 °C in the incubator. Cell numbers, Lipofectamine® RNAiMAX and miRNA concentrations were optimized to the CD34LC system. Lipofectamine® RNAiMAX / miRNA complexes were prepared based on the manufacturer's guidelines. For 0.5 x 10^6 cells, 3 µL of Lipofectamine® RNAiMAX and 2.5 µL of each miRNA (10 µM) were separately diluted with 25 µL Opti-MEM® I Reduced Serum Medium. Diluted Lipofectamine® RNAiMAX was added to the diluted miRNA at a ratio of 1:1 (v/v). Lipofectamine® RNAiMAX / miRNA complexes were incubated for 5 min at room temperature. 50 µL of the complexes were dripped carefully on the cells of each well. The final transfection concentration was 50 nM per miRNA. Cells were incubated at 37 °C and 5 % CO_2 for 24 h. The transfection efficiency of CD34LC was calculated by the amount of BLOCK-iT™ Alexa Fluor® Red Fluorescent Control - positive cells per all $CD1a^+$ cells using flow cytometry analysis. Furthermore, all cells were analyzed by flow cytometry for surface molecules (see 3.1.7) and the $CD1a^+$ enriched cell fraction (see 3.1.2) was examined by qPCR (see 3.2.8) and TaqMan® MicroRNA Assays (see 3.2.9).

3.1.7 Flow cytometry analysis

Surface and intracellular immunofluorescence staining was employed in order to analyze $CD34^+$ hematopoietic stem cell and $CD1a^+$ cell enrichment as well as CD34LC phenotype and treatment effects. Staining antibodies used in this study are summarized in Table 5 and Table 6. Viability of the cells was examined by 7-AAD staining. Intracellular staining was performed according to the BD Cytofix/Cytoperm™ kit protocol (Beckton Dickinson GmbH; Heidelberg, Germany). Unless otherwise specified, 5×10^4 cells for surface staining and 2×10^5 cells for intracellular staining, respectively, were stained successively with the indicated antibodies in a total volume of 100 µL FACS buffer at 4 °C for 25 min. Washing steps were executed in 1 mL FACS buffer (400 x g, 4 °C, 2 min). Cells were measured and analyzed utilizing a FACSCanto™ flow cytometer and FACSDiva™ software (Beckton Dickinson GmbH; Heidelberg, Germany) or FlowJo 7.6.1 software (FlowJo, LLC Data Analysis Software; Ashland, USA), respectively. For statistics, relative fluorescence index (rFI) was calculated as follows:

$$rFI = \frac{MFI_{target} - MFI_{isotype\ control}}{MFI_{isotype\ control}}$$

(MFI = mean fluorescence intensity)

3.1.8 Purity control of $CD34^+$ hematopoietic stem cells and $CD1a^+$ cell enrichment

$CD34^+$ enriched cells (see 3.1.4) were stained with 0.15 µg/mL PE-conjugated anti-CD34 mAb or 2.5 µg/mL PE-conjugated anti-IgG1 for isotype control for 15 min at room temperature.

$CD1a^+$ enriched cells (see 3.1.2) were stained with an antibody mix containing IgG2a-APC, IgG1-RD1 and 7-AAD or CD14-APC, CD1a-RD1 and 7-AAD, respectively. The final concentrations of the respective antibodies for $CD1a^+$ enriched cells are listed in Table 15.

3.1.9 Phenotypic characterization of CD34LC

Expression of specific surface molecules was assessed by staining with unconjugated mouse monoclonal antibodies against Langerin (CD207), FcεRI, TLR2, CD80, CD83, CD86 and MHC II. Unspecific mouse IgG2b was used as an isotype control antibody. FITC-conjugated goat-anti-mouse IgG polyclonal antibody was used as secondary antibody. Free antigen-binding sites were blocked with 2.5 mg/mL mouse serum for 15 min at 4 °C. Finally, an antibody mix containing IgG2a-APC, IgG1-RD1 and 7-AAD or CD14-APC, CD1a-RD1 and 7-AAD was added. Antibody concentrations used for surface staining are listed in Table 15 below.

Table 16 Antibody concentrations used for surface staining

	Antibody	Stock concentration (mg/mL)	Working concentration (µg/mL)	Volume per test (µL)	Final concentration (µg/mL)
1st	IgG2b, κ	1.0	50	5	2.5
	Langerin (CD207)	0.2	50	5	2.5
	FcεRIα	1.0	50	5	2.5
	TLR2*	n.s.	(1/10 dilution)	5	-
	CD83	0.2	50	5	2.5
2nd	Goat anti-mouse IgG, Fc_γ	1.0	50	5	2.5
Mix	IgG2a-APC	0.011	2.75	5	0.14
	CD14-APC	0.011	2.75	5	0.14
	IgG1-RD1	1.0	50	3	1.5
	CD1a-RD1	0.25	25	3	0.75
	7-AAD	1.0	50	2	1.0

* Ascites preparation. Antibody concentration is not specified (n.s.).

Intracellular staining of transcription factors was performed with monoclonal antibodies against PU.1 followed by a FITC-conjugated goat-anti-mouse IgG polyclonal antibody. Free antigen-binding sites were blocked with 5 mg/mL mouse serum for 15 min at 4 °C. Finally, IgG2a-APC and IgG1-RD1 or CD14-APC and CD1a-RD1 antibody mixes were added (see Table 16).

Table 17 Antibody concentrations used for intracellular staining

Antibody		Stock concentration (mg/mL)	Working concentration (µg/mL)	Volume per test (µL)	Final concentration (µg/mL)
1st	IgG1, κ	1.0	50	10	5
	PU.1	2.0	50	10	5
2nd	Goat anti-mouse IgG, Fc_γ	1.0	50	10	5
Mix	IgG2a-APC	0.011	2.75	5	0.14
	CD14-APC	0.011	2.75	5	0.14
	IgG1-RD1	1.0	50	5	2.5
	CD1a-RD1	0.25	25	5	1.25

3.2 Molecular biological methods

3.2.1 Determination of RNA and DNA concentrations and purity

Concentrations and purity of nucleic acids were determined by using the spectrophotometer Synergy™ HT Multi-Mode Microplate Reader (BioTek Germany; Bad Friedrichshall, Germany). Concentrations were calculated by measuring RNA and DNA at the wave length λ = 260 nm. Purity was assessed by a $\lambda_{260/280}$ ratio. Values were calculated with the Gen5™ software (BioTek Germany; Bad Friedrichshall, Germany). 2 µL of each nucleic acid and of nuclease-free water for RNA reference or 5 mM Tris/HCL buffer for DNA reference were measured.

3.2.2 RNA isolation

RNA of $CD1a^+$ enriched cells (see 3.1.2) was extracted via the phenol/chloroform method utilizing TRIzol® reagent in accordance with the manufacturer's protocol. RNA was resuspended in 25 µL nuclease-free water. DNA contaminations were removed using DNA-free™ DNA Removal Kit (Ambion®, Life Technologies GmbH; Darmstadt, Germany) following the manufacturer's instructions. RNA concentration and purity were evaluated (see 3.2.1). RNA was reverse transcribed to cDNA for gene expression experiments (see 3.2.3), subject to TaqMan® MicroRNA Assays (see 3.2.9) or stored at -70 °C.

3.2.3 Reverse transcription for gene expression experiments

RNA was reverse transcribed into complementary DNA (cDNA). 1 µg RNA in 15.5 µL nuclease-free water was denaturated at 65 °C for 3 min and was then transferred directly on ice. 34.5 µL of a reverse transcription reaction mix (see Table 17) was added to RNA and incubated at 37 °C for 1 h. The reaction was stopped at 95 °C for 3 min. The cDNA volume was adjusted to 100 µL with nuclease-free water. cDNAs were kept on ice for immediate use in qPCR or were stored at -20 °C.

Table 18 Components of reverse transcription reaction mix

Reagent	Final concentration
5x reverse transcription buffer	1x
dNTP	4 µM (each)
DTT	100 µM
Random primer	2.4 ng/µL
RNAsin	1 U/µL
SuperScript™ Reverse Transcriptase	4 U/µL
Final volume	34.5 µL

3.2.4 Culture of bacterial cDNA clones

cDNA clones provide the full length cDNA of interest incorporated in a vector and cloned into a host cell. In this study, cDNA clones from *E. coli* DH10B (genotype) carrying full length cDNA of the human *FCER1A* (see Table 8) were used for creating quantitative real-time PCR (qPCR) standard curves. For amplification, an inoculum of the bacterial glycerol stock was dropped into 4 mL liquid LB medium supplemented with 25 µg/mL chloramphenicol for selection. Aerobic growth occurred overnight at 37 °C in an orbital incubator shaker at 170 rpm (Innova 4000, New Brunswick Scientific; Enfield, USA). The plasmid DNA was isolated (see 3.2.5).

3.2.5 Isolation of plasmid DNA

For plasmid isolation, 4 mL of bacterial overnight culture were harvested and processed using Invisorb® Spin Plasmid Mini Two (STRATEC Biomedical AG; Birkenfeld, Germany) according to the manufacturer's instructions. Plasmid DNA was eluted with 75 µL 5 mM Tris/HCL buffer (pH 8.5) and the concentration was determined as described in 3.2.1. The plasmids were subject to restriction enzyme digestion or were stored at -20 °C.

3.2.6 Restriction enzyme digestion

Plasmid DNA was dissected via specific restriction enzyme digestion using EcoRI and XhoI (New England Biolabs; Frankfurt am Main, Germany) at 37 °C overnight following the manufacturer's instructions. Products were analyzed by agarose gel electrophoresis (see 3.2.7).

3.2.7 Agarose gel electrophoresis

For analysis of restriction enzyme digestion products or qPCR products, DNA was supplemented with Gel Loading Dye Blue, (6x) (New England Biolabs; Frankfurt am Main, Germany) and loaded on a 2 % agarose gel (w/v) containing 0.5 µg/mL ethidium bromid. Gels were run in 1x TAE buffer for 45 to 60 min between 90 and 110 volt. DNA was visualized by ImageQuant™ LAS 4000 (GE Healthcare; Chalfont St. Giles, Great Britain). Assessment of DNA fragment size was achieved by using Quick-Load® 2-Log DNA Ladder (0.1-10.0 kb) (New England Biolabs; Frankfurt am Main, Germany).

3.2.8 Quantitative real-time PCR (qPCR) for gene expression experiments

Gene expression is a strongly regulated process to control cellular activities, structure and responsiveness to the environment. Regulation of gene expression determines the amount and the timing of the synthesis of a gene product by the production of mRNA copies of the DNA sequence of a gene. qPCR has become a common technique to monitor gene expression *in vitro* and is based on standard end-point PCR. mRNA is reverse transcribed into cDNA and its amplification is measured after every cycle in "real-time" utilizing a fluorescence dye that intercalates and signals in double stranded DNA sequences (*e.g.* SYBR® Green) or a fluorescence labeled specific oligonucleotide probe that hybridizes with specific complementary DNA sequences (*e.g.* TaqMan® MicroRNA Assays, see 3.2.9). Accumulating amplification products lead to a gain of fluorescence intensity. When amplification-based signals exceed the background fluorescence the so called cycle threshold (Ct) has been reached. The Ct provides the number of cycles that are needed to rise above this threshold.

In this study, iTag™ SYBR® Green Supermix with ROX™ was used. The mix includes SYBR® Green I (DNA-dye-complex absorption λ_{max} = 497 nm; emission λ_{max} = 520 nm) and

ROX™, a passive reference dye to normalize non-PCR-related fluctuations in fluorescence. StepOnePlus™ qPCR devices and StepOne™ Software v2.2.2 (Applied Biosystems® by Life Technologies GmbH; Darmstadt, Germany) were used to execute qPCR and to analyze the results. Amplicons are synthetically created DNA molecules mimicking the gene sequence specific for a certain primer pair. Amplicons and primers (see Table 7) were designed using Primer Express 3.0.1 software (Applied Biosystems® by Life Technologies GmbH; Darmstadt, Germany). Primer specificity for target genes was verified by NCBI/Primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/).

Absolute quantification of the gene expression transcripts was achieved by using standard curves with a defined number of molecules of an amplicon calculated by its nanomolar quantity (indicated by the manufacturer) as follows:

$$\text{Molecules} = x \text{ mole} * 6.022 * 10^{23}$$

Amplicon stock solutions were adjusted to 3×10^{13} molecules/µL in nuclease-free water and stored at 4 °C. A standard dilution series was prepared in six concentrations reaching from 3×10^{8} molecules/µL to 3×10^{3} molecules/µL. Six standard curves of each primer pair were prepared. The mean Ct values were plotted against the concentrations (see Figure 2).

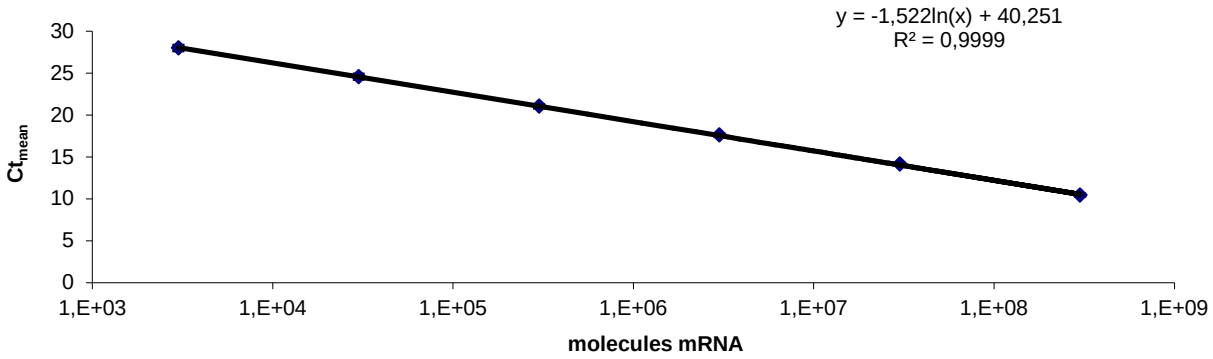

Figure 2 ***Standard curve for actin.*** Dilution series of the amplicon for actin were prepared as described in the text. Mean Ct values of n=6 experiments were used to generate the regression line.

The linear function of the regression line was used to calculate the amounts of molecules in cDNA samples:

$$y = -1.522 * \ln(X) + 40.251$$

$$x = \text{EXP}((y-40.251)/(-1.522))$$

Results were normalized to 10^3 molecules actin. The efficiency of primers was greater than 80 %.

The PCR master mix (see Table 18) was prepared in a volume of 9 µL and distributed into 96-well plates. 1 µL of target cDNA (see 3.2.3), standard dilution series or nuclease-free water for a non-template control was added to the corresponding qPCR master mix. Samples and controls were measured in triplicates. Cycling conditions are summarized in Table 19. Melting curve analysis was performed to detect primer dimers or PCR byproducts.

Table 19 Components of qPCR reaction mix for gene expression experiments. Forward and reverse primers were mixed together (1:1).

Reagent	Volume	Final concentration
iTaq™ SYBR® Green Supermix with ROX™ (2x)	5.0 µL	1x
Primer mix (5 µM)	0.4 µL	0.2 µM
cDNA / standard dilution / nuclease-free water	1.0 µL	10 %
Nuclease-free water	3.6 µL	-
Final volume	10.0 µL	

Table 20 Thermal cycling conditions for qPCR for gene expression experiments (40 cycles)

Step	Duration	Temperature
Initial denaturation	10 min	95 °C
Denaturation	15 s	95 °C
Annealing and elongation	1 min	60 °C
Melting curve	15 s	+0.3 °C (until 95 °C)

3.2.9 TaqMan® MicroRNA Assays

TaqMan® MicroRNA Assays (Life Technologies GmbH; Darmstadt, Germany) were performed to detect and quantify mature miRNA. Generally shortness of mature miRNA molecules (~ 22 nucleotides) leads to difficulties in the reverse transcription reaction with classical random hexamer or oligo-desoxythymidine primers. Therefore, the kit provides small miRNA-specific stem loop primers specific for the reverse transcription of mature miRNA only. The stem loop primer/miRNA complex leads to a 3' extension of the synthesized cDNA that enables the hybridization of the reverse qPCR primer. The forward primer is complementary to the miRNA sequence. Amplification of cDNA in the qPCR is monitored using specific TaqMan® MGB probes containing a reporter dye (FAM™ dye) and a nonfluorescent quencher that suppresses the reporter fluorescence signal. The probe anneals specifically to complementary amplified DNA sequences between forward and reverse primer sites. In the next polymerization step, hybridized probes were cleaved by a hot start DNA polymerase and the reporter dye is separated from the probe. The fluorescence of the isolated reporter dye is no longer suppressed by the quencher molecule and can be detected by the qPCR device.

TaqMan® MicroRNA Assays for miRNA-155-5p, miRNA-146a-5p and RNU48 (endogenous control) were executed following the manufacturer's instructions. Reverse transcription PCR was performed with 10 ng total RNA isolated from TRIzol® samples (see 3.2.2) per 15 µL reaction utilizing the Mastercycler nexus gradient from Eppendorf (thermal cycling conditions: 30 min, 16 °C; 30 min, 42 °C; 5 min, 85 °C; ∞, 4 °C). The products were diluted 1:1 (v/v) with nuclease-free water and were subject to TaqMan® qPCR reaction or stored at -20 °C for up to one week. qPCR was performed in triplicates with 1µL cDNA per 9 µL reaction mix (see Table 20). StepOne™ plus machine and StepOne™ Software v2.2.2 (Applied Biosystems® by Life Technologies GmbH; Darmstadt, Germany) were used for processing (thermal cycling conditions see Table 21) and evaluation of TaqMan® MicroRNA Assays.

Table 21 Composition of qPCR reaction mix for TaqMan® MicroRNA Assays

Component	Volume per reaction	Final concentration
TaqMan® small RNA Assays (20x)	0.5 µL	1x
TaqMan® Universal PCR MasterMix II (2x), no UNG	5.0 µL	1x
Nuclease-free water	3.5 µL	3.5 %
cDNA	1.0 µL	1.0 %
Final volume	10.0 µL	

Table 22 Thermal cycling conditions for qPCR for TaqMan® MicroRNA Assays (40 cycles)

Step	Duration	Temperature
Initial denaturation	10 min	95 °C
Denaturation	15 s	95 °C
Annealing and elongation	1 min	60 °C

4 Results

In contrast to epidermal LC from healthy subjects, those from AD express high levels of FcεRI (Bieber, de la Salle *et al.* 1992, Wollenberg, Kraft *et al.* 1996). AD skin is highly colonized with *S.a.* that can be recognized by TLR on LC. We could show that TLR2 engagement decreases FcεRI in *in vitro* generated LC (Herrmann, Koch *et al.* 2013), but there are still open questions as to how FcεRI is regulated in detail. LC represent only ~2-5 % of the cells in human skin. For LC research, sufficient human skin material is rarely available and LC isolation procedure easily activates these sensitive cells. Therefore a human cell model of *in vitro* generated CD34LC was chosen to investigate FcεRI regulation on human LC.

4.1 *In vitro* generated CD34LC represent a suitable model to investigate human FcεRI regulation

Prior to FcεRI investigation, *in vitro* generated $CD34^+$ hematopoietic stem cell derived LC (CD34LC, see 3.1.4) have to be confirmed as a suitable cell model. Characteristically, LC differ from DC *inter alia* by the expression of Langerin. CD34LC are expected to express high levels of Langerin as well as CD1a. To begin with, an immature phenotype corresponding to LC in their epidermal environment is a prerequisite for stimulation experiments. Cell surface staining and flow cytometry analysis was performed to characterize CD34LC phenotype and maturation state. Cells were gated by their morphology, their viability by 7-AAD staining and by their CD1a expression (see Figure 3 A and B). $CD1a^+$ cells were further analyzed for their Langerin and their CD83 expression (see Figure 3 C).

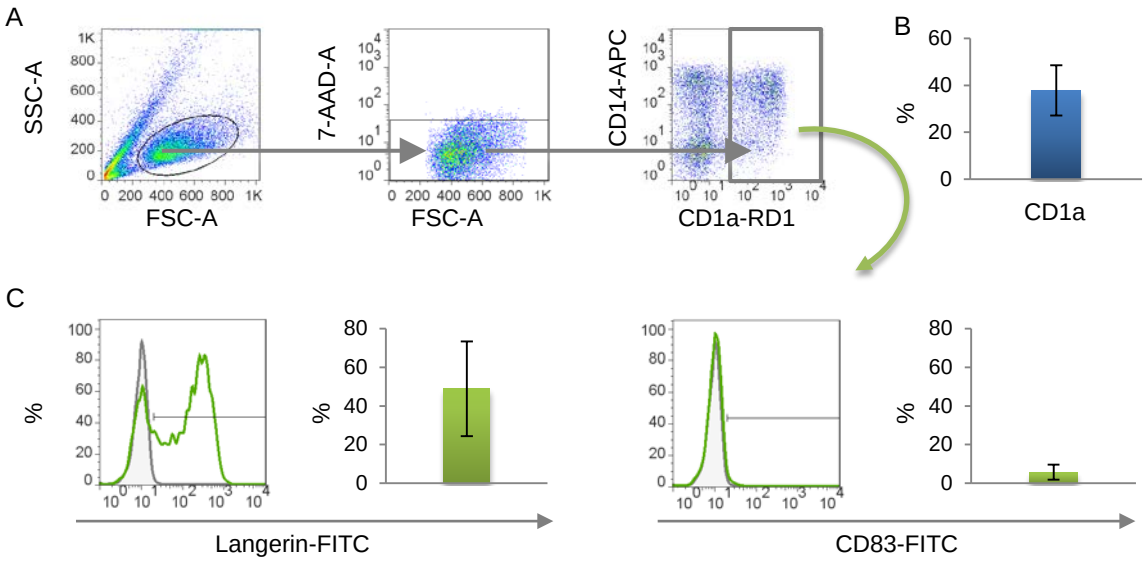

Figure 3 ***In vitro generated CD34LC show an immature LC phenotype.*** PBMC were isolated from cord blood using density gradient medium followed by $CD34^+$ hematopoietic stem cell enrichment using MACS® Technology. CD34LC were generated by cultivating $CD34^+$ hematopoietic stem cells for 8 to 12 days as described in methods. 5 x 10^4 cells were stained with 7-AAD, anti-CD1a-RD1 and anti-CD14-APC to identify viable DC population (A, B). Antibodies against Langerin, CD83 (green) and IgG2b (grey) for isotype control were counterstained by FITC-labeled goat anti-mouse IgG antibody (C). Cells were analyzed using Flow Cytometer FACSCanto™. Mean values ± SD of CD1a, Langerin and CD83 expression of n=8 experiments are depicted (B, C). (SD = standard deviation)

At day 8 to 12, *in vitro* generated CD34LC contained about 30 to 50 % of $CD1a^+$ cells which expressed 50 % (± 25 %) Langerin. The immature phenotype of the cells was confirmed by low or absent CD83 surface expression.

In conclusion, *in vitro* generated CD34LC cells were appropriate for further investigations by their LC-like phenotype and their immaturity. For FcεRI research, the receptor should be expressed on CD34LC, too. Therefore, FcεRI surface expression on CD34LC was verified next by flow cytometry analysis.

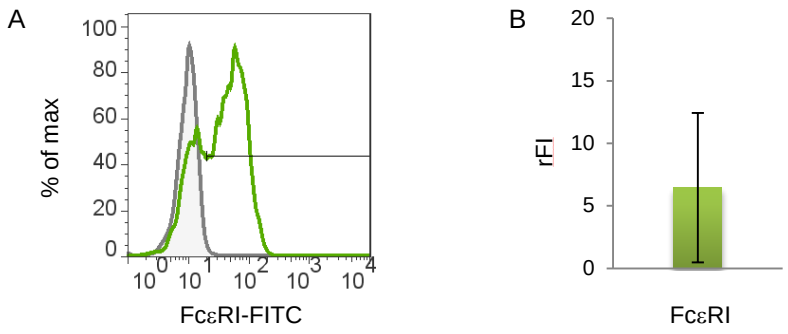

Figure 4 ***In vitro generated CD34LC express FcεRI.*** Cells were generated as described in Figure 3. Viable CD1a$^+$ cells were stained with anti-FcεRI (green) and anti-IgG2b (grey) for isotype control and were counterstained with FITC-labeled goat anti-mouse IgG. Cells were analyzed using Flow Cytometer FACSCanto™. Mean values ± SD of n=8 experiments are shown. (SD = standard deviation)

FcεRI showed a heterogeneous, donor-dependent surface expression. For further studies, only experiments with rFI higher than 1.5 were evaluated.

