Role of E-cadherin in Langerhans cell homeostasis & Analysis of an IL-17A-driven psoriasis mouse model

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Preface

The present PhD thesis is the result of research performed between September 2014 and August 2019 in the group of Prof. Dr. Björn E. Clausen in the Institute for Molecular Medicine of the Johannes Gutenberg University Mainz, Germany. The obtained data have been presented in four publications:

1. *E-cadherin is dispensable to maintain Langerhans cells in the epidermis*
   Anna Brand, Nathalie Diener, Sonja P. Zahner, Christoph Tripp, Ronald A. Backer, Khalad Karram, Aimin Jiang, Ira Mellman, Patrizia Stoitzner and Björn E. Clausen
   Journal of Investigative Dermatology (2019) (article in press)

2. *Monitoring Skin Dendritic Cells in Steady State and Inflammation by Immunofluorescence Microscopy and Flow Cytometry*
   Julia L. Ober-Blöbaum, Daniela Ortner, Bernhard Haid, **Anna Brand**, Christoph Tripp, Björn E. Clausen*, and Patrizia Stoitzner* (equal contribution)
   Methods in Molecular Biology (2017) 1559:37-52

   Gradual development of psoriatic skin lesions by constitutive low-level expression of IL-17A
   Christian Wohn, **Anna Brand** , Kelly van Ettinger, Inge Brouwers-Haspels, Ari Waisman, Jon D. Laman, and Björn E. Clausen
   Cellular Immunology (2016) 308:57-65

3. *Antagonization of IL-17A Attenuates Skin Inflammation and Vascular Dysfunction in Mouse Models of Psoriasis*
   Rebecca Schüler*, **Anna Brand***, Sabrina Klebow, Johannes Wild, Flavio P. Veras, Elisabeth Ullmann, Siyer Roohani, Frank Kolbinger, Sabine Kossmann, Christian Wohn, Andreas Daiber, Thomas Münzel, Philip Wenzel, Ari Waisman, Björn E. Clausen* and Susanne Karbach* (***both first and last authors contributed equally**)
   Journal of Investigative Dermatology (2019) 139(3):638-647

Abbreviations

Ag	Antigen
AngII	Angiotensin II
AMP	Antimicrobial peptides
APC	Antigen-presenting cell
BM	Bone marrow
BMDC	Bone marrow-derived dendritic cell
Ca^{2+}	Calcium
CCL	C-C chemokine ligand
CCR	C-C chemokine receptor
CD	Cluster of differentiation
cDC	Conventional dendritic cell
CHS	Contact hypersensitivity
CKI	Casein kinase 1
CVD	Cardiovascular disease
DAMP	Damage-associated molecular patterns
Defb	β-defensin
DETC	Dendritic epidermal T cell
DC	Dendritic cell
dDC	Dermal dendritic cell
DNA	Deoxyribonucleic acid
DSS	Dextran sodium sulfate
DT	Diphtheria toxin
DTA	Diphtheria toxin subunit A
DTR	Diphtheria toxin receptor
EC	Extracellular cadherin
EMT	Epithelial to mesenchymal transition
FACS	Fluorescence-activated cell sorting
FDA	Food and drug administration
FITC	Fluorescein isothiocyanate
FLT3L	FMS-like tyrosine kinase 3 ligand
GSK-3β	Glycogen synthase kinase 3 beta
H&E	Hematoxylin and Eosin
HLA	Human leukocyte antigen
IEL	Intraepithelial lymphocyte

IFN	Interferon
IKK2	Inhibitor kappa B kinase 2
IL	Interleukin
ILC	Innate lymphoid cell
IMQ	Imiquimod
Ind	Inducible
KC	Keratinocyte
KLRG1	Killer-cell lectin like receptor G1
LC	Langerhans cell
LN	Lymph node
MHC	Major histocompatibility complex
moDC	Monocyte-derived dendritic cell
NET	Neutrophil extracellular traps
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B-cells
NK	Natural killer
PAMP	Pathogen-associated molecular pattern
pDC	Plasmacytoid dendritic cell
PI3K	Phosphoinositide 3-kinase
PSORS	Psoriasis susceptibility
RNA	Ribonucleic acid
ROS/RNS	Reactive oxygen and nitrogen species
RT-PCR	Reverse transcription-polymerase chain reaction
SCID	Severe combined immunodeficient
STAT	Signal transducer and activator of transcription 3
TCR	T cell receptor
Th	T helper
TLR	Toll-like receptor
TNF	Tumor necrosis factor
Treg	Regulatory T cell
Wnt	Wingless-Int

1 Introduction

1.1 The skin as an immunological barrier

As the largest organ of the human body, the skin's primary function is to serve as a physical barrier protecting the internal organs from invading pathogens and water loss (Bos and Kapsenberg, 1986). Far from being a simple mechanical barrier, the skin contains a network of immune cells and molecular mediators known as the “skin immune system” (Abdallah et al., 2017). It is composed of three main layers: the epidermis, the dermis, and subcutaneous tissue also known as hypodermis (Figure 1).

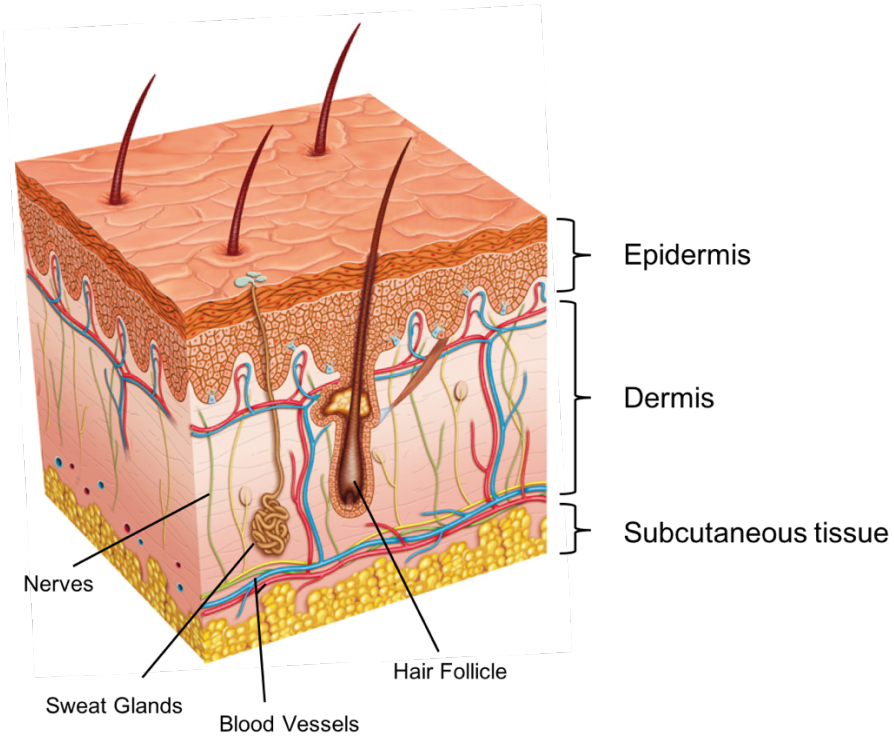

Figure 1: Schematic representation of the different skin layers. The skin is composed of three layers: the epidermis, dermis and subcutaneous tissue. The dermis contains hair follicles, glands, nerves and blood vessels (adapted from http://www.justaboutskin.com).

The outermost epithelial layer, the epidermis, is mainly composed of keratinocytes (KC) which are generated by local proliferation of stem cells. In addition, it harbors a number of other cell types such as melanocytes, Langerhans cells (LC), and Merkel cells. The epidermis is further subdivided into four layers: stratum basale (basal layer), stratum spinosum (spinous cell layer), stratum granulosum, and stratum corneum (corneal layer) (Nestle et al., 2009a). In the stratum basale epidermal cells are constantly renewed. It

consists of one layer of undifferentiated KC, which by proliferation and differentiation move to the stratum spinosum where they start to mature and synthesize keratin (Nestle et al., 2009a). In the stratum granulosum tight junctions are formed and KC produce high amounts of keratin and lipids (Heath and Carbone, 2013). The uppermost layer of the epidermis, the corneal layer, consists of dead KC that provide the physical barrier of the skin. The dermis, beneath the epidermis, is a thick layer of fibrous and elastic connective tissue. It is made mostly of collagen, giving the skin its flexibility and strength. The dermis also contains nerve endings, hair follicles, sweat glands and many different leukocyte populations, thereby integrating the skin along with the nervous and immune system (Nestle et al., 2009a). The subcutaneous tissue represents the deepest and innermost layer of the skin. It is mostly made up of fat, connective tissue, and larger blood vessels and nerves. This subcutaneous tissue serves as energy storage and plays an important role in the thermoregulation of the body (Arda et al., 2014).

Together, all three layers of the skin form an effective barrier to the external environment and are important contributors to maintaining homeostasis. This essential function depends on the cross-talk between several immune sentinels, present in the different layers of the skin, and the interplay between innate and adaptive immune responses (Abdallah et al., 2017).

1.1.1 Differences of human skin versus murine skin

Although the layers of human and murine skin are similar, differences in the anatomy and cellular components can be observed. For example, the dermis of human skin is substantially thicker than mouse dermis and contains fewer hair follicles, which follow an asynchronous hair cycle. Mouse dermis, on the other hand, contains a cutaneous muscle layer, the panniculus carnosus (Gudjonsson et al., 2007), and the interfollicular regions are shorter compared to human skin. Mouse epidermis generally comprises only three cell layers (<25 µm) whereas the epidermis of the human skin (>100 µm) is much thicker, consisting of 6-10 cell layers (Gudjonsson et al., 2007, Wong et al., 2011). Furthermore, the mouse skin has a faster epidermal turnover and can therefore regenerate without scar formation (Takeo et al., 2015). The cellular components also differ between murine and human skin. While the epidermis of mice contains dendritic epidermal T cells (DETC), this population is absent in the epidermis of humans. However, despite these differences, mouse and human skin show many similarities and share common molecular and immunological pathways (Wagner et al., 2010).

1.1.2 Skin immune cells

The skin immune system comprises a complex network of cells, regulating both immunity against invading pathogens and tolerogenic mechanisms to ensure the maintenance of skin immune homeostasis. Furthermore, the skin is colonized by commensal bacteria, which are in constant contact with skin cells and thereby also contribute to normal skin function (Mann, 2014).

Keratinocytes are the first sensors of pathogen invasion and the initiators of inflammation. They can therefore be considered as immune activators (McKenzie and Sauder, 1990). As a reaction to pathogen invasion, KC secrete a variety of cytokines, chemokines and antimicrobial peptides. This results either in direct neutralization of the pathogen, or the activation of immune cells to induce a protective immune response (Abdallah et al., 2017). Also located in the epidermis are leukocytes, i.e. immune cells, including LC and $CD8^+$ tissue-resident memory T cells. LC, which represent 2-4% of the epidermal cell population (Vishwanath et al., 2006), build a dense network and are in close association with KC and the first immune cells to come in contact with invading pathogens. As sentinels of the immune system, LC capture antigens (Ag) and migrate to skin-draining lymph nodes (LN), where they either prime naïve T cells to become Ag-specific effector T cells or induce tolerance by promoting T cell apoptosis or the development of regulatory T cells (Treg) (Maldonado and von Andrian, 2010). The dermis, in contrast to the epidermis, is composed of several specialized immune cells such as tissue-resident macrophages, neutrophils, mast cells, B cells, innate lymphoid cells (ILC), $\gamma\delta^+$ T cells as well as $CD4^+$ T cells with a regulatory or memory phenotype. Moreover, the dermis harbors monocytes and different dendritic cell (DC) subsets (Heath and Carbone, 2013) (Figure 2).

Macrophages are mononuclear phagocytes, which are found in the dermal layer of the skin (Ginhoux and Jung, 2014). They are part of the innate immune system and have a high phagocytic function and degradative potential, allowing them to clear foreign and damaged cells. Skin-resident macrophages are embryo-derived and seed the skin prenatally (Guilliams et al., 2014). They are self-renewing, maintain tissue homeostasis and dampen initiation of inflammation. The second source of macrophages are bone marrow (BM)-derived monocytes that leave the circulation and migrate towards sites of inflammation where they differentiate into mature macrophages (Mann ER, 2014).

Neutrophils are also a part of the innate skin immune system. They have crucial functions since they migrate from the blood to the site of inflammation significantly faster than monocytes (Tsepkolenko et al., 2019). Neutrophils bind the exposed Fc-region of bound

antibodies, which triggers the cell to engulf opsonized pathogens by phagocytosis. Neutrophils are also able to release antimicrobial proteins from granules into the extracellular space to further fight the infection. Recently, another method of killing pathogens has been described for neutrophils. The release of DNA and serine proteases, known as neutrophil extracellular traps (NET), allows neutrophils to catch and immobilize microbes for phagocytosis (Shao et al., 2019, Urban et al., 2009).

Mast cells are mainly located in the upper dermal part of the skin, where they can easily encounter, respond, and protect from infections and stress caused by wound healing (Matejuk, 2018). Originally, mast cells were known as typical allergy-inducing cells since they contain histamine. Nevertheless, recent studies proved their critical role in vital processes such as wound healing, skin inflammation and immune tolerance (Ng, 2010, Sayed et al., 2008). Moreover, mast cell-derived tumor necrosis factor (TNF) drives migration of dermal DC to skin-draining LN in a murine model of contact hypersensitivity (Nguyen and Soulika, 2019, Suto et al., 2006).

B cells in the dermis are responsible for the production of antibodies which activate the complement system to initiate direct elimination of pathogens (Egbuniwe et al., 2015). In normal skin, they are rarely present, however, B cell infiltrates have been observed in chronic inflammatory skin conditions including leishmaniasis and atopic dermatitis (AD) (Geiger et al., 2010, Simon et al., 2008). They also have antibody-independent roles, acting as Ag-presenting cells. They express high level of MHCII and can present Ag to $CD4^+$ T cells (Rivera et al., 2001).

Innate lymphoid cells (ILC) represent a heterogeneous group of immune cells that produce cytokines and coordinate immunity and inflammation at body surfaces, such as the skin (Simoni et al., 2018). They can be divided into three subsets based on their dependence on specific transcription factors and the expression of distinct cytokines: group 1 ILC (ILC1 and natural killer (NK) cells), group 2 ILC (ILC2) and group 3 ILC (ILC3 and lymphoid tissue inducer cells) (Kim, 2015). Another group of innate lymphocytes in the skin are γδ T cells. Mouse skin harbors two populations of γδ T cells: *dermal and epidermal γδ T* cells which can be distinguished by their expression of different Vγ TCR segments. Dermal γδ T cells express Vγ4 (Cai et al., 2011) whereas epidermal γδ T cells (DETC) are $V\gamma5^+$ and are exclusively located in the epidermis (Macleod and Havran, 2011). DETC can secrete a variety of cytokines and chemokines that are important in cutaneous inflammation and the crosstalk between DETC, KC and LC (Macleod and Havran, 2011).

Furthermore, the skin harbors different types of *$CD4^+$ and $CD8^+$ T cells,* which are important in both the steady state and during inflammatory responses. They are

characterized by their expression of the αβ T cell receptor (αβ TCR) that recognizes Ag presented on MHCI or MHCII molecules (Matejuk, 2018).

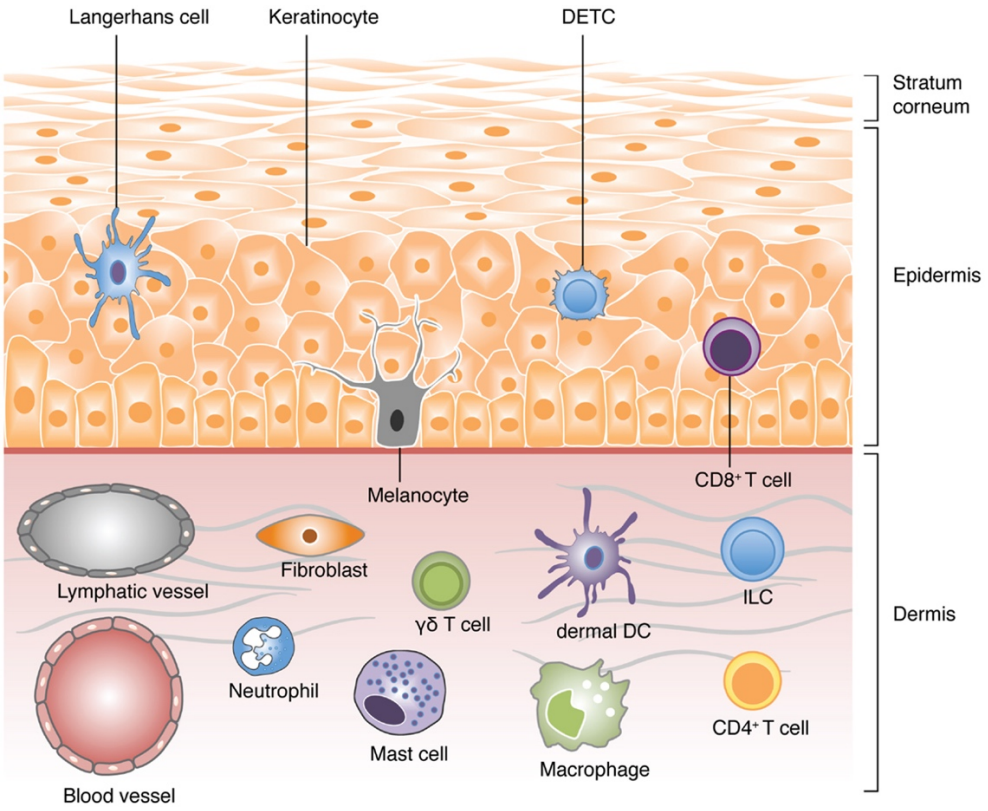

Figure 2: Schematic view of the different cell types populating the skin. The superficial part of the epidermis, known as the stratum corneum, is composed of dead KC and acts as first mechanical/physical barrier. The epidermis comprises mainly KC with few melanocytes. The major immune cells in this compartment are LC, DETC (only in mouse skin), and $CD8^+$ T cells. The highly vascularized dermis consists of fibroblasts, T cells ($CD4^+$ αβ, and γδ), ILC, dermal DC (dDC), macrophages, mast cells, and neutrophils (Chong et al., 2013).

1.1.3 Basic DC functions

DC are a heterogeneous family of professional Ag-presenting cells and key regulators in balancing immunity versus tolerance. Therefore, they are strategically positioned at epithelial borders to the environment where they constantly probe their environment for invading pathogens. At the same time, DC are also critical for maintaining tolerance towards both harmless foreign as well as self-Ag (Clausen and Stoitzner, 2015, Romani et al., 2012, Steinman et al., 2003). DC exist in two phenotypically and functionally

distinct states: immature and mature DC (Schuler et al., 1985). Upon Ag-recognition through specialized receptors of pathogen- or damage-associated molecular patterns (PAMP and DAMP), immature DC, transform into mature DC. This maturation is correlated with the upregulation of MHCII and costimulatory molecules like CD80 and CD86. Upregulation of the chemokine receptor CCR7 acquires their ability to migrate to tissue-draining LN (Ohl et al., 2004). In LN, these mature DC instruct naïve T cells to differentiate into appropriate effector or regulatory T cells (Romani et al., 2012). Therefore, DC process the encountered Ag for presentation on their cell surface using MHC for recognition by the T cell receptor (TCR) on T cells (Banchereau et al., 2000). Ag presented by DC can be either endogenous or exogenous, depending on their entry pathway. Exogenous Ag, for example, are taken up and processed in endosomes. Subsequently, presentation on MHCII molecules results in the activation of Ag-specific $CD4^+$ T cells. In contrast, intracellular endogenous Ag, for example, are produced in the cytoplasm of DC. Here, these Ag are degraded into peptides and presented on MHCI molecules, resulting in the activation of Ag-specific $CD8^+$ T cells (Guermonprez et al., 2002). DC are able to present exogenous Ag on MHCI molecules via a process called cross-presentation. This allows DC to activate Ag-specific $CD8^+$ T cells against exogenous Ag (Allan et al., 2006, Joffre et al., 2012).

1.1.4 DC subsets in the skin

DC can be subdivided into conventional DC (cDC) and plasmacytoid DC (pDC). Although, healthy skin contains very few pDC, they enter inflamed skin to promote wound healing through type-I interferons. Furthermore, they mediate the proinflammatory reaction that develops after TLR7 stimulation, for example, during viral infections or psoriasis (Clausen and Stoitzner, 2015, Reizis et al., 2011).

Skin DC, except for LC, arise from the DC-committed BM progenitors in response to a specific hematopoietin, FMS-like tyrosine kinase 3 ligand (FLT3L) and are continuously renewed by BM-derived pre-DC (Merad et al., 2013). LC, in contrast, arise from yolk sac-derived myeloid progenitors and fetal liver-derived monocytes (Hoeffel et al., 2012). In steady state mouse skin, three major DC subsets can be distinguished: i) Langerin$^+$Epcam$^+$CD103^{neg} epidermal LC, ii) Langerin$^+$Epcam^{neg}CD103$^{neg/+}$ (cDC1) and iii) Langerin^{neg}Epcam^{neg}CD103^{neg}CD11b$^+$ dermal DC (cDC2) (Henri et al., 2010a, Kim T. G. et al., 2017) (Figure3). Additionally, monocyte-derived DC (moDC) and monocyte-derived LC (moLC) can be found in inflamed skin. They differentiate upon microbial or inflammatory stimuli from circulating Ly6C^{hi} blood monocytes and infiltrate

inflamed or infected tissues dependent on the chemokine receptor CCR2 (Dominguez and Ardavin, 2010). Recent experiments demonstrate that the initial wave of moLC reconstitution after UV radiation are short-term LC that are transient and replaced by a second wave of steady-state precursor-derived long-term LC (Seré et al., 2012). Similar to other DC subsets, moDC and moLC are APC and able to cross-present Ag via MHCI to $CD8^+$ T cells (Cheong et al., 2010). Therefore, they serve as a reservoir to supplement the cDC network during inflammatory conditions in order to warrant efficient Ag presentation during acute inflammation (Leon et al., 2007).

The identification of phenotypically distinct DC subsets in the skin leads to the hypothesis that each subpopulation has a particular function ("division of labor"). This hypothesis has been challenged by several reports demonstrating the "multitasking" capacities of DC subsets (Pulendran, 2006). The functional plasticity of DC suggests a possible mechanism of crosstalk between distinct DC subsets determined by the signals the DC receive from their environment (Pulendran et al., 2008).