In AD, skin is highly colonized with a plethora of bacteria which can be sensed by LC via PPR such as TLR. Before the link between TLR engagement and FcεRI regulation was examined, TLR2 expression on CD34LC was checked.

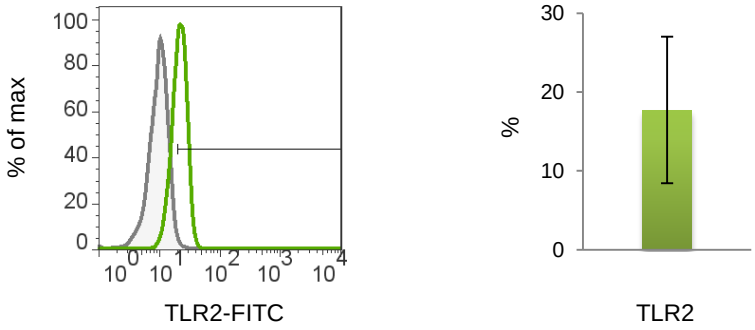

Figure 5 ***In vitro generated CD34LC express TLR2.*** Cells were generated as described in Figure 3. Viable CD1a$^+$ cells were stained with anti-TLR2 (green) and anti-IgG2b (grey) for isotype control and were counterstained with FITC-labeled goat anti-mouse IgG. Cells were analyzed using Flow Cytometer FACSCanto™. Mean values ± SD of n=8 experiments are shown. (SD = standard deviation)

Flow cytometry analysis revealed that TLR2 is expressed on CD34LC surface and may provide the basis for stimulation experiments with TLR2 ligands.

In a next approach, the functionality of surface TLR2 was analyzed. This can be monitored by the TLR2-induced regulation of several costimulatory molecules. Here, the DC maturation marker CD83 as well as the C-C motif chemokine receptor 6 (CCR6) and the C-C motif chemokine receptor 7 (CCR7) were measured after TLR1/2 ligation with P3C. CCR6 is typically expressed in immature DC and is involved in the recruitment of DC to the inflammation sites. In contrast, CCR 7 is upregulated in activated DC and facilitates the migration to the lymph nodes.

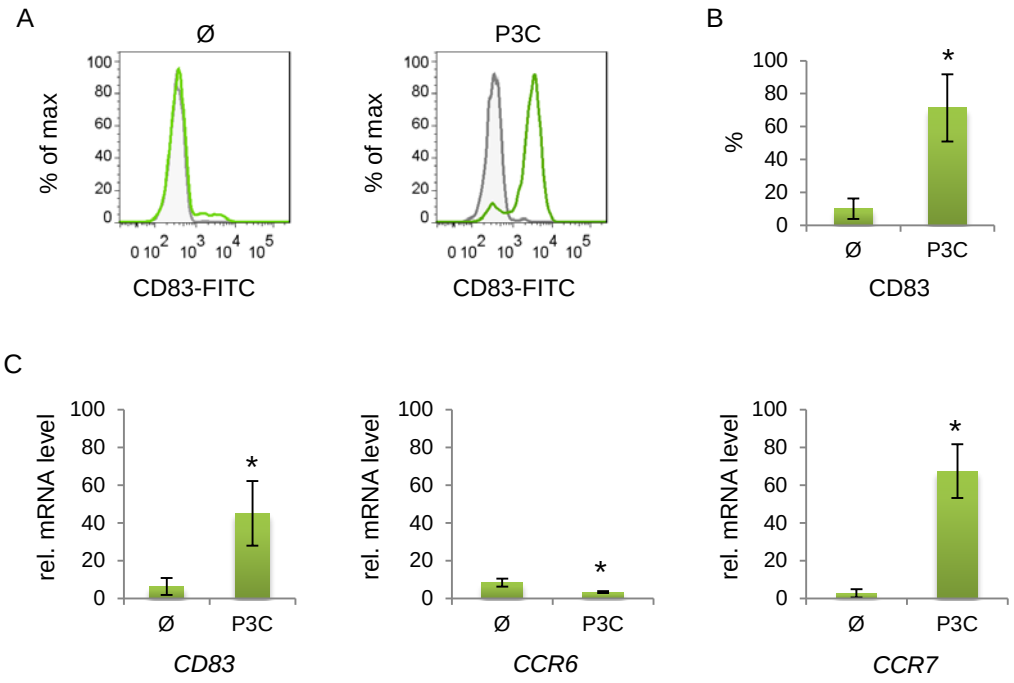

Figure 6 ***TLR2 engagement results in the maturation of in vitro generated CD34LC.*** CD34LC were generated as described in Figure 3. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 1.0 µg/mL P3C for 24 h (A, B). 5 x 10^4 cells were stained against 7-AAD, CD1a-RD1, CD14-APC and CD83 (green) or IgG2b for isotype control (grey). Representative Histograms of CD83 expression are illustrated (A). Mean values ± SD of percentage of CD83 surface expression of untreated vs. treated cells are shown (B, n=8). Total RNA was prepared from TRIzol® samples of MACS® enriched $CD1a^+$ cells. 1 µg of total RNA was used to reverse transcribe mRNA into cDNA. qPCR was performed in triplicates with 1 µL cDNA per reaction. *CD83*, *CCR6* and *CCR7* mRNA was quantified using global standard curves of *CD83*, *CCR6*, *CCR7* and of *ACTB* for normalization. Mean values ± SD of relative mRNA levels from n=8 experiments are shown (C). Statistical significance was assessed by paired sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. *p < 0.05; (SD = standard deviation)

TLR2 ligation resulted in strong and significant upregulation of CD83 surface protein and mRNA levels compared to untreated cells. *CCR6* mRNA levels of treated cells decreased to less of 50 % compared to untreated cells. *CCR7* was strongly induced. These findings revealed that *in vitro* generated CD34LC were functional LC which mature due to TLR2 engagement.

As evident from Figure 3 to Figure 6, *in vitro* generated CD34LC initially showed the required immature LC phenotype that expresses heterogeneous amounts of surface FcεRI. CD34LC maturation upon TLR2 engagement confirmed that the cells themselves and the TLR2 receptors were functional.

In conclusion, *in vitro* generated CD34LC were comparable to LC of the skin. By virtue of their phenotype and of their functionality they provide a suitable cell model for investigations of FcεRI.

4.2 FcεRI is downregulated upon stimulation of CD34LC with TLR ligands

In AD, FcεRI is expressed on skin LC. Concomitantly, AD skin is highly colonized with *S.a.* (Boguniewicz and Leung 2010) that can be recognized by LC via TLR, especially via TLR2 heterodimers (Biedermann 2006). To get more insight in the regulation of FcεRI upon different TLR engagement, CD34LC were stimulated with a set of TLR2 ligands. For ligand binding, TLR2 forms heterodimers with TLR1 and TLR6. TLR1/2 heterodimers recognize triacylated lipopeptides like P3C (Buwitt-Beckmann, Heine *et al.* 2006) while TLR2/6 heterodimers interact with diacylated lipopeptides such as FSL-1 (Skabytska, Wolbing *et al.* 2014) or with lipoteichoic acid (LTA). Here, CD34LC were stimulated with FSL-1, LTA and P3C and FcεRI surface expression was examined by flow cytometry analysis.

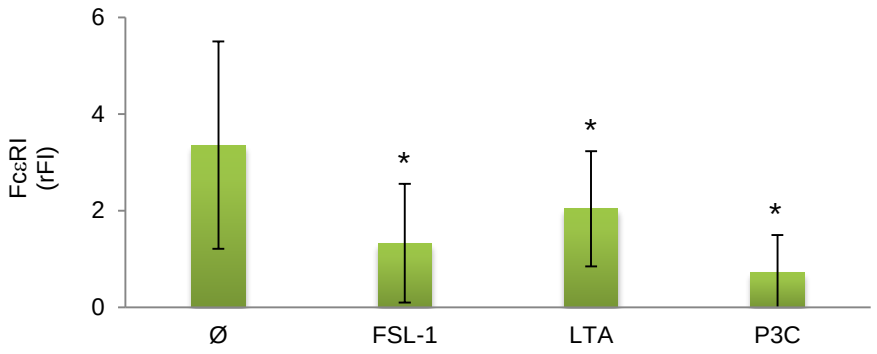

Figure 7 ***FcεRI surface expression is downregulated by the stimulation of CD34LC with TLR2 ligands.*** CD34LC were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 16.6 x 10^{-3} µg/mL FSL-1, 1.0 µg/mL LTA or 1.0 µg/mL P3C for 24 h. FcεRI cell surface expression of viable $CD1a^+$ cells was analyzed by flow cytometry as described. Mean rFI values ± SD from n=7-8 experiments are shown. Statistical significance was assessed by paired-sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. *$p < 0.05$; (SD = standard deviation)

FcεRI surface expression was significantly reduced by the engagement of TLR2/6 heterodimers with FSL-1 (-64 % ± 13 % of unstimulated) and LTA (-49 % ± 25 % of unstimulated), but most effectively after TLR1/2 stimulation with P3C (-82 % ± 10 % of unstimulated).

The decrease of surface FcεRI can be preceded by a downregulation of FCER1 transcripts. On human LC, FcεRI builds a trimeric complex which is composed of one α- and two γ-chains (Kraft and Kinet 2007). Surface expression validation of the γ-chains by flow cytometry analysis was not practical because of the deficient epitopes of the almost intracellularly assembled chains. Therefore, qPCR analysis of *FCER1A* and *FCER1G* gene expression of FSL-1, LTA and P3C stimulated cells was performed to examine TLR-mediated effects on both receptor chain transcripts.

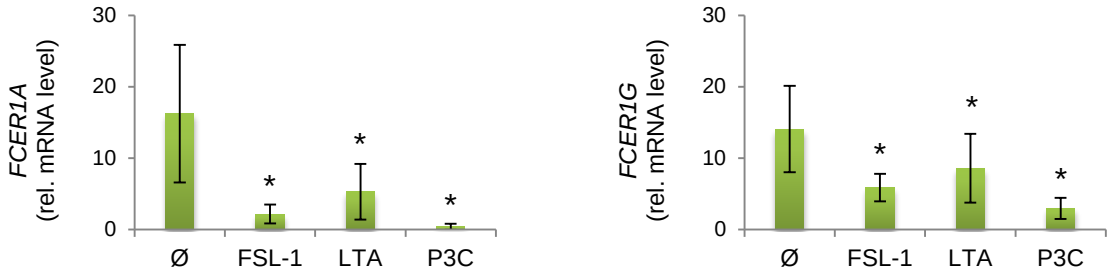

Figure 8 ***FCER1A and FCER1G mRNA transcription levels decrease upon TLR-mediated stimulation of CD34LC.*** CD34LC were harvested between d9 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 16.6 x 10^{-3} µg/mL FSL-1, 1.0 µg/mL LTA or 1.0 µg/mL P3C for 24 h. qPCR was performed as described in Figure 6. *FCER1A* and *FCER1G* mRNA was quantified using global standard curves of *FCER1A*, *FCER1G* and of *ACTB* for normalization. Relative mRNA levels ± SD from n=8 experiments are shown. Statistical significance was assessed by paired-sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. *p < 0.05; (SD = standard deviation)

TLR-mediated stimulation of CD34LC resulted in a significant downregulation of *FCER1A* and *FCER1G* mRNA transcripts. *FCER1A* mRNA was hardly detectable upon stimulation with P3C. *FCER1G* transcripts were downregulated less strongly compared to the α-chain transcripts. In line with the results of the FcεRI cell surface expression (see Figure 7), the strongest reduction was obtained by the TLR1/2 ligand P3C (-84 % ± 10 % of unstimulated) followed by FSL-1 (-44 % ± 14 % of unstimulated) and LTA (-41 % ± 14 % of unstimulated).

In summary, FcεRI was downregulated on the protein and on the transcriptional level by different TLR2 heterodimer ligands.

So far, only synthetically derived or modified TLR2 ligands were tested, but skin harbors other TLR ligands, too. *In vivo*, skin immune cells have to cope additionally with complete bacterial synthesis and cell envelope products. Therefore, stimulation experiments with heat-killed bacterial strains of *S.a.* (Pansorbin®) and of *S.p.* (M49 vaccine POD 591) and the TLR4 ligand LPS derived from *Escherichia coli* (*E. coli*) were performed next. Based on the results above, P3C was taken as positive control. FcεRI expression of *S.a.*, *S.p,* LPS and P3C treated cells was analyzed by flow cytometry and qPCR.

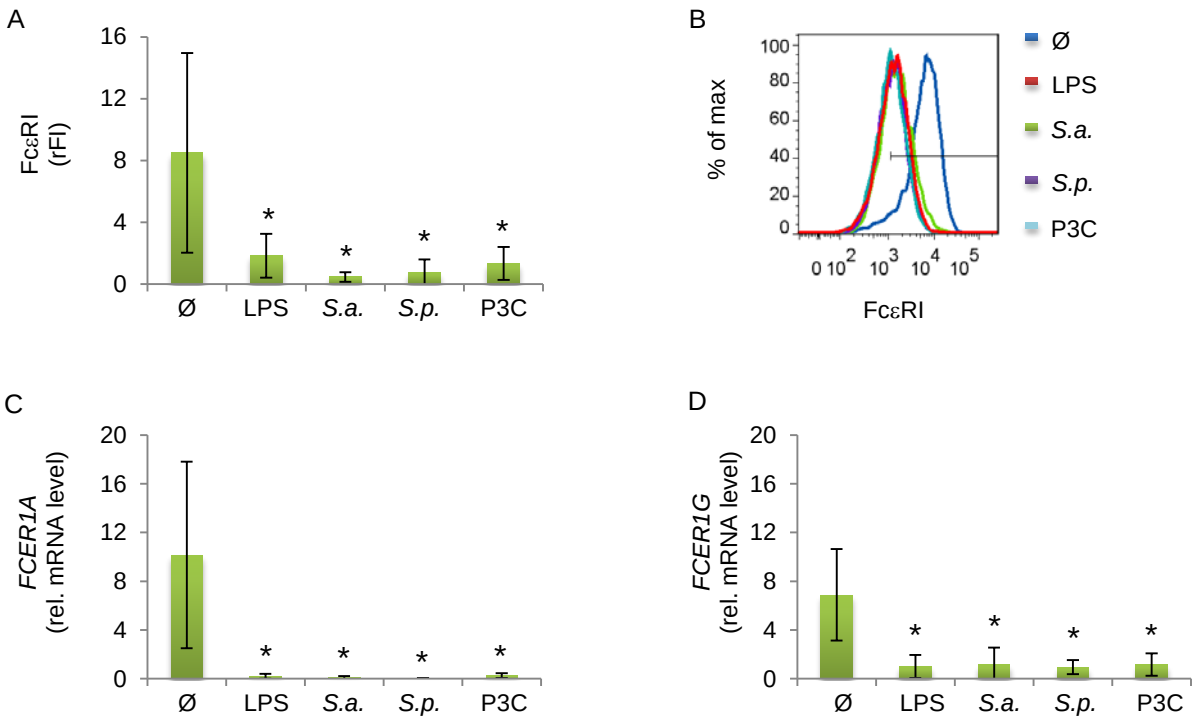

Figure 9 ***FcεRI is downregulated by stimulation of CD34LC with LPS and heat-killed bacteria.*** CD34LC were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 0.1 µg/mL LPS, 10 µL/mL *S.a.*, 6.5 x 10^9 cells/mL *S.p.* or 1.0 µg/mL P3C for 24 h. FcεRI cell surface expression of viable $CD1a^+$ cells was analyzed by flow cytometry as described. Mean values ± SD from n=5-7 experiments are shown (A). Representative histograms of FcεRI surface expression of untreated, LPS, *S.a.*, *S.p.* and P3C treated cells are depicted in B. qPCR was performed as described in Figure 6. *FCER1A* and *FCER1G* mRNA was quantified using global standard curves of *FCER1A, FCER1G* and of *ACTB* for normalization. Relative mRNA levels ± SD from n=6-7 experiments are shown (C, D). Statistical significance was assessed by paired-sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. *p < 0.05; (SD = standard deviation)

FcεRI expression was severely and significantly downregulated on protein and on transcriptional levels. *FCER1A* mRNA was decreased with LPS and P3C by 98 % (± 3 % and ± 1 %) and was almost undetectable after stimulation with *S.a.*, *S.p.* and P3C. *FCER1G* mRNA was strongly downregulated after stimulation of CD34LC with LPS and the heat-killed Gram-positive bacteria. Here, the positive control P3C was significantly reduced, too.

Taken together, stimulation of CD34LC with synthetically derived specific TLR ligands or with complete heat-killed bacteria resulted in all cases in a significant and strong downregulation of FcεRI on protein and mRNA level. How this decrease is regulated in CD34LC will be the aim of the next part of this work.

4.3 *FCER1A*-associated transcription factor PU.1 is decreased after TLR-mediated stimulation of CD34LC

As obtained from the data above, stimulation of CD34LC with several TLR ligands resulted in a significant reduction of FcεRI on protein and on transcriptional levels. In the following approach, the transcriptional regulation of the FcεRI α-chain will be scrutinized.

Transcription factors are regulatory elements that control the synthesis of a gene transcript by binding to a gene's promotor region. They can operate as transcriptional enhancers or repressors. Data obtained from the literature describe that the transcription factors PU.1, Yy1 and Gata-1 as well as Hmgb1, Hmgb2 and Sp1 enhance *FCER1A* gene expression by binding to its proximal promotor region, while *Elf-1* acts as a transcriptional repressor (Kanada, Nakano *et al.* 2008, Nishiyama, Hasegawa *et al.* 2002). First, the expression of these *FCER1A*-associated transcription factors in unstimulated FcεRI$^+$ CD34LC was analyzed by qPCR.

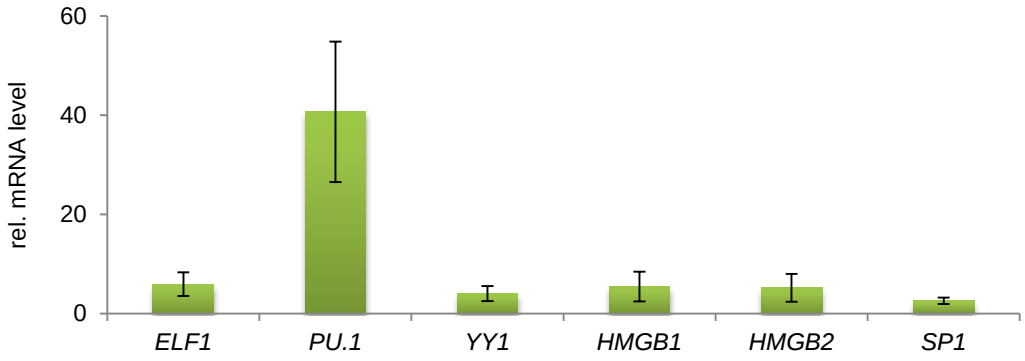

Figure 10 ***Transcription factor PU.1 is predominantly expressed in CD34LC.*** CD34LC were harvested between d8 and d12. qPCR was performed as described in Figure 6. mRNA levels of the indicated transcription factors were quantified using global standard curves of the respective transcription factor and of *ACTB* for normalization. Mean values of relative mRNA levels ± SD from n=8 experiments are shown.

The *FCER1A* related transcription factor *PU.1* showed the highest mRNA expression level with a relative mRNA expression of 44 (± 14 SD) molecules per 10^3 molecules actin. *ELF1*, *HMGB1*, *HMGB2*, *SP1* and *YY1* were only weakly expressed. *GATA1* mRNA was not detected (data not shown). This indicated that PU.1 may be the most influencing transcription factor in FcεRI regulation in CD34LC. For further evidence, the indicated transcription factors were analyzed by qPCR in FSL-1, LTA and P3C stimulated CD34LC, too.

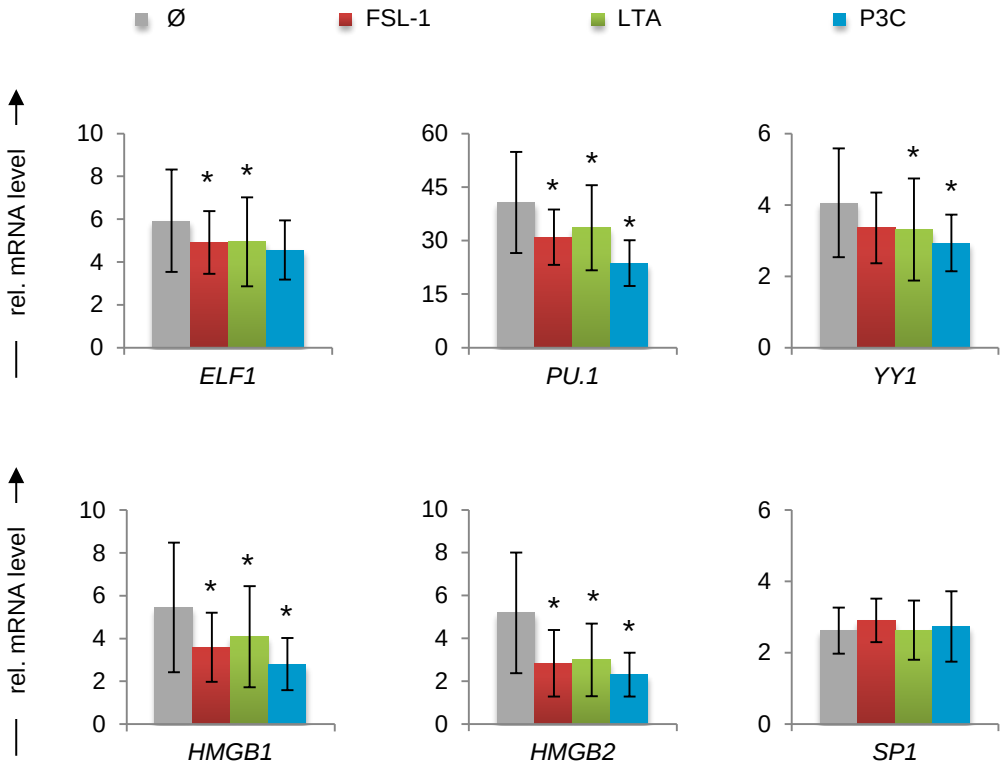

Figure 11 ***EFL1, PU.1, YY1, HMGB1 and HMGB2 are downregulated upon TLR2-mediated stimulation of CD34LC.*** CD34LC were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 16.6 x 10^{-3} µg/mL FSL-1, 1.0 µg/mL LTA or 1.0 µg/mL P3C for 24 h. qPCR was performed as described in Figure 6. mRNA levels of the indicated transcription factors were quantified using global standard curves of the respective transcription factor and of *ACTB* for normalization. Mean values of relative mRNA levels ± SD from n=7-8 experiments are shown. Statistical significance was assessed by paired-sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. *p ≤ 0.05; (SD = standard deviation)

PU.1, *HMGB1* and *HMGB2* mRNA expression was significantly and most strongly decreased after stimulation with P3C followed by FSL-1 and LTA. *ELF1* and *YY1* were downregulated to a lesser extent after TLR2 engagement. *SP1* mRNA was not changed by the TLR-mediated stimulation of CD34LC.

To evaluate the relationship between the expression of FcεRI and its respective transcription factor on CD34LC, Pearson correlations with *FCER1A* of untreated, FSL-1, LPS and P3C treated cells were analyzed.