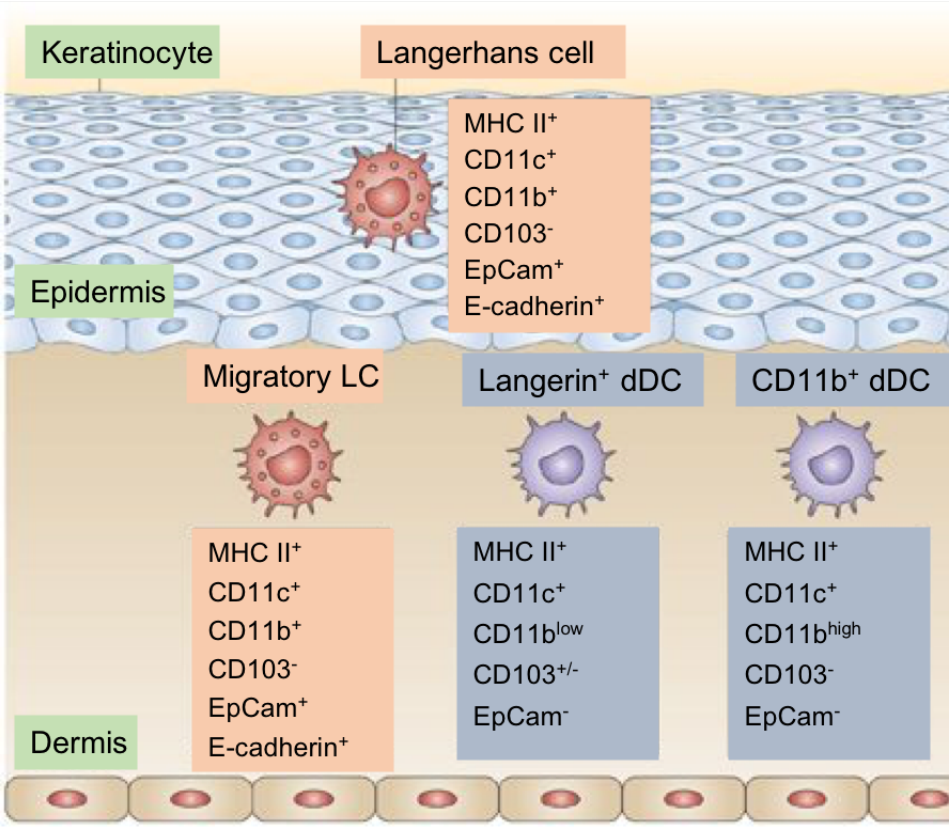

Figure 3: Dendritic cell populations in the mouse skin. In steady state, the skin contains two main populations of $Langerin^+$ DC: LC in the epidermis and $Langerin^+$ DC in the dermis. Migrating LC on their way to the LN can also be found in the dermis. LC and dermal $Langerin^+$

DC can be distinguished based on their expression of CD11b, EpCAM and CD103. The skin also contains dermal CD11b^{+}Langerin^{neg} DC (adapted from Henri et al., 2010a, Merad et al., 2008).

1.1.4.1 Langerhans cells

LC were initially identified by Paul Langerhans in 1868 and were thought to be a part of the peripheral nervous system due to their dendritic shape (Langerhans, 1868). However, later studies placed them within the hematopoietic system as cells efficiently acquiring and processing Ag (Schuler et al., 1985). LC are a special subset of Ag presenting cells in the epidermis that share a common ontogeny with macrophages but function as DC (Ginhoux and Merad, 2010, Ginhoux et al., 2006, Merad et al., 2008). LC arise from yolk sac-derived myeloid progenitors around embryonic day 18 and are largely replaced by fetal liver-derived monocytes during late embryogenesis (Clausen and Stoitzner, 2015, Hoeffel et al., 2012). After birth, LC precursors acquire their DC morphology and phenotype and undergo a massive proliferation burst, expanding their numbers by 10-20-fold (Chorro et al., 2009). In contrast to other DC, LC are radio-resistant and self-renewal through life without any contribution from BM precursors (Merad et al., 2002). Although, ontogenetically LC and macrophages are closely related, LC migrate to LN where they prime naïve T cells to induce regulatory or effector responses while macrophages do not migrate and stay tissue-resident. LC share features with macrophages but clearly function as DC (Otsuka et al., 2018). Thus, they are a unique cell type and probably best considered to be a special subset of DC.
Originally LC have been identified by their characteristic organelles, the Birbeck granules, and the expression of Langerin/CD207 (Birbeck, 1963, Valladeau et al., 2000). Langerin is a membrane bound C-type lectin receptor that recognizes mannosylated ligands (PAMP) on the surface of pathogens, including viruses, bacteria and fungi. Upon Ag encounter, receptor-mediated endocytosis by LC is induced, followed by transport of Langerin to Birbeck granules where it becomes part of the Ag processing pathway (Figdor et al., 2002). LC acquire their Langerin expression only 2-3 days after birth, whereas CD11c and MHC-II expression is present immediately after birth (Tripp et al., 2004). To date, LC (CD11c^{+}MHCII^{+}Langerin^{+}) are identified based on their expression of EpCam and can be thereby distinguished from dermal Langerin^{+} DC. Furthermore, LC constitutively express E-cadherin, an adhesion molecule which has been described to anchor LC to neighboring KC (Tang et al., 1993). LC are in close contact with KC via homophilic E-cadherin binding and together they build up the first line of defense against

invading pathogens. LC extend their dendrites through epidermal tight junctions to the stratum corneum in a dynamic process to capture Ag. Thus, LC survey their environment not only within the skin but also on the skin surface without disturbing barrier integrity (Kubo et al., 2009, Ouchi et al., 2011).

Upon Ag uptake and activation, immature LC undergo maturation which includes the upregulation of surface MHCII and costimulatory molecules like CD40, CD80 and CD86. Mature LC downregulate E-cadherin and migrate to the LN to initiate T cell-mediated immune responses (Jakob and Udey, 1998). The function of LC in regulating immunity and tolerance is still controversial. In mice, the inducible depletion of LC by diphtheria toxin (DT) injection has been shown to ameliorate the inflammatory response in contact hypersensitivity (Bennett et al., 2005). In contrast, Langerin-DTA mice, which constitutively lack LC from birth, exhibit an increased disease severity, suggesting a tolerogenic function of LC (Kaplan et al., 2005). Additional studies demonstrated that selective depletion of LC in a low-dose *Leishmania major* infection model was responsible for the reduced Treg cell immigration and the enhanced Th1 response, resulting in attenuated disease (Kautz-Neu et al., 2011). Similar to DC, LC exhibit a high functional plasticity and become tolerogenic or immunogenic depending on the nature of the encountered pathogen.

1.1.4.2 Dermal dendritic cells

Dermal Langerin$^+$ cDC1 (Langerin$^+$ dDC) were long considered to be migrating LC but recent studies have shown that they represent a distinct DC population. In contrast to LC, Langerin$^+$ dDC are radiosensitive. In irradiated BM chimeras Langerin$^+$ dDC are replaced by donor cells whereas LC remain of host origin (Merad et al., 2002, Poulin et al., 2007). Moreover, in mouse models where Langerin$^+$ cells are depleted, dDC display different repopulation kinetics as compared to LC. Here Langerin$^+$ dDC can be detected after a few days of depletion, while LC are still absent (Nagao et al., 2009).

Skin DC are functionally diverse and can induce different types of immune responses. Langerin$^+$ dDC are able to capture dead cell debris and express various receptors for the recognition of intracellular pathogens, including viruses. The most prominent function of this DC subset is the cross-presentation of pathogen-derived or self-Ag via MHCI molecules (Edwards et al., 2003). However, Langerin$^+$ dDC also ensure peripheral tolerance to skin Ag by deletion of self-reactive T cells (Henri et al., 2010b).

Dermal CD11b$^+$ cDC2 (CD11b$^+$ dDC) preferentially present Ag to CD4$^+$ T cells and can induce the activation of memory T cells in non-lymphoid tissues (Wakim et al., 2008).

Moreover, they can induce protective Th2- and Th17-type immunity in the skin. However, $CD11b^+$ DC not only prime $CD4^+$ T helper cells but also induce the formation of $Foxp3^+$ Treg (Guilliams et al., 2010). This demonstrates that specific DC subsets indeed exert specialized functions but it also highlights the functional plasticity of DC and the possible mechanism of crosstalk between the different subsets.

1.2 The cell adhesion molecule E-cadherin

E-cadherin (encoded by the gene *Cdh1*) is a transmembrane glycoprotein that mediates Ca^{2+}-dependent cell-cell adhesion and plays a pivotal role in embryonic development (Gumbiner, 2005). The E-cadherin molecule contains an ectodomain composed of 5 extracellular cadherin repeats (EC1-5), a transmembrane region, and a cytoplasmic tail (Figure 4). This cytoplasmic tail can be further subdivided into a β-catenin-binding domain and a membrane proximal cytoplasmic/conserved domain. This domain is important for p120-catenin binding, resulting in the stabilization of the E-cadherin/catenin complex at the cell surface (Van den Bossche et al., 2012, van Roy and Berx, 2008) (Figure 4). E-cadherin is well characterized as adherens junction protein, which contributes to the maintenance of the epithelial barrier integrity through homophilic interactions (Van den Bossche and Van Ginderachter, 2013). Next to its role in cell adhesion, E-cadherin can also bind β-catenin, the central component of the canonical Wingless-Int (Wnt) signaling pathway (Jakob et al., 1999, Staal et al., 2008). In the absence of a Wnt signal, cytoplasmic β-catenin is either bound to E-cadherin or phosphorylated by casein kinase 1 (CKI) and glycogen synthase kinase 3 beta (GSK-3β), which targets it for ubiquitination and proteasomal degradation (Figure 4). Once Wnt binds its receptor Frizzled, β-catenin phosphorylation is inhibited, leading to the accumulation of β-catenin in the cytoplasm and its translocation into the nucleus where it activates the expression of different target genes (Staal et al., 2008, Van den Bossche et al., 2012). E-cadherin binding protects β-catenin from proteasomal degradation, providing a sustained pool of β-catenin that can be released upon E-cadherin downregulation (Tian et al., 2011).

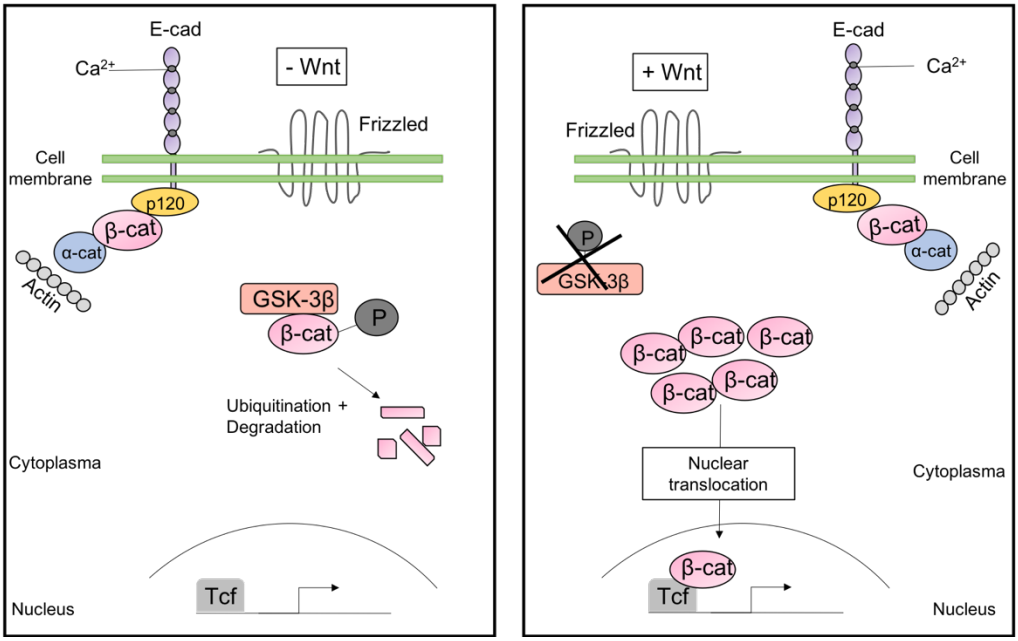

Figure 4: E-cadherin/β-catenin complex and Wnt signaling. β-catenin interacts with the cytoplasmic tail of E-cadherin and is sequestered at the cell membrane. When Wnt signals are absent, free β-catenin forms a complex with GSK3β in the cytoplasm and is phosphorylated. Phosphorylated β-catenin is subsequently degraded through the ubiquitination-proteasome degradation system. Wnt ligand binding to Frizzled receptor leads to the inhibition of GSK3β activity, which results in the accumulation of β-catenin in the cytoplasm and its translocation to the nucleus where it regulates target gene transcription.

E-cadherin is best characterized in epithelial cells, whereas little is known about its role and function in immune cells. Over the last years it became clear that E-cadherin is expressed on a variety of leukocytes including LC, macrophages and γδ T cells, and therefore can function as a potential immunological regulator (Van den Bossche et al., 2012). LC express high levels of E-cadherin and attach themselves to the surrounding KC via homophilic E-cadherin binding. During LC mobilization and migration from the epidermis to skin-draining LN, E-cadherin is downregulated on LC (Jakob and Udey, 1998, Tang et al., 1993). However, whether this is cause or consequence of LC mobilization and maturation remains unknown. Moreover, downregulation of E-cadherin is a molecular hallmark of a genetic program called epithelial-to-mesenchymal-transition (EMT), which has been suggested to control LC development, adhesion and migration (Hieronymus et al., 2015, Konradi et al., 2014, Sagi and Hieronymus, 2018).

E-cadherin can also interact in a heterophilic manner with the killer-cell lectin like receptor G1 (KLRG1) and the integrin CD103, which are expressed by different DC, NK and T cell subpopulations (Cepek et al., 1994, Rosshart et al., 2008). Upon KLRG1

binding, TCR signaling on T cells and NK cell cytotoxicity get inhibited indicating that E-cadherin$^+$ cells can attenuate inflammatory immune responses (Beyersdorf et al., 2007). Furthermore, E-cadherin-CD103 binding could be important in facilitating the cross-talk between E-cadherin$^+$ LC and CD103$^+$ Treg (Suffia et al., 2005) or CD103$^+$CD8$^+$ tissue-resident memory T cells (Topham and Reilly, 2018).

It has been shown that bone marrow-derived DC (BMDC) express E-cadherin. *In vitro* experiments demonstrated that disruption of E-cadherin binding on BMDC results in their phenotypic maturation (including the upregulation of MHCII, CD80 and CD86) without the production of inflammatory cytokines. This E-cadherin cluster disruption leads to a tolerogenic DC phenotype that promotes the induction of IL-10–producing Treg. Moreover, these E-cadherin-matured DC prevent the induction of experimental autoimmune encephalomyelitis (EAE) in mice (Jiang et al., 2007). In addition, mice with a CD11c-specific β-catenin deficiency are more susceptible to DSS-induced colitis, which was accompanied by an increased Th17 cell number and a reduced Foxp3$^+$ Treg frequency in the intestine (Manicassamy et al., 2010). Together, these data suggest that E-cadherin and β-catenin play a crucial role in the regulation of LC maturation as well as function and might be important in orchestrating inflammatory immune responses in the skin.

1.3 Psoriasis

Psoriasis is a chronic inflammatory skin disease which affects 2-3% of the human population worldwide (Mohd Affandi et al., 2018). The most common form of psoriasis is psoriasis vulgaris, affecting 90% of the patients (Raychaudhuri et al., 2014). The disease is manifested by chronic erythematous and scaly plaques (Langley et al., 2005) (Figure 5), which have extensive emotional and psychosocial effects on patients (Kim W. B. et al., 2017). Although the etiology of psoriasis is unknown, it has a strong genetic component and also environmental factors, such as stress, trauma or infections, play an important role in the development of the disease (Di Meglio et al., 2014)

1.3.1 Genetic background

Population studies clearly indicate that the incidence of psoriasis is greater among first-degree and second-degree relatives of patients than among the general population. That genetic components play a role in psoriasis is further supported by studies among twins, showing a risk of psoriasis that is two to three times as high among monozygotic

as compared to dizygotic twins (Farber et al., 1974). The major genome region linked to psoriasis is called psoriasis susceptibility 1 (PSORS1). The human leukocyte Ag (HLA)-Cw6 allele, which is located in the *PSORS1* locus, was found to be strongly associated with psoriasis, as 60% of all psoriasis patients carry this allele (Perera et al., 2012). In agreement, patients who are homozygous for the gene have a 2.5-fold increased chance to develop psoriasis compared to patients with the heterozygous allele (Gudjonsson et al., 2003).

Moreover, the immunogenetics of IL-23 are strongly associated with the development of psoriasis. IL-23 signals through a heterodimeric receptor expressed by both innate and adaptive immune cells. Single nucleotide polymorphisms in the regions coding for the IL-23 cytokine as well as the IL-23R have been identified to promote the risk of psoriasis (Rendon and Schakel, 2019).

1.3.2 Histological features

Psoriatic plaques are characterized by epidermal changes due to hyperproliferation and dysfunctional differentiation of KC. This is leading to retention of the nuclei in the stratum corneum, elongation of the epidermal rete ridges and loss of the granular layer (Figure 5). Lesional skin also harbors a massive leukocyte infiltrate, as well as, marked vascularization and dilation of blood vessels resulting in erythema (Lowes et al., 2014). The cellular infiltrate mainly consists of activated $CD4^+$ and $CD8^+$ T cells, monocytes, and DC. Also neutrophils transmigrate through the epidermis and form a histopathological hallmark of psoriatic lesions, called Munro microabscesses (Lowes et al., 2014).

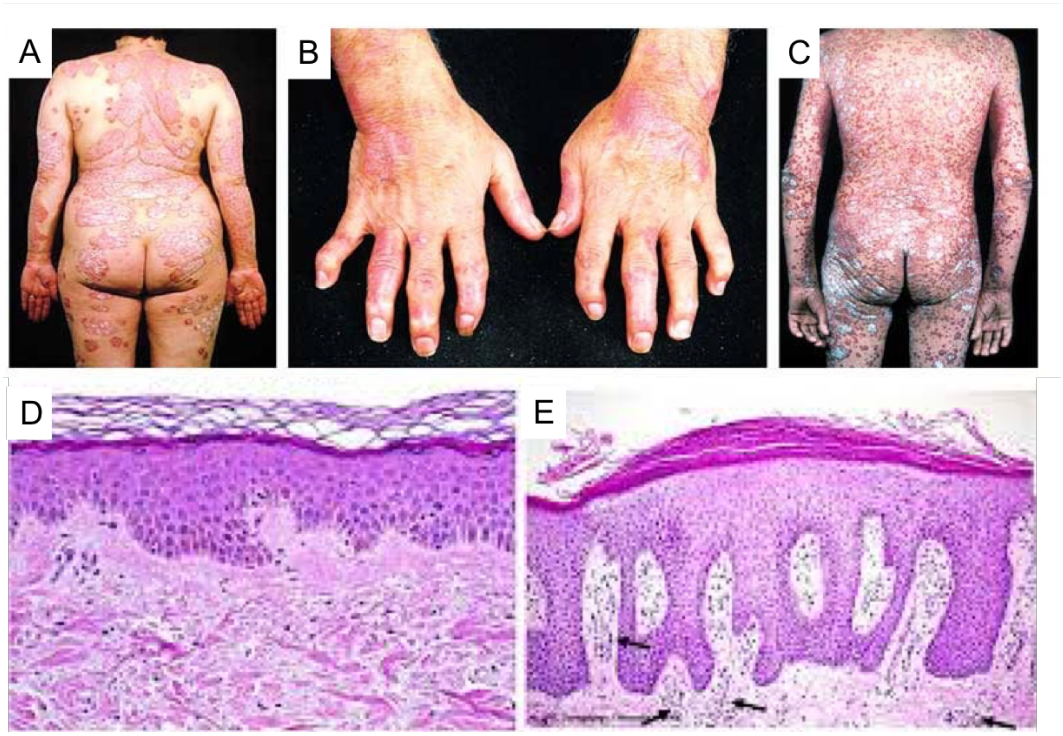

Figure 5: Clinical and Histologic Features of Psoriasis. (A-C) Psoriatic patients with red and scaly plaques. (D) Hematoxylin and Eosin staining (H&E) of a non-lesional and (E) lesional skin biopsy. The epidermis is depicted in dark and the dermis in light pink. Lesional psoriatic skin shows thickened epidermis (acanthosis) and elongated rete ridges. Retention of nuclei (parakeratosis) is seen as thickened stratum corneum. The dermis of the lesional biopsy displays an increase of infiltrating immune cells (indicated with arrows) (adapted from Nestle et al., 2009b).

1.3.3 Pathogenesis

Despite the fact that psoriasis was considered as a disease caused by hyperproliferation of aberrant KC, clinical and experimental studies from the last years highlighted the important role of the immune system in the pathogenesis of psoriasis. Although it is considered a T cell-mediated inflammatory disease, the pathogenesis involves cells belonging to both the adaptive and the innate immune system, as well as, non-immune cells (Surcel et al., 2019) (Figure 6).

DC are thought to play a major role in the initial phase of the disease by recognizing antimicrobial peptides (AMP), which are secreted by KC in response to injury, mechanical stress and other environmental triggers. LL-37, β-defensins, and S100 proteins are the most studied psoriasis-associated AMP (Morizane and Gallo, 2012, Rendon and Schakel, 2019). LL-37 is released by damaged KC and, bound to DNA,

stimulates TLR9 in pDC. These pDC are present in high numbers at early stages of psoriasis, and their activation results in the production of type I IFN. The release of IFNα by pDC activates cDC and their migration to skin-draining LN. Furthermore, cDC can be directly activated by stressed KC, due to the release of IL-1β, IL-6 or TNF (Perera et al., 2012). These stressed KC release chemokines such as CXCL1, CXCL2, and CXCL8, that mediate the recruitment of circulating neutrophils, which is associated with the release of NET. Psoriatic NET consist of DNA strands associated with antimicrobial peptides such as LL-37 (Di Domizio and Gilliet, 2019, Lande et al., 2015, Lande et al., 2007). In the skin, cDC secrete cytokines like IL-12 or IL-23 which activates γδ T cells and innate lymphocytes to produce IL-17. Dependent on the secreted cytokine by cDC in the LN, naïve T cells differentiate into Th1 or Th17 cells, which are subsequently directed into the skin tissue where they release inflammatory cytokines like IL-17 and IL-22. These mediators further activate KC and induce the production of antimicrobial peptides (LL-37), proinflammatory cytokines (TNF, IL-1β, IL-6), and chemokines, hence maintaining and propagating inflammation (Nestle et al., 2009a) (Figure 6).

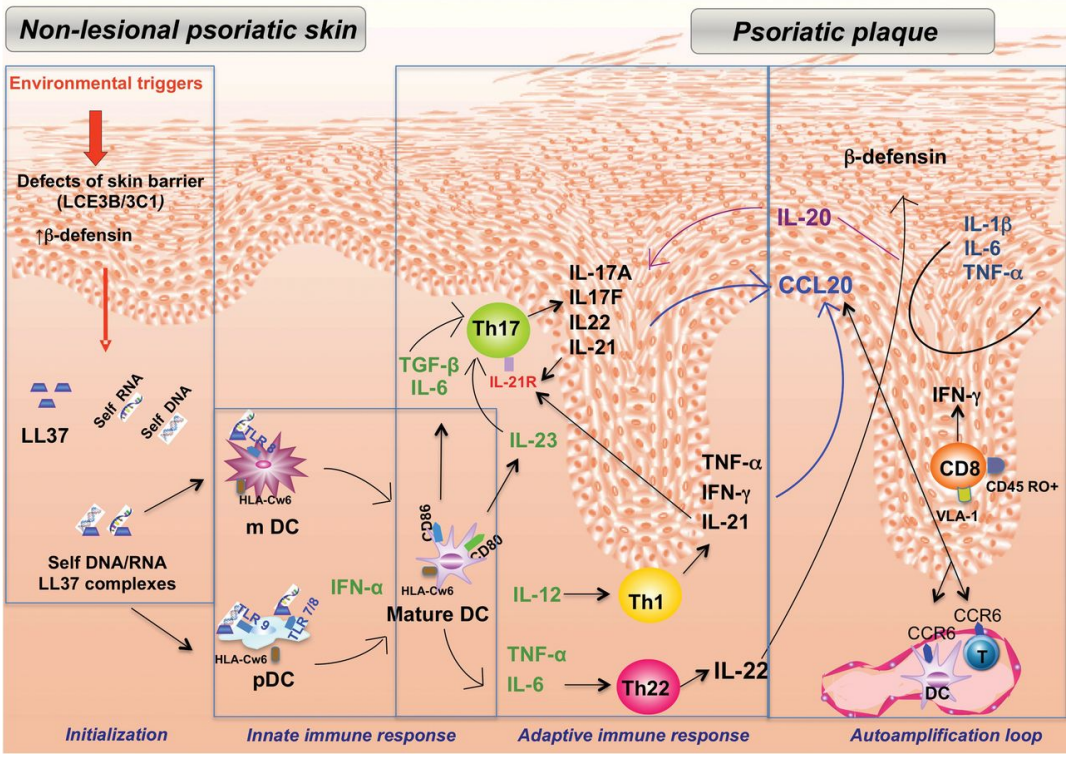

Figure 6: Schematic view of the pathogenesis of psoriasis. Both environmental triggers and genetic defects that alter the skin barrier, contribute to the formation of self-DNA/RNA and LL-37 complexes, leading to the synthesis of IFNα by pDC and maturation of cDC.

Mature cDC in turn, produce multiple cytokines that promote differentiation and expansion of Th1 (i.e. IL-12), Th17 (i.e. IL-6, TGF-β1 and IL-23) and Th22 (i.e. TNF and IL-6) cells. Both Th1 and Th17 cytokines induce KC to produce CC-chemokine ligand (CCL) 20, a chemoattractant for CCR6-expressing cDC and T cells, thus promoting the accumulation of these cells in the psoriatic skin. Th17-related cytokines stimulate cDC and proliferating KC to make IL-20, a cytokine that promotes KC proliferation. KC produce inflammatory cytokines, such as IL-1β, IL-6 and TNF, thus contributing to enhance DC activation and expand the local inflammation (adapted from Monteleone et al., 2011).

1.3.4 The IL-23/IL-17 axis in psoriasis

Many different cytokines are involved in the pathogenesis of psoriasis, but especially the IL-23/IL-17 axis has been shown to play a crucial role in the development of the disease. IL-23 is a heterodimer consisting of a p19 and a p40 subunit. It is mainly produced by DC and is required for the differentiation and expansion of Th17 cells which subsequently secrete cytokines such as IL-17 and IL-22 (Girolomoni et al., 2017).

IL-23 signals via a heterodimeric receptor complex, that predominantly activates signal transducer and activator of transcription 3 (STAT3), leading to IL-23-dependant gene expression (Teng et al., 2015). The critical role of IL-23 signaling in the pathogenesis of psoriasis has also been demonstrated in humans and mouse models of psoriasis. In the skin of psoriatic patients, IL-23 is increased and the IL-23 receptor is upregulated on dDC and LC (Girolomoni et al., 2017). In line with this observation, mice that constitutively express the p19 subunit, develop severe skin inflammation (Piskin et al., 2006, Wiekowski et al., 2001), whereas IL-23p19 knock-out mice lack IL-17-producing T cells (Girolomoni et al., 2017). Moreover, intradermal injection of IL-23 induces a psoriasis-like skin phenotype in mice (Zheng et al., 2007).

Numerous reports show that psoriatic plaques also contain increased numbers of IL-17-producing cells with increased levels of Th17 cytokines (Chiricozzi et al., 2016, Martin et al., 2013). Th17 cells are localized in the dermis of psoriasis patients and IL-17 mRNA expression increases with disease development and severity (Golden et al., 2013). For several years, it has been hypothesized that the primary source of IL-17 in psoriasis are Th17 cells. However, data from the last years indicate that additional important cellular sources of IL-17 are γδ T cells and innate lymphoid cells (Keijsers et al., 2014).

1.3.5 Mouse models of psoriasis

Psoriasis pathogenesis has been studied intensively. However, the mechanisms of disease development are still not completely understood. Psoriasis seems to be restricted to humans. Therefore, a large number of animal models was developed to mimic different aspects of the human disease, and to study the cellular and molecular interactions during disease pathogenesis (Bochenska et al., 2017). The current models, being used to study psoriasis, can be divided into three major types: xenograft (humanized), genetically engineered (transgenic), and acute (inducible) models.

Table 1: Selected psoriasis mouse models

	Mouse model		Model description	Advantages	Limitations	References
Xenotransplantation	Human skin on SCID mice		Transplantation of human psoriatic or non-lesional skin onto immunodeficient mice	• Most closely mimics the phenotypic and genetic characteristics of the human disease	• Technically difficult • Lack of systemic effects • Comorbidities cannot be studied	Nestle et al., 2005
	Human skin on AGR129 mice					Boyman et al., 2004
	Human skin on athymic nude mice					Krueger et al., 1981
Transgenic	Overexpression	K14-p40	Epidermal overexpression of IL-12p40 subunit	• Limits gene expression to specific cell populations or tissues • Allows investigation and testing of gene-specific effects • Often more chronic disease and comorbidities can be studied	• Often results in early death due to whole body involvement • Single gene alterations do not recapitulate complex, multigenic disease • Transgene may affect more than target gene	Kopp et al., 2003
		K14-IL-17A	Epidermal overexpression of IL-17A or IL-17C			Croxford et al., 2014
		K5-IL-17C				Johnston et al., 2013
		K5-Stat3C	Epidermal overexpression of constitutively active Stat3			Sano et al., 2003
	Knock out	K14-IKK2	Epidermal deletion of IKK2			Stratis et al., 2006
		IL1Rn-/-	Deletion of IL1R			Shepherd et al., 2004
Inducible/ acute	Imiquimod		Strong immune activation via TLR7	• Easy to use • Inducible disease at specific age or time point • Investigation of early events during disease initiation	• Lack of chronicity • Comorbidities cannot be studied • No standardized protocols • unintended effects of the topical agent	Van der Fits et al., 2009
	IL-23		Intradermal injection of IL-23 activates TH17 cells and ILC			Kopp et al., 2003

1.3.5.1 Xenotransplantation model

Different humanized mouse models have been established in which skin from psoriasis patients is transplanted onto immunodeficient mice. A few weeks after transplantation, murine skin develops clinical and histological features of psoriasis. Common mouse strains used for this method are severe combined immunodeficient (SCID) or AGR129

mice (Boyman et al., 2004, Nestle et al., 2005), both allowing engraftment without undergoing tissue rejection due to the absence of B and T cells. However, AGR129 mice also lack type I and II IFN receptors on a RAG-$2^{-/-}$ background, which results in impaired NK cell activity and B and T cell deficiency. Transplanted human tissues develop into psoriatic plaques because of the expansion of resident immune cell populations found in the donor skin (Hawkes et al., 2018). The usage of human-derived tissue is a main advantage, as this most closely mimics the immunologic and genetic basis of the human disease. However, a huge disadvantage of this model is the lack of systemic effects, meaning that only the skin phenotype can be analyzed.

1.3.5.2 Transgenic mouse models

Various genetically engineered mouse models have been designed to study the pathogenesis of psoriasis. In most of these models, either increased expression or knockout of specific genes has been achieved by using promotors mainly for the keratin 5 and keratin 14 genes (Bochenska et al., 2017). These genetically modified mouse models make it possible to clarify the relationship and role of specific cytokines, growth factors, and mediators of the inflammatory response during disease development. Induction or deletion of crucial signaling pathways for example TNF/nuclear factor 'kappa-light-chain-enhancer' of activated B-cells (NF-κB) or IL-23/IL-17 alters skin homeostasis that leads to a skin phenotype with features of human psoriasis (Gudjonsson et al., 2007, Shepherd et al., 2004, Stratis et al., 2006). The specific deletion of NF-κB kinase inhibitor kappa B kinase 2 (IKK2) in KC (K-14-IKK2 mice) results in skin inflammation with hallmarks of human psoriasis, which was found to be dependent on TNF (Stratis et al., 2006). Similar psoriatic skin inflammation could be observed when STAT3 was constitutively active in KC (K5-STAT3C mice) (Sano et al., 2005). Moreover, mice that express the p40 subunit under the control of the K14 promoter (K14-p40 mice) develop an inflammatory skin disease similar to psoriasis, demonstrating the strong association between IL-23 and the disease (Kopp et al., 2003). In line with this, KC-specific overexpression of members of the IL-17 family (K5-IL-17C and K14-IL-$17A^{ind/+}$mice) leads to a severe psoriasis-like phenotype in mice (Croxford et al., 2014, Johnston et al., 2013), confirming a strong contribution of IL-17 to disease development.

1.3.5.3 Acute IMQ-induced psoriasis-like skin disease

In humans, imiquimod (IMQ), a TLR7/8 ligand and potent immune activator, is used for topical treatment of genital and perianal warts caused by human papilloma virus. The clinical indications have additionally been expanded to treat other virus-associated skin abnormalities, such as actinic keratoses as well as superficial basal cell carcinomas (Bianchi et al., 2003, Geisse et al., 2002, Miller et al., 1999, van der Fits et al., 2009). The IMQ mouse model is based on clinical observations that topical treatment of cancerous skin lesions with *Aldara*^{TM} cream, which contains 5% of IMQ, aggravates psoriasis in patients. The exacerbation of psoriasis was accompanied by a massive induction of lesional type I interferon from TLR7-expressing pDC (Gilliet et al., 2004). Daily topical application of *Aldara*^{TM} cream on mouse skin has been shown to induce a psoriasis-like skin inflammation that resembles human psoriasis in terms of the phenotypical and histological characteristics. This includes erythema, scaling, thickening of the epidermis due to KC hyperproliferation, and abnormal differentiation of KC (van der Fits et al., 2009). The lesions contain inflammatory skin immune cell infiltrates consisting of neutrophils, $CD4^+$ T cells, DC and pDC. The IMQ-induced psoriasis model also depends on the IL-23/IL-17 axis, since IL-23p19 and IL-17RA deficient mice are protected from the IMQ-induced inflammation (van der Fits et al., 2009). The main mechanism of IMQ-induced inflammation in mice is due to the IMQ-mediated activation of TLR7/8 which is expressed by monocytes, macrophages, and DC (Schon and Schon, 2007). Activation of TLR7 induces MyD88 signaling pathway, resulting in the production of inflammatory cytokines and chemokines (Hemmi et al., 2002). Additionally, IMQ interacts with adenosine receptors to induce TLR7/8 independent inflammatory responses. Receptor binding leads to the suppression of anti-inflammatory cyclic adenosine monophosphate formation which results in a higher production of proinflammatory mediators (Schon et al., 2006). The early inflammation caused by IMQ is driven by DC. Both, LC and dDC, have been reported to be sources of IL-23 that activates IL-17- and IL-22-producing T cells (Wohn et al., 2013, Yoshiki et al., 2014). Studies in $TCR\beta^{-/-}$ mice, that lack αβ T cells, indicated that IL-17- and IL-22-producing αβ T cells are dispensable for the development of skin inflammation in this model since they respond to IMQ comparable as WT mice (Pantelyushin et al., 2012). In contrast, mice lacking γδ T cells display markedly reduced inflammation upon IMQ treatment, indicating that γδ T cells are required for the development of the skin inflammation. γδ T cells infiltrate the skin after topical applications of IMQ and have been identified as main producers of IL-17 in the IMQ model (Cai et al., 2011, Pantelyushin et al., 2012).

Cutaneous IL-17 induces the hyperproliferation of KC, which express the IL-17R. Thus, IL-17 drives the psoriasis-like skin inflammation in this model (Moos et al., 2019). Moreover, IMQ treated mice that lack the cytokine IL-22 develop a milder psoriasiform skin inflammation, demonstrating the importance of this cytokine in the development of the disease (Pantelyushin et al., 2012).

The IMQ mouse model closely recapitulates the complex cellular interactions, cytokines and inflammatory pathways initiating psoriatic plaque formation, and is therefore a major breakthrough in dissecting the early molecular and cellular players promoting the development of the disease. However, the IMQ model lacks the chronic nature of the disease, which limits testing novel therapeutic interventions (Wohn et al., 2016).

1.3.6 Therapeutic concepts

Although there is no cure for psoriasis yet, various treatment options and new therapies have been developed over the last years. The choice of therapy for psoriasis patients is determined by disease severity and comorbidities. Psoriatic patients are categorized into three groups (mild, moderate or severe psoriasis), depending on the clinical severity of the lesions, the percentage of affected body surface area, and patient quality of life (Rendon and Schakel, 2019). Mild to moderate psoriasis can be treated topically with a combination of corticosteroids, vitamin D analogues, and phototherapy. Corticosteroids have an anti-inflammatory effect by inhibiting proinflammatory cytokines like TNF. Long-term treatment with corticosteroids can cause side effects like thinning of the skin (Rhen and Cidlowski, 2005). Due to their inhibition of KC proliferation and differentiation, Vitamin D analogues are successful in the treatment of psoriasis patients (van de Kerkhof, 1995). These therapies are often combined with phototherapy to slow the growth of the affected skin cells (Parrish and Jaenicke, 1981).

The new insights discussed above into the immunopathogenesis of psoriasis resulted in the development of various biologic agents that target key molecules associated with the inflammatory process of psoriasis (Figure 7). Moderate to severe psoriasis often requires such a systemic treatment when the patients are unresponsive to topical agents or phototherapy. The clinical efficacy of multiple TNF antagonists (e.g. Adalimumab (Humira®; AbbVie), Etanercept (Amgen), and Infliximab (Janssen)) underscore the importance of this cytokine in the development and maintenance of psoriatic skin lesions. Since the IL-23/IL-17 axis is critically involved in psoriasis, monoclonal antibodies (mAb) targeting these cytokines have been developed for the treatment of psoriatic patients. Ustekinumab (Stelara®; Janssen), a human mAb, which binds the p40 subunit of

IL-12/IL-23 (Campa et al., 2016) has been shown to be efficient during different phase III clinical trials. Comparable effects can be achieved by targeting the p19 subunit with the mAb Gruselumab (Tremfya®; Janssen) and Tildrakizumab (Merck). Targeting IL-17 with Secukinumab (Cosentyx®; Novartis) was recently approved by the Food and Drug Administration (FDA) for the treatment of moderate-to-severe psoriasis (Hawkes et al., 2017). Other IL-17 antibodies (e.g Ixekizumab (Taltz®; Eli Lily), and Brodalumab (Kyntheum®; Amgen)) show the same results as the majority of the patients reached a 75 % reduction of the clinical score (Campa and Menter, 2016, Hawkes et al., 2017).
Although biological therapy revolutionized the treatment of moderate to severe forms of psoriasis one problem is the loss of effectiveness over time. For example, in a cohort of 650 patients treated with anti-TNF, lack of efficacy has been observed in 14% of the patients after 28 months (Esposito et al., 2013, Olszewska et al., 2018). Therefore, improved biologics need to be developed to provide long-term treatment of psoriasis patients.

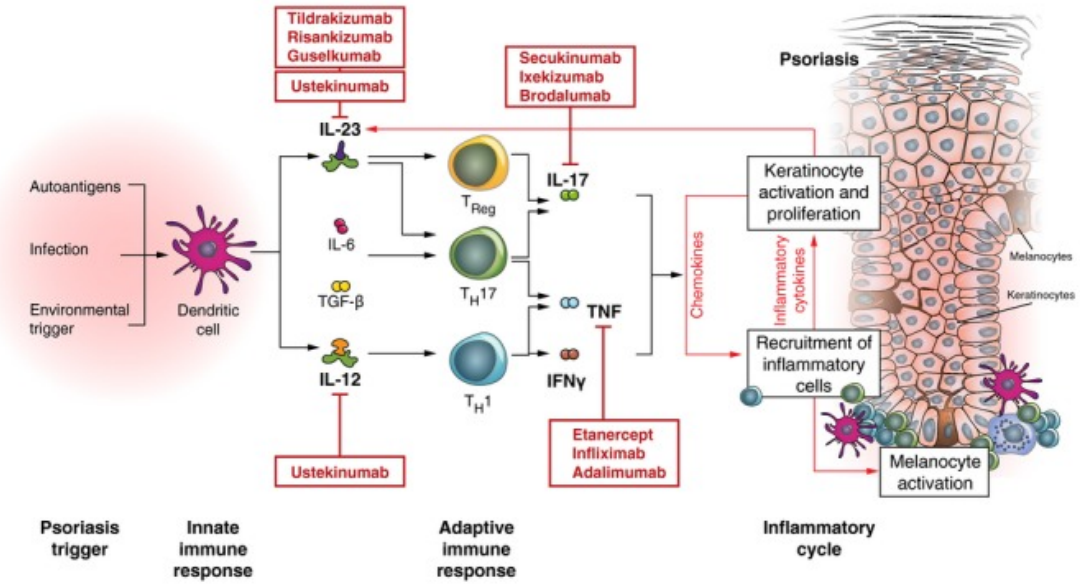

Figure 7: Diversity of targeted therapies in the pathogenesis of psoriasis. Due to the importance of immunological mechanisms in the pathogenesis of the disease, therapeutic research has been directed towards drugs that either block or inhibit these pathways. Currently available or biological therapies under development are: anti-TNF agents (Etanercept, Infliximab, Adalimumab), IL-17 inhibitors (Secukinumab, Ixekizumab, and Brodalumab), IL-23 inhibitors (Guselkumab), and IL-12/23 inhibitors (Ustekinumab) (Girolomoni et al., 2017).

1.3.7 Cardiovascular disease as a comorbidity of psoriasis

Psoriasis is associated with several comorbidities including arthritis, metabolic syndrome and, in particular, cardiovascular disease (CVD) (Oliveira Mde et al., 2015). It has been suggested that these immune-mediated chronic inflammatory processes are an independent risk factor for certain comorbidities associated with psoriasis. For example, in psoriasis, chronic inflammatory responses together with the associated production of Th1 and Th17 cytokines promotes systemic inflammation connected to the development of atherosclerosis, a chronic inflammatory disease of blood vessels that likely causes CVD (Hansson, 2005). Innate, as well as, adaptive immune responses have been identified during the course of atherosclerosis, and involve both the Th1 and Th17 pathways (Hansson and Libby, 2006). Furthermore, other cardiovascular risk factors have been linked to psoriasis and could partially explain the observed association between psoriasis and increased CVD risk. Patients suffering from psoriasis have an increased frequency of high blood pressure, which can be related to an increased level of angiotensin-converting enzyme, endothelin-1 (ET-1) (Salihbegovic et al., 2015). Moreover, C-reactive protein levels were significantly elevated in patients with psoriasis as compared to healthy controls. This increase of C-reactive protein is a risk factor for cardiovascular disease and directly related to the severity of the disease (Strober et al., 2008).

Therefore, combined dermatological-cardiological treatment should be considered for psoriasis patients. This should include regular blood pressure controls and adequate therapy in the case of hypertension, to limit this life-limiting comorbidity of psoriasis.

1.3.8 IL-17A as a key cytokine linking psoriasis to cardiovascular disease

As discussed above, many cytokines are involved in the development of psoriasis but recent studies identified IL-17A as the major effector cytokine driving the pathogenesis. However, IL-17A has not only been identified as a key factor in psoriasis, it also contributes to the development of vascular dysfunction and hypertension and has been therefore suggested to connect psoriasis and cardiovascular comorbidity (Karbach et al., 2014, Schüler et al., 2019). In the vasculature, IL-17A is an essential cytokine contributing to angiotensin II (AngII)-induced vascular dysfunction and hypertension. Thus, mice lacking IL-17A display reduced vascular inflammation and attenuated vascular dysfunction in response to AngII treatment (Madhur et al., 2010). Furthermore,

overexpression of IL-17A in KC induces systemic vascular inflammation, endothelial dysfunction, and arterial hypertension, thus leading to increased mortality. Thereby, inflammatory myeloid cells play a crucial role and depletion of neutrophils by anti-GR-1 antibody injection and antagonization of TNF and IL-6 partially attenuates oxidative stress in those mice (Karbach et al., 2014). Additionally, psoriasis patients with moderate to severe disease have significantly elevated serum IL-17A levels and those patients appear to be at the highest risk for developing and dying from CVD indicating that IL-17A links psoriasis to CVD.

2 Aims of the study

The cell adhesion molecule E-cadherin is a major component of adherens junctions and marks LC, the only DC population of the epidermis. There, LC build a dense network with KC via E-cadherin binding and downregulation of E-cadherin coincides with LC migration. To date, it is unknown whether E-cadherin is just a marker identifying LC or whether it plays a role in regulating LC homeostasis and function. Therefore, the first aim of this thesis was to investigate E-cadherin-mediated cell adhesion and signaling in LC *in vivo*. To aim this, we have crossed CD11c-Cre mice to E-cadherin$^{fl/fl}$ mice to obtain conditional knockouts of E-cadherin in all CD11c^{+} cells. LC of these mice, have been analyzed regarding their migration and function in steady state and inflammation using contact hypersensitivity and psoriasis models.

In the second part of this thesis we focused on the role of IL-17A in psoriasis and cardiovascular disease. Patients with severe psoriasis suffer from an increased risk of cardiovascular mortality. IL-17A plays a central role in the pathogenesis of psoriasis (Di Cesare et al., 2009) and also contributes to the development of vascular dysfunction and hypertension (Madhur et al., 2010) and might connect both diseases. To further understand the role of IL-17A in linking psoriatic skin inflammation and cardiovascular disease, we have generated a mouse model with constitutive low-level expression of this cytokine. This has been achieved by crossing CD11c-Cre mice to IL-17A$^{ind/ind}$ mice (Wohn et al., 2016). These transgenic mice have been analyzed for the development of psoriatic skin lesions and their vascular function. Furthermore, we wanted to test whether anti-IL-17A therapy could ameliorate the vascular dysfunction in different mouse models of psoriasis. With this approach we hoped to clarify whether an adequate anti-inflammatory therapy of psoriatic skin lesions could in parallel result in an improvement of the associated vascular disease.