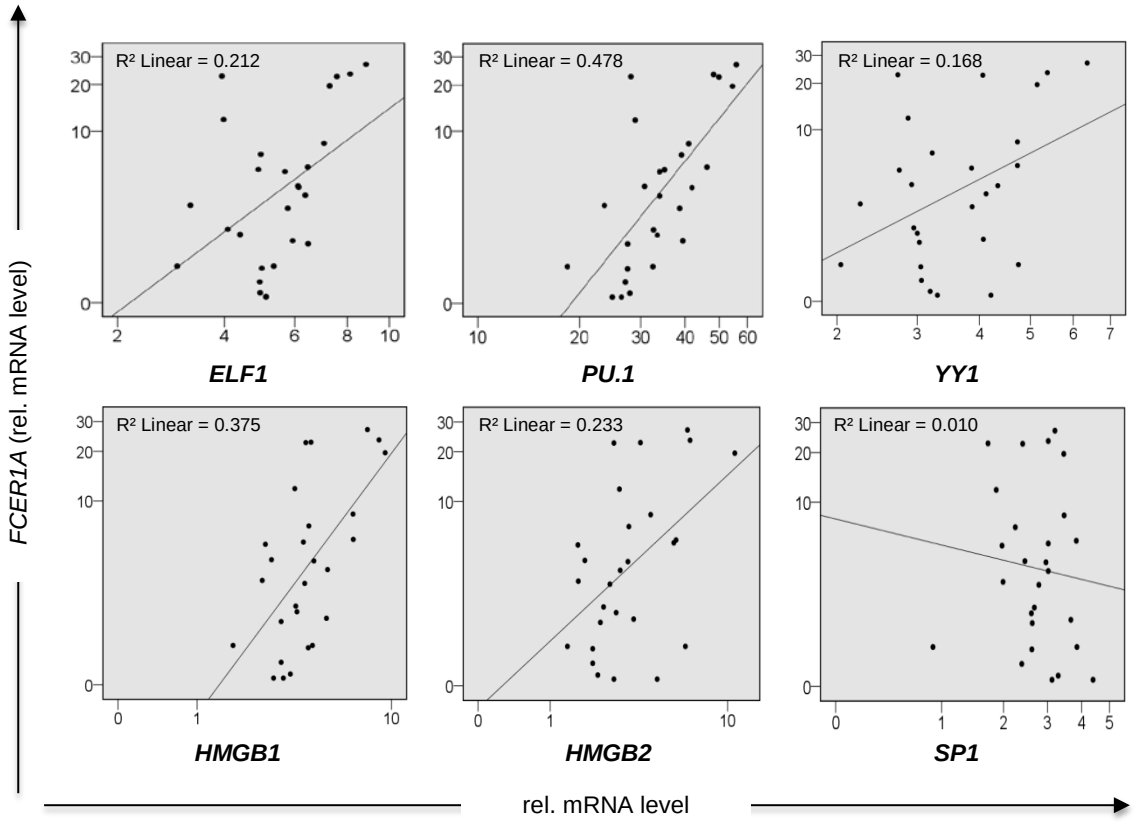

Figure 12 ***Expression of ELF1, PU.1, YY1, HMGB1 and HMGB2 correlates with FCER1A mRNA expression.*** CD34LC were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 16.6 x 10^{-3} µg/mL FSL-1, 1.0 µg/mL LTA or 1.0 µg/mL P3C for 24 h. qPCR was performed as described in Figure **6**. mRNA levels of the indicated transcription factors were quantified using global standard curves of the respective transcription factors and of ACTB for normalization. Relative mRNA levels of *FCER1A* were plotted against relative mRNA levels of indicated transcription factors. Statistical analyses of correlations are summarized in Table 22. Pearson correlations blots were generated using IBM SPSS Statistics 22 software.

Pearson correlation coefficients and significances of data depicted in Figure 12 were calculated and summarized in the following table.

Table 23 Summary of Pearson correlations and significances as depicted in Figure 12. Pearson correlation coefficients and significances were calculated using IBM SPSS Statistics 22 software.

		ELF1	***PU.1***	***YY1***	***HMGB1***	***HMGB2***	***SP1***
FCER1A	Pearson correlation	0.560**	0.682**	0.513**	0.649**	0.537**	0.082
	Sig. (2-tailed)	0.002	0,000	0.006	0.000	0.004	0.685
	N	27	27	27	27	27	27

** Correlation significance at level 0.01 (2-tailed).
* Correlation significance at level 0.05 (2-tailed).

The mRNA expression of the *FCER1A*-associated transcription factors *PU.1*, *EFL1*, *YY1, HMGB1* and *HMGB2* positively correlated with the *FCER1A* transcripts. *PU.1* achieved the highest correlation coefficient with 0.682. Significances were obtained for all transcription factors except *SP1* that neither correlated with *FCER1A*.

According to Figure 9, FcεRI expression decreased significantly with LPS and the heat killed bacteria, too. Therefore, FcεRI-associated transcription factors were examined again by qPCR after treatment of CD34LC with LPS, *S.a.*, *S.p.* and P3C.

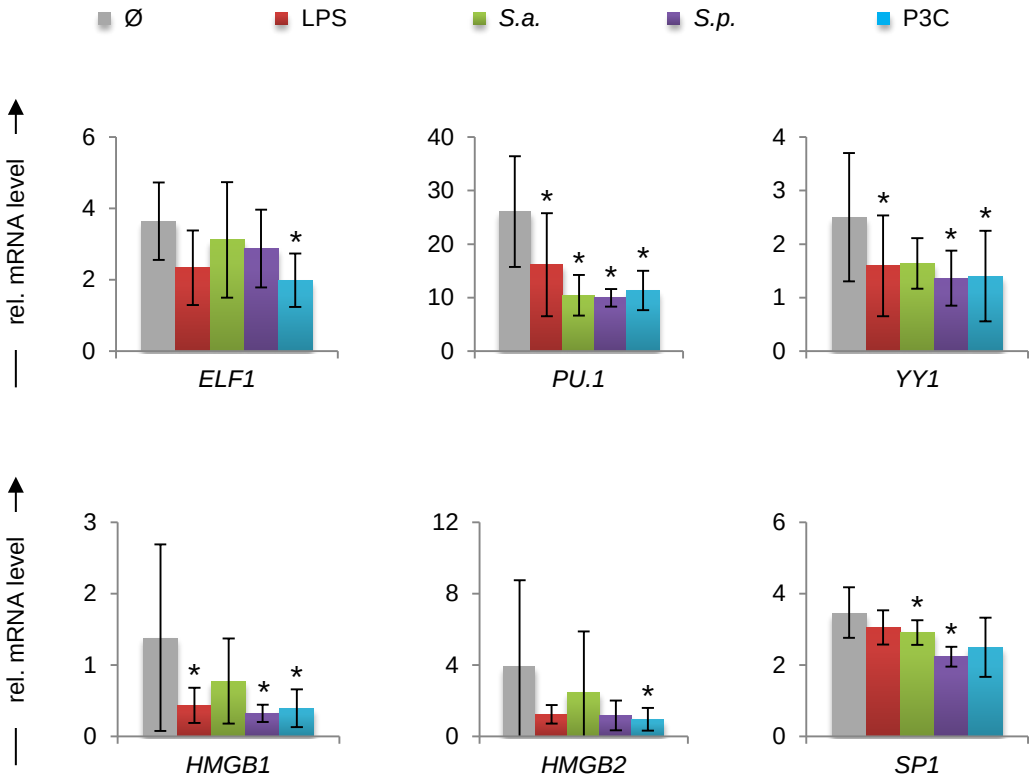

Figure 13 ***PU.1 is significantly decreased upon stimulation of CD34LC with LPS, S.a. and S.p..*** Cells were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 0.1 µg/mL LPS, 10 µL/mL *S.a.*, 6.5 x 10^9 cells/mL *S.p.* or 1.0 µg/mL P3C for 24 h. qPCR was performed as described in Figure 6**.** mRNA levels of the indicated transcription factors were quantified using global standard curves of the respective transcription factor and of *ACTB* for normalization. Relative mRNA levels ± SD from n=6-7 experiments are shown. Statistical significance was assessed by paired-sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. * $p < 0.05$; (SD = standard deviation)

Only the transcription factor *PU.1* was downregulated significantly by all tested stimulants. The expression of *YY1* was diminished equally by LPS, *S.p.* and P3C. *HMGB1* expression was decreased significantly by LPS, *S.p.* and P3C, while *HMGB2* showed a significant reduction only with P3C. Overall, *HMGB1* and *HMGB2* exhibited a very heterogeneous expression. Less regulatory effects on mRNA levels were attained for the transcription factors *ELF1* and *SP1*.

In summary, *PU.1* was the only transcription factor that decreased significantly with all investigated stimuli.

Again, Pearson correlations of *FCER1A* and the indicated transcription factors were analyzed in untreated, LPS, *S.a.*, *S.p.* and P3C treated CD34LC.

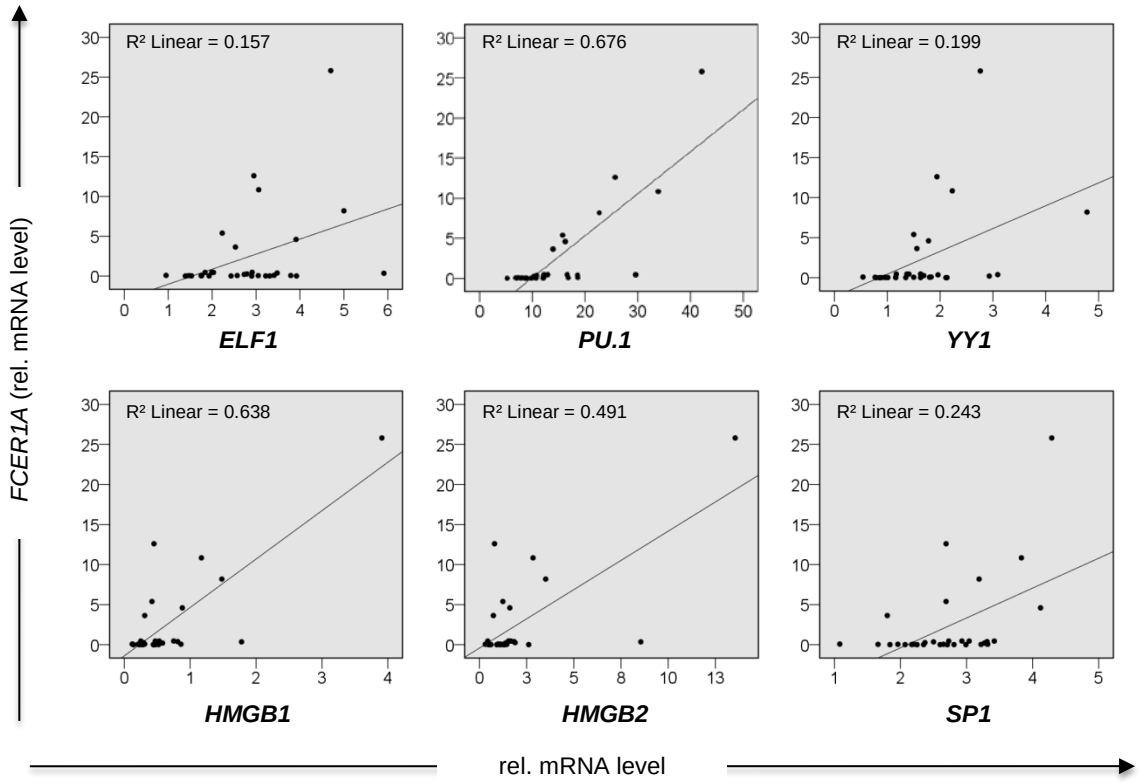

Figure 14 ***Expression of ELF1, PU.1, YY1, HMGB1 and HMGB2 correlates with FCER1A mRNA expression.*** CD34LC were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 0.1 µg/mL LPS, 10 µL/mL *S.a.*, 6.5 x 10^9 cells/mL *S.p.* or 1.0 µg/mL P3C for 24 h. qPCR was performed as described in Figure 6. mRNA levels of the indicated transcription factors were quantified using global standard curves of the respective transcription factor and of *ACTB* for normalization. Relative mRNA levels of *FCER1A* were plotted against relative mRNA levels of the indicated transcription factor. Statistical analyses of correlations are summarized in Table 23. Pearson correlations blots were generated using IBM SPSS Statistics 22 software.

Pearson correlation coefficients and significances of data depicted in Figure 14 were calculated and summarized in the following table.

Table 24 Summary of Pearson correlations and significances as depicted in Figure 14. Pearson correlation coefficients and significances were calculated using IBM SPSS Statistics 22 software.

		ELF1	***PU.1***	***YY1***	***HMGB1***	***HMGB2***	***SP1***
FCER1A	Pearson correlation	0.396**	0.822**	0.446**	0.799**	0.700**	0.493
	Sig. (2-tailed)	0.025	0.000	0.010	0.000	0.000	0.004
	N	32	32	32	32	32	32

** Correlation significance at level 0.01 (2-tailed).

* Correlation significance at level 0.05 (2-tailed).

The correlation analysis revealed the highest correlation of *FCER1A* with *PU.1* (0.822) followed by *HMGB1* (0.799) and *HMGB2* (0.700). Correlation coefficients of *ELF1*, *YY1* and *SP1* were less than 0.5.

Taken together so far, *PU.1* showed the highest expression of all *FCER1A* related transcription factors investigated in this study and may therefore contribute most strongly to the regulation of FcεRI. Stimulation of CD34LC with TLR2 and TLR4 ligands as well as with heat-killed bacteria resulted in a significant decrease of *PU.1* which correlated with the *FCER1A* mRNA expression. Therefore, PU.1 protein expression was further investigated by intracellular staining with anti-PU.1 and flow cytometry to analyze the expression of the transcription factor on protein level after TLR2 stimulation of CD34LC with P3C.

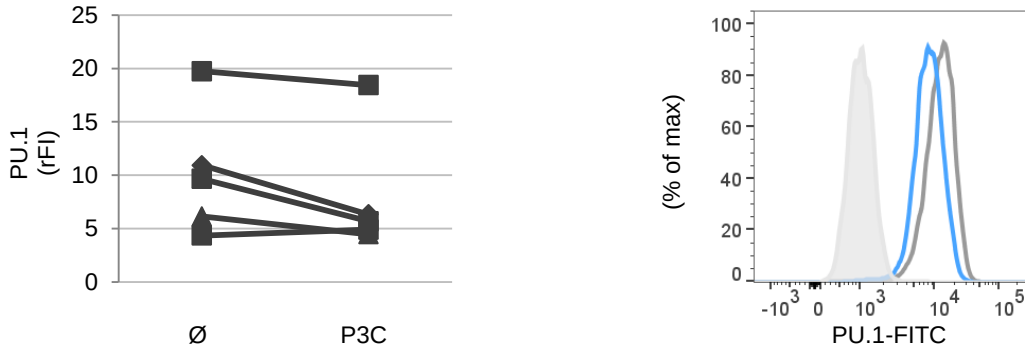

Figure 15 ***Intracellular PU.1 expression is reduced after TLR-mediated stimulation.*** CD34LC were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 1.0 µg/mL P3C for 24 h. 2 x 10^5 cells were prepared for intracellular staining according to the BD Cytofix/Cytoperm™ kit protocol. Viable $CD1a^+$ cells were stained with anti-PU.1 and followed by FITC-labeled goat anti-mouse IgG. Intracellular PU.1 of n=5 experiments was analyzed by flow cytometry using Flow Cytometer FACSCanto™ (A). Representative histograms of intracellular PU.1 expression of untreated (grey line) and P3C-treated cells (blue line) are depicted in B (Isotype control = grey tinted).

The expression of PU.1 protein level was reduced up to one third after TLR1/TLR2 heterodimer engagement with P3C. These results were in line with the reduced *PU.1* mRNA expression level.

Comparing the *FCER1A*-associated transcription factor investigated in this study, *PU.1* was most strongly expressed in CD34LC. *PU.1* mRNA expression was significantly downregulated after engagement of TLR1/2 (P3C), TLR2/6 (FSL-1, LTA), TLR4 (LPS) and with preparations of *S.a.* and *S.p.*. On protein level, PU.1 was reduced up to 30 % after stimulation of CD34LC with P3C. Furthermore, *PU.1* expression correlated with the expression of *FCER1A*. In sum, among the here investigated transcription factors, PU.1 may contribute most strongly to the regulation of FcεRI in CD34LC. It will be elucidated in the next part of this work how PU.1 itself is regulated in CD34LC upon stimulation.

4.4 Regulation of the transcription factor PU.1

The results obtained above suggest that PU.1 is involved in the regulation of the expression of FcεRI. PU.1 expression itself can be controlled on different levels. First, the transcription of PU.1 is regulated by several - in part tissue specific - transcription factors or transcriptional enhancers, as described *e.g.* for CCAAT-enhancer-binding proteins (C/EBP), c-jun or Runx1 (Behre *et al.* 1999, Leddin, Perrod *et al.* 2011). The latter is only expressed in early myeloid cell development and will not be included in this study. Secondly, post-transcriptional mechanisms such as protein-protein interactions like those of Gata-1 and PU.1, phosphorylation status of the PU.1 protein or *PU.1* mRNA binding by micro-RNA (miRNA) can influence the protein function and the protein formation. Additionally, recent studies revealed that the cytokine interleukin 33 (IL-33) enhances PU.1 expression in murine mast cells (Ito *et al.* 2015) most probably by using one of the above mentioned regulatory mechanisms.

4.4.1 C/EBPα and Gata-1 are not involved in the regulation of the transcription factor PU.1 in CD34LC

The function of C/EBPα is controversly discussed in the literature. In murine myeloid cells, C/EPBα is described to bind to an upstream regulatory element of the *PU.1* promotor and thereby promotes *PU.1* auto-activation through opening chromatin structures (Leddin, Perrod *et al.* 2011). Former studies revealed that C/EBPα competes with c-Jun, a co-activator of *PU.1*, and thereby inhibits *PU.1* transcription in myeloid U937 cells (Reddy, Iwama *et al.* 2002).

Moreover, protein-protein interactions as described for the Gata-1 zink finger domains and PU.1 Ets domains can negatively influence the DNA binding capacity of PU.1 (Nerlov, Querfurth *et al.* 2000), too.

Here, the expression of *C/EBPA* and *GATA-1* in FSL-1, LTA and P3C treated CD34LC was examined by qPCR.

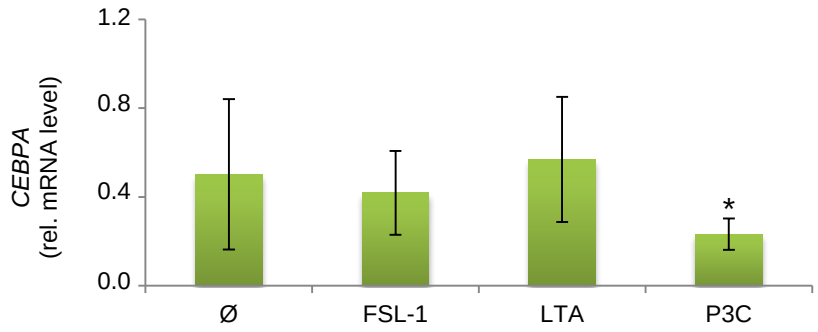

Figure 16 ***CEBPA is only marginally expressed in CD34LC.*** CD34LC were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 16.6 x 10^{-3} µg/mL FSL-1, 1.0 µg/mL LTA or 1.0 µg/mL P3C for 24 h. qPCR was performed as described in Figure 6. mRNA levels of *CEBPA* were quantified using global standard curves of *CEBPA* and of *ACTB* for normalization. Mean values of relative mRNA levels ± SD from n=6-7 experiments are shown. Statistical significance was assessed by paired-sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. *p ≤ 0.05; (SD = standard deviation)

CEBPA was only negligibly expressed in CD34LC and showed a significant regulation only with P3C. *GATA1* mRNA was not detectable in any case (data not shown).

Recent studies reported an upregulation of PU.1 by IL-33 in murine mast cells (Ito, Egusa *et al.* 2015). Here, IL-33 treated CD34LC (kind gift of Tim Stroisch and Dr. Takanobu Kan) were analyzed for their *PU.1* and additionally for *FCER1A* and *FCER1G* expression.

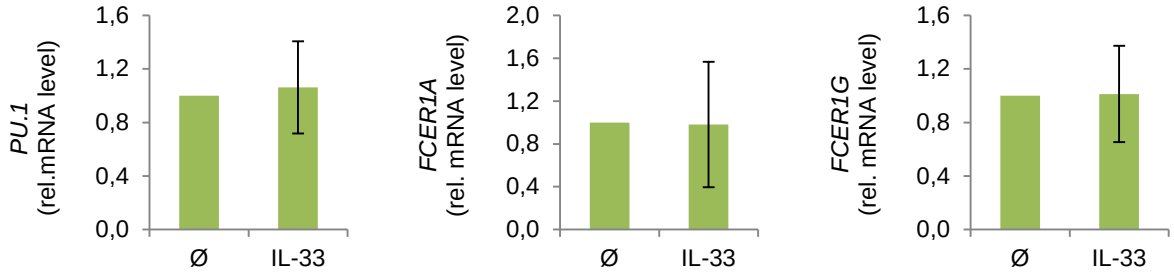

Figure 17 ***IL-33 has no influence on PU.1 expression.*** CD34LC were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 10 ng/mL IL-33 for 24 h. qPCR was performed as described in Figure 6. Mean values of relative mRNA levels ± SD from n=9 experiments are shown. Statistical significance was assessed by paired-sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. *p ≤ 0.05; (SD = standard deviation)

Neither *PU.1* nor *FCER1A* and *FCER1G* mRNA levels were affected by IL-33 treatment.

The lack of *GATA1* expression and the very low *C/EBPA* mRNA levels in CD34LC renders it unlikely that these transcription factors are involved in PU.1-mediated regulation of FcεRI in CD34LC. IL-33 neither emerged as a candidate for PU.1 regulation in these cells. Therefore, in the next step the contribution of miRNA to PU.1 regulation in CD34LC was investigated.

4.4.2 The transcription factor PU.1 is regulated by miRNA-155

Since PU.1 regulation is not controlled by the above investigated factors in CD34LC, *PU.1*-targeting miRNA-155 was analyzed.

In general, miRNA are short single stranded regulatory nucleic acids. They are highly conserved in animals and plants and play a pivotal role in post-transcriptional modulation of gene expression by binding target mRNA. miRNA-155 was reported to down-regulate PU.1 expression by binding to the 3' UTR of *PU.1* mRNA (Martinez-Nunez, Louafi *et al.* 2009). Another miRNA, namely miRNA-146a, plays a pivotal role in desensitization against TLR dependent stimuli (Jurkin, Schichl *et al.* 2010, Nahid, Pauley *et al.* 2009) and is constitutively expressed in DC subsets. Since CD34LC were treated with several TLR ligands, miRNA-146a expression was analyzed in this work, too.

Here, TaqMan® MicroRNA Assay of miRNA-155 and miRNA-146a was performed to analyze the expression of those miRNA in FSL-1, LTA, LPS, *S.a.*, *S.p.* and P3C treated CD34LC.