3 Results

3.1 E-cadherin is dispensable to maintain Langerhans cells in the epidermis

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E-Cadherin is Dispensable to Maintain Langerhans Cells in the Epidermis

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The cell adhesion molecule E-cadherin is a major component of adherens junctions and marks Langerhans cells (LC), the only dendritic cell (DC) population of the epidermis. LC form a dense network and attach themselves to the surrounding keratinocytes via homophilic E-cadherin binding. LC activation, mobilization, and migration require a reduction in LC E-cadherin expression. To determine whether E-cadherin plays a role in regulating LC homeostasis and function, we generated CD11c-specific E-cadherin knockout mice (CD11c-Ecad^{del}). In the absence of E-cadherin–mediated cell adhesion, LC numbers remained stable and similar as in control mice, even in aged animals. Intriguingly, E-cadherin–deficient LC displayed a dramatically changed morphology characterized by a more rounded cell body and fewer dendrites than wild-type cells. Nevertheless, maturation and migration of LC lacking E-cadherin was not altered, neither under steady-state nor inflammatory conditions. Accordingly, CD11c-Ecad^{del} and control mice developed comparable contact hypersensitivity reactions and imiquimod-triggered psoriatic skin inflammation, indicating that E-cadherin on LC does not influence their ability to orchestrate T cell-mediated immunity. In conclusion, our data demonstrate that E-cadherin is dispensable to maintain LC in the epidermis and does not regulate LC maturation, migration, and function.

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INTRODUCTION

Dendritic cells (DC) are specialized antigen presenting cells strategically positioned at epithelial borders to the environment, including the skin. Here, they play a critical role inducing effective immune responses to fight off invading pathogens. At the same time, in particular in the steady-state, DC are also critical for maintaining tolerance toward both harmless foreign as well as self-antigens (Romani et al., 2012; Steinman et al., 2003). Upon antigen uptake, immature DC transform into mature DC and migrate to tissue-draining lymph nodes (LN), where they instruct naïve T cells to differentiate into appropriate effector or regulatory T cells (Romani et al., 2012). Although DC were originally considered primarily as potent activators of protective immunity, in the meantime it has become clear that DC play an essential role in balancing immunity and tolerance.

In mouse skin, three major DC subsets can be distinguished: Langerin^{+}Epcam^{+}CD103^{neg} epidermal Langerhans cells (LC), Langerin^{+}Epcam^{neg}CD103^{+}, and Langerin^{neg}Epcam^{neg}CD103^{neg}CD11b^{+} dermal DC (dDC) (Henri et al., 2010). LC are unique, as they share a common embryonic origin with tissue-resident macrophages (Hoeffel et al., 2012) and self-renew in situ in the steady-state without any contribution from bone marrow precursors (Merad et al., 2002), yet functionally they represent typical conventional DC (Clausen and Stoitzner, 2015). Moreover, LC are the only DC population in the epidermis and form a dense network among the surrounding keratinocytes, creating the first line of defense against invading pathogens. On the other hand, LC act as negative regulators of the anti-*Leishmania* response (Kautz-Neu et al., 2011), and their role in inducing inflammatory skin diseases like contact dermatitis or psoriasis remains controversial.

E-cadherin is a transmembrane glycoprotein that mediates Ca^{2+}-dependent cell-cell adhesion and is a major component of adherens junctions that contribute to the maintenance of epithelial barrier integrity (Van den Bossche and Van Ginderachter, 2013). Notably, LC attach themselves to the surrounding keratinocytes via homophilic E-cadherin binding, and E-cadherin is downregulated upon LC mobilization and emigration from the epidermis (Jakob and Udey, 1998; Tang et al., 1993). In addition, E-cadherin ligation on LC inhibits the upregulation of maturation markers like CD86 in vitro, suggesting that E-cadherin–mediated keratinocyte cell adhesion may represent a regulatory mechanism that prevents uncontrolled LC maturation in vivo (Riedl et al., 2000). Next to epidermal LC, bone marrow-derived DC (BMDC) express high levels of E-cadherin (Jiang et al., 2007). Beyond its homophilic binding, E-cadherin can also engage in heterophilic interactions with KLRG1 and CD103, which

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Abbreviatons: BMDC, bone marrow-derived DC; CHS, contact hypersensitivity; DC, dendritic cells; dDC, dermal DC; EMT, epithelial-to-mesenchymal-transition; IMQ, imiquimod; LC, Langerhans cells; LN, lymph nodes; sdLN, skin-draining LN; TLR, toll-like receptor

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are expressed by DC, Natural killer, and T cell subpopulations (Cepek et al., 1994; Rosshart et al., 2008). Upon ligation, KLRG1 inhibits TCR signaling and Natural killer cell cytotoxicity, which could be a way for E-cadherin$^+$ cells to attenuate inflammatory immune responses in situ (Beyersdorf et al., 2007; Ito et al., 2006). Similarly, E-cadherin–CD103 binding may facilitate the cross-talk between certain DC subsets and CD103$^+$ regulatory T cells (Suffia et al., 2005) or CD103$^+$CD8$^+$ tissue-resident memory T cells (Topham and Reilly, 2018).

Intracellularly, E-cadherin binds to β-catenin, the central component of the canonical Wnt signaling pathway (Jakob et al., 1999; Staal et al., 2008). Thereby E-cadherin protects β-catenin from proteasomal degradation, providing a sustained pool of β-catenin that can be released upon E-cadherin downregulation (Tian et al., 2011), while β-catenin stabilizes E-cadherin expression on the cell surface (and hence adherens junctions). Previous studies revealed a crucial role for E-cadherin and β-catenin in the regulation of DC maturation and function (Mellman and Clausen, 2010). In particular, in vitro disruption of E-cadherin binding in BMDC results in their phenotypic maturation, that is, β-catenin–mediated upregulation of MHCII and costimulatory molecules without activation of pro-inflammatory cytokine secretion, leading to a tolerogenic DC phenotype that promotes the induction of IL-10–producing T cells (Jiang et al., 2007). Here, we addressed whether E-cadherin downregulation is a cause or a consequence of LC maturation and migration and whether it contributes to the modulation of LC function in the steady-state and during inflammation.

RESULTS

Deletion of E-cadherin in BMDC does not lead to phenotypic maturation and activation

BMDC express high levels of E-cadherin (Figure 1a), and previous studies demonstrated that mechanical disruption of E-cadherin binding ("cluster disruption") leads to their phenotypic maturation via the release and nuclear translocation of membrane-bound β-catenin (Jiang et al., 2007). Thus, we were interested in whether the lack of E-cadherin affects BMDC maturation and cytokine production. Therefore, we analyzed the expression levels of the costimulatory molecules CD80 and CD86 on BMDC generated from CD11c-Cre x E-cadherin$^{fl/fl}$ (CD11c-Ecad^{del}) and wild-type (Cre-negative) control mice (Figure 1a). We detected a similar expression of both maturation markers on BMDC lacking E-cadherin as compared to controls. Treatment of BMDC with LPS induced the expected downregulation of E-cadherin associated with DC maturation in wild-type cells and equally high expression of CD80 and, in particular, CD86 in both groups. To determine differences in cytokine production, we measured the levels of IL-1β, IL-6, TNF-α, IL-12p70 and IL-10 in the supernatants of cells treated with different toll-like receptor (TLR) ligands. Whereas unstimulated cells also failed to produce pro- and anti-inflammatory cytokines in the absence of E-cadherin, the stimulation of different TLR resulted in a high production of cytokines, which was similar in the supernatants of CD11c-Ecad^{del} and control BMDC (Figure 1b). Taken together, the deletion of E-cadherin in BMDC does not lead to spontaneous maturation nor enhanced cytokine production.

To exclude that antigen uptake, processing, and presentation of DC lacking E-cadherin were altered, we investigated whether E-cadherin expression on DC influences their T cell priming capacity. To this aim, immature BMDC were loaded with ovalbumin (OVA) protein and cocultured with naïve Cell Tracer Violet-labeled OVA-specific CD4$^+$ T cells (OT-II). After four days, we did not observe any differences in T cell proliferation between cells cocultured with E-cadherin–deficient and control BMDC (Supplementary Figure S1), suggesting that E-cadherin does not govern DC-induced T cell priming and proliferation in vitro.

E-cadherin is highly expressed by epidermal LC but also by Langerin$^+$ dDC

LC are thought to be the only skin DC population expressing E-cadherin (Romani et al., 2010). Thus, when we first compared E-cadherin expression levels by the different skin DC subsets of CD11c-Ecad^{del} and control mice, as expected, LC expressed high levels of E-cadherin, which were efficiently decreased in CD11c-Ecad^{del} mice (Figure 2a). Notably, we also found detectable, albeit much lower, E-cadherin expression on Langerin$^+$ and Langerin^{neg} dDC. While this likely represents unspecific background staining in the latter, the low E-cadherin expression on Langerin$^+$ dDC appears to be genuine, as it is significantly reduced in the CD11c-Ecad^{del} mice (Figure 2a). Moreover, E-cadherin sequesters cytoplasmic β-catenin to the cell membrane and protects it from proteasomal degradation; sustaining a pool of β-catenin that can be released upon E-cadherin downregulation (Jiang et al., 2007; Mellman and Clausen, 2010; Staal et al., 2008). Therefore, we analyzed whether the loss of E-cadherin affects the expression of β-catenin. Indeed, both LC and Langerin$^+$ dDC lacking E-cadherin expressed significantly lower levels of β-catenin, as compared with the control cells (Figure 2b). In line with the presumably unspecific residual E-cadherin staining, β-catenin expression was unaffected in Langerin^{neg} dDC from the CD11c-Ecad^{del} mice. Taken together, these data reveal that next to epidermal LC, Langerin$^+$ dDC also express some E-cadherin.

Lack of E-cadherin leads to a more rounded LC cell body and less dendrites

Since E-cadherin is downregulated during LC migration, we wondered whether the loss of E-cadherin had any influence on LC homeostasis in the CD11c-Ecad^{del} mice. Therefore, we prepared epidermal sheets and analyzed the LC network by immunofluorescence microscopy of MHCII (Figure 3a). Unexpectedly, in the absence of E-cadherin–mediated cell adhesion, we detected comparable and stable numbers of LC as in epidermal sheets of control mice (Figure 3b), even in aged animals as quantified by flow cytometry (Figure 3c). Intriguingly, E-cadherin–deficient LC displayed a dramatically altered morphology with more rounded cell bodies and fewer dendrites than wild-type cells (Figure 3a, enlarged detail). Moreover, E-cadherin–deficient LC may be less tightly anchored in the epidermis than wild-type cells, as suggested by the higher absolute LC number

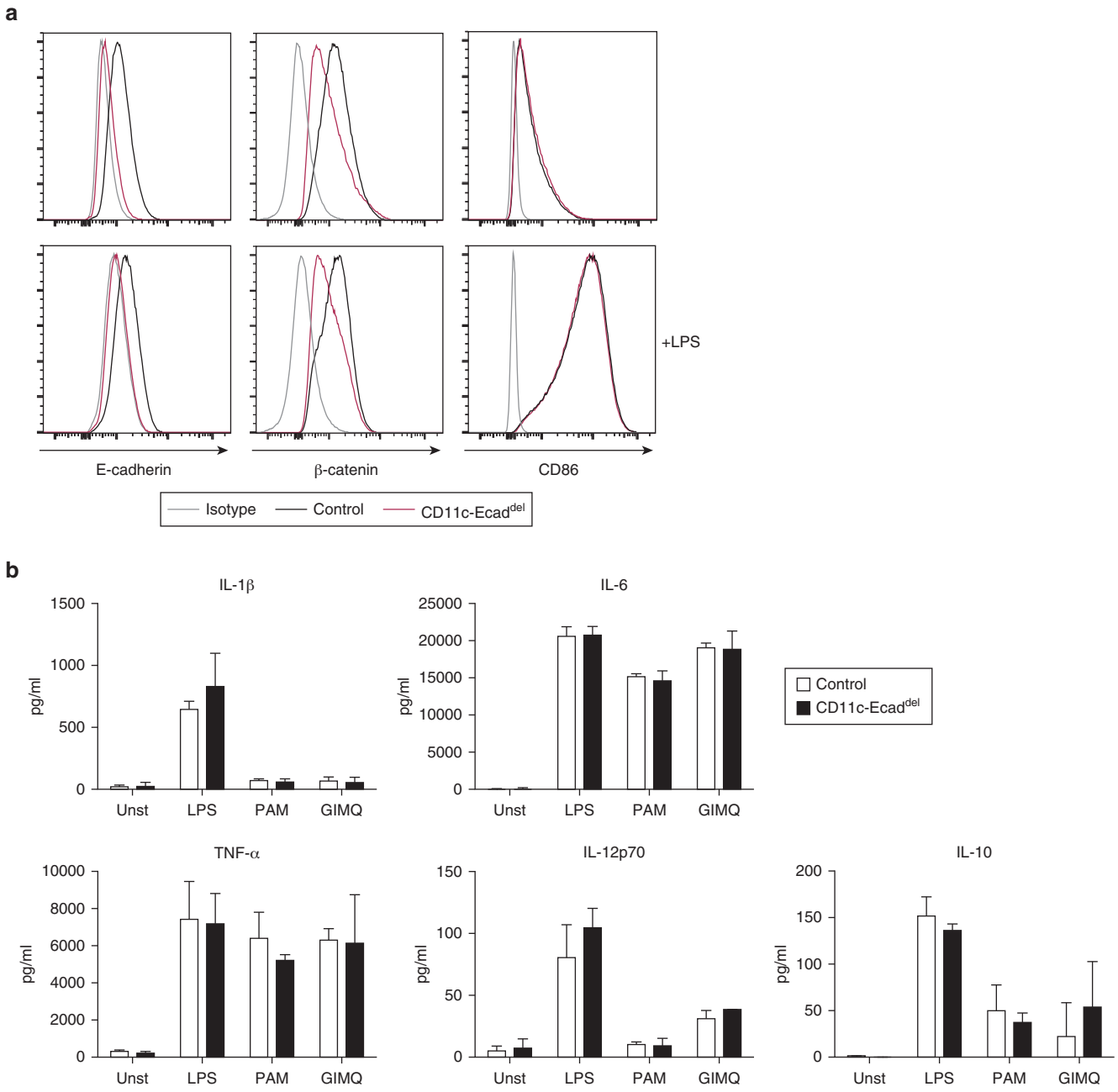

Figure 1. Loss of E-cadherin has no impact on BMDC maturation and cytokine production. (**a**) Upper row: Representative histograms showing E-cadherin, β-catenin, and CD86 expression of BMDC generated from CD11c-Ecad^{del} and control mice. Lower row: E-cadherin, β-catenin, and CD86 expression of BMDC after stimulation with LPS (1 μg/ml) for 12 hours. (**b**) Cytokines produced by untreated and different TLR-ligand (LPS: TLR4, PAM [Pam_3Cys-Ser-$[Lys]_4$]: TLR1/2, and Gardiquimod [GIMQ]: TLR7) stimulated BMDC. Values are the mean ± standard error of the mean, and the data are representative of two independent experiments ($n = 3$ mice). LPS, lipopolysaccharide; Unst, unsimulated.

released from collagenase IV-digested skin (3,063 ± 171.1 versus 1,612 ± 327.3 LC, $P = 0.0041$) as compared to the equal maximum number of LC detectable after a more vigorous Liberase digestion (2,916 ± 312.2 versus 3,886 ± 329.7, $P = 0.0651$) (Supplementary Figure S2).

Thus, although we identified comparable numbers of LC in the skin of CD11c-Ecad^{del} and control mice, the cells seem to be more loosely attached to the keratinocytes, which could enhance their maturation and turnover in the absence of E-cadherin. Therefore, we analyzed LC of CD11c-Ecad^{del} and control mice regarding their maturation and proliferation in the steady-state by flow cytometry. We confirmed similar absolute LC numbers in the skin of CD11c-Ecad^{del} and control mice (2,608 ± 315.8 versus 3,560 ± 678.9, $P = 0.1771$) (Figure 3d). Furthermore, the expression of CD80 and CD86 on LC was not altered, indicating that the loss of E-cadherin does not influence LC maturation (Figure 3d). The presence of a similar frequency of Ki67$^+$ LC in the skin demonstrates that E-cadherin deficiency does not affect LC proliferation and turnover (Figure 3e).

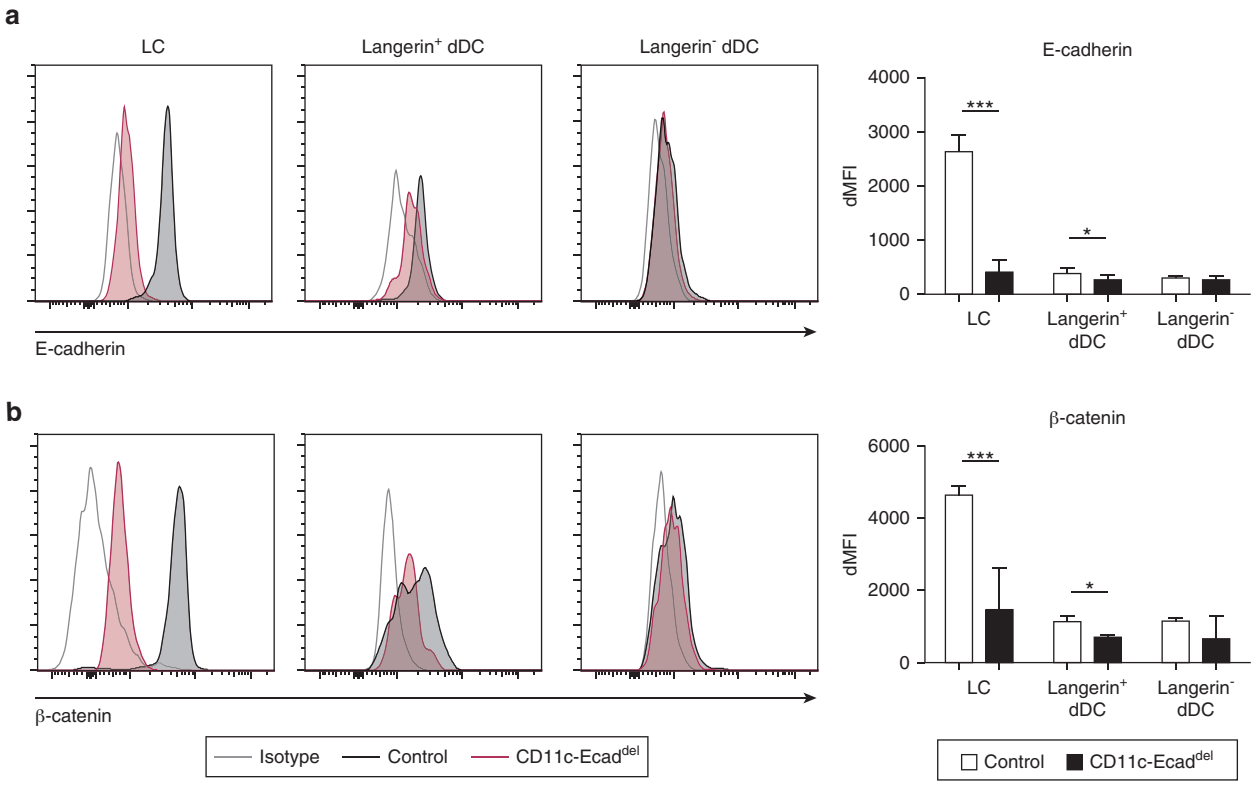

Figure 2. E-cadherin and β-catenin expression on DC subsets in the skin of CD11c-Ecad^{del} and control mice. Single-cell suspensions of skin from CD11c-Ecad^{del} and control mice were obtained by enzymatic digestion. (**a**) Histograms of E-cadherin and (**b**) β-catenin expression by different DC subsets pre-gated on MHCII^{+}CD11c^{hi} cells. Values are the mean ± standard error of the mean, and the data are representative of three independent experiments ($n = 3 - 4$ mice). dDC, dermal DC; LC, Langerhans cells.

E-cadherin deficiency does not enhance LC emigration from the skin

LC attach themselves via homophilic E-cadherin binding to the surrounding keratinocytes (Tang et al., 1993) and downregulate E-cadherin during their migration, but whether this is cause or consequence of LC mobilization and maturation remains unknown. Moreover, E-cadherin downregulation on LC is associated with a broader genetic program known as epithelial-to-mesenchymal transition (EMT) (Hieronymus et al., 2015; Konradi et al., 2014; Sagi and Hieronymus, 2018). Thus, we determined whether the loss of E-cadherin influences EMT marker expression and LC migration. First, we isolated LC from the skin and skin-draining LN (sdLN) of CD11c-Ecad^{del} and control mice and analyzed the EMT markers N-cadherin and EpCam (Figure 4b). While EpCam was down- and N-cadherin upregulated on migratory LC in sdLN, we detected a similar expression of both EMT markers between CD11c-Ecad^{del} and control mice. Next, we investigated steady-state migratory DC by flow cytometry, without observing any differences in neither LC numbers nor other DC subsets (Figure 4a). To assess LC migration in vitro, we split ears into dorsal and ventral halves, cultured them dermal side down, and quantified the LC that had crawled out of the tissue. After 48 hours, we detected similar LC numbers in the medium of skin explants from CD11c-Ecad^{del} and control mice (Figure 4c). Although LC migration was similar in the steady-state, inflammation could trigger enhanced emigration of LC lacking E-cadherin out of the skin. Therefore, we applied FITC contact sensitizer onto the ears of the mice and analyzed the sdLN for incoming FITC^{+} LC. After 24, 48, 72, and 96 hours, we identified a similar amount of LC in the LN of CD11c-Ecad^{del} and control mice, suggesting that the lack of E-cadherin also does not facilitate the mobilization of LC to LN during inflammation (Figure 4d).

E-cadherin on LC has no impact on Trinitrochlorobenzene-induced contact hypersensitivity

Although LC numbers, maturation, and migration were similar in CD11c-Ecad^{del} and control mice, the fact that LC and T cells can interact via E-cadherin–KLRG1/CD103 signaling could influence the ability of E-cadherin–deficient LC to govern T cell-mediated immunity. Thus, we tested the mice in contact hypersensitivity (CHS), a Th1/Th17-mediated delayed-type hypersensitivity reaction to a topically applied hapten. Five days after sensitization with 450 μg trinitrochlorobenzene on the shaved abdomen, the mice were challenged with 45 μg trinitrochlorobenzene on one ear. Comparable ear swelling reactions up to 96 hours after hapten challenge in CD11c-Ecad^{del} and control mice indicate that E-cadherin on LC does not influence their ability to regulate T cell-mediated immune responses (Figure 5a). In line with this conclusion, CD11c-Ecad^{del} mice contained similar numbers of LC (Figure 5b) and lymphocyte subpopulations (Supplementary Figure S3) in the ears and ear-draining LN after the elicitation of CHS.

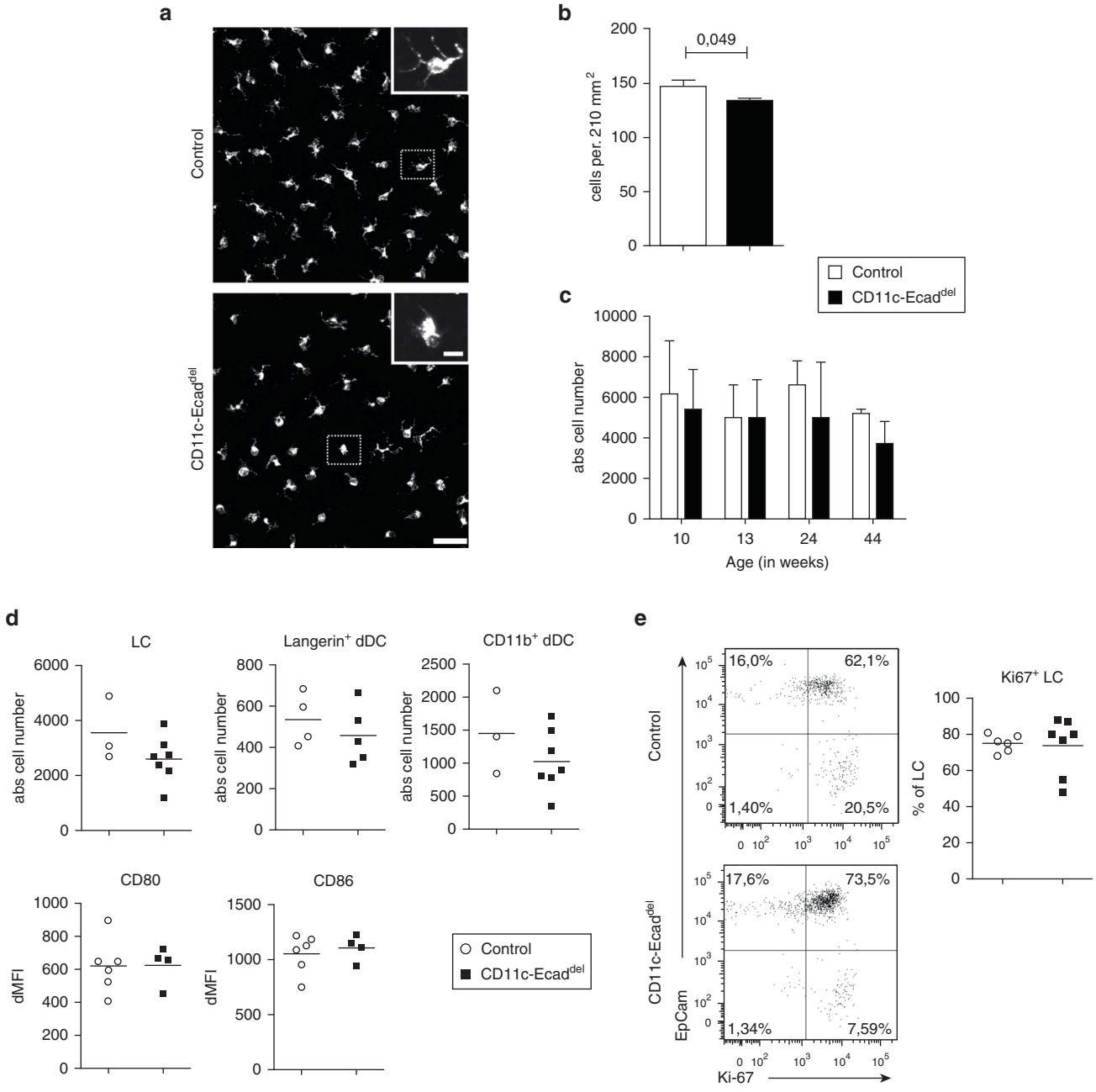

Figure 3. Deletion of E-cadherin leads to a more rounded LC body and less dendrites. (**a**) Epidermal sheets of 14 week-old control and CD11c-Ecad^{del} mice stained for MHCII and analyzed by fluorescence microscopy. (**b**) LC counts in epidermal sheets. (**c**) Absolute LC numbers from both ears of CD11c-Ecad^{del} and control mice at the indicated ages as determined by FACS. Data are representative of two independent experiments ($n = 3 - 4$). (**d**) FACS analysis of skin single-cell suspensions gated on live CD45$^+$MHCII$^+$CD11c$^+$ cells. Upper row: Absolute cell numbers of LC, Langerin$^+$ dDC, and CD11b$^+$ dDC. Lower Row: CD80 and CD86 expression on LC. Values are the mean ± standard error of the mean. (**b**, **c**) or the mean (**d, e**) and the data are representative of three independent experiments ($n = 4 - 6$). (**e**) Percentages of Ki67$^+$ LC in the skin. Data are representative of two independent experiments ($n = 6 - 7$). dDC, dermal DC; LC, Langerhans cells.

Deletion of E-cadherin on LC does not influence imiquimod-induced psoriasis

Psoriasis represents another inflammatory skin disease, which may be triggered and/or perpetuated by DC (Nestle et al., 2009). Topical application of Aldara cream containing the TLR 7/8-ligand imiquimod (IMQ) induces skin inflammation closely resembling human plaque-type psoriasis (van der Fits et al., 2009). Thus, next we investigated whether E-cadherin on LC affects psoriasis-like skin disease in mice. After painting IMQ onto the back skin and ears of CD11c-Ecad^{del} and control animals for six consecutive days, we could not detect any differences in skin thickness, erythema, and scaling during the course of disease (Figure 5c) nor in the cellular skin infiltrates (Figure 5d). These data indicate that E-cadherin on LC plays no role in regulating IMQ-induced psoriatic skin inflammation.

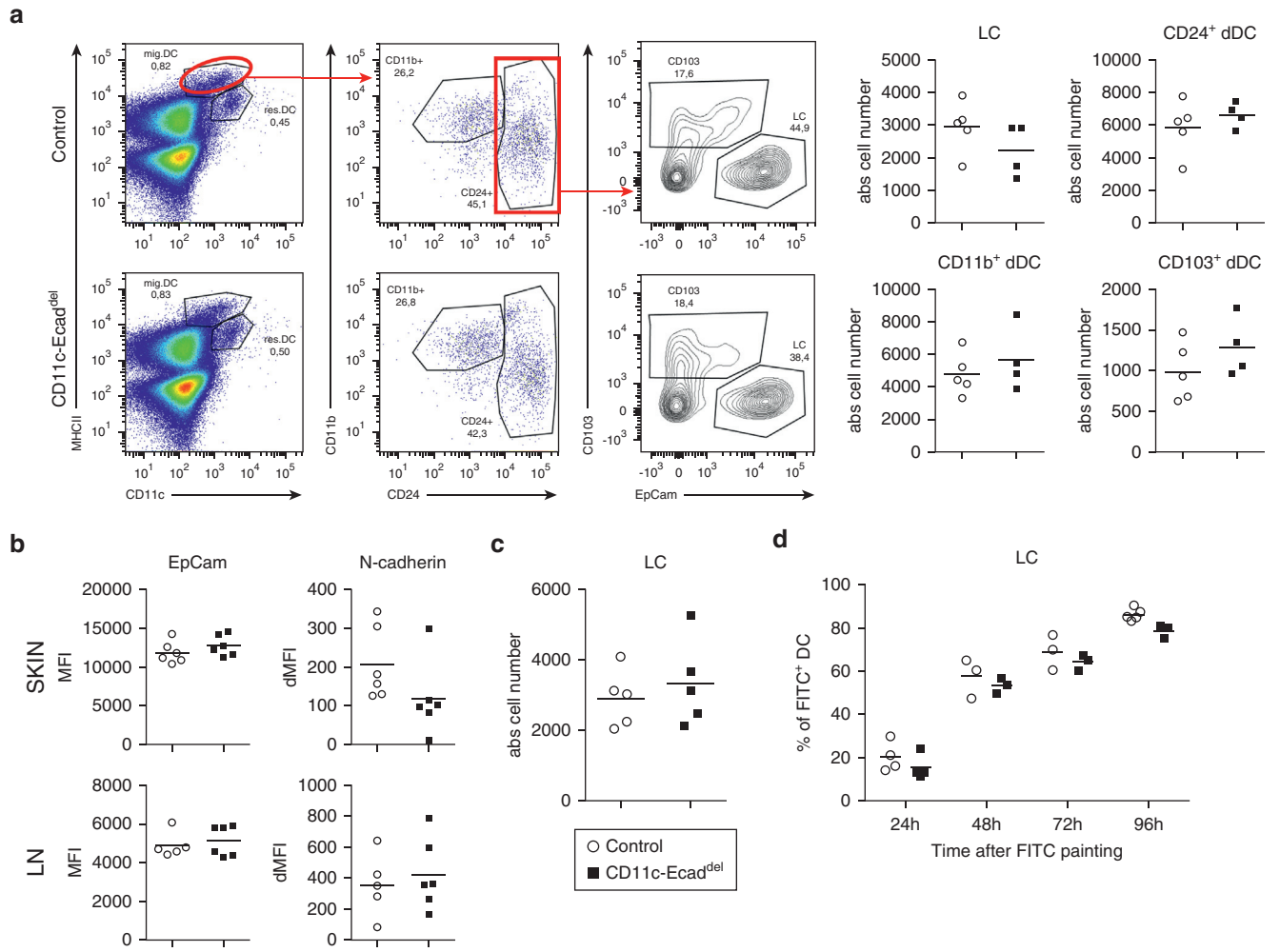

Figure 4. Similar migration of E-cadherin−deficient LC to skin-draining LN during steady-state and inflammation. (**a**) DC subsets in the LN of CD11c-Ecad^{del} mice analyzed by flow cytometry. Data are representative of three independent experiments ($n = 4 - 5$). (**b**) EpCam and N-cadherin expression on LC in the skin and LN of CD11c-Ecad^{del} and control mice. (**c**) Whole skin explants from CD11c-Ecad^{del} and control mice were incubated for 48 hours in culture medium, and LC numbers in the medium ("crawl outs") were analyzed by flow cytometry. (**d**) FITC was painted onto the ears of the mice, and ear-draining LN were analyzed for FITC^{+} migratory LC at the indicated time points. Values are the mean, and the data are representative of two independent experiments ($n = 3 - 5$). dDC, dermal DC; LC, Langerhans cells; LN, lymph nodes.

DISCUSSION

Contrary to general assumption, here we demonstrate that E-cadherin expression on LC is dispensable for their maintenance in the epidermis. On the other hand, LC lacking E-cadherin display a dramatically altered morphology characterized by more rounded cell bodies and fewer dendrites. Apart from that, the loss of E-cadherin does not influence LC turnover, maturation, migration nor function, that is, their ability to induce T cell-mediated immune responses.

E-cadherin has primarily been described as a cell adhesion molecule and central component of adherens junctions (van Roy and Berx, 2008). Since the seminal discovery by the Udey lab that LC and keratinocytes are linked to each other via homophilic E-cadherin binding (Tang et al., 1993), it is generally accepted that E-cadherin is essential for LC seeding and maintenance in the epidermis (Jakob and Udey, 1998). This concept is further supported by the pivotal role of TGF-β1 and TGF-β receptor signaling for LC differentiation, including the induction of E-cadherin expression, and LC positioning in the epidermis (Bobr et al., 2012; Kel et al., 2010). The ligation of E-cadherin on immature LC also inhibits their maturation in vitro (Riedl et al., 2000). In contrast, our data reveal that E-cadherin on LC is dispensable to maintain the cells in the epidermis but is required for the formation of their dendrites. Owing to the relatively late expression of the *CD11c* promoter (Kel et al., 2010), we do not know whether E-cadherin also plays any role during early seeding of the epidermis during ontogeny (Chorro et al., 2009; Hoeffel et al., 2012). Without E-cadherin, the LC fail to acquire their typical dendritic morphology and instead exhibit a more rounded phenotype with very few remaining dendrites. Notably, wild-type LC display a unique behavior characterized by rhythmic extension and retraction of their dendrites into the intercellular spaces between keratinocytes in both the steady-state and amplified during inflammation (Nishibu et al., 2006). Whether this impaired dynamic behavior of

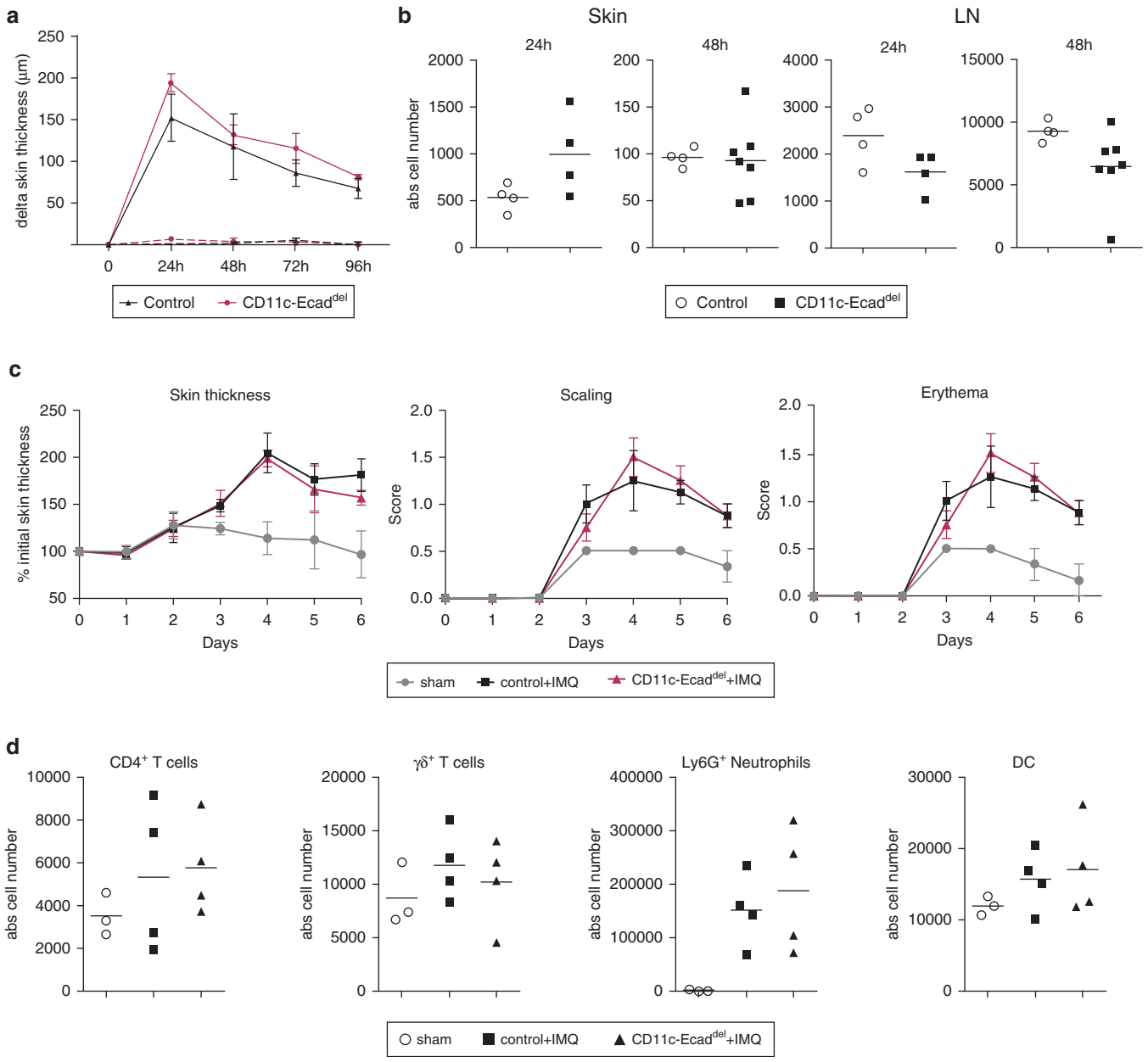

Figure 5. E-cadherin on LC has no impact on CHS and IMQ-induced psoriatic skin disease. (**a**) CD11c-Ecad^{del} (red line) and littermate controls (black line) were sensitized with 450 µg trinitrochlorobenzene on the shaved abdomen and challenged with either 45 µg trinitrochlorobenzene on their ears five days later or treated with vehicle alone (dotted lines). Data (mean ± standard error of the mean) depict ear swelling over 96 hours and are representative of four independent experiments ($n = 4 - 5$). (**b**) LC numbers in the skin and sdLN 24 and 48 hours after hapten challenge. Data (mean) are representative of two independent experiments ($n = 4$). (**c**) 7 week-old CD11c-Ecad^{del} and control mice were treated for six days with Aldara cream containing 5% IMQ. Skin thickness, scaling and erythema was evaluated daily. (**d**) The cellular skin infiltrate was analyzed on day seven by FACS. Values are the mean ± standard error of the mean (**c**) and the data are representative of two independent experiments ($n = 4$). IMQ, imiquimod; LC, Langherhans cells; LN, lymph nodes.

E-cadherin–deficient LC compromises their ability to acquire antigens from the surface of the skin (Ouchi et al., 2011) and to interact with the cutaneous microbiota and/or their surveillance of invading pathogens (Naik et al., 2015; Naik et al., 2012) remain to be investigated.

Although LC are present at similar numbers in epidermal sheets as compared to age-matched wild-type mice, in the absence of E-cadherin LC may still exhibit a higher turnover and enhanced migration behavior. This hypothesis is further corroborated by their altered morphology and less dendritic phenotype, which suggests that E-cadherin–deficient LC may be less tightly anchored within the epidermis. Moreover, during their mobilization from the skin LC downregulate E-cadherin, but whether this can cause or is merely a consequence of their maturation/migration remains elusive (Bobr et al., 2012; Kel et al., 2010; Tang et al., 1993). Although we expected a higher LC turnover in CD11c-Ecad^{del} mice, the frequency of proliferating (Ki67^{+}) LC in the epidermis is similar to that of control animals. Moreover, LC migration from the skin to sdLN in both the steady-state and during inflammation is not altered in the absence of E-cadherin.

The downregulation of E-cadherin is a molecular hallmark of a genetic program called EMT, which plays important roles in embryonic development, wound healing, and tumor invasion (Sagi and Hieronymus, 2018). Indeed, it has been suggested that EMT also controls LC development, adhesion, and/or migration. For one, LC maturation leads to a switch from an adhesive to a motile state that is similar to EMT (Hieronymus et al., 2015). Second, during their mobilization, LC downregulate a set of epithelial genes, including E-cadherin and EpCam, while they upregulate the mesenchymal marker N-cadherin, known to be involved in cell migration (Konradi et al., 2014). Evidence of the similar expression and regulation of EpCam and N-cadherin indicates that this genetic program is not induced in E-cadherin−deficient LC. Hence, these data establish that the downregulation and/or lack of E-cadherin alone does not trigger EMT nor initiate or enhance LC mobilization and migration.

So far, it has been assumed that in mouse skin LC are the only DC population expressing E-cadherin (Borkowski et al., 1994; Tang et al., 1993). Here, we demonstrate that next to LC, the small subset of Langerin^{+} dDC expresses E-cadherin, albeit at much lower levels. At the time E-cadherin on skin DC was first studied, the Langerin^{+} dDC subset had not yet been identified (Bursch et al., 2007; Ginhoux et al., 2007; Poulin et al., 2007). Hence, any E-cadherin^{+} DC detected in the dermis was considered to be a migrating LC on its way to the sdLN (Clausen and Stoitzner, 2015; Romani et al., 2012). To confirm *bona fide* E-cadherin expression by Langerin^{+} dDC, we analyzed the expression of β-catenin in the different skin DC subsets in CD11c-Ecad^{del} and control mice. Whereas β-catenin is expressed by all skin DC, the lack of E-cadherin results in its reduced presence in both LC and Langerin^{+} dDC but not in Langerin^{-} dDC of CD11c-Ecad^{del} mice.

During our analysis of LC numbers and their turnover and/or migration in the skin, we observed that different enzymes greatly affect the number of LC released from the tissue. Specifically, a milder digestion with collagenase IV, which lacks any additional trypsin activity, releases more LC from the skin of CD11c-Ecad^{del} than control mice. On the other hand, digestion with Liberase, containing trypsin activity, leads to the isolation of similar LC numbers from CD11c-Ecad^{del} and control skin, but more total LC than collagenase IV digestion. For one, this finding indicates that the choice of tissue processing and/or digestion greatly influences the LC yields and is of general importance when assessing absolute LC numbers in the skin. Second, despite similar turnover and migration, it suggests that without E-cadherin LC adhere less tightly to the keratinocytes and can be released more easily from the epidermis by enzymatic digestion than wild-type cells.

E-cadherin is best characterized as an adhesion molecule in epithelial cells, including keratinocytes (Charest et al., 2009; Young et al., 2003), whereas little is known about its role and function in immune cells. It is becoming increasingly clear that E-cadherin is also expressed on a variety of leukocytes like LC, macrophages, and γδ T cells, and therefore may be a potential immunological regulator (Van den Bossche et al., 2012). Moreover, E-cadherin is present on BMDC, and the disruption of homophilic E-cadherin binding leads to their phenotypic maturation, that is the upregulation of MHCII, costimulatory molecules, and CCR7 in the absence of any pro-inflammatory cytokine production (Jiang et al., 2007). Notably, this phenotypic maturation is mediated via β-catenin, which disengages from E-cadherin and translocates into the nucleus where it regulates gene transcription. In contrast, E-cadherin−deficient BMDC showed no spontaneous maturation, suggesting that the mechanical disruption of homophilic E-cadherin contacts induces a maturation program that is not activated in cells lacking E-cadherin. While E-cadherin cluster disruption of wild-type BMDC leads to a sudden release of membrane-bound β-catenin, this pool of β-catenin is absent in E-cadherin−deficient BMDC. This conclusion is in line with the lack of spontaneous or enhanced LC mobilization and migration in CD11c-Ecad^{del} mice.

Moreover, β-catenin has been suggested to promote a tolerogenic and/or regulatory DC phenotype in vivo via the induction of IL-10−producing T cells (Jiang et al., 2007), and mice with a CD11c-specific deletion of β-catenin display enhanced Th1/Th17 responses and disease susceptibility in chemically-induced colitis (Manicassamy et al., 2010). Despite the lack of spontaneous maturation and mobilization of LC in CD11c-Ecad^{del} mice in the steady-state, without the membrane-bound pool of β-catenin E-cadherin−deficient LC (and/or E-cadherin^{-}Langerin^{+} dDC) might trigger exacerbated immune reactions in the skin. Therefore, we investigated their capacity to induce CHS, a T cell-mediated cutaneous immune response in which the role of LC and Langerin^{+} dDC remains controversial (Clausen and Stoitzner, 2015). CD11c-Ecad^{del} and control mice mount similar T cell responses and hence ear swelling reactions, indicating that E-cadherin signaling in LC (and Langerin^{+} dDC) does not govern their function even under inflammatory conditions. In light of the multiple E-cadherin−dependent pathways potentially affected by the lack of E-cadherin on LC, this is quite an unexpected finding (Van den Bossche and Van Ginderachter, 2013). Besides the release of β-catenin, E-cadherin−dependent PI3K activation may induce an anti-inflammatory LC phenotype (Fukao et al., 2002). Furthermore, E-cadherin can bind to KLRG1, an inhibitory receptor expressed on mature T cells and Natural killer cells (Banh et al., 2009). The engagement of KLRG1 inhibits Natural killer cell cytotoxicity, cytokine production, and antigen-induced T cell proliferation (Gründemann et al., 2006; Ito et al., 2006), while simultaneous "reverse" signaling impairs the ability of E-cadherin expressing DC to release inflammatory cytokines (Banh et al., 2009). Nevertheless, the percentage and absolute cell numbers of CD4, CD8, and γδ T cells from the skin of the CD11c-Ecad^{del} and control mice are similar, indicating that the binding of LC E-cadherin to KLRG1 does not govern CHS responses.