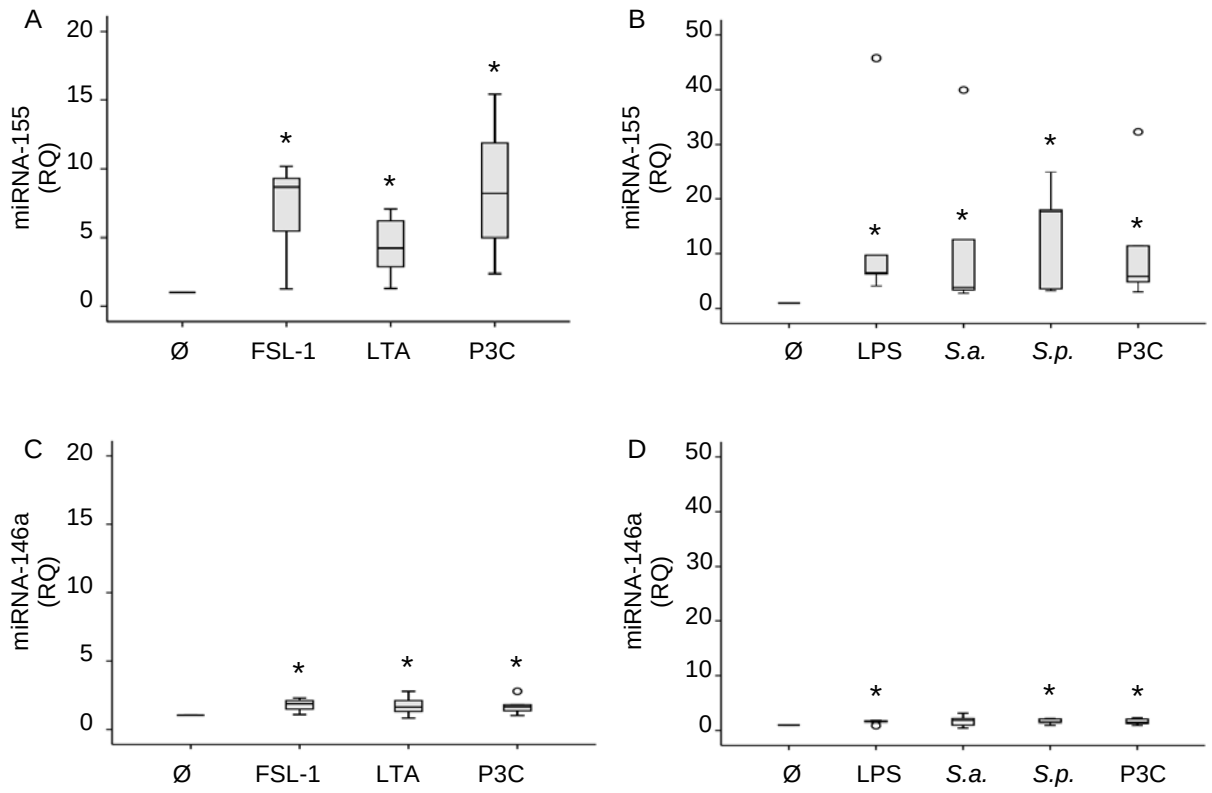

Figure 18 ***miRNA-155 is induced by stimulation of CD34LC with TLR ligands and heat-killed bacteria.*** CD34LC were harvested between d8 and d12. 1 x 10^6 cells/mL were left untreated (∅) or were treated with 16.6 x 10^{-3} µg/mL FSL-1, 1.0 µg/mL LTA or 1.0 µg/mL P3C (A, C), 0.1 µg/mL LPS, 10 µL/mL *S.a.*, 6.5 x 10^9 cells/mL *S.p.* or 1.0 µg/mL P3C (B, D) for 24 h. Total RNA preparation was performed as described in Figure 6. 10 ng RNA were subject to Taqman® MicroRNA Assays of miRNA-155 (A, B) and miRNA-146a (C, D). Boxplots of relative miRNA levels ± SD from n=6-7 experiments and statistical significances assessed by paired sample Wilcoxon signed rank test were generated using IBM SPSS Statistics 22 software.ᵒ =outlier; *p < 0.05; (SD = standard deviation)

Taqman® MicroRNA Assays of miRNA-155 revealed a strong and significant upregulation of miRNA-155 after stimulation of CD34LC with specific TLR ligands. TLR1/TLR2 heterodimer engagement with P3C caused the strongest effect with mean values up to 13-fold, followed by FSL-1 (7.1 ± 3.2 -fold) and LTA (4.4 ± 2.2 -fold) for TLR2/TLR6 engagement. Among the heat-killed bacteria, *S.p.* was the most powerful inducer of miRNA-155 (17.2 ± 12.7 -fold). miRNA-146a was less strongly induced after TLR1/TLR2, TLR2/TLR6 and TLR4 engagement as well as after stimulation with *S.a.* and *S.p.*.

To evaluate the relationship between the expression of miRNA-155 and miRNA-146a, respectively, and PU.1 as well as FcεRIα, Pearson correlation analyses for miRNA-155, miRNA146a, *PU.1* and *FCER1A* of untreated, FSL-1, LTA, LPS, *S.a.*, *S.p.* and P3C treated CD34LC were performed.

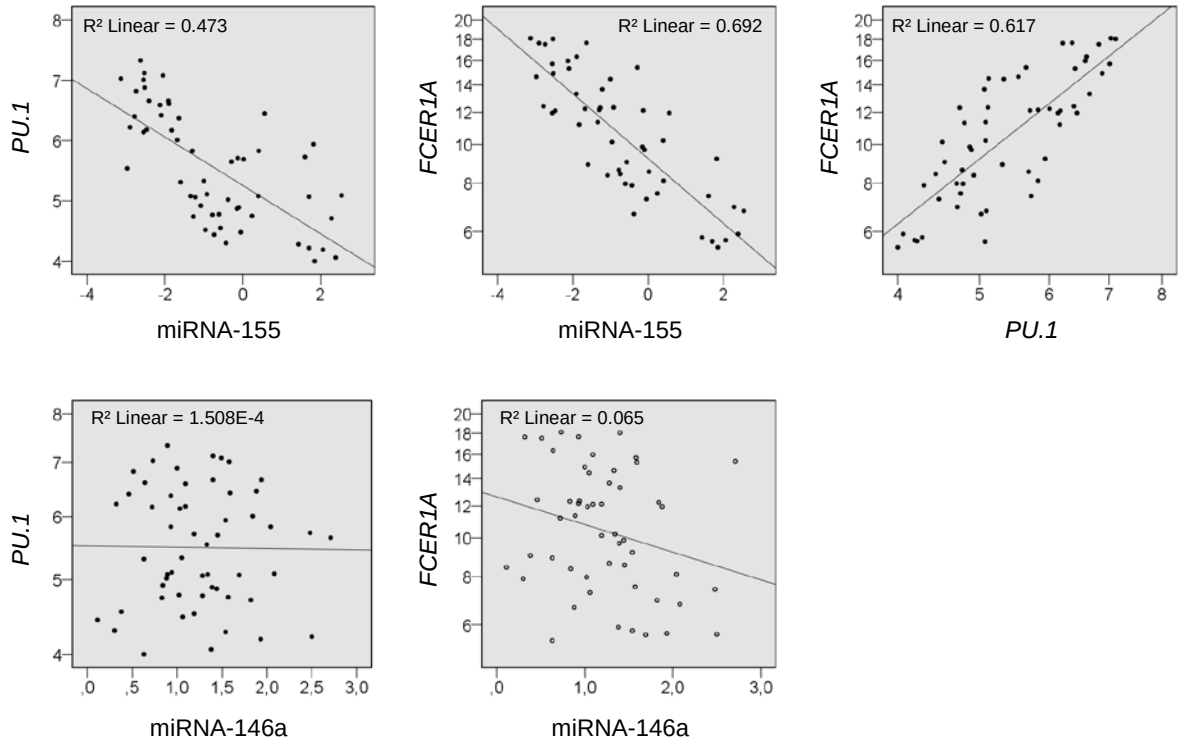

Figure 19 ***Expression of miRNA-155, but not miRNA-146a correlates with PU.1 and FCER1A mRNA.*** Cells were treated as described in Figure 18. ΔΔCT values of *PU.1*, *FCER1A* were plotted against miRNA-155 and miRNA-146a or against each other. Statistical analyses of correlations are summarized in Table 24. Pearson correlation plots were generated using IBM SPSS Statistics 22 software.

Pearson correlation coefficients and significances of data depicted in Figure 19 were calculated and summarized in the following table.

Table 25 Summary of Pearson correlations and significances as depicted in Figure 19. Pearson correlation coefficients and significances were calculated using IBM SPSS Statistics 22 software.

		miRNA-155	***PU.1***	***FCER1A***	**miRNA-146a**
miRNA-155	Pearson correlation	1	-0.688**	-0.807**	0.476**
	Sig. (2-tailed)		0.000	0.000	0.000
	N	55	55	52	55
PU.1	Pearson correlation	-0.688**	1	0.790**	-0.020
	Sig. (2-tailed)	0.000		0.000	0.883
	N	55	58	55	55
FCER1A	Pearson correlation	-0.807**	0.790**	1	-0.239
	Sig. (2-tailed)	0.000	0.000		0.088
	N	52	55	55	52

** Correlation significance at level 0.01 (2-tailed).
* Correlation significance at level 0.05 (2-tailed).

Correlation analysis of miRNA-155, *PU.1* and *FCER1A* mRNA expressions revealed significant negative correlations between miRNA-155 and *PU.1* mRNA as well as *FCER1A* mRNA. As expected, a significant positive correlation was found between mRNA of *PU.1* and *FCER1A*. miRNA-146a expression did not correlate with *PU.1* or *FCER1A*.

In conclusion, the data above revealed that miRNA-155 was strongly induced upon stimulation of CD34LC with several TLR ligands, while PU.1 and FcεRI decreased in inverse proportion. Thus, miRNA-155 seems to regulate PU.1 and indirectly FcεRI expression in a CD34LC activation-dependent manner. The regulation of PU.1 by miRNA-155 will be proved by transfection experiments with pre-miRNA-molecules in CD34LC.

4.5 Ectopic miRNA-155 is sufficient for downregulation of *PU.1* and FcεRI

As observed from Figure 18, miRNA-155 is induced by the stimulation of CD34LC with several TLR ligands or heat-killed bacteria. Transcription factor PU.1 and FcεRI were downregulated in line with miRNA-155 upregulation. Transfection of CD34LC with synthetically derived pre-miRNA molecules for miRNA-155 and miRNA-146a was established to evaluate miRNA function independent of stimulation conditions. Synthetically derived pre-miRNA are released into the cytoplasm of the target cells where they are transformed into mature and functional miRNA molecules using the endogenous pre-miRNA processing pathway.

4.5.1 miRNA transfection is successfully established in CD34LC

The first challenge in transfection experiments is to get the desired product into the chosen cells. In particular, transfection of immature CD34LC is tricky because of their capacity to mature rapidly upon several stimuli. Therefore a mild transfection method with lipid particles was selected and the transfection efficiency (TE) was proved in a first step.

TE was examined by flow cytometry analysis. CD34LC were transfected with Block-It-AF555, an unspecific miRNA coupled to an AlexaFluor®-555 fluorochrome. To exclude others than $CD1a^+$ cells, the efficiency was calculated only by the percentage of Block-It-$AF555^+/CD1a^+$ cells in relation to all $CD1a^+$ cells (see equation).

$$TE = \frac{\text{Block-IT-AF555}^+ \text{ / CD1a}^+}{\text{All CD1a}^+}$$

Figure 20 ***Equation for the calculation of the transcription efficiency in CD1a$^+$ CD34LC.*** Block-ItAF555 - transfected cell (see 3.1.6) were gated on their $CD1a^+$ cell population. The ratio of Block-It-$AF555^+/CD1a^+$ to all $CD1a^+$ cells was calculated.

To evaluate the kinetics of CD34LC transfection with Block-It-AF555, TE was analyzed by flow cytometry prior to transfection (0 h) as well as 2, 4 and 24 h after transfection.

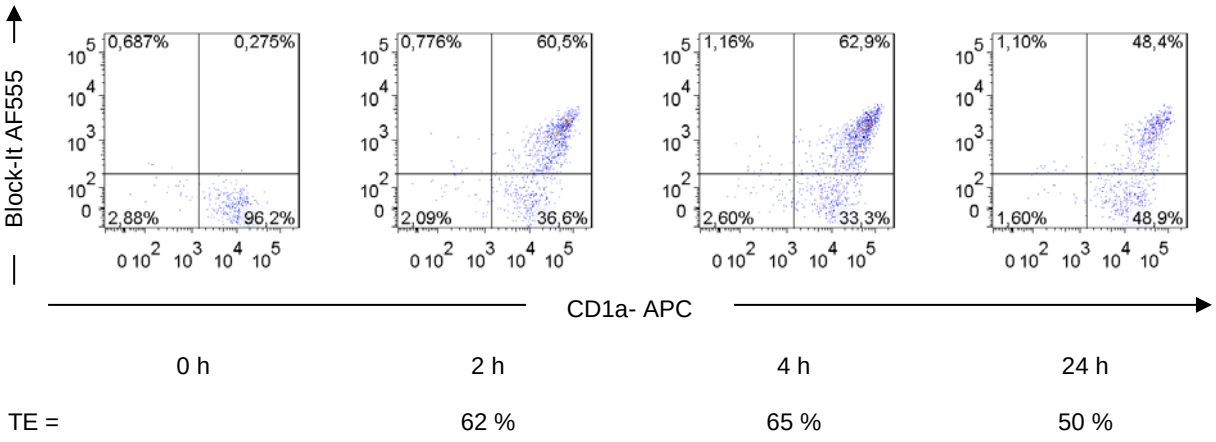

Figure 21 ***Transfection efficiency of CD34LC.*** CD34LC were harvested between d8 and d12. 0.5 x 10^6 cells/mL were transfected with 50 nm Block-It-AF555 fluorescence oligo for 2, 4 and 24 h. Cells were stained with anti-CD1a-APC and were subject to flow cytometry analysis using Flow Cytometer FACSCanto™.

The time course of CD34LC transfection with Block-It-AF555 showed the highest TE within 2 h and 4 h. After 24 h 50 % of all $CD1a^+$ were still Block-It-$AF555^+$. Since Block-It-AF555 is no pre-miRNA and will not be processed by the cells' machinery, it is just an indication of effective transfection. In summary, transfection experiments of this study showed heterogeneous TE reaching from 14 % to 50 % after 24 h.

For further validations, Taqman® MicroRNA Assays were performed to confirm specificity and quantity of every individual transfection with specific pre-miRNA. First, kinetics of specific pre-miRNA-155 transfection should provide information of the evolving time and the stability of mature miRNA in the cells. Therefore, CD34LC were transfected with pre-miRNA-155 and with Block-It-AF555 for 0.5, 1, 2, 4 and 24 h and were analyzed by Taqman® MicroRNA Assays for miRNA-155 at every time point.

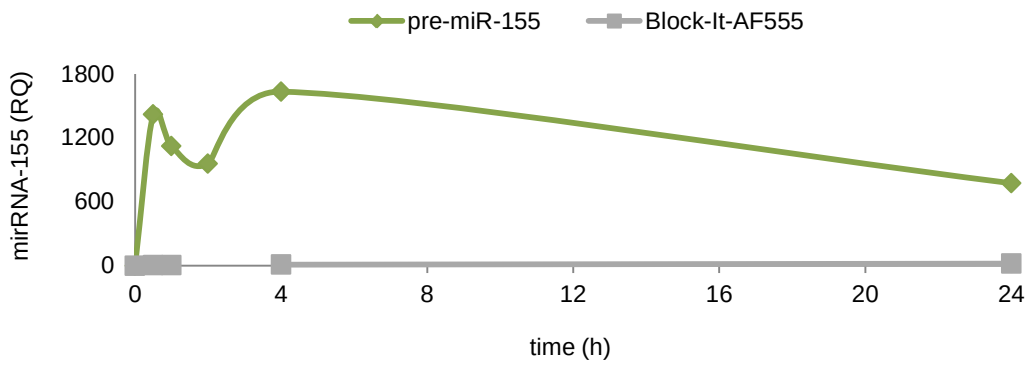

Figure 22 ***Ectopic miRNA-155 is stable up to 24 h.*** CD34LC were harvested between d8 and d12. 0.5 x 10^6 cells/mL were transfected with 50 nm pre-miRNA-155 or 50 nm Block-It-AF555 for 0.5, 1, 2, 4 and 24 h. Total RNA was prepared as described in Figure 6. 10 ng RNA were subject to Taqman® MicroRNA Assays for miRNA-155.

Time course experiments of pre-miRNA-155 transfection in CD34LC revealed that the amounts of detected miRNA-155 via Taqman® MicroRNA Assays reached a very high peak after 4 h and were stable for at least 24 h. Transfection with Block-It-AF555 was used as control miRNA and for calculation of the transfection efficiency by flow cytometry analysis (see Figure 20). Block-It-miRNA transfection showed no effect on miRNA-155 expression. Next, the specifity of pre-miRNA-155 and pre-miRNA-146a transfection was checked by Taqman® MicroRNA Assays.

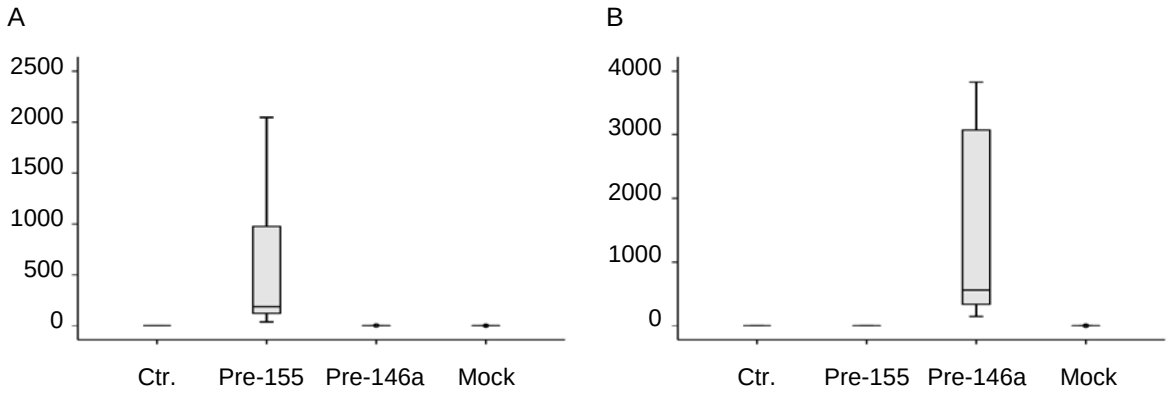

Figure 23 ***Transfections of pre-miRNA-155 and pre-miRNA-146a are specific.*** CD34LC were harvested between d8 and d12. 0.5 x 10^6 cells/mL were transfected with control miRNA (Ctr.), 50 nm pre-miRNA-155 (Pre-155), 50 nm pre-miRNA-146a (Pre-146a) or with Lipofectamine only (mock) for 24 h. RNA preparation was performed as described in Figure 6. 10 ng RNA were subject to Taqman® MicroRNA Assays of miRNA-155 (A) and miRNA-146a (B). Boxplots of n=7-10 (mock n=5) experiments were created using IBM SPSS Statistics 22 software.

Transfections with pre-miRNA-155 and pre-miRNA-146a showed a high increase of mature miRNA-155 respective miRNA-146a. To exclude side effects and to confirm the specificity of the transfections, the primers of a respective Taqman® MicroRNA Assay were used for both pre-miRNA transfections.

As evident from Figure 21 to Figure 23, transfection of CD34LC with pre-miRNA-155 and pre-miRNA-146a was established successfully. Taqman® MicroRNA Assays of both miRNA confirmed a successful, specific and sufficient transfection of CD34LC with pre-miRNA-155 and pre-miRNA-146a. Therefore, basic requirements for the following analysis of the influence of miRNA-155 on *PU.1* and FcεRI in CD34LC were met.

4.5.2 miRNA-155 down-regulates PU.1 and FcεRI

miRNA-155 has been validated for binding *PU.1* mRNA in human DC (Martinez-Nunez, Louafi *et al.* 2009). In the previous section, pre-miRNA transfection in CD34LC was successfully established. Next, the influence of ectopic miRNA-155 on *PU.1* and subsequently FcεRI expression will be investigated.

CD34LC were transfected with pre-miRNA-155 and pre-miRNA-146a. Expression of *PU.1* and FcεRI was analyzed by qPCR and flow cytometry.

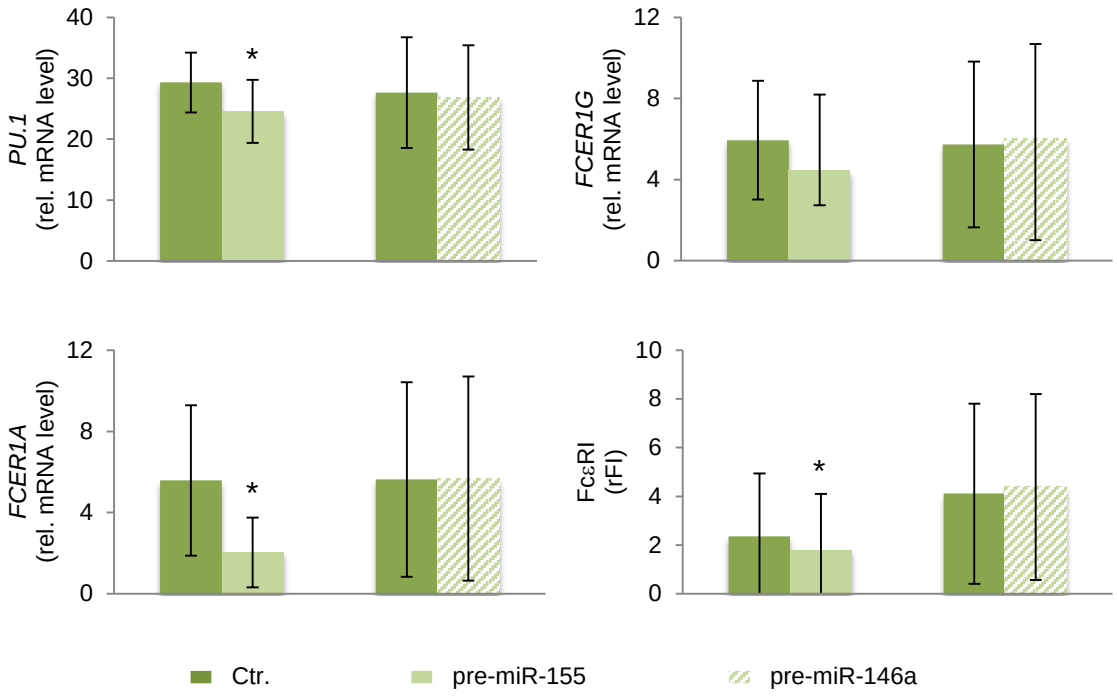

Figure 24 ***PU.1 and FCER1A are downregulated by ectopic miRNA-155.*** CD34LC were harvested between d8 and d12. 0.5 x 10^6 cells/mL were transfected with 50 nm pre-miRNA-155 (pre-miR-155), 50 nm pre-miRNA-146a (pre-miR-146a) or 50 nm control miRNA (Ctr.) for 24 h. qPCR was performed as described in Figure 6. FcεRI cell surface expression of viable $CD1a^+$ cells was analyzed by flow cytometry as described. Mean values ± SD of relative miRNA levels from n=9-10 experiments are shown. Statistical significance was assessed by paired-sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. *p ≤ 0.05; (SD = standard deviation)

Transfection of CD34LC with miRNA-155 precursor molecules resulted in a significant downregulation of *PU.1* and of FcεRI. Precursors of miRNA-146a did not influence *PU.1* nor FcεRI expression. *FCER1G* tended to decrease only with miRNA-155, but this was not significant. However, FcεRI decreased after miRNA-155 transfection most likely due to the downregulation of the *FCER1A* transcript. This may be caused by a miRNA-155 – induced *PU.1* reduction.

Finally, other FcεRI related transcription factors were analyzed by qPCR after pre-miRNA-155 and pre-miRNA-146a transfection of CD34LC.