DC have been implicated in the pathogenesis of psoriasis, but the roles for specific DC subsets, in particular, LC are not well understood. Intriguingly, LC are severely diminished in human psoriatic skin lesions (Glitzner et al., 2014). In agreement with an anti-inflammatory role during active psoriasis, the depletion of LC during this phase aggravates psoriasis symptoms in mice (Glitzner et al., 2014; Terhorst

et al., 2015). On the other hand, the depletion of LC before the onset of disease has no effect, indicating that LC are dispensable to drive psoriatic plaque formation in mice (Glitzner et al., 2014; Wohn et al., 2013), while others reported that LC can produce the IL-23 essential to trigger the IL-17–mediated inflammatory cascade (Singh et al., 2016; Yoshiki et al., 2014). Recently, it became clear that dermal γδ T cells are the major IL-17 producers in the skin during the initiation of psoriasiform disease (Cai et al., 2011; Pantelyushin et al., 2012). Furthermore, dendritic epidermal γδ T cells, which express E-cadherin, are elevated in psoriatic skin, but their role in disease progression remains elusive (Lee et al., 1994). E-cadherin on dendritic epidermal γδ T cells controls their activation by acting as an inhibitory receptor (Uchida et al., 2011), suggesting that E-cadherin binding between LC and dendritic epidermal γδ T cells may confine psoriatic skin inflammation. In contrast, after topical application of the TLR7-agonist IMQ, we did not observe any differences in ear swelling, redness, and scaling, which is in line with similar cellular infiltrates in the skin of CD11c-Ecad^{del} and control mice. This indicates that E-cadherin interactions of LC and dendritic epidermal γδ T cells are not involved in IMQ-induced skin disease.

Taken together, despite many and plausible indications, and contrary to common conviction, our data establish that E-cadherin is dispensable to maintain immature LC in the epidermis and does not regulate LC maturation, migration, nor function during steady-state and inflammation.

MATERIAL AND METHODS

Mice

E-cadherin$^{fl/fl}$ mice (Boussadia et al., 2002) were crossed to CD11c-Cre (Caton et al., 2007) to obtain conditional knockouts with the specific deletion of E-cadherin in all CD11c^{+} cells (CD11c-Ecad^{del}). All the experiments were performed with 8 − 14 week-old, sex, and age-matched animals and Cre-negative littermate controls. All the mice were housed and treated in accordance with the relevant laws and institutional guidelines of the Central Animal Facility of the University Medical Center Mainz.

Cell preparation

The LN, belly skin, and ears were mechanically disrupted and digested with 400 U/ml collagenase IV (Worthington Biochemical Corp., Lakewood, NJ) and the belly skin and ears were additionally digested with 100 U/ml hyaluronidase (Sigma-Aldrich, St. Louis, MO) and 0.5 U/ml DNase (Promega, Madison, WI) in RPMI for 30 − 60 minutes at 37 °C. For Liberase digestion, the ears were digested with 0.15 mg/ml Liberase and 0.12 mg/ml DNase I (both from Roche, Basel, Switzerland) for 45 minutes at 37 °C. EDTA (10 mM) was added for 5 minutes at room temprature. Subsequently, the cells were filtered through 70 μm cell strainers (BD Biosciences, San Jose, CA) to obtain single-cell suspensions. For experiments where dermis and epidermis were separated, the ears were pretreated with trypsin and EDTA (0.5% for ventral, 0.3% for dorsal sides) containing 10 U/ml DNase for 1 hour at 37 °C shaking. The epidermis was mechanically separated from the dermis, and both were digested as described above.

Flow cytometry

LN and skin cells were pre-incubated in FACS-buffer containing Fc-Block (Biocompare, South San Francisco, CA) for 10 minutes and then surface-stained with various combinations of fluorescence-conjugated antibodies at 4 °C for 30 minutes. For intracellular staining, the cells were fixed with 2% paraformaldehyde, permeabilized with 0.1% Saponin and incubated with appropriate antibodies for 60 minutes at 4 °C. Flow cytometric acquisition was performed on a FACS Canto II (BD) and analyzed using FlowJo software (Treestar, Ashland, OR).

Epidermal sheets

Epidermal sheets were prepared as previously described (Ober-Blöbaum et al., 2017). The ears were split into dorsal and ventral halves and floated with the dermal side facing down on 500 μl of 20 mM EDTA solution for 90 minutes at 37 °C. The epidermis was detached from the dermis and fixed in 4% paraformaldehyde for 20 minutes at room temprature. The epidermal sheets were washed twice and stained with directly conjugated primary antibodies (MHCII-PE and Langerin-FITC).

Additional Methods

Antibodies, generation of BMDC, T cell proliferation assay, cytokine detection, mouse skin explant cultures, FITC painting, CHS, and IMQ-induced psoriatic skin inflammation are described in the Supplementary Material and Methods.

Statistical analysis

The results were analyzed using GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA). Statistical significance between the two groups was assessed by using a two-tailed Student's *t*-test. Psoriasis area and severity index scores and CHS curves were analyzed by a two-way analysis of variance with a Bonferroni post-hoc test. *P*-values less than 0.05 were considered significant ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Error bars are presented as the standard error of the mean.

Data availability statement

No datasets were generated or analyzed during this study.

CONFLICT OF INTEREST

IM is a full-time employee and officer of Genentech and a Roche stock holder. The other authors state no conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization: BEC; Data curation: AB, BEC; Formal analysis: AB; Funding acquisition: BEC; Investigation: AB, ND, SPZ, CT; Methodology: AB; Project administration: AB, BEC; Resources: BEC, KK; Software: KK; Supervision: BEC; Validation: AB, BEC; Visualization: AB, KK, BEC; Writing − original draft: AB, BEC; Writing − review & editing: SPZ, RAB, PS, AJ, and IM.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2019.06.132.

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SUPPLEMENTARY MATERIALS AND METHODS

Generation of Bone marrow dendritic cells

Bone marrow dendritic cells (BMDC) were generated in vitro from femurs and tibia of CD11c-Ecad^{del} and control mice, and red blood cells were depleted by 5 minutes of incubation with tetrabutylammonium chloride solution. To obtain single-cell solutions, cells were filtered through 70 µm cell strainers. A total of $1.5x10^6$ cells per ml were cultured in RPMI 1640 with 5% fetal calf serum, 10,000 U/ml penicillin and streptomycin and 50 mM β-mercaptoethanol in the presence of 20 ng/ml GM-CSF. After 8 −10 days, the cells were further matured by overnight stimulation with LPS (Lipopolysaccharide, TLR4 ligand) (1 µg/ml), GIMQ (Gardiquimod, TLR7 ligand) (1 µg/ml), PAM (Pam_3Cys-Ser-[Lys])$_4$, TLR1/2 ligand) (1 µg/ml) or left untreated.

Antibodies

The following anti-mouse monoclonal antibodies (clone) from BD Biosciences, BioLegend (San Diego, CA) and eBioscience (San Diego, CA) were used for flow cytometry: CD11c (N418), MHC-II (M5/114), CD45 (30-F11), CD11b (M1/70), CD103 (M290), CD24 (M1/69), Langerin (929F3), EpCam (G8.8), E-cadherin (24E10; Cell Signaling), β-catenin (L54E2; Cell Signaling), N-cadherin (EPR22397-264; Abcam), CD40 (3/23), CD80 (16-10A1), CD86 (GL1), CD4 (L3T4), CD8α (53-6.7), TCRβ (H57-597), and TCRγδ (eBio GL3).

T cell proliferation assay

BMDC were differentiated as described above. Immature BMDC were loaded with 500 µg/ml OVA protein and cocultured with naïve ($CD4^+CD25^{neg}$) Cell Tracer Violet -labeled OVA-specific T cells (OT-II) at a ratio of 1:10 for 4 days. T cell proliferation (Cell Tracer Violet dilution) was monitored using flow cytometry.

Cytokine detection

Cell culture supernatant levels of IL-1β, IL-6, IL-10, IL-12p70 and TNF-α were determined by Luminex Multiplex Assays according to the manufacturer's instructions (ThermoFischer, Waltham, MA).

Mouse skin explant cultures ("crawl outs")

Ears were cut off at the base, and the skin was split into dorsal and ventral halves (Stoitzner et al., 2003). Both halves were cultured at 37 °C in 24-well plates dermal side down in RPMI containing 10% fetal calf serum, 50 µg/ml gentamycin, 10,000 U/ml penicillin and streptomycin and 50 mM β-mercaptoethanol. After two to four days, the emigrant DC were harvested and analyzed by flow cytometry.

FITC painting

Both ears were painted with 25 µl FITC solution (5 mg/ml FITC in dibutylphalate:acetone [1:1]) and after 24, 48, 72, and 96 hours draining, the cervical lymph nodes were collected, enzymatically digested to obtain a single-cell suspension and analyzed for the frequency of incoming $FITC^+$ migratory DC by flow cytometry.

Contact hypersensitivity

Mice were sensitized by painting 15 µl of trinitrochlorobenzene (450 µg per mouse) in acetone:olive oil (4:1) on the shaved abdomen. To elicit CHS, the animals were challenged five days later with 15 µl of trinitrochlorobenzene (45 µg per mouse) in acetone:olive oil (4:1) onto the ears. As a readout of the adaptive immune response, the amount of ear swelling was measured daily before and up to 96 hours after hapten challenge, and the results were normalized to the ear thickness before sensitization.

Imiquimod (IMQ)-induced psoriatic skin inflammation

The Aldara/IMQ mouse model of psoriatic skin inflammation was performed as described previously (van der Fits et al., 2009; Wohn et al., 2013; Wohn et al., 2014). After shaving the back of the mice, Aldara cream was applied to the ears and back skin for six consecutive days. The severity of inflammation was evaluated daily using Psoriasis Area and Severity Index scoring, including erythema, scaling, and thickening of the skin (0: none; 1: slight, 2: moderate; 3: marked; 4: highly marked).

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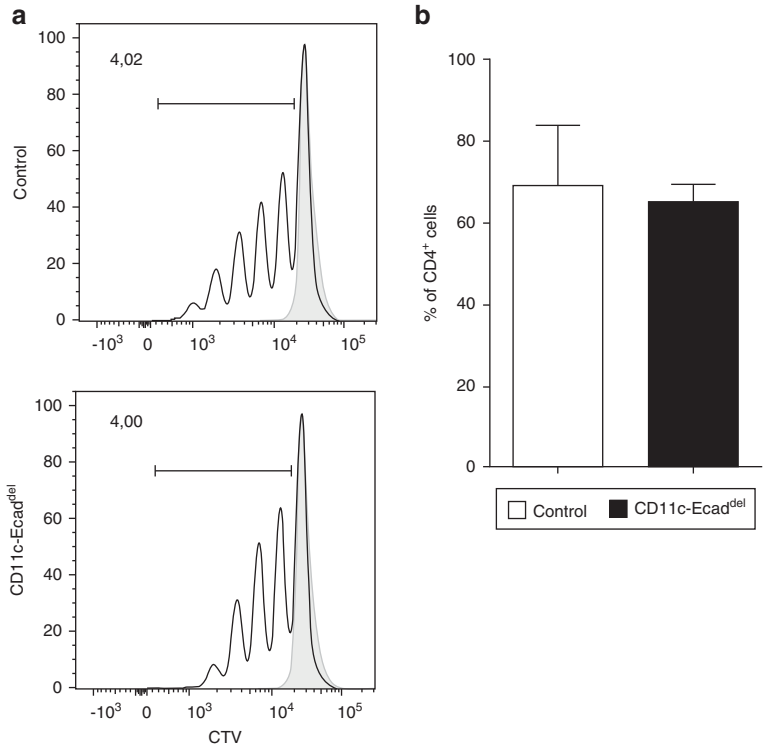
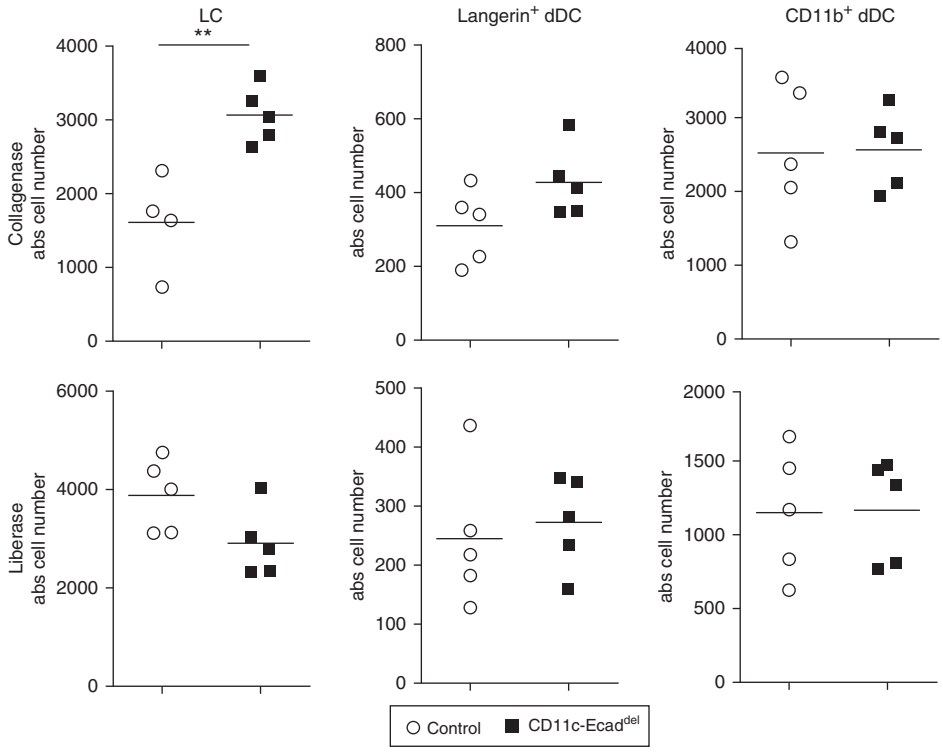
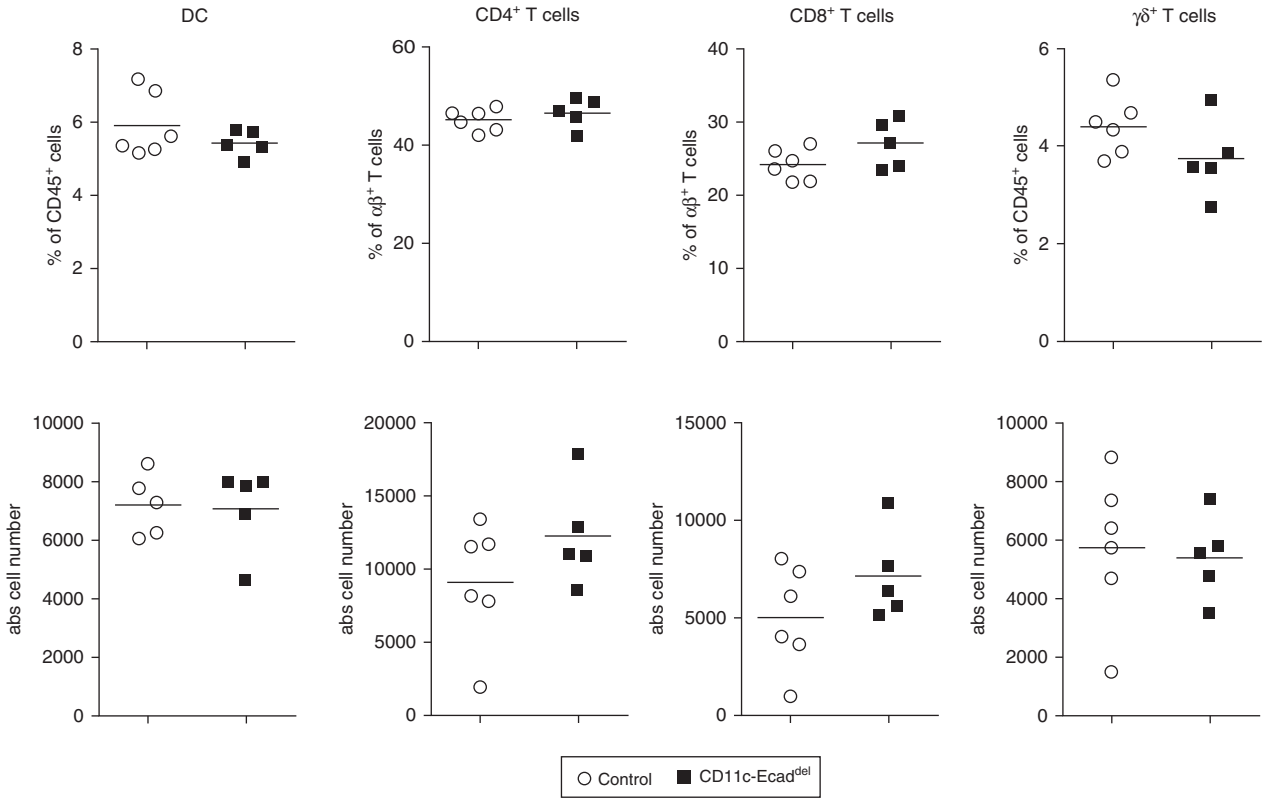


Supplementary Figure S1. E-cadherin expression on Bone marrow dendritic cells does not influence their T cell priming capacity. Bone marrow dendritic cells cultured with CTV-labeled $CD4^+$ T cells at a ratio of 1:10 purified from OT-II mice. T cell proliferation was evaluated by CTV dilution after four days. Values are the mean ± standard error of the mean, and the data are representative of two independent experiments ($n = 3$ mice). CTV, Cell Tracer Violet.



Supplementary Figure S2. The type of enzymatic digestion greatly influences the frequency and absolute number of LC and dDC subsets recovered from the skin. FACS analysis of total skin single-cell suspensions gated on live $CD45^+MHCII^+CD11c^+$ cells from CD11c-$Ecad^{del}$ and control mice. Absolute cell numbers of LC, Langerin$^+$ dDC, and $CD11b^+$ dDC in the ears from the same mouse digested either with collagenase IV (upper row) or Liberase (lower row). Values are the mean ± standard error of the mean, and the data are representative of three independent experiments ($n = 3 - 7$ mice). dDC, dermal dendritic cells; LC, Langerhans cells.



Supplementary Figure S3. CD11c-Ecad^{del} and control mice contain similar numbers of DC and lymphocyte subpopulations in the ears and ear-draining lymph nodes after contact hypersensitivity induction. FACS analysis of total skin single-cell suspensions gated on live CD45$^+$ cells from CD11c-Ecad^{del} and control mice. Percentages (upper row) and absolute cell numbers (lower row) of DC, CD4$^+$ T cells, CD8$^+$ T cells and γδ T cells. The data are representative of two independent experiments ($n = 5$ mice). DC, dendritic cells.

3.2 Monitoring skin dendritic cells in steady state and inflammation by immunofluorescence microscopy and flow cytometry

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Chapter 3

Monitoring Skin Dendritic Cells in Steady State and Inflammation by Immunofluorescence Microscopy and Flow Cytometry

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Abstract

Skin dendritic cells (DC) are strategically positioned at the body's second largest epithelial border to the environment. Hence they are the first antigen presenting cells that encounter invading pathogens and environmental antigens, including contact sensitizers and carcinogens penetrating the skin. Moreover, DC have the unique ability to induce immunity or tolerance and thus take center stage in regulating innate and adaptive immune responses. Skin DC can be divided into several phenotypically and functionally distinct subtypes. The three main subsets are Langerin^{+} epidermal Langerhans cells (LC) and Langerin^{+} as well as Langerin^{neg} dermal DC. In the steady state skin DC form a dense network to survey the periphery for pathogens and harmful substances breaching the cutaneous barrier. During inflammation DC become rapidly activated and start their migration to skin-draining lymph nodes where they initiate antigen-specific T cell responses. The homeostasis and mobilization of DC in the skin can be visualized by immunofluorescent staining of epidermal and dermal sheet preparations or skin sections. Here, we describe in detail how inflammation can be induced in the skin with tape stripping or FITC painting and how the skin DC network can be monitored using immunofluorescence microscopy and flow cytometry.

Key words Dendritic cells, Epidermal and dermal sheets, FITC painting, Immunofluorescence microscopy, Langerhans cells, Skin inflammation, Skin cryosections, Tape stripping

1 Introduction

The skin can be divided into an outer epidermis and the underlying dermis that are separated by a basement membrane. Together they form a strong mechanical barrier that protects the host from physical stress and a wide variety of environmental threats, including chemicals and pathogens. In addition, the skin harbors a heterogeneous population of dendritic cells (DC), professional antigen presenting

Note: In the printed book and online version of chapter 3, an asterisk (*) was mistakenly omitted after the author Patrizia Stoitzner's name. It has now been inserted to show that she and Björn E. Clausen have contributed equally to this chapter.

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cells that orchestrate the immunological barrier and are central regulators of innate and adaptive immune responses [1, 2]. In the steady state, DC continuously scrutinize the skin for invading pathogens and along the way sample self- and environmental antigens. Through an unknown mechanism a small fraction of the cells undergoes spontaneous maturation, facilitating their chemokine receptor CCR7-mediated migration to the T cell areas of skin-draining lymph nodes. During migration, the DC upregulate the expression of surface MHC/peptide complexes and cell adhesion molecules enabling efficient recognition and interaction with naïve antigen-specific T cells. Encounter of these *phenotypically mature* DC with T cells recognizing self- or harmless environmental antigens leads to T cell anergy, deletion or induction of regulatory T cells (Treg) (*tolerizing function*) [3, 4]. Pathogen invasion together with proinflammatory signals trigger the *functional maturation* of DC, which now also upregulate expression of costimulatory molecules and, importantly, proinflammatory cytokines. Together, these mediate the activation and proliferation of naïve antigen-specific T cells as well as their polarization towards appropriate T helper (Th) type-1, Th2 or Th17 effector cells (*sensitizing function*) [5–7].

To date, we can distinguish five phenotypically distinct subsets among the CD11c$^+$MHC-II$^+$ DC that reside in healthy mouse skin using multi-color flow cytometry [8, 9]. The only DC population present in the epidermis are Langerhans cells (LC), which are characterized by the expression of the C-type lectin Langerin (CD207), the β_2-integrin CD11b and the cell adhesion molecule EpCam. In addition to transmigrating LC, the dermis contains a small population of Langerin$^+$ DC that can be unambiguously recognized by expression of the chemokine receptor XCR1, lack CD11b and EpCam, and can be further subdivided into a CD103$^+$ and negative subset. The by far largest population of DC present in the skin are Langerin^{neg}CD11b$^+$ dermal DC and, finally, the dermis harbors a minor population of Langerin^{neg}XCR1^{neg} DC that are uniquely identified by high expression levels of the chemokine receptor CX_3CR1. During their low-level migration to skin-draining lymph nodes in the steady state, emigrating LC are replaced from a local precursor, while dermal DC are replenished from blood-borne precursors [10, 11]. To compensate for the increased loss of cutaneous DC due to their enhanced mobilization during inflammation, large numbers of monocytes infiltrate the skin where they differentiate into CD11c$^{neg\text{-}low}$CD11b$^+$CD64$^+$ monocyte-derived DC as well as LC [9, 12]. However, these cells mainly activate skin-resident T cells and disappear after resolution of the inflammation.