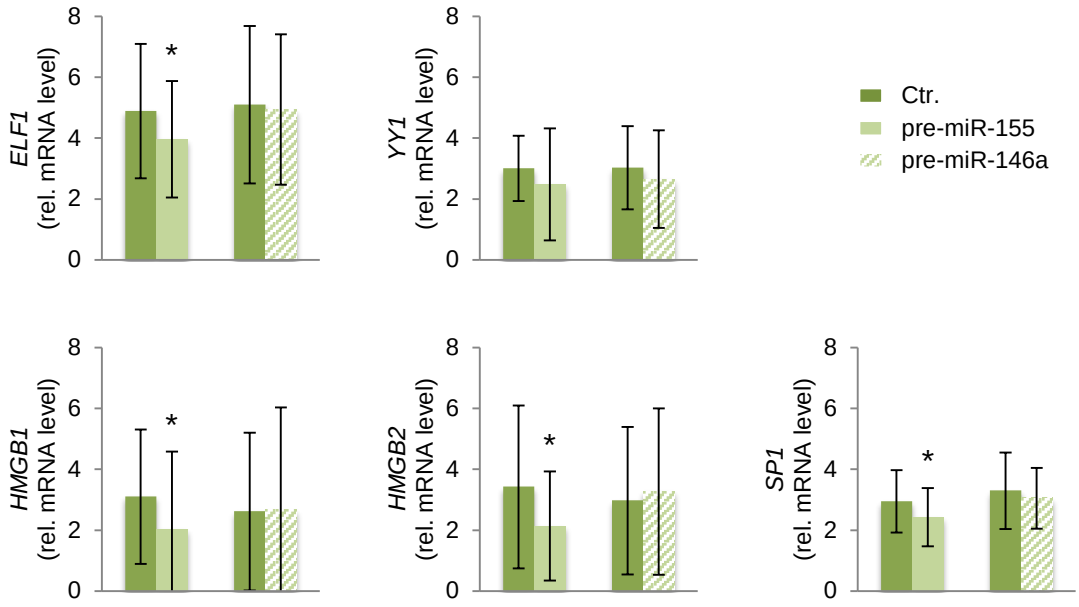

Figure 25 ***EFL1, HMGB1, HMGB2 and SP1 decrease upon transfection with ectopic miRNA-155.*** CD34LC were harvested between d8 and d12. 0.5 x 10^6 cells/mL were transfected with 50 nm pre-miRNA-155 (pre-miR-155), 50 nm pre-miRNA-146a (pre-miR-146a) or 50 nm control miRNA (Ctr.) for 24 h. qPCR was performed as described in Figure 6. Mean values ± SD of relative miRNA levels from n=9-10 experiments are shown. Statistical significance was assessed by paired-sample Wilcoxon signed rank test using IBM SPSS Statistics 22 software. *p ≤ 0.05; (SD = standard deviation)

After transfection of CD34LC with pre-miRNA-155, FcεRIα-associated transcription factors *ELF1*, *HMGB1*, *HMGB2* and *SP1* showed significant decreases along with *PU.1* and FcεRI downregulation. This suggests a contribution of those transcription factors to the regulation of FcεRI in CD34LC. To elucidate the relationship between ectopic miRNA-155 and miRNA-146a, Pearson correlation analysis was performed.

Table 26 Summary of Pearson correlations and significances of *FCER1A*, *FCER1G* and denoted transcription factors after pre-miRNA transfections. Pearson correlation coefficients and significances were calculated using IBM SPSS Statistics 22 software.

		FCER1A	***FCER1G***	***ELF1***	***PU.1***	***YY1***	***HMBG1***	***HMGB2***	***SP1***
miRNA-155	Pearson correlation	-0.558**	-0.490*	-0.449*	-0.440*	-0.354	-0.347	-0.491*	-0.690**
	Sig. (2-tailed)	0.006	0.018	0.032	0.036	0.098	0.104	0.017	0.000
	N	23	23	23	23	23	23	23	23
miRNA-146a	Pearson correlation	0.101	0.100	0.082	0.104	-0.095	-0.046	-0.011	0.141
	Sig. (2-tailed)	0.6634	0.6660	0.7237	0.6547	0.6816	0.8422	0.9631	0.5419
	N	21	21	21	21	21	21	21	21

** Correlation significance at level 0.01 (2-tailed).

* Correlation significance at level 0.05 (2-tailed).

Pearson correlation analysis revealed significant negative correlations for miRNA-155 with *FCER1A, FCER1G*, *PU.1* and the transcription factors *ELF1*, *HMGB2* and *SP1*. miRNA-146a did not correlate with *FCER1A* or any of the transcription factors investigated here. The results implicate again that miRNA-155 is participating in FcεRI regulation via PU.1 targeting. Other FcεRIα–associated transcription factors may contribute to the downregulation of FcεRI.

To conclude, the induction of miRNA-155 with ectopic precursor molecules proved to be a sufficient model to investigate FcεRI function. In CD34LC, *PU.1* and subsequently FcεRI were downregulated significantly by ectopic miRNA-155, but not by miRNA-146a. Downregulation of FcεRI was most likely caused by the decrease of *FCER1A*, since *FCER1G* was not changed significantly by miRNA-155 nor by miRNA-146a. Even if there is no evidence for being a target of miRNA-155 so far, *ELF1*, *HMBG2*, and *SP1* expression changed significantly in pre-miRNA-155 transfected CD34LC, too.

4.6 Summary of the results

In this work, an *in vitro* generated CD34$^+$ stem cell-derived Langerhans cell model (CD34LC) was validated for the investigation of FcεRI regulation upon activation of the cells. FcεRI was downregulated after engagement of CD34LC with a set of TLR ligands and inactivated bacterial strains of *S.a.* and *S.p.*. Among the investigated FcεRIα-related transcription factors, PU.1 was the most predominant one. PU.1 was downregulated significantly with all tested stimuli and its expression correlated with those of *FCER1A*. Concerning the regulation of PU.1 itself, miRNA-155 emerged as a post-transcriptional regulator of PU.1, while other regulatory elements such as C/EBPα, Gata-1 and IL-33 could be excluded in CD34LC. miRNA-155 was strongly induced upon stimulation of CD34LC, while PU.1 as well as FcεRI expressions decreased in inverse proportion. miRNA-146a was only weakly affected and lacked any influence on PU.1 or FcεRI expression. To confirm the regulatory influence of miRNA-155 on PU.1 and in succession on FcεRI expression independently of TLR activation, transfection experiments with pre-miRNA-155 and pre-miRNA-146 were established in CD34LC. Here, ectopic miRNA-155, but not miRNA-146, was sufficient to significantly down-regulate *PU.1* and subsequently FcεRIα independent of any stimuli.

Finally, this study uncovered that TLR signaling is linked to the regulation of FcεRI via miRNA-155 and PU.1 in CD34LC.

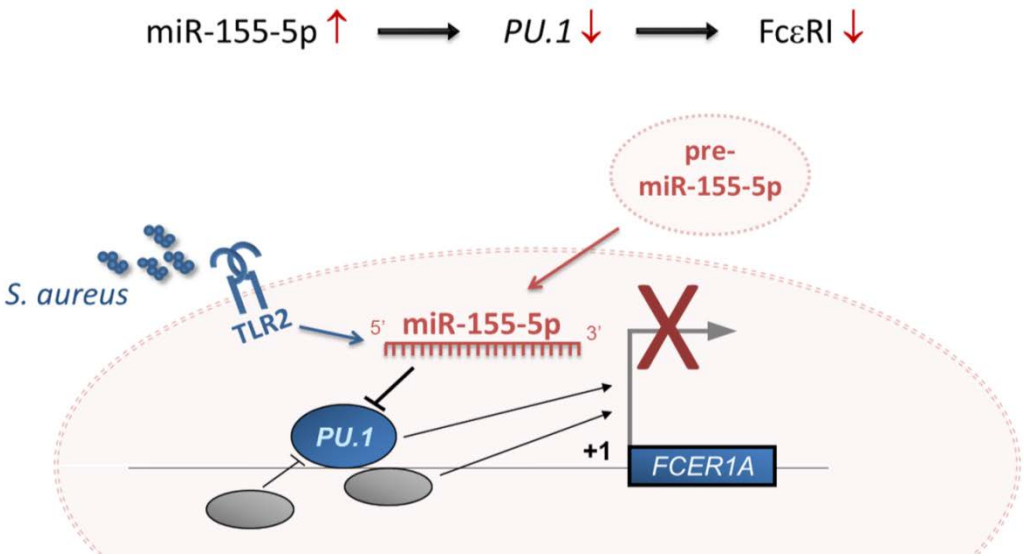

Figure 26 ***Scheme of miRNA-155-dependent regulation of FcεRI in human LC.*** TLR-induced or ectopic miRNA-155 reduces PU.1 and subsequently FcεRI expression.

5 Discussion

FcεRI is a crucial structure involved in IgE mediated allergic reactions, which is usually expressed on mast cells and basophiles. In the context of atopic dermatitis (AD), FcεRI is also expressed on epidermal LC and inflammatory dendritic epidermal cells (IDEC) and is involved in the pathophysiology of the disease (Bieber, de la Salle *et al.* 1992, Wollenberg, Kraft *et al.* 1996). Activation of LC by cross-linking of the receptor via allergen bound IgE may lead to either inflammatory immune responses via cytokine release and T cell activation or may also contribute to regulatory processes. AD skin is highly colonized with *Staphylococcus aureus* (*S.a.*) (Boguniewicz and Leung 2010) which can be recognized by pattern recognition receptors (PRR) like Toll-like receptors (TLR). We have shown previously that TLR-mediated activation on *in vitro* generated LC results in a downregulation of FcεRI expression (Herrmann, Koch *et al.* 2013). However, the mechanism governing the expression of FcεRI on LC in the context of AD is still unclear.

The aim of this work was to study the molecular mechanisms of the regulation of the FcεRI expression on human LC. Molecular aspects acting on the regulation of FcεRI expression were investigated.

5.1 *In vitro* generated CD34LC are comparable to skin-derived LC

CD34LC were generated from $CD34^+$ hematopoietic stem cells. Immature CD34LC express high amounts of Langerin and no CD83. TLR2 expression was sufficient for activation of CD34LC as shown by elevated surface protein levels of the DC maturation marker CD83. Additionally, the chemokine receptor CCR6, representative for immature DC, was downregulated while the maturation-associated chemokine receptor CCR7 was upregulated upon activation of CD34LC by TLR ligands. Thus, *in vitro* generated CD34LC are comparable to immature skin LC. Advantageously, CD34LC provide sufficient amounts of immature LC compared to isolated skin LC which are often restricted first in the number of the donors and secondly in the number of isolated LC. Moreover, skin LC often get activated by the isolation process and would not be suitable for further investigations in the context of this study.

FcεRI was heterogeneously expressed on the CD34LC. This could be explained by a differential donor dependent genetic background like single nucleotide polymorphisms for

FcεRI (Potaczek *et al.* 2009, Weidinger *et al.* 2010). Additionally, existing diseases, drug treatments, certain pregnancy conditions or yet-to-be-defined factors that could not be worked out within the scope of the study may contribute to this heterogeneity. However, variable FcεRI expression on CD34LC mirrors those of epidermal LC of skin preparations from healthy and atopic donors (Jurgens, Wollenberg *et al.* 1995).

In summary, *in vitro* generated CD34LC are comparable to immature skin LC and provide a suitable cell model to investigate TLR engagement-dependent FcεRI regulation.

5.2 FcεRI is downregulated by TLR engagement

CD34LC were stimulated with different TLR ligands as well as with bacterial lysates from *S.a.* and *S.p*. and FcεRI was downregulated strongly on the transcriptional and on the protein level. In contrast, elevated FcεRI expression levels were observed in AD (Bieber, de la Salle *et al.* 1992, Boguniewicz and Leung 2010, Wollenberg, Kraft *et al.* 1996) in spite of *S.a.* predominance, which would initiate TLR2-mediated responses of LC. Usually, activated LC leave the skin and migrate to local lymph nodes. It could be speculated that the high FcεRI expression as observed on AD LC is found on those resident skin LC that have not yet seen or even did not react to any TLR stimuli for reasons of habituation to the continuity of the bacterial attraction. *In vitro* generated cells were grown without any microbial stimuli by working under sterile conditions and therefore retain FcεRI expression. Another reason could be single nucleotide polymorphisms (SNP) in the *FCER1A* promotor region that go along with an upregulation of the receptor (Potaczek *et al.* 2013, Potaczek, Nishiyama *et al.* 2009, Weidinger, Gieger *et al.* 2008) in AD. Unfortunately, the origin of the blood samples used in this study did not allow for information about a putative atopic background and/or a current immunotherapy of the donors. In monocyte-derived DC, FcεRIα has been reported to reach the cell surface in dependency of FcεRIγ, which is expressed predominantly in DC from atopic individuals compared to non-atopic ones (Novak *et al.* 2003). In CD34LC, *FCER1G* mRNA expression levels were comparable to those of *FCER1A* in unstimulated cells, but were downregulated to a lesser extent upon stimulation. Thus, *FCER1A* seems to be the limiting factor of FcεRI surface expression in CD34LC.

5.3 Transcription factor PU.1 is involved in the downregulation of *FCER1A*

Regulation of FcεRI has been investigated in human mast cells (Inage *et al.* 2014). The transcription factors PU.1, Yy1, Gata-1, Hmgb1, Hmgb2 and Sp1 are described as enhancers of the proximal promotor of *FCER1A*, while Elf-1 represses its transcription by competing with PU.1 and Yy1 for the same binding site in the promotor sequence (Hasegawa, Nishiyama *et al.* 2003, Nishiyama, Hasegawa *et al.* 2002). PU.1 is a key factor in hematopoiesis and is highly expressed in lymphoid and myeloid progenitors (Scott *et al.* 1997, Scott *et al.* 1994). Among the transcription factors investigated here, PU.1 was most abundantly expressed in CD34LC. Its expression exceeded the mRNA levels of *ELF1, YY1*, *HMGB1*, *HMGB2* and *SP1* up to 4-fold. *GATA1* was not expressed. The very low expression levels of *ELF1* were unexpected, because ChIP-chip and co-transfection assays revealed PU.1 as a positive upstream regulatory element of the *ELF1* promotor (Calero-Nieto *et al.* 2010). One explanation could be an impeded *ELF1* promotor accessibility for PU.1 or a reduced promotor activity in CD34LC. However, the extend of the inhibitory influence of Elf-1 on the FcεRI expression still remains questionable since *ELF1* mRNA was in part reduced upon stimulation of the cells, too.

Stimulation experiments of CD34LC with TLR1/2 and TLR2/6 heterodimer ligands or with bacterial lysates of *S.a.* and *S.p.* revealed a significant downregulation of the transcription factor PU.1 in all cases. *YY1*, *HMGB1* and *HMGB2* expression was decreased by all stimuli, too. Only *SP1* showed little or no effect upon TLR engagement. Analysis of the *FCER1A*-associated transcription factors showed a positive correlation between *FCER1A* expression and the transcription factors *ELF1, PU.1, YY1, HMGB1* and *HMGB2*. Taken together, PU.1 may be the most influencing factor accompanied by Yy1, Hmgb1 and Hmgb2. One should keep in mind that the expression of FcεRI could be biased by the location and the phosphorylation status of the transcription factor proteins. As already mentioned in the introduction, the human FCER1A promotor consists of a proximal promotor cooperating with Elf-1, PU.1, Yy1 and Gata-1, while PU.1 and Yy1 have an inhibitory effect on the distal promotor element (Hasegawa, Nishiyama *et al.* 2003). The opposing function of PU.1 and Yy1 on the two promotor sites may have no influence in the CD34LC model, since the activity of the distal promotor element is IL-4 dependent and CD34LC are generated in the absence of IL-4.

Concerning the human α-chain promotor, there are several known SNP associated with allergic diseases and serum IgE levels (Weidinger, Gieger *et al.* 2008). A strong promotor

activation is described for the -315T and -66T allele sequences that allow the binding of Hmgb1 and Hmgb2 as well as several Gata-1 molecules. A weaker activity is reported for the -315C and the -66C allele sequences promoting Sp1 binding and less Gata-1 binding (Kanada, Nakano *et al.* 2008). Since *GATA1* and *SP1* transcripts are not accordingly expressed very weakly, PU.1 may be in fact the most important factor for the FcεRI expression in CD34LC.

5.4 miRNA-155 is sufficient for downregulation of FcεRI via its transcription factor PU.1

FCER1A is only one of at least 3000 genes regulated by PU.1 which is a key factor determining hematopoiesis and cell fate (Burda *et al.* 2010). Its expression is crucial for the differentiation and development of several lymphoid and myeloid cell lineages (Iwasaki *et al.* 2005, Scott, Fisher *et al.* 1997, Scott, Simon *et al.* 1994). PU.1 dysregulation can lead to severe leukemic diseases (reviewed in (Turkistany and DeKoter 2011)). Therefore, a precise control of PU.1 expression and function is indispensable.

The PU.1 promotor exhibits binding sites for several transcription factors like Sp1, Gata-1, Oct-1 and at least for PU.1 itself (Burda, Laslo *et al.* 2010). A sufficient and tissue-specific PU.1 expression depends on an additional upstream regulatory element (URE). Mouse experiments showed a strong decrease of PU.1 expression after deletion of the URE (Rosenbauer *et al.* 2006). The URE itself contains binding sites for several transcription and co-factors like C/EBP, Elf-1, Runx-1 or PU.1 itself. Binding of C/EBPα to the URE opens downstream chromatin structures for the binding of PU.1. Runx-1 is only required in early blood cell development and is not further required in myeloid cells (Leddin, Perrod *et al.* 2011). Here, the investigated transcription factors *ELF1* and *SP1* showed only minor expression and in the case of *SP1* no regulation upon stimulation of the cells. *C/EBPA* was expressed only negligibly in CD34LC. Because of its very low expression, *C/EBPA* expression differences between unstimulated and stimulated cell should be judged very carefully. PU.1 is constitutively expressed highly in CD34LC and is downregulated due to TLR engagement. This effect could be due to an altered availability of the promotor, the URE binding sites or the phosphorylation status of promotor- or URE-related factors. Additionally, PU.1 activity can be influenced by protein-protein interactions with other transcription factors like Gata-1, Gata-2 or C/EBPα (Nerlov, Querfurth *et al.* 2000, Reddy,

Iwama *et al.* 2002). *GATA1* and *GATA2* mRNA could not be detected in CD34LC and the very low expression of *C/EBPA* transcripts indicated a lack of sufficient protein amounts. Therefore it is unlikely that these mechanisms play major roles in TLR-mediated regulation of PU.1 in CD34LC.

Besides its transcriptional regulation, PU.1 protein synthesis can be regulated post-transcriptionally by binding of miRNA-155 to the 3' UTR of *PU.1* mRNA (Martinez-Nunez, Louafi *et al.* 2009). In CD34LC, miRNA-155 was induced and PU.1 decreased upon TLR engagement. The downregulation of PU.1 by miRNA-155 was confirmed by ectopic miRNA-155 expression independent of any stimulation. Since miRNA-146a is expressed constitutively in human LC and is highly involved in endotoxin-induced tolerance (Nahid, Satoh *et al.* 2011), this miRNA was investigated, too. miRNA-146a is positively controlled by NF-κB and PU.1, while C/EBPα is a dominant negative regulator of miRNA-146a (Jurkin, Schichl *et al.* 2010). It targets *inter alia* TLR down-stream signaling molecules like IRAK1 (IL-1R-associated kinase-1) and TRAF6 (TNFR-associated factor 6) (Taganov, Boldin *et al.* 2006). Keratinocytes and skin of patients with AD show an elevated level of miRNA-146a that is assumed to control inflammation by affecting NF-κB dependent inflammatory immune responses (Rebane, Runnel *et al.* 2014). Here, miRNA-146a expression was only little affected after stimulation of CD34LC with TLR ligands and ectopic miRNA-146a revealed no significant effect on *FCER1A*, *FCER1G*, *PU.1* or FcεRIα-associated transcription factors. In the context of PU.1 dependent regulation of the FcεRI expression in CD34LC, miRNA-146a plays no functional role.

Compared to healthy skin, AD skin exhibits elevated miRNA-155 levels mainly provided by dermal infiltrating $CD4^+CD3^+$ T and to a lesser extent by DC (Sonkoly, Janson *et al.* 2010). In total AD skin samples, miRNA-155 levels are highest in lesional skin followed by perilesional skin and lowest in normal skin (Ma *et al.* 2015). The results obtained in this study would suggest a reduction of PU.1 in the case of miRNA-155 binding, but PU.1 itself has been described to be elevated in AD, too (Ma *et al.* 2014). In PBMC of AD patients *PU.1* mRNA levels correlate with SCORing atopic dermatitis index (SCORAD) and with IgE levels (Ma, Xue *et al.* 2014). In murine bone marrow derived mast cells (BMMC) *PU.1* mRNA and protein levels are increased upon IL-33 treatment (Ito, Egusa *et al.* 2015), an interleukin that is upregulated upon house dust mice (HDM) or staphylococcal enterotoxin B (SEB) exposure (Savinko *et al.* 2012) in the human disease. In mice, IL-33 is positively regulated by miRNA-155 in chronic allergen challenged type 2 innate lymphoid cells (ILC2)

(Johansson *et al.* 2016). This would suggest that miRNA-155-induced PU.1 expression via IL-33 is more powerful than a concurrent downregulation of PU.1 through direct miRNA-155/mRNA interaction. In CD34LC, IL-33 receptor protein ST2 was not detectable on protein and on mRNA levels (personal communication, Tim Stroisch) and IL-33 did neither affect *PU.1* nor *FCER1A or FCER1G* expression.

However, *in vivo* the expression of transcription factors like C/EBPα and Gata-1 as well as the IL-33 receptor and IL-33 itself may be different and therefore influence the expression of FcεRI in healthy and in the disease.

Post-transcriptionally, alterations in the 3' UTR of PU.1 may influence target gene expression. A functional SNP in the 3'UTR (rs1057233) has been described in peripheral blood leukocytes of systemic lupus erythematosus patients and enhances PU.1 transcription (Hikami *et al.* 2011). *In silico* alignment using CLUSTAL-O (1.2.4) multiple sequence alignment (http://www.ebi.ac.uk/Tools/services) revealed no known SNP in the PU.1's 3' UTR (https://www.ncbi.nlm.nih.gov/snp/?term=SPI1) that affects the binding site for miRNA-155 (see Figure 27).

```
rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ------------------------------------------------------------
rs1057221        ------------------------------------------------------------
mir155_site      --------------CUCCCCGCUGGCCAUAGCAUUAA----------------------
rs1057233        ------------------------------------------------------------
PU.1 3'UTR       ccggccccgccaggcctccccgctggccatAGCATTAagccctcgcccggcccggacaca
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------
```

Figure 27 ***Up to date, no SNP of PU.1 3' UTR has been located in the miRNA-155 binding region.*** PU.1 3' UTR SNP sequences taken from https://www.ncbi.nlm.nih.gov/snp/?term=SPI1 were aligned with PU.1 mRNA (GenBank: X52056.1) and the miRNA-155 pairing side (http://www.targetscan.org) using CLUSTAL O (1.2.4) multiple sequence alignment (http://www.ebi.ac.uk/Tools/services). The representative detail of the binding region of miRNA-155 and the PU.1`s 3' UTR of the alignment is depicted (for complete alignment see attachment 8.1).

Moreover, the expression of cell type specific trans-acting factors like mRNA-binding proteins (RBP) can influence miRNA binding efficacy (Nam *et al.* 2014). RBB-induced local changes in the mRNA facilitate miRNA binding and function. This mechanism is well explained by the p27 tumor suppressor, whose miRNA (miR-221 and miR-222) are able to act on the p27 target mRNA only after RBP Pumilio-1 catalyzed local changes (Kedde *et al.* 2010, Nam, Rissland *et al.* 2014).

In summary, a miRNA-155 overcoming the *PU.1* level or alterations of the *PU.1*'s 3'UTR by not yet discovered SNP or RBP may affect the dialogue between miRNA-155 and *PU.1* in AD.

On behalf of the expression of FcεRIγ and FcεRIα-associated transcription factors, online miRNA target prediction tools (http://www.microrna.org, http://www.targetscan.org) did not identify conserved binding sites of miRNA-155 nor of miRNA-146a for *FCER1G*, *ELF1*, *HMGB1*, *HMGB2* and *SP1*. Only for *YY1*, a putative binding of miRNA-146a (5p or 3p n/a) was found using http://www.microrna.org prediction tool. However, it could not be validated with the help of another miRNA target validation tool (http://mirtarbase.mbc.nctu.edu.tw). Nevertheless, miRNA-146a transfection showed a slightly, but not significant reduced *YY1* expression and therefore may not have a strong relevance in CD34LC. Despite finding no or only one questionable target site in the above mentioned FcεRIα-associated transcription factors, their expressions were analyzed after transfection of CD34LC with pre-miRNA-155 and pre-miRNA-146a. Significant and correlative reductions were achieved only for *ELF1*, *HMBG1* and *SP1* by ectopic miRNA-155. All three factors were still expressed weakly in CD34LC even in unstimulated cells. However, FcεRI regulation is not achieved by one factor alone and therefore they all may contribute to its regulation. Compared to pre-miRNA-155, ectopic miRNA-146a did not reveal any clear effect on the expression of the transcription factors investigated here.

5.5 Conclusion

In conclusion, PU.1 was downregulated by miRNA-155 in CD34LC. In line with its function as a positive transcription factor of FcεRI, PU.1 decrease was accompanied by a reduction of FcεRI expression. This supports the thesis of a functional TLR – miRNA-155 – PU.1 -

FcεRI pathway in CD34LC. In AD, this pathway may be altered or interrupted at different points. First, TLR expression, susceptibility or activation can be impaired in resident epidermal LC. Secondly, miRNA-155 – dependent PU.1 binding and regulation could be impaired by a failed miRNA/mRNA pairing or may be overcome by a superior number of PU.1 molecules. Thirdly, SNP in the *FCER1A* promotor may cause a constitutively high expression of the receptor. Even if miRNA-155, PU.1 and FcεRI have all been described to be elevated in AD, it does not mean that this happens at same time and even not in the same cells. Nevertheless, it is very likely that the high expression of FcεRI in the disease is regulated rather multifactorially and very individually. This keeps the research in this field challenging and very exciting.

5.6 Perspectives

The observations made in this study revealed the involvement of miRNA-155, the transcription factor PU.1 and TLR2/TLR4 in the regulation of FcεRI in human primary LC.

These findings open new questions concerning differences in the local expression *in vivo* of PU.1 and miRNA-155 between epidermal and mucosal LC, the influence of different environmental conditions like UV exposure, pH values, temperatures or the connection to other regulatory pathways involved in the regulation of FcεRI. Moreover, the investigation of miRNA expression profiles of acute and chronic AD episodes may provide new biomarkers for the disease and may open new possibilities for the development of individual treatment strategies.

During the last decade, miRNA therapeutics formulated as anti-miRNA or as miRNA mimics have evolved as candidates in the drug development. However, even though there are first promising results of preclinical studies (reviewed in (Rupaimoole and Slack 2017)), successful application of miRNA as therapeutics is very challenging. In general, miRNA target several mRNA, which can be involved in physiological, but also in pathological pathways. The involvement of miRNA-155 in lymphoma and solid tumors makes miRNA-155 a candidate tool for the treatment of those diseases. While studies with anti-miR-155 are promising, miRNA-155 mimicking drugs might be problematic, because of the oncogenic nature of this special miRNA. Provided that oncogenicity, toxicity and off-target effects will be eliminated and an efficacious delivery system can be established, a local low-dose treatment with miRNA-155 mimic might drive skin LC to downregulate FcεRI and might thus

diminishing inflammatory responses in AD skin. Finally, further tests could verify whether combinations of miRNA-155 mimics and established drugs facilitate the therapeutic success.

6 Summary

Atopic dermatitis (AD) is a multifactorial severe skin disease with increasing incidence in western countries. In the disease, Langerhans cells (LC) play a pivotal role in bridging innate and adaptive immunity through sensing invading pathogens by pattern recognition receptors (PRR) like Toll-like receptors (TLR) and presenting them to naïve T cells. A hallmark of skin LC of atopic individuals is the expression of the high-affinity receptor for IgE, FcεRI. Our former studies revealed that TLR mediated activation of LC results in a downregulation of FcεRI expression (Herrmann, Koch *et al.* 2013), thus influencing the course of AD by a cross-talk of those receptors.

The aim of this study was to shed light on the regulatory mechanisms involved in FcεRI expression on activated human LC. Therefore, $CD34^+$ hematopoietic stem cell-derived LC (CD34LC) were used for stimulation assays with TLR ligands as well as with heat-killed *Staphylococcus aureus* (*S.a.*) and *Streptococcus pyogenes* (*S.p.*) to mimic the pathogens and the commensals colonizing human skin. Stimulation of CD34LC with all ligands resulted in a significant downregulation of FcεRI. Analysis of the factors promoting the expression of FcεRI revealed that the main transcription factor PU.1 and the less expressed transcription factors Yy1, Elf-1, Hmgb1 and Hmgb2 were significantly downregulated upon activation of the cells with the ligands used here.

Since PU.1 was the strongest expressed transcription factor in CD34LC, its regulation was further investigated. PU.1 is *inter alia* controlled by micro-RNA-155 (miRNA-155), a miRNA that is induced in macrophages and DC upon activation. The expression of miRNA-155 was examined and, additionally, CD34LC were transfected with pre-miRNA-155 molecules to confirm the effect of those miRNA on the expression of FcεRI. In summary, a high induction of miRNA-155 was observed under all stimulation conditions followed by a decrease of PU.1, which was further confirmed by ectopic miRNA-155. In line with PU.1 downregulation, FcεRI expression also decreased with all stimuli and with ectopic miRNA-155, too.

Another important immunological miRNA is miRNA-146a, which is expressed constitutively on LC and facilitates endotoxin-induced tolerance. miRNA-146a analysis was included in this study to check a putative involvement of this miRNA in the regulation of FcεRI expression. In contrast to miRNA-155, miRNA-146a was only weakly induced upon stimulation of CD34LC. Additionally, no effect of ectopic miRNA-146a on FcεRI expression was detected.

In conclusion, the results of this thesis revealed a regulatory network between TLR and FcεRI involving the TLR-dependent induction of miRNA-155, which in turn controls the translation of PU.1 and in succession the expression of FcεRI.

7 References

Allam, J. P., Niederhagen, B., Bucheler, M., Appel, T., Betten, H., Bieber, T., Berge, S. and Novak, N. (2006). "Comparative analysis of nasal and oral mucosa dendritic cells." Allergy **61**(2): 166-17210.1111/j.1398-9995.2005.00965.x.

Allam, J. P., Peng, W. M., Appel, T., Wenghoefer, M., Niederhagen, B., Bieber, T., Berge, S. and Novak, N. (2008). "Toll-like receptor 4 ligation enforces tolerogenic properties of oral mucosal Langerhans cells." J Allergy Clin Immunol **121**(2): 368-374 e36110.1016/j.jaci.2007.09.045.

Angel, C. E., Chen, C. J., Horlacher, O. C., Winkler, S., John, T., Browning, J., Macgregor, D., Cebon, J. and Dunbar, P. R. (2009). "Distinctive localization of antigen-presenting cells in human lymph nodes." Blood **113**(6): 1257-126710.1182/blood-2008-06-165266.

Ariizumi, K., Shen, G.-L., Shikano, S., Xu, S., Ritter, R., Kumamoto, T., Edelbaum, D., Morita, A., Bergstresser, P. R. and Takashima, A. (2000). "Identification of a Novel, Dendritic Cell-associated Molecule, Dectin-1, by Subtractive cDNA Cloning." Journal of Biological Chemistry **275**(26): 20157-2016710.1074/jbc.M909512199.

Banerjee, A., Schambach, F., Dejong, C. S., Hammond, S. M. and Reiner, S. L. (2010). "Micro-RNA-155 inhibits IFN-gamma signaling in CD4+ T cells." Eur J Immunol **40**(1): 225-23110.1002/eji.200939381.

Behre, G., Whitmarsh, A. J., Coghlan, M. P., Hoang, T., Carpenter, C. L., Zhang, D. E., Davis, R. J. and Tenen, D. G. (1999). "c-Jun is a JNK-independent coactivator of the PU.1 transcription factor." J Biol Chem **274**(8): 4939-4946,

Bender, A., Sapp, M., Schuler, G., Steinman, R. M. and Bhardwaj, N. (1996). "Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood." J Immunol Methods **196**(2): 121-135,

Bieber, T. (2008). "Atopic dermatitis." N Engl J Med **358**(14): 1483-1494, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=18385500

Bieber, T., De La Salle, H., Wollenberg, A., Hakimi, J., Chizzonite, R., Ring, J., Hanau, D. and De La Salle, C. (1992). "Human epidermal Langerhans cells express the high affinity receptor for immunoglobulin E (Fc epsilon RI)." J Exp Med **175**(5): 1285-1290, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1533242

Biedermann, T. (2006). "Dissecting the role of infections in atopic dermatitis." Acta Derm Venereol **86**(2): 99-10910.2340/00015555-0047.

Birbeck, M. S., Breathnach, A. S. and Everall, J. D. (1961). "An Electron Microscope Study of Basal Melanocytes and High-Level Clear Cells (Langerhans Cells) in Vitiligo**From the Chester Beatty Research Institute, Royal Cancer Hospital, London, S.W. 3, and the Departments of Anatomy, and Dermatology, St. Mary's Hospital Medical School (University of London) London, W. 2, England." Journal of Investigative Dermatology **37**(1): 51-64http://dx.doi.org/10.1038/jid.1961.80.

Blum, J. S., Wearsch, P. A. and Cresswell, P. (2013). "Pathways of antigen processing." Annu Rev Immunol **31**: 443-47310.1146/annurev-immunol-032712-095910.

Boguniewicz, M. and Leung, D. Y. (2010). "Recent insights into atopic dermatitis and implications for management of infectious complications." J Allergy Clin Immunol **125**(1): 4-13; quiz 14-15S0091-6749(09)01748-5 [pii]10.1016/j.jaci.2009.11.027.

Borkowski, T. A., Letterio, J. J., Farr, A. G. and Udey, M. C. (1996). "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 **184**(6): 2417-2422,

Breton, G., Lee, J., Zhou, Y. J., Schreiber, J. J., Keler, T., Puhr, S., Anandasabapathy, N., Schlesinger, S., Caskey, M., Liu, K. and Nussenzweig, M. C. (2015). "Circulating precursors of human CD1c+ and CD141+ dendritic cells." J Exp Med **212**(3): 401-41310.1084/jem.20141441.

Breton, G., Zheng, S., Valieris, R., Tojal Da Silva, I., Satija, R. and Nussenzweig, M. C. (2016). "Human dendritic cells (DCs) are derived from distinct circulating precursors that are precommitted to become CD1c+ or CD141+ DCs." J Exp Med **213**(13): 2861-287010.1084/jem.20161135.

Burda, P., Laslo, P. and Stopka, T. (2010). "The role of PU.1 and GATA-1 transcription factors during normal and leukemogenic hematopoiesis." Leukemia **24**(7): 1249-125710.1038/leu.2010.104.

Buwitt-Beckmann, U., Heine, H., Wiesmuller, K. H., Jung, G., Brock, R., Akira, S. and Ulmer, A. J. (2006). "TLR1- and TLR6-independent recognition of bacterial lipopeptides." J Biol Chem **281**(14): 9049-905710.1074/jbc.M512525200.

Calero-Nieto, F. J., Wood, A. D., Wilson, N. K., Kinston, S., Landry, J. R. and Gottgens, B. (2010). "Transcriptional regulation of Elf-1: locus-wide analysis reveals four distinct promoters, a tissue-specific enhancer, control by PU.1 and the importance of Elf-1 downregulation for erythroid maturation." Nucleic Acids Res **38**(19): 6363-637410.1093/nar/gkq490.

Carotta, S., Dakic, A., D'amico, A., Pang, S. H., Greig, K. T., Nutt, S. L. and Wu, L. (2010). "The transcription factor PU.1 controls dendritic cell development and Flt3 cytokine receptor expression in a dose-dependent manner." Immunity **32**(5): 628-64110.1016/j.immuni.2010.05.005.

Caux, C., Dezutter-Dambuyant, C., Schmitt, D. and Banchereau, J. (1992). "GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells." Nature **360**(6401): 258-26110.1038/360258a0.

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

Cella, M., Jarrossay, D., Facchetti, F., Alebardi, O., Nakajima, H., Lanzavecchia, A. and Colonna, M. (1999). "Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon." Nat Med **5**(8): 919-92310.1038/11360.

Ceppi, M., Pereira, P. M., Dunand-Sauthier, I., Barras, E., Reith, W., Santos, M. A. and Pierre, P. (2009). "MicroRNA-155 modulates the interleukin-1 signaling pathway in activated human monocyte-derived dendritic cells." Proc Natl Acad Sci U S A **106**(8): 2735-274010.1073/pnas.0811073106.

Chen, H., Ray-Gallet, D., Zhang, P., Hetherington, C. J., Gonzalez, D. A., Zhang, D. E., Moreau-Gachelin, F. and Tenen, D. G. (1995). "PU.1 (Spi-1) autoregulates its expression in myeloid cells." Oncogene **11**(8): 1549-1560,

Chicha, L., Jarrossay, D. and Manz, M. G. (2004). "Clonal type I interferon-producing and dendritic cell precursors are contained in both human lymphoid and myeloid progenitor populations." J Exp Med **200**(11): 1519-152410.1084/jem.20040809.

Chopin, M., Seillet, C., Chevrier, S., Wu, L., Wang, H., Morse, H. C., 3rd, Belz, G. T. and Nutt, S. L. (2013). "Langerhans cells are generated by two distinct PU.1-dependent transcriptional networks." J Exp Med **210**(13): 2967-298010.1084/jem.20130930.

Collin, M., Mcgovern, N. and Haniffa, M. (2013). "Human dendritic cell subsets." Immunology **140**(1): 22-3010.1111/imm.12117.

Colonna, M., Trinchieri, G. and Liu, Y. J. (2004). "Plasmacytoid dendritic cells in immunity." Nat Immunol **5**(12): 1219-122610.1038/ni1141.

Costinean, S., Sandhu, S. K., Pedersen, I. M., Tili, E., Trotta, R., Perrotti, D., Ciarlariello, D., Neviani, P., Harb, J., Kauffman, L. R., Shidham, A. and Croce, C. M. (2009). "Src homology 2 domain-containing inositol-5-phosphatase and CCAAT enhancer-binding protein beta are targeted by miR-155 in B cells of Emicro-MiR-155 transgenic mice." Blood **114**(7): 1374-138210.1182/blood-2009-05-220814.

Cremer, T. J., Ravneberg, D. H., Clay, C. D., Piper-Hunter, M. G., Marsh, C. B., Elton, T. S., Gunn, J. S., Amer, A., Kanneganti, T. D., Schlesinger, L. S., Butchar, J. P. and Tridandapani, S. (2009). "MiR-155 induction by F. novicida but not the virulent F. tularensis results in SHIP down-regulation and enhanced pro-inflammatory cytokine response." PLoS One **4**(12): e850810.1371/journal.pone.0008508.

Doulatov, S., Notta, F., Eppert, K., Nguyen, L. T., Ohashi, P. S. and Dick, J. E. (2010). "Revised map of the human progenitor hierarchy shows the origin of macrophages and dendritic cells in early lymphoid development." Nat Immunol **11**(7): 585-59310.1038/ni.1889.

Drummond, R. A. and Brown, G. D. (2011). "The role of Dectin-1 in the host defence against fungal infections." Curr Opin Microbiol **14**(4): 392-39910.1016/j.mib.2011.07.001.

Dzionek, A., Fuchs, A., Schmidt, P., Cremer, S., Zysk, M., Miltenyi, S., Buck, D. W. and Schmitz, J. (2000). "BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood." J Immunol **165**(11): 6037-6046,

Ebner, S., Ehammer, Z., Holzmann, S., Schwingshackl, P., Forstner, M., Stoitzner, P., Huemer, G. M., Fritsch, P. and Romani, N. (2004). "Expression of C-type lectin receptors by subsets of dendritic cells in human skin." Int Immunol **16**(6): 877-88710.1093/intimm/dxh088.

Ebralidze, A. K., Guibal, F. C., Steidl, U., Zhang, P., Lee, S., Bartholdy, B., Jorda, M. A., Petkova, V., Rosenbauer, F., Huang, G., Dayaram, T., Klupp, J., O'brien, K. B., Will, B., Hoogenkamp, M., Borden, K. L., Bonifer, C. and Tenen, D. G. (2008). "PU.1 expression is modulated by the balance of functional sense and antisense RNAs regulated by a shared cis-regulatory element." Genes Dev **22**(15): 2085-209210.1101/gad.1654808.

Eis, P. S., Tam, W., Sun, L., Chadburn, A., Li, Z., Gomez, M. F., Lund, E. and Dahlberg, J. E. (2005). "Accumulation of miR-155 and BIC RNA in human B cell lymphomas." Proc Natl Acad Sci U S A **102**(10): 3627-363210.1073/pnas.0500613102.

Elton, T. S., Selemon, H., Elton, S. M. and Parinandi, N. L. (2013). "Regulation of the MIR155 host gene in physiological and pathological processes." Gene **532**(1): 1-1210.1016/j.gene.2012.12.009.

Enk, A. H., Angeloni, V. L., Udey, M. C. and Katz, S. I. (1993). "Inhibition of Langerhans cell antigen-presenting function by IL-10. A role for IL-10 in induction of tolerance." J Immunol **151**(5): 2390-2398,

Faraoni, I., Antonetti, F. R., Cardone, J. and Bonmassar, E. (2009). "miR-155 gene: a typical multifunctional microRNA." Biochim Biophys Acta **1792**(6): 497-50510.1016/j.bbadis.2009.02.013.

Flacher, V., Bouschbacher, M., Verronese, E., Massacrier, C., Sisirak, V., Berthier-Vergnes, O., De Saint-Vis, B., Caux, C., Dezutter-Dambuyant, C., Lebecque, S. and Valladeau, J. (2006). "Human Langerhans cells express a specific TLR profile and differentially respond to viruses and Gram-positive bacteria." J Immunol **177**(11): 7959-7967,

Fujita, H., Shemer, A., Suarez-Farinas, M., Johnson-Huang, L. M., Tintle, S., Cardinale, I., Fuentes-Duculan, J., Novitskaya, I., Carucci, J. A., Krueger, J. G. and Guttman-Yassky, E. (2011). "Lesional dendritic cells in patients with chronic atopic dermatitis and psoriasis exhibit parallel ability to activate T-cell subsets." J Allergy Clin Immunol **128**(3): 574-582 e571-51210.1016/j.jaci.2011.05.016.

Ginhoux, F., Tacke, F., Angeli, V., Bogunovic, M., Loubeau, M., Dai, X. M., Stanley, E. R., Randolph, G. J. and Merad, M. (2006). "Langerhans cells arise from monocytes in vivo." Nat Immunol **7**(3): 265-27310.1038/ni1307.

Guilliams, M., Ginhoux, F., Jakubzick, C., Naik, S. H., Onai, N., Schraml, B. U., Segura, E., Tussiwand, R. and Yona, S. (2014). "Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny." Nat Rev Immunol **14**(8): 571-57810.1038/nri3712.

Guttman-Yassky, E., Lowes, M. A., Fuentes-Duculan, J., Whynot, J., Novitskaya, I., Cardinale, I., Haider, A., Khatcherian, A., Carucci, J. A., Bergman, R. and Krueger, J. G. (2007). "Major differences in inflammatory dendritic cells and their products distinguish atopic dermatitis from psoriasis." J Allergy Clin Immunol **119**(5): 1210-121710.1016/j.jaci.2007.03.006.

Hambleton, S., Salem, S., Bustamante, J., Bigley, V., Boisson-Dupuis, S., Azevedo, J., Fortin, A., Haniffa, M., Ceron-Gutierrez, L., Bacon, C. M., Menon, G., Trouillet, C., Mcdonald, D., Carey, P., Ginhoux, F., Alsina, L., Zumwalt, T. J., Kong, X. F., Kumararatne, D., Butler, K., Hubeau, M., Feinberg, J., Al-Muhsen, S., Cant, A., Abel, L., Chaussabel, D., Doffinger, R., Talesnik, E., Grumach, A., Duarte, A., Abarca, K., Moraes-Vasconcelos, D., Burk, D., Berghuis, A., Geissmann, F., Collin, M., Casanova, J. L. and Gros, P. (2011). "IRF8 mutations and human dendritic-cell immunodeficiency." N Engl J Med **365**(2): 127-13810.1056/NEJMoa1100066.

Haniffa, M., Bigley, V. and Collin, M. (2015). "Human mononuclear phagocyte system reunited." Semin Cell Dev Biol **41**: 59-6910.1016/j.semcdb.2015.05.004.

Hasegawa, M., Nishiyama, C., Nishiyama, M., Akizawa, Y., Takahashi, K., Ito, T., Furukawa, S., Ra, C., Okumura, K. and Ogawa, H. (2003). "Regulation of the human Fc epsilon RI alpha-chain distal promoter." J Immunol **170**(7): 3732-3738, http://www.ncbi.nlm.nih.gov/pubmed/12646639

Heinz, L. X., Platzer, B., Reisner, P. M., Jorgl, A., Taschner, S., Gobel, F. and Strobl, H. (2006). "Differential involvement of PU.1 and Id2 downstream of TGF-beta1 during Langerhans-cell commitment." Blood **107**(4): 1445-145310.1182/blood-2005-04-1721.

Herrmann, N., Koch, S., Leib, N., Bedorf, J., Wilms, H., Schnautz, S., Fimmers, R. and Bieber, T. (2013). "TLR2 down-regulates FcepsilonRI and its transcription factor PU.1 in human Langerhans cells." Allergy **68**(5): 621-62810.1111/all.12145.

Hikami, K., Kawasaki, A., Ito, I., Koga, M., Ito, S., Hayashi, T., Matsumoto, I., Tsutsumi, A., Kusaoi, M., Takasaki, Y., Hashimoto, H., Arinami, T., Sumida, T. and Tsuchiya, N. (2011). "Association of a functional polymorphism in the 3'-untranslated region of SPI1 with systemic lupus erythematosus." Arthritis Rheum **63**(3): 755-76310.1002/art.30188.

Hoeffel, G., Ripoche, A. C., Matheoud, D., Nascimbeni, M., Escriou, N., Lebon, P., Heshmati, F., Guillet, J. G., Gannage, M., Caillat-Zucman, S., Casartelli, N., Schwartz, O., De La Salle, H., Hanau, D., Hosmalin, A. and Maranon, C. (2007). "Antigen crosspresentation by human plasmacytoid dendritic cells." Immunity **27**(3): 481-49210.1016/j.immuni.2007.07.021.

Hoogenkamp, M., Krysinska, H., Ingram, R., Huang, G., Barlow, R., Clarke, D., Ebralidze, A., Zhang, P., Tagoh, H., Cockerill, P. N., Tenen, D. G. and Bonifer, C. (2007). "The Pu.1 locus is differentially regulated at the level of chromatin structure and noncoding transcription by alternate mechanisms at distinct developmental stages of hematopoiesis." Mol Cell Biol **27**(21): 7425-743810.1128/MCB.00905-07.

Hunger, R. E., Sieling, P. A., Ochoa, M. T., Sugaya, M., Burdick, A. E., Rea, T. H., Brennan, P. J., Belisle, J. T., Blauvelt, A., Porcelli, S. A. and Modlin, R. L. (2004). "Langerhans cells utilize CD1a and langerin to efficiently present nonpeptide antigens to T cells." J Clin Invest **113**(5): 701-70810.1172/jci19655.

Inage, E., Kasakura, K., Yashiro, T., Suzuki, R., Baba, Y., Nakano, N., Hara, M., Tanabe, A., Oboki, K., Matsumoto, K., Saito, H., Niyonsaba, F., Ohtsuka, Y., Ogawa, H., Okumura, K., Shimizu, T. and Nishiyama, C. (2014). "Critical Roles for PU.1, GATA1, and GATA2 in the expression of human FcepsilonRI on mast cells: PU.1 and GATA1 transactivate FCER1A, and GATA2 transactivates FCER1A and MS4A2." J Immunol **192**(8): 3936-394610.4049/jimmunol.1302366.

Ito, T., Egusa, C., Maeda, T., Numata, T., Nakano, N., Nishiyama, C. and Tsuboi, R. (2015). "IL-33 promotes MHC class II expression in murine mast cells." Immun Inflamm Dis **3**(3): 196-20810.1002/iid3.59.

Iwasaki, H., Somoza, C., Shigematsu, H., Duprez, E. A., Iwasaki-Arai, J., Mizuno, S., Arinobu, Y., Geary, K., Zhang, P., Dayaram, T., Fenyus, M. L., Elf, S., Chan, S., Kastner, P., Huettner, C. S., Murray, R., Tenen, D. G. and Akashi, K. (2005). "Distinctive and indispensable roles of PU.1 in maintenance of hematopoietic stem cells and their differentiation." Blood **106**(5): 1590-160010.1182/blood-2005-03-0860.