In light of their phenotypic diversity, major research efforts have been aimed at dissecting the unique functions of the different skin DC subsets in balancing immunity and tolerance (reviewed in [1]). While there is overwhelming evidence for a functional specialization of the various skin DC populations (*division of labor*) [13], it is becoming increasingly clear that a given DC subset exerts a particular function

that may differ depending on the context, i.e., the type of inflammation. For example, the different skin DC populations exhibit functional redundancy during contact hypersensitivity [14, 15], while LC act as negative regulators of the anti-*Leishmania* immune response [16], are essential to induce antigen-specific Th17 responses after epicutaneous *Candida albicans* infection [17], and mediate cross-tolerance towards epicutaneously applied ovalbumin [18]. On the other hand, Langerin$^+$ dermal DC promote *C. albicans*-specific Th1 and efficiently cross-present fungal antigens to activate cytotoxic T cell (CTL) responses [17]; they are also responsible for cross-priming CTL responses following deposition of the model antigen ovalbumin in the skin [18].

In all of these studies, advanced multi-color flow cytometry is essential to distinguish and analyze the small number of cells of individual DC subsets that can be purified from/are present in the skin [8, 9, 11]. However, during their life cycle, the morphology, tissue distribution, mobilization, and migration are important parameters of skin DC function, which can only be visualized by immunofluorescence microscopy. Here we describe in detail how to monitor the skin DC network in the steady state and during inflammation induced by tape stripping or FITC painting.

2 Materials

2.1 Tape Stripping of Murine Ear Skin

1. Sex- and weight-matched mice (*see* **Notes 1** and **2**).
2. Ketamine, xylazine for anesthesia.
3. Syringes.
4. 25–30 gauge needles.
5. 3 M™ Transpore™ Surgical Tape.

2.2 FITC Painting

1. Sex- and weight-matched mice (*see* **Notes 1** and **2**).
2. Ketamine, xylazine for anesthesia.
3. FITC (Fluorescein isothiocyanate isomer I).
4. Dimethyl sulfoxide (DMSO).
5. Dibutyl phthalate (DBP).
6. Acetone.
7. Syringes.
8. 25–30 gauge needles.

2.3 Epidermal and Dermal Sheet Preparation from Murine Ear Skin

2.3.1 Preparation of Epidermal and Dermal Skin Biopsies

1. Sex- and weight-matched mice (*see* **Notes 1** and **2**).
2. Carbon dioxide (CO_2) for euthanizing animals.
3. Scissors.
4. Two surgical splinter forceps.
5. 24-well plates.

6. 0.1 M phosphate buffer (8.9 g $Na_2HPO_4 \times 2\ H_2O$ plus 6.8 g KH_2PO_4 in 500 ml distilled water, pH 6.8).
7. 0.5 M ammonium thiocyanate solution (ATC) (1.9 g ATC in 50 ml 0.1 M phosphate buffer, pH 6.8).
8. Two surgical fine curved forceps.
9. Phosphate buffered saline (PBS).
10. Glass petri dishes.
11. Acetone.
12. PBS supplemented with 1% bovine serum albumin (1% BSA/PBS, dissolve 1 g BSA in 100 ml PBS).

2.3.2 Epidermal Sheet Preparation of Whole Ear on Tape

1. Sex- and weight-matched mice (*see* **Notes 1** and **2**).
2. Carbon Dioxide (CO_2) for euthanizing animals.
3. Scissors.
4. Two surgical fine curved forceps.
5. Depilation cream.
6. 15 ml Falcon tubes.
7. 12-well plates.
8. 20 mM EDTA solution (372 mg in 50 ml phosphate buffer, pH 7.2).
9. Phosphate buffered saline (PBS).
10. 5 cm petri dishes.
11. Tesa tape "crystal clear" (*see* **Note 3**).
12. Optional: Acetone.
13. Glycine solution (3% BSA/200 mM glycine/PBS, dissolve 3 g BSA and 1.5 g glycine in 100 ml PBS).
14. Optional: 0.1% Sudan black in 70% ethanol.

2.4 Immunofluorescence Staining of Murine Ear Skin Sheets

2.4.1 Immunofluorescence Staining of Epidermal and Dermal Sheets

1. Freshly prepared or thawed epidermal and dermal sheets.
2. One fine curved forceps.
3. 96-well plate.
4. Primary and secondary antibodies for immunofluorescence staining.
5. 1% BSA/PBS.
6. VECTASHIELD® Antifade Mounting Medium (Vector Laboratories).
7. Glass microscopic slides and coverslips.

2.4.2 Immunofluorescent Staining of Epidermal Sheets on Tape

1. Epidermal sheets on tape, prepared fresh or taken from the fridge.
2. Fine curved forceps (Dumont 7).
3. Phosphate buffered saline (PBS).

4. Glycine solution (3% BSA/200 mM glycine/PBS, dissolve 3 g BSA and 1.5 g glycine in 100 ml PBS).
5. Fc-block.
6. Saponin solution (10% w/v saponin in PBS).
7. Parafilm.
8. Humid chamber (an airtight, lightproof plastic box with a wet tissue at the bottom).
9. Blocking solution: 1× PBS containing 3% FCS and 0.02% Tween 20.
10. Staining solution: 1× PBS containing 1% BSA and 0.25% saponin (saponin is only necessary for intracellular staining, for example, for Langerin).
11. Primary and secondary antibodies for immunofluorescence staining.
12. ProLong® Gold Antifade Mountant with DAPI.
13. Microscope slides, superfrost and coverslips.

2.5 Preparation and Immunofluorescent Staining of Cryosections of Murine Skin

1. Sex- and weight-matched mice (*see* **Notes 1** and **2**).
2. Carbon dioxide (CO_2) for euthanizing animals.
3. Scissors.
4. One splinter forceps.
5. Freezing microtome.
6. Colorless Neg50-Frozen-Section-Medium (Thermo Fischer Scientific).
7. Poly-L-lysine coated slides.
8. Humid chamber.
9. Glass cuvette.
10. Acetone.
11. Hydrophobic slide marker (PAP-pen, Sigma-Aldrich).
12. 1% BSA/PBS.
13. Primary and secondary antibodies for immunofluorescence staining.
14. VECTASHIELD® Antifade Mounting Medium (Vector Laboratories).
15. Glass microscopic slides and coverslips.

2.6 Preparation of Migratory Skin DC from Lymph Nodes for FACS Analysis

1. Fine curved forceps (Dumont 7).
2. Fine scissors.
3. 1× phosphate buffered saline (PBS).
4. 1× PBS containing 2 mM EDTA.
5. 500 mM EDTA solution.

6. Digestion mix (RPMI 1640, containing 200 U/ml collagenase IV and 0.5 U/ml DNase I).
7. FACS buffer (PBS containing 2 mM EDTA, 3% FCS, and 0.02% thimerosal).
8. 1.5 ml Eppendorf tubes.
9. 50 ml Falcon tubes.
10. 15 ml Falcon tubes.
11. 70 μm cell strainer.
12. Fc-block.
13. Primary antibodies to stain for skin-derived DC subsets in lymph nodes.

3 Methods

3.1 Induction of Skin Inflammation by Tape Stripping

Mice are anesthetized with a mixture of 80–120 mg/kg ketamine and 5–10 mg/kg xylazine in PBS. The dorsal side of the ear is tape stripped by repeated application and removal of 3 M™ Transpore™ Surgical Tape. For each stripping a fresh piece of tape is lightly pressed onto the ear and pulled off [19]. Tape stripping causes

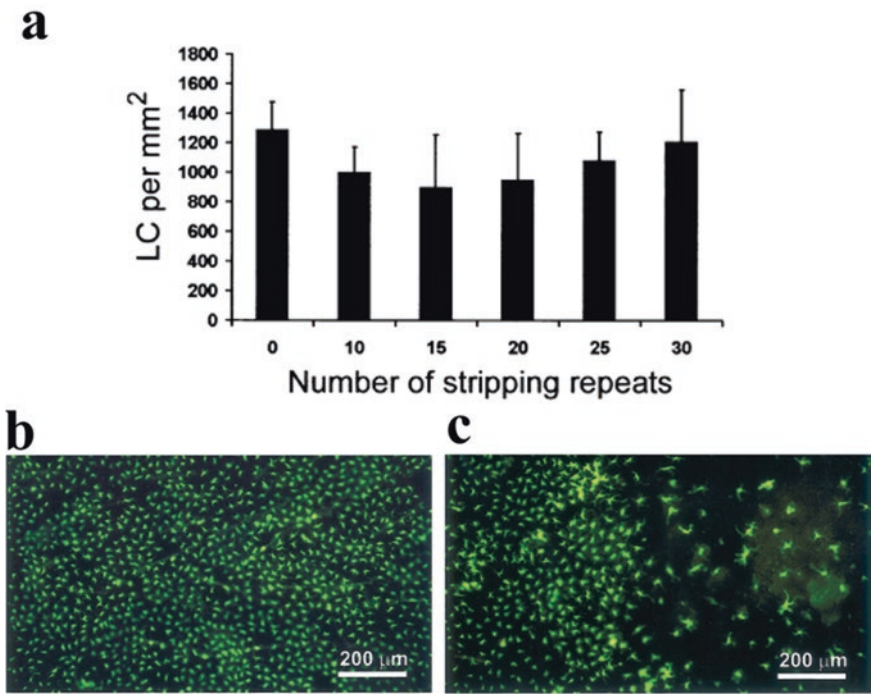

Fig. 1 Tape stripping induces emigration of LC from epidermis. (**a**) Number of stripping repeats affects the network of LC in the epidermis. (**b, c**) Epidermal sheets from steady state skin (**b**) and tape stripped skin (**c**) were stained with antibodies against MHC-class II. Tape stripping induces emigration of LC from epidermis. [Reproduced from Holzmann S, J Invest Dermatol, 2004 with permission from Nature Publishing Group.]

inflammation in the skin and emigration of immune cells like LC and dermal DC (Fig. 1). Experimental parameters e.g., kind of tape and number of stripping repeats, are crucial to the outcome of the procedure. For induction of mild inflammation we recommend 8 strippings, for strong inflammation 12 strippings (*see* **Note 4**).

3.2 Induction of Skin Inflammation by FITC Painting

5 mg FITC are dissolved in 100 ml DMSO. Acetone and DBP are mixed in a 1:1 ratio. The DMSO/FITC is diluted 10-fold in the Acetone/DBP mixture and mixed well. Mice are anesthetized with a mixture of 80–120 mg/kg ketamine and 5–10 mg/kg xylazine in PBS. Both ears (and optional the back) are shaved carefully and painted with 25 µl FITC solution each (optional: paint 100 µl FITC solution on the back, i.e., on the posterior flank, lateral to the spine). After the FITC solution has dried mice are put back into their cages. After 24, 48, 72, and/or 96 h (*see* **Note 5**) mice are sacrificed and the ears are cut off to prepare epidermal sheets as depicted in Fig. 2 (*see* Subheading 3.3 and 3.4), cryosections

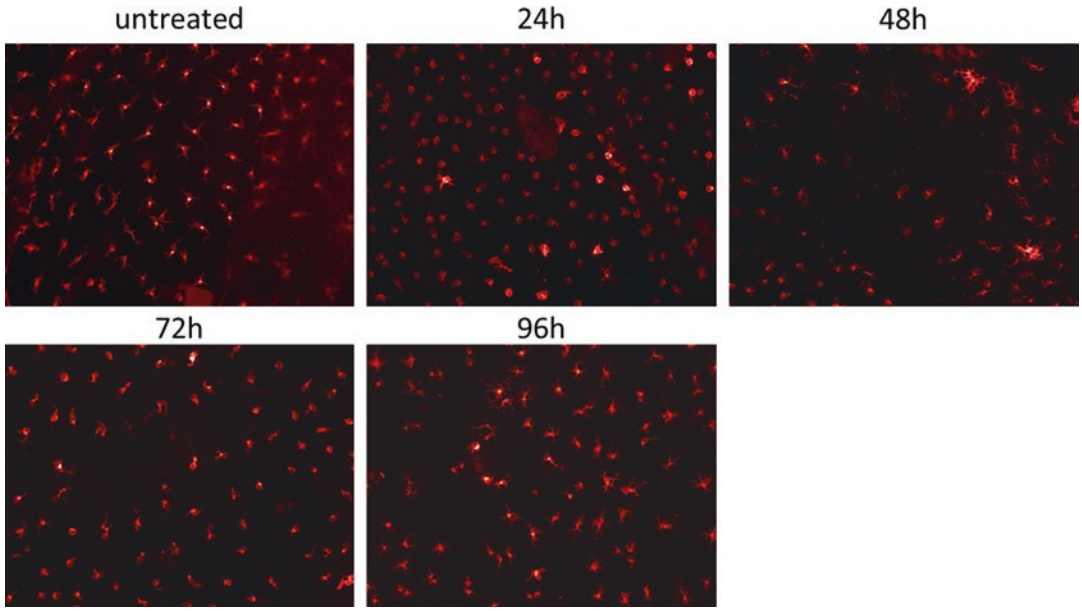

Fig. 2 LC emigration from epidermis after FITC painting. After 24 h LC (stained with MHC-class II in *red*) round up and start to leave the epidermis. 48 h after FITC painting most of the LC have left, while new LC start to repopulate the epidermis. After 72 h new LC have filled in most of the blanks and after 96 h new LC close the gaps by extending their dendrites. The LC network is re-established and the epidermis appears comparable to untreated control samples

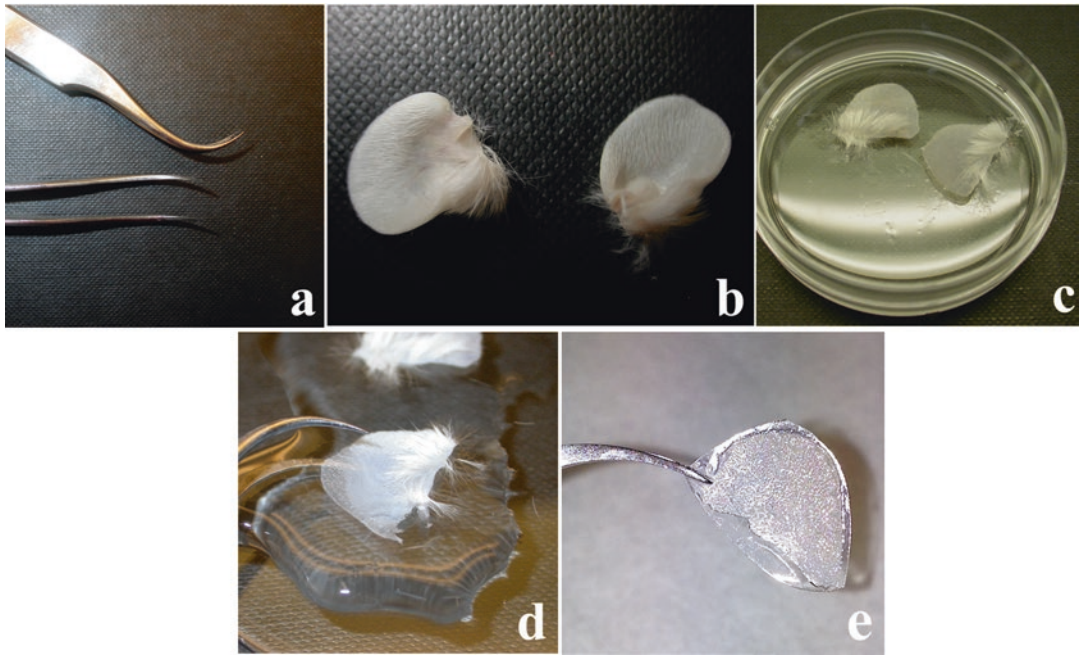

Fig. 3 Epidermal and dermal sheet preparation. Sheets are prepared with thin curved forceps (**a**) from ear skin (**b**). Dorsal ear halves are floated on ATC to split epidermis from dermis (**c**). Epidermis can be peeled off with forceps after incubation with ATC on a drop of PBS (**d**). Alternatively, Tesa tape can be applied to the epidermis before separating from the dermis to obtain whole ear specimens for immune-fluorescence staining (**e**)

(Subheading 3.5) or migratory skin DC from skin-draining lymph nodes for FACS analysis (*see* Subheading 3.6).

3.3 Monitoring of the Epidermal and Dermal DC Network—Epidermal and Dermal Sheet Preparation: Two Alternative Techniques

3.3.1 Preparation of Epidermal and Dermal Sheets

Mice are sacrificed and ears are cut off at their base. The cartilage-containing ventral ear halves can be removed from the dorsal ear halves with two surgical splinter forceps. Then the dorsal skin is floated epidermal side up on 500 µl 0.5 M ammonium thiocyanate solution for 20 min at 37 °C in a 24-well plate (one ear per well) (Fig. 3a–c). This chemically digests the basement membrane and allows the separation of epidermis and dermis [20]. Thereafter the skin is transferred onto a drop of PBS in a petri dish and the epidermis is separated from the dermis using two surgical curved forceps (Fig. 3d, *see* **Note 6**). The epidermal and dermal tissues are fixed in acetone for 20 min at room temperature in glass petri dishes. After fixation tissues are washed in a 24-well plate for 10 min twice in PBS and twice in 1% BSA/PBS to remove the acetone. Epidermal and dermal sheets can be used instantly for immunofluorescence staining or can be frozen in a drop of PBS and wrapped in aluminum foil for storage at −20 °C (*see* **Note 7**).

3.3.2 Epidermal Sheet Preparation of Whole Ear on Tape

Mice are sacrificed and the ears are cut off at their base. Depilation cream is applied onto both sides of the ears and incubated for 3 min at room temperature. Subsequently the ears are washed twice in 10 ml PBS by vortexing vigorously (*see* **Note 8**). Blot the ears on paper to dry and remove any remaining hair. The ears are split into the cartilage-containing ventral side and the dorsal side using two forceps (Fig. 3a–c). Both halves are stretched out on a reversed 5 cm petri dish with the dermal side facing downwards. A small piece (~3–4 cm) of tape is applied onto the epidermal side of each half and rubbed on (*see* **Note 9**). Next, the tape—with the dorsal or ventral half of the ear sticking to it—is carefully taken off the petri dish and is trimmed to leave only a small rim around the ear (Fig. 3c). Now the halves are floated with the dermal sides facing down on 500 µl of 20 mM EDTA solution for 1.5 h at 37 °C in a 12-well plate (one half per well) to digest the basement membrane and to allow the separation of epidermis and dermis. Thereafter, the tape—with the ear halves sticking to it—is transferred with the tape side down into a 5 cm petri dish filled with ~2 ml PBS. The dermis can now be detached from the epidermis by carefully peeling it off. The epidermis remains glued to the tape. For fixation the epidermis is floated—epidermis side down—on 500 µl of either 4% PFA or ice-cold acetone for 20 min at room temperature (PFA) or −20 °C (acetone) in a 12-well plate (one half per well). After fixation the epidermal sheets are washed twice in PBS. Afterwards 500 µl of Glycine solution is added per well and the sheets are incubated for at least 1 h (and up to 1 week) at 4 °C to reduce potential background fluorescence prior to immunofluorescence staining (*see* **Note 10**).

3.4 Monitoring of the Epidermal and Dermal DC Network—Immunofluorescent Staining of Epidermal Sheets: Two Alternative Techniques

Freshly prepared or thawed epidermal and dermal sheets are cut into smaller pieces of 5 × 5 mm and used for immunofluorescence staining. Stained sheets can be preserved on microscopic slides in VECTASHIELD® Antifade Mounting Medium for long-term storage at 4 °C for up to 1–2 years.

3.4.1 Immunofluorescence Staining of Epidermal and Dermal Sheets

One-Step Staining Procedure/Direct Staining

Sheets can be incubated with one or a mixture of primary antibodies, which are directly conjugated with different fluorescence dyes. Antibody staining solutions are prepared in 1% BSA/PBS and 100 µl of antibody solution is used per well containing one piece of an epidermal or dermal sheet. The tissue is incubated for 1 h at

37 °C or overnight at 4 °C (*see* **Note 11**). The 96-well plates are covered with a lid or Parafilm during incubation to prevent any fluid evaporation. After incubation with antibody the sheets are washed three times for 10 min in 100 µl 1% BSA/PBS in 96-well plates to remove unbound antibody. The sheets are carefully transferred from well to well with fine curved forceps. After washing steps, the sheets are embedded in VECTASHIELD® Antifade Mounting Medium on glass microscope slides and overlaid with slide coverslips.

Two- and Three-Step Staining Procedure/Indirect Staining

Besides using directly fluorochrome-coupled antibodies one has the option to use unconjugated primary antibodies, which are detected in a two or three step staining procedure. These staining techniques allow amplifying the fluorescent signal in case the detected molecule is only weakly expressed in the tissue. For the two-step staining procedure, sheets are incubated with the primary antibody followed by a fluorochrome-coupled secondary antibody directed against the host species of the primary antibody. For the three step staining a biotinylated secondary antibody against the host species of the primary antibody is used followed by a third amplification step with streptavidin, which binds to biotin, coupled to a fluorochrome (*see* **Note 12**). As described above stainings are performed in 100 µl antibody solution for 1 h at 37 °C. After each staining step the sheets are washed three times for 10 min in 100 µl 1% BSA/PBS in the 96 well plate, followed by embedding in VECTASHIELD® Antifade Mounting Medium on glass microscope slides.

Double Staining

Sometimes it is helpful to counterstain cells to simultaneously visualize two markers on the same cell, e.g., to visualize DC within the pool of $CD45^+$ leukocytes. In order to achieve this, both molecules can be stained concurrently with a mix of two fluorescently conjugated antibodies. When an indirect staining has to be performed, we advise to first stain the molecule that is indirectly labeled. It is important to block open binding sites of the secondary antibody with 10% serum or 100 µg/ml Ig from the host species of the secondary antibody for 15 min at 37 °C (*see* **Note 13**). Then the counterstaining with another antibody can be performed. Species- or isotype-specific secondary antibodies can also be used to avoid cross-reactivity (*see* **Note 12**). An example for a Langerin and MHC-class II double staining is shown in Fig. 4.