Johansson, K., Malmhall, C., Ramos-Ramirez, P. and Radinger, M. (2016). "MicroRNA-155 is a critical regulator of type 2 innate lymphoid cells and IL-33 signaling in experimental models of allergic airway inflammation." J Allergy Clin Immunol, 10.1016/j.jaci.2016.06.03510.1016/j.jaci.2016.06.035.

Jurgens, M., Wollenberg, A., Hanau, D., Delasalle, H. and Bieber, T. (1995). "Activation of Human Epidermal Langerhans Cells by Engagement of the High-Affinity Receptor for Ige, Fc-Epsilon-Ri." Journal of Immunology **155**(11): 5184-5189, <Go to ISI>://A1995TF68600013

Jurkin, J., Schichl, Y. M., Koeffel, R., Bauer, T., Richter, S., Konradi, S., Gesslbauer, B. and Strobl, H. (2010). "miR-146a is differentially expressed by myeloid dendritic cell subsets and desensitizes cells to TLR2-dependent activation." J Immunol **184**(9): 4955-496510.4049/jimmunol.0903021.

Kanada, S., Nakano, N., Potaczek, D. P., Maeda, K., Shimokawa, N., Niwa, Y., Fukai, T., Sanak, M., Szczeklik, A., Yagita, H., Okumura, K., Ogawa, H. and Nishiyama, C. (2008). "Two different transcription factors discriminate the -315C>T polymorphism of the Fc epsilon RI alpha gene: binding of Sp1 to -315C and of a high mobility group-related molecule to -315T." J Immunol **180**(12): 8204-8210, http://www.ncbi.nlm.nih.gov/pubmed/18523286

Kanitakis, J., Hoyo, E., Perrin, C. and Schmitt, D. (1993). "Electron-microscopic observation of a human epidermal Langerhans cell in mitosis." J Dermatol **20**(1): 35-39,

Kanitakis, J., Petruzzo, P. and Dubernard, J. M. (2004). "Turnover of epidermal Langerhans' cells." N Engl J Med **351**(25): 2661-266210.1056/nejm200412163512523.

Kaplan, D. H., Li, M. O., Jenison, M. C., Shlomchik, W. D., Flavell, R. A. and Shlomchik, M. J. (2007). "Autocrine/paracrine TGFbeta1 is required for the development of epidermal Langerhans cells." J Exp Med **204**(11): 2545-255210.1084/jem.20071401.

Kapsenberg, M. L. (2003). "Dendritic-cell control of pathogen-driven T-cell polarization." Nat Rev Immunol **3**(12): 984-99310.1038/nri1246.

Kashihara, M., Ueda, M., Horiguchi, Y., Furukawa, F., Hanaoka, M. and Imamura, S. (1986). "A monoclonal antibody specifically reactive to human Langerhans cells." J Invest Dermatol **87**(5): 602-607,

Kedde, M., Van Kouwenhove, M., Zwart, W., Oude Vrielink, J. A., Elkon, R. and Agami, R. (2010). "A Pumilio-induced RNA structure switch in p27-3' UTR controls miR-221 and miR-222 accessibility." Nat Cell Biol **12**(10): 1014-102010.1038/ncb2105.

Kerschenlohr, K., Decard, S., Przybilla, B. and Wollenberg, A. (2003). "Atopy patch test reactions show a rapid influx of inflammatory dendritic epidermal cells in patients with extrinsic atopic dermatitis and patients with intrinsic atopic dermatitis." J Allergy Clin Immunol **111**(4): 869-874,

Kinet, J. P. (1999). "The high-affinity IgE receptor (Fc epsilon RI): from physiology to pathology." Annu Rev Immunol **17**: 931-97210.1146/annurev.immunol.17.1.931.

Kingston, D., Schmid, M. A., Onai, N., Obata-Onai, A., Baumjohann, D. and Manz, M. G. (2009). "The concerted action of GM-CSF and Flt3-ligand on in vivo dendritic cell homeostasis." Blood **114**(4): 835-84310.1182/blood-2009-02-206318.

Klechevsky, E., Morita, R., Liu, M., Cao, Y., Coquery, S., Thompson-Snipes, L., Briere, F., Chaussabel, D., Zurawski, G., Palucka, A. K., Reiter, Y., Banchereau, J. and Ueno, H. (2008). "Functional specializations of human epidermal Langerhans cells and CD14+ dermal dendritic cells." Immunity **29**(3): 497-51010.1016/j.immuni.2008.07.013.

Kraft, S. and Kinet, J. P. (2007). "New developments in FcepsilonRI regulation, function and inhibition." Nat Rev Immunol **7**(5): 365-37810.1038/nri2072.

Kraft, S., Novak, N., Katoh, N., Bieber, T. and Rupec, R. A. (2002). "Aggregation of the high-affinity IgE receptor Fc(epsilon)RI on human monocytes and dendritic cells induces NF-kappaB activation." J Invest Dermatol **118**(5): 830-83710.1046/j.1523-1747.2002.01757.x.

Krol, J., Loedige, I. and Filipowicz, W. (2010). "The widespread regulation of microRNA biogenesis, function and decay." Nat Rev Genet **11**(9): 597-61010.1038/nrg2843.

Kurts, C., Cannarile, M., Klebba, I. and Brocker, T. (2001). "Dendritic cells are sufficient to cross-present self-antigens to CD8 T cells in vivo." J Immunol **166**(3): 1439-1442,

Langerhans, P. (1868). "Über die Nerven der menschlichen Haut." Virchows Archiv: The European Journal of Pathology 1868 **44**: 325-337,

Leddin, M., Perrod, C., Hoogenkamp, M., Ghani, S., Assi, S., Heinz, S., Wilson, N. K., Follows, G., Schonheit, J., Vockentanz, L., Mosammam, A. M., Chen, W., Tenen, D. G., Westhead, D. R., Gottgens, B., Bonifer, C. and Rosenbauer, F. (2011). "Two distinct auto-regulatory loops operate at the PU.1 locus in B cells and myeloid cells." Blood **117**(10): 2827-283810.1182/blood-2010-08-302976.

Lee, J., Breton, G., Oliveira, T. Y., Zhou, Y. J., Aljoufi, A., Puhr, S., Cameron, M. J., Sekaly, R. P., Nussenzweig, M. C. and Liu, K. (2015). "Restricted dendritic cell and monocyte progenitors in human cord blood and bone marrow." J Exp Med **212**(3): 385-39910.1084/jem.20141442.

Lee, R. C., Feinbaum, R. L. and Ambros, V. (1993). "The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14." Cell **75**(5): 843-854,

Lin, S., Cicala, C., Scharenberg, A. M. and Kinet, J. P. (1996). "The Fc(epsilon)RIbeta subunit functions as an amplifier of Fc(epsilon)RIgamma-mediated cell activation signals." Cell **85**(7): 985-995, http://www.ncbi.nlm.nih.gov/pubmed/8674126

Lowes, M. A., Chamian, F., Abello, M. V., Fuentes-Duculan, J., Lin, S. L., Nussbaum, R., Novitskaya, I., Carbonaro, H., Cardinale, I., Kikuchi, T., Gilleaudeau, P., Sullivan-Whalen, M., Wittkowski, K. M., Papp, K., Garovoy, M., Dummer, W., Steinman, R. M. and Krueger, J. G. (2005). "Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a)." Proc Natl Acad Sci U S A **102**(52): 19057-1906210.1073/pnas.0509736102.

Lu, C., Huang, X., Zhang, X., Roensch, K., Cao, Q., Nakayama, K. I., Blazar, B. R., Zeng, Y. and Zhou, X. (2011). "miR-221 and miR-155 regulate human dendritic cell development, apoptosis, and IL-12 production through targeting of p27kip1, KPC1, and SOCS-1." Blood **117**(16): 4293-430310.1182/blood-2010-12-322503.

Ma, L., Xue, H. B., Guan, X. H., Shu, C. M., Zhang, J. H. and Yu, J. (2014). "Possible pathogenic role of T helper type 9 cells and interleukin (IL)-9 in atopic dermatitis." Clin Exp Immunol **175**(1): 25-3110.1111/cei.12198.

Ma, L., Xue, H. B., Wang, F., Shu, C. M. and Zhang, J. H. (2015). "MicroRNA-155 may be involved in the pathogenesis of atopic dermatitis by modulating the differentiation and function of T helper type 17 (Th17) cells." Clin Exp Immunol **181**(1): 142-14910.1111/cei.12624.

Maraskovsky, E., Daro, E., Roux, E., Teepe, M., Maliszewski, C. R., Hoek, J., Caron, D., Lebsack, M. E. and Mckenna, H. J. (2000). "In vivo generation of human dendritic cell subsets by Flt3 ligand." Blood **96**(3): 878-884,

Martinez-Nunez, R. T., Louafi, F., Friedmann, P. S. and Sanchez-Elsner, T. (2009). "MicroRNA-155 modulates the pathogen binding ability of dendritic cells (DCs) by down-regulation of DC-specific intercellular adhesion molecule-3 grabbing non-integrin (DC-SIGN)." J Biol Chem **284**(24): 16334-1634210.1074/jbc.M109.011601.

Maurer, D., Fiebiger, E., Reininger, B., Ebner, C., Petzelbauer, P., Shi, G. P., Chapman, H. A. and Stingl, G. (1998). "Fc epsilon receptor I on dendritic cells delivers IgE-bound multivalent

antigens into a cathepsin S-dependent pathway of MHC class II presentation." J Immunol **161**(6): 2731-2739,

Mcgovern, N., Schlitzer, A., Gunawan, M., Jardine, L., Shin, A., Poyner, E., Green, K., Dickinson, R., Wang, X. N., Low, D., Best, K., Covins, S., Milne, P., Pagan, S., Aljefri, K., Windebank, M., Miranda-Saavedra, D., Larbi, A., Wasan, P. S., Duan, K., Poidinger, M., Bigley, V., Ginhoux, F., Collin, M. and Haniffa, M. (2014). "Human dermal CD14(+) cells are a transient population of monocyte-derived macrophages." Immunity **41**(3): 465-47710.1016/j.immuni.2014.08.006.

Mcgreal, E. P., Rosas, M., Brown, G. D., Zamze, S., Wong, S. Y., Gordon, S., Martinez-Pomares, L. and Taylor, P. R. (2006). "The carbohydrate-recognition domain of Dectin-2 is a C-type lectin with specificity for high mannose." Glycobiology **16**(5): 422-43010.1093/glycob/cwj077.

Mizumoto, N. and Takashima, A. (2004). "CD1a and langerin: acting as more than Langerhans cell markers." J Clin Invest **113**(5): 658-66010.1172/jci21140.

Morelli, A. E., Rubin, J. P., Erdos, G., Tkacheva, O. A., Mathers, A. R., Zahorchak, A. F., Thomson, A. W., Falo, L. D., Jr. and Larregina, A. T. (2005). "CD4+ T cell responses elicited by different subsets of human skin migratory dendritic cells." J Immunol **175**(12): 7905-7915,

Moseman, E. A., Liang, X., Dawson, A. J., Panoskaltsis-Mortari, A., Krieg, A. M., Liu, Y.-J., Blazar, B. R. and Chen, W. (2004). "Human Plasmacytoid Dendritic Cells Activated by CpG Oligodeoxynucleotides Induce the Generation of CD4⁺CD25⁺ Regulatory T Cells." The Journal of Immunology **173**(7): 4433-444210.4049/jimmunol.173.7.4433.

Nagasawa, M., Schmidlin, H., Hazekamp, M. G., Schotte, R. and Blom, B. (2008). "Development of human plasmacytoid dendritic cells depends on the combined action of the basic helix-loop-helix factor E2-2 and the Ets factor Spi-B." Eur J Immunol **38**(9): 2389-240010.1002/eji.200838470.

Nahid, M. A., Pauley, K. M., Satoh, M. and Chan, E. K. (2009). "miR-146a is critical for endotoxin-induced tolerance: IMPLICATION IN INNATE IMMUNITY." J Biol Chem **284**(50): 34590-3459910.1074/jbc.M109.056317.

Nahid, M. A., Satoh, M. and Chan, E. K. (2011). "Mechanistic role of microRNA-146a in endotoxin-induced differential cross-regulation of TLR signaling." J Immunol **186**(3): 1723-173410.4049/jimmunol.1002311.

Nam, J. W., Rissland, O. S., Koppstein, D., Abreu-Goodger, C., Jan, C. H., Agarwal, V., Yildirim, M. A., Rodriguez, A. and Bartel, D. P. (2014). "Global analyses of the effect of different cellular contexts on microRNA targeting." Mol Cell **53**(6): 1031-104310.1016/j.molcel.2014.02.013.

Nerlov, C., Querfurth, E., Kulessa, H. and Graf, T. (2000). "GATA-1 interacts with the myeloid PU.1 transcription factor and represses PU.1-dependent transcription." Blood **95**(8): 2543-2551, http://www.ncbi.nlm.nih.gov/pubmed/10753833

Nimmerjahn, F. and Ravetch, J. V. (2007). "Fc-receptors as regulators of immunity." Adv Immunol **96**: 179-20410.1016/s0065-2776(07)96005-8.

Nishiyama, C., Hasegawa, M., Nishiyama, M., Takahashi, K., Akizawa, Y., Yokota, T., Okumura, K., Ogawa, H. and Ra, C. (2002). "Regulation of human Fc epsilon RI alpha-chain gene expression by multiple transcription factors." J Immunol **168**(9): 4546-4552, http://www.ncbi.nlm.nih.gov/pubmed/11971001

Nishiyama, C., Ito, T., Nishiyama, M., Masaki, S., Maeda, K., Nakano, N., Ng, W., Fukuyama, K., Yamamoto, M., Okumura, K. and Ogawa, H. (2005). "GATA-1 is required for expression of Fc{epsilon}RI on mast cells: analysis of mast cells derived from GATA-1 knockdown mouse bone marrow." Int Immunol **17**(7): 847-85610.1093/intimm/dxh278.

Novak, N. (2012). "An update on the role of human dendritic cells in patients with atopic dermatitis." J Allergy Clin Immunol **129**(4): 879-88610.1016/j.jaci.2012.01.062.

Novak, N. and Bieber, T. (2005). "The role of dendritic cell subtypes in the pathophysiology of atopic dermatitis." J Am Acad Dermatol **53**(2 Suppl 2): S171-17610.1016/j.jaad.2005.04.060.

Novak, N. and Bieber, T. (2008). "2. Dendritic cells as regulators of immunity and tolerance." J Allergy Clin Immunol **121**(2 Suppl): S370-374; quiz S41310.1016/j.jaci.2007.06.001.

Novak, N., Bieber, T. and Katoh, N. (2001). "Engagement of Fc epsilon RI on human monocytes induces the production of IL-10 and prevents their differentiation in dendritic cells." J Immunol **167**(2): 797-804,

Novak, N., Koch, S., Allam, J. P. and Bieber, T. (2010). "Dendritic cells: bridging innate and adaptive immunity in atopic dermatitis." J Allergy Clin Immunol **125**(1): 50-59S0091-6749(09)01736-9 [pii]
10.1016/j.jaci.2009.11.019.

Novak, N., Kraft, S., Haberstok, J., Geiger, E., Allam, P. and Bieber, T. (2002). "A reducing microenvironment leads to the generation of FcepsilonRIhigh inflammatory dendritic epidermal cells (IDEC)." J Invest Dermatol **119**(4): 842-84910.1046/j.1523-1747.2002.00102.x.

Novak, N., Tepel, C., Koch, S., Brix, K., Bieber, T. and Kraft, S. (2003). "Evidence for a differential expression of the FcepsilonRIgamma chain in dendritic cells of atopic and nonatopic donors." J Clin Invest **111**(7): 1047-105610.1172/jci15932.

Novak, N., Valenta, R., Bohle, B., Laffer, S., Haberstok, J., Kraft, S. and Bieber, T. (2004). "FcepsilonRI engagement of Langerhans cell-like dendritic cells and inflammatory dendritic epidermal cell-like dendritic cells induces chemotactic signals and different T-cell phenotypes in vitro." J Allergy Clin Immunol **113**(5): 949-95710.1016/j.jaci.2004.02.005.

O'connell, R. M., Chaudhuri, A. A., Rao, D. S. and Baltimore, D. (2009). "Inositol phosphatase SHIP1 is a primary target of miR-155." Proc Natl Acad Sci U S A **106**(17): 7113-711810.1073/pnas.0902636106.

O'connell, R. M., Taganov, K. D., Boldin, M. P., Cheng, G. and Baltimore, D. (2007). "MicroRNA-155 is induced during the macrophage inflammatory response." Proc Natl Acad Sci U S A **104**(5): 1604-160910.1073/pnas.0610731104.

Pasquinelli, A. E., Reinhart, B. J., Slack, F., Martindale, M. Q., Kuroda, M. I., Maller, B., Hayward, D. C., Ball, E. E., Degnan, B., Muller, P., Spring, J., Srinivasan, A., Fishman, M., Finnerty, J., Corbo, J., Levine, M., Leahy, P., Davidson, E. and Ruvkun, G. (2000). "Conservation of the sequence and temporal expression of let-7 heterochronic regulatory RNA." Nature **408**(6808): 86-8910.1038/35040556.

Potaczek, D. P., Michel, S., Sharma, V., Zeilinger, S., Vogelberg, C., Von Berg, A., Bufe, A., Heinzmann, A., Laub, O., Rietschel, E., Simma, B., Frischer, T., Genuneit, J., Illig, T. and Kabesch, M. (2013). "Different FCER1A polymorphisms influence IgE levels in asthmatics and non-asthmatics." Pediatr Allergy Immunol **24**(5): 441-44910.1111/pai.12083.

Potaczek, D. P., Nasta Ek, M., Wojas-Pelc, A. and Undas, A. (2014). "The relationship between total serum IgE levels and atopic sensitization in subjects with or without atopic dermatitis." Allergol Int **63**(3): 485-48610.2332/allergolint.13-LE-0660.

Potaczek, D. P., Nishiyama, C., Sanak, M., Szczeklik, A. and Okumura, K. (2009). "Genetic variability of the high-affinity IgE receptor alpha-subunit (FcepsilonRIalpha)." Immunol Res **45**(1): 75-8410.1007/s12026-008-8042-0.

Poulin, L. F., Reyal, Y., Uronen-Hansson, H., Schraml, B. U., Sancho, D., Murphy, K. M., Hakansson, U. K., Moita, L. F., Agace, W. W., Bonnet, D. and Reis E Sousa, C. (2012). "DNGR-1 is a specific and universal marker of mouse and human Batf3-dependent dendritic cells in lymphoid and nonlymphoid tissues." Blood **119**(25): 6052-606210.1182/blood-2012-01-406967.

Probst, H. C., Mccoy, K., Okazaki, T., Honjo, T. and Van Den Broek, M. (2005). "Resting dendritic cells induce peripheral CD8+ T cell tolerance through PD-1 and CTLA-4." Nat Immunol **6**(3): 280-28610.1038/ni1165.

Rebane, A. and Akdis, C. A. (2014). "MicroRNAs in allergy and asthma." Curr Allergy Asthma Rep **14**(4): 42410.1007/s11882-014-0424-x.

Rebane, A., Runnel, T., Aab, A., Maslovskaja, J., Ruckert, B., Zimmermann, M., Plaas, M., Karner, J., Treis, A., Pihlap, M., Haljasorg, U., Hermann, H., Nagy, N., Kemeny, L., Erm, T., Kingo, K., Li, M., Boldin, M. P. and Akdis, C. A. (2014). "MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes." J Allergy Clin Immunol **134**(4): 836-847 e81110.1016/j.jaci.2014.05.022.

Reddy, V. A., Iwama, A., Iotzova, G., Schulz, M., Elsasser, A., Vangala, R. K., Tenen, D. G., Hiddemann, W. and Behre, G. (2002). "Granulocyte inducer C/EBPalpha inactivates the myeloid master regulator PU.1: possible role in lineage commitment decisions." Blood **100**(2): 483-490,

Reinhart, B. J., Slack, F. J., Basson, M., Pasquinelli, A. E., Bettinger, J. C., Rougvie, A. E., Horvitz, H. R. and Ruvkun, G. (2000). "The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans." Nature **403**(6772): 901-90610.1038/35002607.

Rissoan, M. C., Duhen, T., Bridon, J. M., Bendriss-Vermare, N., Peronne, C., De Saint Vis, B., Briere, F. and Bates, E. E. (2002). "Subtractive hybridization reveals the expression of immunoglobulin-like transcript 7, Eph-B1, granzyme B, and 3 novel transcripts in human plasmacytoid dendritic cells." Blood **100**(9): 3295-330310.1182/blood-2002-02-0638.

Rissoan, M. C., Soumelis, V., Kadowaki, N., Grouard, G., Briere, F., De Waal Malefyt, R. and Liu, Y. J. (1999). "Reciprocal control of T helper cell and dendritic cell differentiation." Science **283**(5405): 1183-1186,

Rodriguez, A., Griffiths-Jones, S., Ashurst, J. L. and Bradley, A. (2004). "Identification of mammalian microRNA host genes and transcription units." Genome Res **14**(10A): 1902-191010.1101/gr.2722704.

Rodriguez, A., Vigorito, E., Clare, S., Warren, M. V., Couttet, P., Soond, D. R., Van Dongen, S., Grocock, R. J., Das, P. P., Miska, E. A., Vetrie, D., Okkenhaug, K., Enright, A. J., Dougan, G., Turner, M. and Bradley, A. (2007). "Requirement of bic/microRNA-155 for normal immune function." Science **316**(5824): 608-61110.1126/science.1139253.

Romani, N., Clausen, B. E. and Stoitzner, P. (2010). "Langerhans cells and more: langerin-expressing dendritic cell subsets in the skin." Immunol Rev **234**(1): 120-14110.1111/j.0105-2896.2009.00886.x.

Rosenbauer, F., Owens, B. M., Yu, L., Tumang, J. R., Steidl, U., Kutok, J. L., Clayton, L. K., Wagner, K., Scheller, M., Iwasaki, H., Liu, C., Hackanson, B., Akashi, K., Leutz, A., Rothstein, T. L., Plass, C. and Tenen, D. G. (2006). "Lymphoid cell growth and transformation are suppressed by a key regulatory element of the gene encoding PU.1." Nat Genet **38**(1): 27-3710.1038/ng1679.

Rupaimoole, R. and Slack, F. J. (2017). "MicroRNA therapeutics: towards a new era for the management of cancer and other diseases." Nat Rev Drug Discov **16**(3): 203-22210.1038/nrd.2016.246.

Saba, R., Sorensen, D. L. and Booth, S. A. (2014). "MicroRNA-146a: A Dominant, Negative Regulator of the Innate Immune Response." Front Immunol **5**: 57810.3389/fimmu.2014.00578.

Saini, H. K., Griffiths-Jones, S. and Enright, A. J. (2007). "Genomic analysis of human microRNA transcripts." Proc Natl Acad Sci U S A **104**(45): 17719-1772410.1073/pnas.0703890104.

Salem, S., Langlais, D., Lefebvre, F., Bourque, G., Bigley, V., Haniffa, M., Casanova, J. L., Burk, D., Berghuis, A., Butler, K. M., Leahy, T. R., Hambleton, S. and Gros, P. (2014). "Functional characterization of the human dendritic cell immunodeficiency associated with the IRF8(K108E) mutation." Blood **124**(12): 1894-190410.1182/blood-2014-04-570879.

Sallusto, F. and Lanzavecchia, A. (1994). "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 **179**(4): 1109-1118,

Savinko, T., Matikainen, S., Saarialho-Kere, U., Lehto, M., Wang, G., Lehtimaki, S., Karisola, P., Reunala, T., Wolff, H., Lauerma, A. and Alenius, H. (2012). "IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors." J Invest Dermatol **132**(5): 1392-140010.1038/jid.2011.446.

Schiavoni, G., Mattei, F., Sestili, P., Borghi, P., Venditti, M., Morse, H. C., 3rd, Belardelli, F. and Gabriele, L. (2002). "ICSBP is essential for the development of mouse type I interferon-producing cells and for the generation and activation of CD8alpha(+) dendritic cells." J Exp Med **196**(11): 1415-1425,

Schotte, R., Nagasawa, M., Weijer, K., Spits, H. and Blom, B. (2004). "The ETS transcription factor Spi-B is required for human plasmacytoid dendritic cell development." J Exp Med **200**(11): 1503-150910.1084/jem.20041231.

Schreibelt, G., Klinkenberg, L. J., Cruz, L. J., Tacken, P. J., Tel, J., Kreutz, M., Adema, G. J., Brown, G. D., Figdor, C. G. and De Vries, I. J. (2012). "The C-type lectin receptor CLEC9A mediates antigen uptake and (cross-)presentation by human blood BDCA3+ myeloid dendritic cells." Blood **119**(10): 2284-229210.1182/blood-2011-08-373944.

Scott, E. W., Fisher, R. C., Olson, M. C., Kehrli, E. W., Simon, M. C. and Singh, H. (1997). "PU.1 functions in a cell-autonomous manner to control the differentiation of multipotential lymphoid-myeloid progenitors." Immunity **6**(4): 437-447,

Scott, E. W., Simon, M. C., Anastasi, J. and Singh, H. (1994). "Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages." Science **265**(5178): 1573-1577,

Serbina, N. V., Salazar-Mather, T. P., Biron, C. A., Kuziel, W. A. and Pamer, E. G. (2003). "TNF/iNOS-Producing Dendritic Cells Mediate Innate Immune Defense against Bacterial Infection." Immunity **19**(1): 59-70http://dx.doi.org/10.1016/S1074-7613(03)00171-7.

Shklovskaya, E., O'sullivan, B. J., Ng, L. G., Roediger, B., Thomas, R., Weninger, W. and Fazekas De St Groth, B. (2011). "Langerhans cells are precommitted to immune tolerance induction." Proc Natl Acad Sci U S A **108**(44): 18049-1805410.1073/pnas.1110076108.

Siegal, F. P., Kadowaki, N., Shodell, M., Fitzgerald-Bocarsly, P. A., Shah, K., Ho, S., Antonenko, S. and Liu, Y.-J. (1999). "The Nature of the Principal Type 1 Interferon-Producing Cells in Human Blood." Science **284**(5421): 1835-183710.1126/science.284.5421.1835.

Skabytska, Y., Wolbing, F., Gunther, C., Koberle, M., Kaesler, S., Chen, K. M., Guenova, E., Demircioglu, D., Kempf, W. E., Volz, T., Rammensee, H. G., Schaller, M., Rocken, M., Gotz, F. and Biedermann, T. (2014). "Cutaneous innate immune sensing of Toll-like receptor 2-6 ligands suppresses T cell immunity by inducing myeloid-derived suppressor cells." Immunity **41**(5): 762-77510.1016/j.immuni.2014.10.009.

Sonkoly, E., Janson, P., Majuri, M. L., Savinko, T., Fyhrquist, N., Eidsmo, L., Xu, N., Meisgen, F., Wei, T., Bradley, M., Stenvang, J., Kauppinen, S., Alenius, H., Lauerma, A., Homey, B., Winqvist, O., Stahle, M. and Pivarcsi, A. (2010). "MiR-155 is overexpressed in patients with atopic dermatitis and modulates T-cell proliferative responses by targeting cytotoxic T lymphocyte-associated antigen 4." J Allergy Clin Immunol **126**(3): 581-589 e581-52010.1016/j.jaci.2010.05.045.

Steinman, R. M. and Cohn, Z. A. (1973). "Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution." J Exp Med **137**(5): 1142-1162,

Strobl, H., Riedl, E., Scheinecker, C., Bello-Fernandez, C., Pickl, W. F., Rappersberger, K., Majdic, O. and Knapp, W. (1996). "TGF-beta 1 promotes in vitro development of dendritic cells from CD34+ hemopoietic progenitors." J Immunol **157**(4): 1499-1507,

Taganov, K. D., Boldin, M. P., Chang, K. J. and Baltimore, D. (2006). "NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses." Proc Natl Acad Sci U S A **103**(33): 12481-1248610.1073/pnas.0605298103.

Tam, W. (2001). "Identification and characterization of human BIC, a gene on chromosome 21 that encodes a noncoding RNA." Gene **274**(1-2): 157-167,

Tam, W., Ben-Yehuda, D. and Hayward, W. S. (1997). "bic, a novel gene activated by proviral insertions in avian leukosis virus-induced lymphomas, is likely to function through its noncoding RNA." Mol Cell Biol **17**(3): 1490-1502,

Tsujimura, H., Tamura, T., Gongora, C., Aliberti, J., Reis E Sousa, C., Sher, A. and Ozato, K. (2003). "ICSBP/IRF-8 retrovirus transduction rescues dendritic cell development in vitro." Blood **101**(3): 961-96910.1182/blood-2002-05-1327.

Turkistany, S. A. and Dekoter, R. P. (2011). "The transcription factor PU.1 is a critical regulator of cellular communication in the immune system." Arch Immunol Ther Exp (Warsz) **59**(6): 431-44010.1007/s00005-011-0147-9.

Valladeau, J., Dezutter-Dambuyant, C. and Saeland, S. (2003). "Langerin/CD207 sheds light on formation of birbeck granules and their possible function in Langerhans cells." Immunol Res **28**(2): 93-10710.1385/ir:28:2:93.

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. and Saeland, S. (2000). "Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules." Immunity **12**(1): 71-81, http://www.ncbi.nlm.nih.gov/pubmed/10661407

Van Der Aar, A. M. G., Sylva-Steenland, R. M. R., Bos, J. D., Kapsenberg, M. L., De Jong, E. C. and Teunissen, M. B. M. (2007). "Cutting Edge: Loss of TLR2, TLR4, and TLR5 on Langerhans Cells Abolishes Bacterial Recognition." The Journal of Immunology **178**(4): 1986-199010.4049/jimmunol.178.4.1986.

Van Rhijn, I., Ly, D. and Moody, D. B. (2013). "CD1a, CD1b, and CD1c in immunity against mycobacteria." Adv Exp Med Biol **783**: 181-19710.1007/978-1-4614-6111-1_10.

Von Bubnoff, D., Bausinger, H., Matz, H., Koch, S., Hacker, G., Takikawa, O., Bieber, T., Hanau, D. and De La Salle, H. (2004). "Human epidermal langerhans cells express the immunoregulatory enzyme indoleamine 2,3-dioxygenase." J Invest Dermatol **123**(2): 298-30410.1111/j.0022-202X.2004.23217.x.

Von Bubnoff, D., Fimmers, R., Bogdanow, M., Matz, H., Koch, S. and Bieber, T. (2004). "Asymptomatic atopy is associated with increased indoleamine 2,3-dioxygenase activity and interleukin-10 production during seasonal allergen exposure." Clin Exp Allergy **34**(7): 1056-106310.1111/j.1365-2222.2004.01984.x.

Wang, J., Ke, X. and Jia, L. (2002). "The role of B7 molecules in T cell anergy." Zhonghua Xue Ye Xue Za Zhi **23**(7): 341-344,

Wang, P., Hou, J., Lin, L., Wang, C., Liu, X., Li, D., Ma, F., Wang, Z. and Cao, X. (2010). "Inducible microRNA-155 feedback promotes type I IFN signaling in antiviral innate immunity by targeting suppressor of cytokine signaling 1." J Immunol **185**(10): 6226-623310.4049/jimmunol.1000491.

Wang, Q. H., Nishiyama, C., Nakano, N., Shimokawa, N., Hara, M., Kanada, S., Ogawa, H. and Okumura, K. (2008). "Suppressive effect of Elf-1 on FcepsilonRI alpha-chain expression in primary mast cells." Immunogenetics **60**(10): 557-56310.1007/s00251-008-0318-y.

Weidinger, S., Baurecht, H., Naumann, A. and Novak, N. (2010). "Genome-wide association studies on IgE regulation: are genetics of IgE also genetics of atopic disease?" Curr Opin Allergy Clin Immunol **10**(5): 408-41710.1097/ACI.0b013e32833d7d2d.

Weidinger, S., Gieger, C., Rodriguez, E., Baurecht, H., Mempel, M., Klopp, N., Gohlke, H., Wagenpfeil, S., Ollert, M., Ring, J., Behrendt, H., Heinrich, J., Novak, N., Bieber, T., Kramer, U., Berdel, D., Von Berg, A., Bauer, C. P., Herbarth, O., Koletzko, S., Prokisch, H., Mehta, D., Meitinger, T., Depner, M., Von Mutius, E., Liang, L., Moffatt, M., Cookson, W., Kabesch, M., Wichmann, H. E. and Illig, T. (2008). "Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus." PLoS Genet **4**(8): e100016610.1371/journal.pgen.1000166.

Wightman, B., Ha, I. and Ruvkun, G. (1993). "Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans." Cell **75**(5): 855-862,

Wilsmann-Theis, D., Koch, S., Mindnich, C., Bonness, S., Schnautz, S., Von Bubnoff, D. and Bieber, T. (2013). "Generation and functional analysis of human TNF-alpha/iNOS-producing dendritic cells (Tip-DC)." Allergy **68**(7): 890-89810.1111/all.12172.

Wollenberg, A., Kraft, S., Hanau, D. and Bieber, T. (1996). "Immunomorphological and ultrastructural characterization of Langerhans cells and a novel, inflammatory dendritic epidermal cell (IDEC) population in lesional skin of atopic eczema." J Invest Dermatol **106**(3): 446-453, http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=8648175

Wollenberg, A., Mommaas, M., Oppel, T., Schottdorf, E. M., Gunther, S. and Moderer, M. (2002). "Expression and function of the mannose receptor CD206 on epidermal dendritic cells in inflammatory skin diseases." J Invest Dermatol **118**(2): 327-33410.1046/j.0022-202x.2001.01665.x.

Wollenberg, A., Wagner, M., Gunther, S., Towarowski, A., Tuma, E., Moderer, M., Rothenfusser, S., Wetzel, S., Endres, S. and Hartmann, G. (2002). "Plasmacytoid dendritic cells: a new cutaneous dendritic cell subset with distinct role in inflammatory skin diseases." J Invest Dermatol **119**(5): 1096-110210.1046/j.1523-1747.2002.19515.x.

Zaba, L. C., Fuentes-Duculan, J., Steinman, R. M., Krueger, J. G. and Lowes, M. A. (2007). "Normal human dermis contains distinct populations of CD11c+BDCA-1+ dendritic cells and CD163+FXIIIA+ macrophages." The Journal of Clinical Investigation **117**(9): 2517-252510.1172/JCI32282.

Ziegler-Heitbrock, L., Ancuta, P., Crowe, S., Dalod, M., Grau, V., Hart, D. N., Leenen, P. J., Liu, Y. J., Macpherson, G., Randolph, G. J., Scherberich, J., Schmitz, J., Shortman, K., Sozzani, S., Strobl, H., Zembala, M., Austyn, J. M. and Lutz, M. B. (2010). "Nomenclature of monocytes and dendritic cells in blood." Blood **116**(16): e74-8010.1182/blood-2010-02-258558.

8 Appendix

8.1 Figures

8.2 Tables

9 Attachment

9.1 Multiple sequence alignment of SNP in *PU.1* mRNA with miRNA-155

```
rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ------------------------------------------------------------
rs1057221        ------------------------------------------------------------
mir155_side      ------------------------------------------------------------
rs1057233        ------------------------------------------------------------
PU.1 mRNA        gtagctcaggggcaggcctgagccctgcacccgccccacgaccgtccagcccctgacgg
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------


rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ------------------------------------------------------------
rs1057221        ------------------------------------------------------------
mir155_side      ------------------------------------------------------------
rs1057233        ------------------------------------------------------------
PU.1             gcaccccatcctgaggggctctgcattggcccccaccgaggcaggggatctgaccgactc
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------


rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ------------------------------------------------------------
rs1057221        ------------------------------------------------------------
mir155_side      ------------------------------------------------------------
rs1057233        ------------------------------------------------------------
PU.1             ggagcccggctggatgttacaggcgtgcaaaatggaagggtttcccctcgtccccctcc
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------


rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ------------------------------------------------------------
rs1057221        ------------------------------------------------------------
mir155_side      ------------------------------------------------------------
rs1057233        ------------------------------------------------------------
```

```
PU.1            atcagaagacctggtgccctatgacacggatctataccaacgccaaacgcacgagtatta
rs528011574     ------------------------------------------------------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         ------------------------------------------------------------
rs371396849     ------------------------------------------------------------
rs562299021     ------------------------------------------------------------

rs116330587     ------------------------------------------------------------
rs182746140     ------------------------------------------------------------
rs531943594     ------------------------------------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            cccctatctcagcagtgatggggagagccatagcgaccattactgggacttccaccccca
rs528011574     ------------------------------------------------------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         ------------------------------------------------------------
rs371396849     ------------------------------------------------------------
rs562299021     ------------------------------------------------------------

rs116330587     ------------------------------------------------------------
rs182746140     ------------------------------------------------------------
rs531943594     ------------------------------------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            ccacgtgcacagcgagttcgagagcttcgccgagaacaacttcacggagctccagagcgt
rs528011574     ------------------------------------------------------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         ------------------------------------------------------------
rs371396849     ------------------------------------------------------------
rs562299021     ------------------------------------------------------------

rs116330587     ------------------------------------------------------------
rs182746140     ------------------------------------------------------------
rs531943594     ------------------------------------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            gcagcccccgcagctgcagcagctctaccgccacatggagctggagcagatgcacgtcct
rs528011574     ------------------------------------------------------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         ------------------------------------------------------------
rs371396849     ------------------------------------------------------------
rs562299021     ------------------------------------------------------------

rs116330587     ------------------------------------------------------------
rs182746140     ------------------------------------------------------------
rs531943594     ------------------------------------------------------------
```

```
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            cgatacccccatggtgccaccccatcccagtcttggccaccaggtctcctacctgccccg
rs528011574     ------------------------------------------------------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         --------------------------------------------------------CG
rs371396849     ------------------------------------------------------------
rs562299021     ------------------------------------------------------------


rs116330587     ------------------------------------------------------------
rs182746140     ------------------------------------------------------------
rs531943594     ------------------------------------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            gatgtgcctccagtacccatccctgtccccagcccagcccagctcagatgaggaggaggg
rs528011574     ------------------------------------------G-----------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         G-----------------GTCGTCCTCTGCAAGGTTGCCCCGG-----------------
rs371396849     ----------------------------------------CCCGG-----------------
rs562299021     ----------------------------GCAAGGTTGCCCCGG-----------------


rs116330587     ------------------------------------------------------------
rs182746140     ------------------------------------------------------------
rs531943594     ------------------------------------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            cgagcggcagagccccccactggaggtgtctgacggcgaggcggatggcctggagcccgg
rs528011574     ----------------------CGGGATGTGGAGGGGGCCTGGAGTCGGGGGAGGGGGC
rs878963605     --------------------------TGTGGAGGGGGCCTGGAGTGGGGGGAGGGGGC
rs878892082     ---------------------------------GGGGGCCTGGAGTGGGGGGAGGGGGC
rs764782105     -------------------------------GACGCCCAGCTGGCGTCCGGGAGCCGGGG
rs10747         ----------------------TGGGGTCTGACGCCCAGCTGGCG--------------
rs371396849     ----------------------TGGGGTCTGACGCCCAGCTGCCGTCCGGGAGCCGGGG
rs562299021     ----------------------TGGGGTCTGACGCCCAGCTGGCGTCCGGGAGCCGGG-


rs116330587     ------------------------------------------------------------
rs182746140     ------------------------------------------------------------
rs531943594     ------------------------------------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            gcctgggctcctgcctggggagacaggcagcaagaagaagatccgcctgtaccagttcct
rs528011574     GGGTGAGGCGAGG------------------------------------------------
rs878963605     GGGTGAGGCGAGGCCCGGC------------------------------------------
rs878892082     GGGTGAGGCGAGGCCCGGCCCGCCA------------------------------------
rs764782105     TGGAGTCCTGGAGGGAGGCGAA--------------------------------------
rs10747         ------------------------------------------------------------
rs371396849     TGGAGTCCT---------------------------------------------------
rs562299021     ------------------------------------------------------------
```

```
rs116330587     ------------------------------------------------------------
rs182746140     ------------------------------------------------------------
rs531943594     ----------------AGGGGAGATCT-----------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            gttggacctgctccgcagcggcgacatgaaggacagcatctggtgggtggacaaggacaa
rs528011574     ------------------------------------------------------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         ------------------------------------------------------------
rs371396849     ------------------------------------------------------------
rs562299021     ------------------------------------------------------------


rs116330587     ------------------------------------------------------------
rs182746140     ------------------------------------------------------------
rs531943594     ------------------------------------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            gggcaccttccagttctcgtccaagcacaaggaggcgctggcgcaccgctggggcatcca
rs528011574     ------------------------------------------------------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         ------------------------------------------------------------
rs371396849     ------------------------------------------------------------
rs562299021     ------------------------------------------------------------


rs116330587     --------------------TGTGCTGCAAGAGGATGGATTG------------------
rs182746140     ------------------------------------------------------------
rs531943594     ------------------------------------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            gaagggcaaccgcaagaagatgacctaccagaagatggcgcgcgcgctgcgcaactacgg
rs528011574     ------------------------------------------------------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         ------------------------------------------------------------
rs371396849     ------------------------------------------------------------
rs562299021     ------------------------------------------------------------


rs116330587     -------------------------AGAATAACTTTACTTGTTTTTTGGGAGGA-----
rs182746140     -----------------------------ATAACTTTACTTGTTTTTTGGGAGGA-GGTT
rs531943594     ------------------------------------------------------------
rs1057221       ------------------------------------------------------------
mir155_side     ------------------------------------------------------------
rs1057233       ------------------------------------------------------------
PU.1            caagacgggcgaggtcaagaaggtgaagaagaagctcacctaccagttcagcggcgaagt
rs528011574     ------------------------------------------------------------
rs878963605     ------------------------------------------------------------
rs878892082     ------------------------------------------------------------
rs764782105     ------------------------------------------------------------
rs10747         ------------------------------------------------------------
rs371396849     ------------------------------------------------------------
```

```
rs562299021      ------------------------------------------------------------


rs116330587      ------------------------------------------------------------
rs182746140      AATGGGTGGGAGGGGTGAGAG------------------------------------------
rs531943594      ------------------------------------------------------------
rs1057221        ------------------------------------------------------------
mir155_side      ------------------------------------------------------------
rs1057233        ------------------------------------------------------------
PU.1             gctgggccgcgggggcctggccgagcggcgccacccgccccactgagcccgcagcccccg
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------


rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ------------------------------------------------------------
rs1057221        ------------------------------------------------------------
mir155_side      --------------CUCCCCGCUGGCCAUAGCAUUAA----------------------
rs1057233        ------------------------------------------------------------
PU.1 3'UTR       ccggccccgccaggcctccccgctggccatAGCATTAagccctcgcccggcccggacaca
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------


rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ------------------------------------------------------------
rs1057221        --------------------------AGGACTGTGGCGGGCCGGGCCTCGCCTCACCCG
mir155_side      ------------------------------------------------------------
rs1057233        ------------------------------------------------------------
PU.1             gggaggacgctcccggggcccagaggcaggactgtggcgggccgggctccgtcacccgcc
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------


rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ------------------------------------------------------------
rs1057221        CCCCCTCCCCCCACTCCA--------------------------------------------
mir155_side      ------------------------------------------------------------
rs1057233        ------------------------------------------------------------
PU.1             c---ctccccccactccaggccccctccacatcccgcttcgcctccctccaggactccac
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
```

```
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------


rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ----------------------------------------------------------GATTT
rs1057221        ------------------------------------------------------------
mir155_side      ------------------------------------------------------------
rs1057233        ------------------------------------------------------------
PU.1             cccggctcccgacgccagctgggcgtcagacccaccggcaaccttgcagaggacgacccg
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------


rs116330587      ------------------------------------------------------------
rs182746140      ------------------------------------------------------------
rs531943594      ACATACGGACTTGAGACTCCCAAGGCAGTACCCCG------------------------
rs1057221        ------------------------------------------------------------
mir155_side      ------------------------------------------------------------
rs1057233        ----------------------------------------------CCCTCTCACCCCTCC
PU.1             gggtactgccttgggagtctcaagtccgtatgtaaatcagatctcccctctcacccctcc
rs528011574      ------------------------------------------------------------
rs878963605      ------------------------------------------------------------
rs878892082      ------------------------------------------------------------
rs764782105      ------------------------------------------------------------
rs10747          ------------------------------------------------------------
rs371396849      ------------------------------------------------------------
rs562299021      ------------------------------------------------------------


rs116330587      ---------------------------------------------
rs182746140      ---------------------------------------------
rs531943594      ---------------------------------------------
rs1057221        ---------------------------------------------
mir155_side      ---------------------------------------------
rs1057233        CACCCATTAACCTCCTCCCAAAAAACAAGTAAAGTT-----------
PU.1             cacccattaacctcctcccaaaaaacaagtaaagttattctcaatcc
rs528011574      ---------------------------------------------
rs878963605      ---------------------------------------------
rs878892082      ---------------------------------------------
rs764782105      ---------------------------------------------
rs10747          ---------------------------------------------
rs371396849      ---------------------------------------------
rs562299021      ---------------------------------------------
```

Figure S1: ***No described SNP of PU.1 3' UTR is located in the miRNA-155 binding region.*** *PU.1* 3' UTR SNP sequences taken from http://www.ebi.ac.uk/Tools/services were aligned with *PU.1* mRNA (GenBank: X52056.1) and the miRNA-155 pairing side (http://www.targetscan.org) using CLUSTAL O (1.2.4) multiple sequence alignment (http://www.ebi.ac.uk/Tools/services).

Publications

Journal Articles

Herrmann, N., Koch, S., **Leib, N.**, Bedorf, J., Wilms, H., Schnautz, S., Fimmers, R., Bieber, T. (2013). "TLR2 down-regulates FcepsilonRI and its transcription factor PU.1 in human Langerhans cells." Allergy **68**(5): 621-62810.1111/all.12145.

Raafat, D., **Leib, N.**, Wilmes, M., Francois, P., Schrenzel, J. and Sahl, H. G. (2017). "Development of in vitro resistance to chitosan is related to changes in cell envelope structure of Staphylococcus aureus." Carbohydr Polym 157: 146-15510.1016/j.carbpol.2016.09.075.

Poster Presentations

Leib, N., Herrmann, N., Koch, S., Iwamoto K., Wilms H., Schnautz S., Fischer D., Ulas, T., Baßler, K., Schultze, J., Bieber, T.: Regulatory mechanisms for the high affinity receptor for IgE (FcεRI) on human Langerhans cells.
5th CK-CARE Team meating, Davos, Switzerland, 2014

Leib, N., Herrmann, N., Koch, S., Schnautz, S., Wilms, H., Iwamoto, K., Bieber, T.: Micro-RNA-mediated transcriptional regulation of the high-affinity receptor for IgE on human Langerhans cells.
44th Annual meeting of the German Society for Immunology (DGfI), Bonn, Germany, 2014
And: Immunosensation cluster science days, Bonn, Germany, 2014

Talks

Leib, N.: Micro-RNA-mediated transcriptional regulation of the high-affinity receptor for IgE (FcεRI) on human Langerhans cells.
30th Symposium of the Collegium Internationale Allergologicum (CIA), Königswinter, Germany, 2014

Awards

Alain L. de Weck Travel Grant Award for the abstract entitled “Micro-RNA-mediated transcriptional regulation of the high-affinity receptor for IgE (FcεRI) on human Langerhans cells”. 30th Symposium of the Collegium Internationale Allergologicum (CIA), Königswinter, Germany, 2014

Declaration (Eidesstattliche Erklärung)

Hiermit erkläre ich an Eides statt, dass ich für meine Promotion keine anderen als die angegebenen Hilfsmittel benutzt habe. Stellen und Zitate, die aus anderen Werken entnommen wurden, sind als solche gekennzeichnet. Diese Arbeit ist weder identisch noch teilidentisch mit einer Arbeit, die an der Rheinischen Friedrich-Wilhelms-Universität Bonn oder einer anderen Hochschule zur Erlangung eines akademischen Grades oder als Prüfungsleistung vorgelegt worden ist. Teile der Dissertation wurden vorab an den unter Punkt 8 „Publications“ aufgeführten Stellen veröffentlicht.

Bonn, 24.07.2017

_____

Nicole Leib