3.4.2 Immunofluorescent Staining of Epidermal Sheets on Tape

Epidermal sheets are washed once in PBS and incubated in Glycine solution containing 0.1% saponin and Fc-block for 5 min at room temperature. Meanwhile the antibody mix is prepared in 60 µl staining buffer per sheet (**Note 14**). A layer of Parafilm is placed at the bottom of a saturated humid chamber and a rectangle for each sheet is drawn on the Parafilm (leaving enough space between the rectangles). A drop of 50 µl staining solution is placed into each

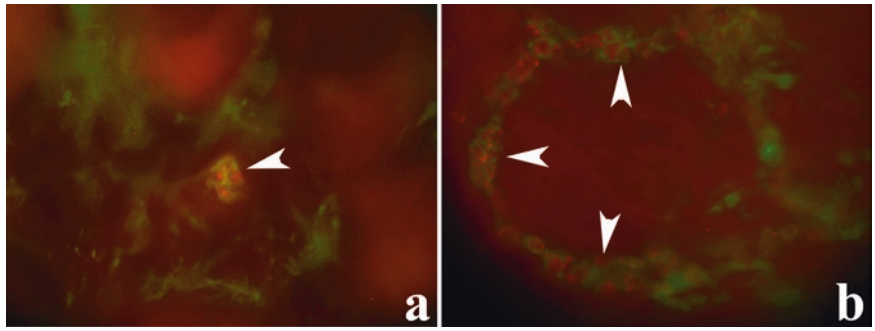

Fig. 4 Dermal sheets with lymphatic vessels containing migratory Langerin^{+} skin DC. Dermal sheets from steady state skin (**a**) and inflammation induced by tape stripping (**b**) were stained with antibodies against Langerin (*red fluorescence*) and MHC-class II (*green fluorescence*). *Arrowheads* indicate lymphatic vessels filled with migratory skin DC. Double positive cells represent LC and Langerin^{+} dermal DC, MHC-class II single positive cells represent Langerin^{neg} dermal DC and macrophages

rectangle and the sheet is placed carefully on top (tape facing upwards), avoiding air bubbles. The remaining 10 µl staining solution is used to fill up any blank area underneath the sheet if necessary. The epidermal sheets can be incubated for 1 h at room temperature or at 4 °C overnight, depending on the antibodies used (*see* **Notes 10** and **11**). After incubation the sheets are washed three times in PBS and placed on glass slides (tape facing downwards). The sheets are encircled with Pap-Pen and mounted with ProLong® Gold Antifade with DAPI. Slides can be stored like this for up to 2 years at 4 °C.

3.5 Monitoring of Skin DC—Preparation and Staining of Cryosections

Mice are sacrificed and ears are cut off at their base. Tissue is embedded in colorless Neg50-Frozen-Section Medium and 8 µm skin sections are prepared from hardened tissue (−30 °C) in a freezing microtome at −27 °C. Cryosections are placed on poly-L-lysine coated microscopic slides, where they are dried for 1 h at room temperature. Afterwards the microscopic slides with sections can be either stored long-term at −20 °C or used instantly for immunofluorescence staining.

3.5.1 Preparation of Cryosections

3.5.2 Staining of Cryosections

Frozen sections are taken out from the freezer and are placed in a humid chamber for 20 min at room temperature. The sections are fixed with acetone for 10 min at room temperature in glass cuvettes. Afterwards acetone needs to evaporate, so let the sections dry for another 10 min. Before staining draw a circle with a hydrophobic slide marker (Pap Pen) around each tissue sections to prevent spilling of antibody solution. The sections are stained by applying 100 µl of antibody solution onto the tissue, which is then incubated for 30 min at 37 °C or overnight at 4 °C (*see* **Note 11**). Antibody dilutions are prepared in 1% BSA/PBS. As described above for sheet staining, the cryosections can be stained with one or a mixture of primary antibodies in a one, two or three step

staining procedure, depending on the expression levels of the molecule to be detected. Incubations should be performed in a closed humid chamber to avoid liquid evaporation. Following antibody incubation sections are washed three times for 5 min in 1% BSA/PBS to remove unbound antibody. At the end of the staining process sections are embedded in VECTASHIELD® Antifade Mounting Medium on glass microscope slides and covered with slide coverslips. Stained sections can be stored at 4 °C for up to 1–2 years.

3.6 Analysis of Migratory Skin DC from Lymph Nodes by Flow Cytometry

3.6.1 Preparation of a Single Cell Suspension

After FITC painting cervical and, if FITC was also applied onto the back, inguinal lymph nodes are isolated and collected in 1.5 ml Eppendorf tubes. 200 µl of digestion mix are added to each tube and the lymph nodes are cut into very small pieces (clean scissors between samples to avoid cross-contamination). Another 800 µl of digestion mix are added and the samples are incubated for 45 min at 37 °C, shaking at ~1000 rpm. After incubation 20 µl 500 mM EDTA solution per 1 ml digestion mix (final concentration 10 mM) are added and the samples are incubated for additional 5 min at room temperature to separate T cell/ DC clusters. The cell suspension is passed over a 70 µm cell strainer and the strainer is rinsed with 10 ml PBS/ 2 mM EDTA. The cells are centrifuged for 5 min at 400 × *g* at 4 °C and the supernatant is discarded. The cells are resuspended in an appropriate volume of PBS/2 mM EDTA and counted. The cell concentration is adjusted to 10^6 cells per 50 µl and 10^6 cells (50 µl) are taken per staining.

3.6.2 Flow Cytometric Analysis of Migratory DC in Skin-Draining Lymph Nodes After FITC Painting

10^6 cells are used per staining and are incubated with Fc-block for 10 min at room temperature. For the detection of migratory DC the cells are stained with an antibody cocktail including fixable Life/dead marker in AmCyan (eBioscience), CD103 in PE (clone 2E7), MHC-II in PerCP-Cy5.5 (clone M5/114.15.2), CD11b in PE-Cy7 (clone M1/70), Langerin in Alexa 647 (clone 929 F3.01, Dendritics), CD11c in APC-Cy7 (clone N418), and CD24 in Pacific blue (clone M1769) in FACS buffer. The cells are incubated with the antibody cocktail for 1 h at 4 °C in the dark. Following this incubation, the cells are washed with PBS/2 mM EDTA, resuspended in 100 µl FACS buffer and acquired immediately with a flow cytometer. An example for the FACS analysis of FITC positive migratory cells is provided in Fig. 5 (*see* **Note 15**).

4 Notes

1. Inflammation and emigration of LC from the epidermis can be induced in every inbred mouse strain; nevertheless, the degree of skin inflammation might vary between strains.
2. We recommend not mixing male and female mice in experimental groups. The development of skin inflammation can

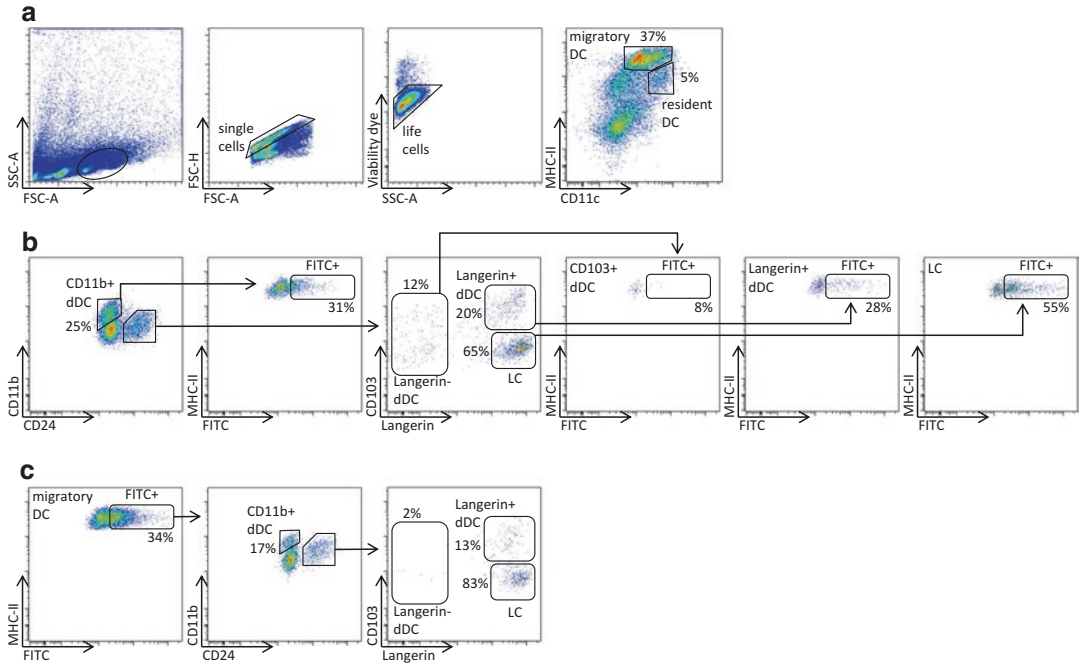

Fig. 5 Flow cytometric analysis of migratory skin DC in skin-draining lymph nodes after FITC painting. (**a**) To identify migratory DC a gate is drawn around mononuclear cells. Single cells are determined with the area and the height of the FSC. Dead cells are excluded by life/dead staining. Migratory DC are defined as MHC-II^{high}CD11c^{int} cells and can be analyzed in two different ways: (**b**) By gating on DC subsets like CD11b^{+} dermal DC, LC and Langerin^{+} dermal DC first and then analyzing frequencies of FITC^{+} cells or (**c**) by gating on FITC^{+} cells within the migratory DC subset first and then dividing them into the respective skin DC subsets. While CD24 mirrors Langerin expression in the epidermis, this correlation is less pronounced in skin-draining lymph nodes [8]. The indicated percentages represent the frequencies of DC subsets of the respective parental gate

vary between the sexes due to differences in skin thickness and weight.

3. According to our experience Tesa tape crystal clear is the only tape that does not tarnish after contact with acetone or alcohol. Furthermore it does not impair fluorescence microscopy since it stays completely transparent.
4. In early experiments using tape stripping LC were physically extracted with help of the tape [21, 22]. Milder tape stripping induces inflammation followed by emigration of LC from the epidermis [19]. Tape stripping triggers the upregulation of mRNA for pro-inflammatory cytokines like IL-1α, IL-1β, and TNF-α in the skin [23], cytokines that are important for the migration of LC [24].
5. First signs of Langerhans cell emigration can be observed after 24 h, when the cells change their morphology to leave the epidermis (Fig. 2).
6. Epidermis should be easily removable from the dermis in one piece. If this is not the case the digestion might be incomplete,

then incubate the skin for another 5–10 min. Make sure that all epidermal pieces are peeled off from the dermis.

7. Epidermal and dermal sheets can be stored long-term at −20 °C without any loss of quality. Freshly prepared and frozen sheets show comparable staining results.
8. If the ears are still highly inflamed depilation can damage the delicate epidermis, in this case the depilation step should be skipped.
9. In case of strong tissue inflammation the skin will be oozing fluid, which prevents it from sticking to the tape properly. In this case the ears can be pre-fixed in ice-cold acetone for 20 min prior to splitting them into halves to dehydrate the tissue.
10. If high background fluorescence is an issue (e.g., after topical FITC painting), the sheets can be exposed to light for a minimum of 4 h prior to immunofluorescence staining. Alternatively, epidermal sheets can be incubated for 20 min in 0.1% Sudan black in 70% ethanol after immunofluorescence staining. This will result in a blackening of the tape, without interfering with the antibody staining.
11. The decision how long primary antibodies are incubated with the sheets depends on the expression levels of the molecules to be detected. We recommend shorter incubation of 1 h at 37 °C for strongly expressed molecules and longer incubation overnight at 4 °C for weakly expressed molecules.
12. Indirect staining in a two or three step procedure can result in unspecific background staining especially in dermal sheets. In order to quench this effect as well as antibody cross-reactivity between species, blocking with normal serum (5% v/v) from the host species of the secondary antibody can be performed before the staining procedure is started. In addition, secondary antibodies should be carefully titrated.
13. Alternatively, the secondary antibody can be replaced by a F(ab) fragment; a monovalent antibody molecule still binding to its antigen but lacking the Fc portion. Using a F(ab) fragment as secondary antibody can improve the staining results, because (1) it eliminates nonspecific binding between Fc portions of antibodies and Fc receptors on cells and (2) F(ab) fragments do not interfere with specific anti-Fc mediated secondary antibody detection.
14. In case of a weak staining result due to low expression of the target molecule, the signal intensity can be increased by replacing the staining solution with a so-called staining enhancer, such as the Thermo Scientific Pierce Immunostain Enhancer.
15. This basic protocol can be used to generally analyze migratory DC after the induction of skin inflammation, e.g., tape stripping, without the possibility to identify $FITC^+$ recent skin immigrants.

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3.3 Gradual development of psoriatic skin lesions by constitutive low-level expression of IL-17A

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Research paper

Gradual development of psoriatic skin lesions by constitutive low-level expression of IL-17A

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ABSTRACT

Psoriasis is a common chronic inflammatory skin disease restricted to humans. The understanding of its pathogenesis has long been hampered by the lack of suitable chronic mouse models. The cytokine IL-17A has emerged as a key player in epithelial immune responses and the defense against extracellular pathogens. Moreover, enhanced expression of IL-17A can turn pathologic and is closely associated with psoriasis. In this study, we generated a novel transgenic mouse model that recapitulates many characteristics of psoriasis. DC-IL-17A^{ind} mice with constitutive low-level expression of IL-17A by CD11c^{+} cells gradually develop skin lesions during adult life. The lesions preferentially occur at sites of mechanical stress and exhibit macroscopic, histologic and genetic hallmarks of psoriatic plaques. Intriguingly, the age of disease onset depends on the levels of IL-17A and disruption of the epidermal barrier by tape-stripping triggers psoriatic plaque formation in the DC-IL-17A^{ind} model. In summary, our results suggest that deregulated IL-17A together with epidermal trauma initiates skin inflammation and lesion formation in mice closely resembling plaque-type psoriasis. Due to the gradual development and chronic nature of disease, DC-IL-17A^{ind} mice provide a unique tool to dissect the pathogenesis of human psoriasis and potentially could serve as a model to validate novel therapeutic strategies.

1. Introduction

Psoriasis is a chronic inflammatory skin disease clinically manifested by demarcated, erythematous, scaly skin lesions [1]. Typical psoriatic plaques are characterized by epidermal changes due to keratinocyte (KC) hyper-proliferation and disturbed differentiation. These pathogenic alterations are the result of environmental and genetic factors and driven by a complex interplay of innate and adaptive immune cells with the cutaneous epithelial cells. Among a range of pro-inflammatory mediators a key role has been attributed to IL-17A, which can be produced by innate cells (γδ T cells, innate lymphocytes, mast cells and neutrophils) as well as adaptive T-helper (Th) 17 cells [2–6]. IL-17R signaling in KC triggers innate immune defense pathways and stimulates the production of antimicrobial peptides (AMP), pro-inflammatory cytokines and chemokines [3–6]. These soluble mediators in turn activate resident immune cells and lead to the activation and recruitment of effector cells such as neutrophils, monocytes, plasmacytoid dendritic cells (DC) and T cells into the skin. Thus, psoriatic plaque formation represents a chronic inflammatory loop in the skin shaped by an aberrant crosstalk between immune cells and KC [1]. Nonetheless, the etiology of the disease remains unknown.

The latter is largely due to the lack of suitable animal models. Many transgenic mouse models of psoriasis have been developed that overexpress a specific gene, for example, a transcription factor or cytokine. While they facilitated defining the functions of particular factors or cell types contributing to the pathophysiology, many of these models mimic only selected aspects of psoriasis [7]. Recently, the development of the imiquimod mouse model represented a major breakthrough in dissecting the molecular and cellular players promoting the development of psoriatic skin lesions [8]. This model of acute skin inflammation closely recapitulates psoriatic plaque formation with its IL-23/IL-17 cytokine axis

Abbreviations: AMP, antimicrobial peptides; BM, bone-marrow; DC, dendritic cells; KC, keratinocytes; LC, Langerhans cells; LDC, Langerin^{+} DC; Tg, transgenic; TLR, toll-like receptor.

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0008-8749/

driven by DC-mediated activation of innate lymphocytes [9–12]. However, a major drawback of the imiquimod model is its lack of chronicity [13].

Therefore, based on the robust association of psoriasis and IL-17A, we sought to generate a chronic psoriasis mouse model by constitutive low-level expression of this cytokine. To this aim, we targeted IL-17A expression to $CD11c^+$ cells that are present at low frequency in healthy skin. In this study, we analyzed this novel DC-IL-$17A^{ind}$ mouse model that is characterized by the gradual development of psoriatic skin lesions in adult life.

2. Material and methods

2.1. Mice

IL-$17A^{ind}$ mice [14,15] were crossed to CD11c-Cre [16] to obtain DC-IL-$17A^{ind/+}$ mice (TG). In selected experiments, homozygous DC-IL-$17^{ind/ind}$ animals (TG/TG) were used to double the amount of constitutive DC IL-17A expression. Mice were kept in IVC cages under SPF conditions and all animal experimentation was conducted in accordance with relevant laws and institutional guidelines.

2.2. Cell preparation

Spleens, lymph nodes and ears were mechanically disrupted and digested with 400 U/mL Collagenase IV (Worthington) and for the ears additionally with 100 U/mL hyaluronidase (Sigma) and 0.1% RNase-free DNase (Promega) in HBSS for 30–60 min at 37 °C. Subsequently, EDTA (final concentration of 2 mM) was added for 5 min. The preparation was filtered through 70-μm cell strainers (BD Falcon) to obtain single-cell suspensions for flow cytometry. The erythrocytes of the spleen were lysed for 10 min with trizma-base-ammonium-chloride (TBAC) solution (155 mM NH_4Cl (Merck), 10 mM $KHCO_3$ (Sigma) and 0.1 mM EDTA).

2.3. Flow cytometry

Before surface staining, cell suspensions were pre-incubated in PBS containing fixable-dead-cell stain (Invitrogen) for at least 15 min. In addition, cells were pre-incubated in FACS buffer containing Fc-Block (Biolegend) for 15 min and then labeled with appropriate cell surface antibodies at 4 °C for 45 min. Subsequently, samples were measured directly on a FACS Canto II or LSRII Fortessa (BD Biosciences) and analyzed using FlowJo software (Treestar). Blood was collected in EDTA cups at the indicated time points by bleeding tail vein or performing heart puncture after sacrifice. Whole blood cells were pre-incubated with Fc-block (Biolegend) and then labeled with anti-mouse monoclonal antibodies at 4 °C for 20 min. Before acquisition, erythrocytes were lysed with BD FACS lysing solution (BD Biosciences). Single cell suspensions were stained with the following antibodies: CD11c (N418), MHC-II (M5/114.15.2), CD45 (30-F11), Ly-6G (1A8), Ly-6C (HK1.4), CD11b (M1/70), CD64 (X54-5/7.1), F4/80 (BM8), CD3 (145-2C11), CD4 (GK1.5) from Biolegend; anti-TCR Vγδ (Uc7-13D5) from BD Biosciences.

2.4. Cytokine detection

Blood was collected at the indicated time points by bleeding tail vein or heart puncture after sacrifice and collected in serum clot activator tubes and serum isolated (5 min at 13,500 rpm). Serum or supernatant levels of IL-17A, IL-6, CXCL1 and CCL2 were determined by Cytometric Bead Assay (CBA) (eBiosciences). The samples were analyzed using a FACS Canto II (BD Biosciences) and FlowCytomix Pro-2.4 software (eBiosciences).

2.5. Quantitative RT-PCR

mRNA was extracted from whole skin material by using the GenElute mammalian total RNA miniprep kit (Sigma–Aldrich). cDNA was synthesized from mRNA with SuperScript II reverse transcriptase (Invitrogen) according to the manufacturer's protocol. TaqMan real-time quantitative PCR assays were designed to determine transcript levels of Keratin-16, S100A7, IL-17A, Defb3, IL-6, CXCL1, CXCL2, iNOS2 and GAPDH. Expression levels were measured using a 7900HT Fast Real Time PCR machine (Applied Biosystems) and normalized to GAPDH. Sequences of PCR primers, and reference numbers of probes (Universal Probe Library, Roche Applied Science), were as follows: IL-17A forward primer 5′-TTT TCA GCA AGG AAT GTG GA, reverse primer 5′-TTC ATT GTG GAG GGC AGA C, probe No. 34, S100A7 forward primer 5′-GCC TCG CTT CAT GGA CAC, reverse primer 5′-CGG AAC AGC TCT GTG ATG TAG T, probe No. 27, Defb3 forward primer 5′-GCC TCG CTT CAT GGA CAC, reverse primer 5′-CGG AAC AGC TCT GTG ATG TAG T, probe No. 2, K16 forward primer 5′-AGC AGG AGA TCG CCA CCT A, reverse primer 5′-AGT GCT GTG AGG AGG AGT GG probe No. 42, IL-6 forward primer 5′-TCT AAT TCA TAT CTT CAA CCA AGA GG, reverse primer 5′-TGG TCC TTA GCC ACT CCT TC, probe No. 78, NOS2 forward primer 5′-GGG CTG TCA CGG AGA TCA, reverse primer 5′-CCA TGA TGG TCA CAT TCT GC, probe No. 76, CXCL1 forward primer 5′-AGA CTC CAG CCA CAC TCC AA, reverse primer 5′-TGA CAG CGC AGC TCA TTG, probe No. 83, CXCL2 forward primer 5′-AAA ATC ATC CAA AAGA TAC TGA ACA A, reverse primer 5′-CTT TGG TTC TTC CGT TGA GG, probe No. 26, GAPDH forward primer 5′-AGC TTG TCA TCA ACG GGA AG, reverse primer 5′-TTT GAT GTT AGT GGG GTC TCG, probe No. 9.

2.6. Histology

Back and ear skin was immersed in TissueTek (Bayer), snap-frozen in liquid nitrogen, and stored at −80 °C until use. Six-micrometer cryosections were stained with H&E or by immunohistological and fluorescent staining according to standard procedures. Sections were scanned with the NanoZoomer 2.0-HT virtual microscope (Hamamatsu) and processed with NDP software. Fluorescent images were taken with a Zeiss LSM 700 microscope. The following antibodies were used: primary antibodies against Gr-1 (RB6-8C5), MHC-II (M5/114.15.2, kindly provided by Pieter Leenen), K14 and K10 (polyclonal, both from Covance). Immunostainings of Gr-1 and MHC-II were followed by incubation with biotin-conjugated secondary rabbit anti-rat antibodies. Visualization of the stainings was accomplished by avidin-poly horseradish peroxidase (Dako) and 3-amino-9-ethylcarbazole (Sigma–Aldrich) as the chromogen, resulting in a bright red staining. Immunofluorescent stainings for K10 and K14 were revealed with secondary antibodies coupled to Alexa 594 (Molecular Probes) and sections were counterstained with DAPI (Sigma) for visualization of nuclei.

2.7. Tape-stripping

The right ear and shaved back skin were tape-stripped 15 times with adhesive tape (Scotch Magic 810). For each stripping, a fresh piece of tape was lightly pressed onto the ear and pulled off. After 96 h mice were euthanized and skin was examined by histology or flow cytometry.

2.8. Generation of BM-DC

DC were generated from murine BM isolated from femur/tibia of the indicated mice. Erythrocytes were lysed with TBAC solution for 7 min and cells were filtered through 70-μm cell strainers (BD Falcon) to obtain single-cell suspensions. Cells were cultured at a density of 1.5×10^6/mL for 8–10 days (37 °C, 10% CO_2) in RPMI 1640 supplemented with 5% FCS Gold (PAA), 0.5% penicillin/streptomycin (Invitrogen), 1% Ultraglutamine (Lonza), 50 mM β-ME (Sigma–Aldrich) and 5% X63 supernatant (containing GM-CSF).

2.9. Statistical analysis

Results were analyzed using GraphPad Prism software. Multiple independent variables were analyzed with 1-way ANOVA; and with student's *t* test or Mann–Whitney for 2 independent samples. A *p*-value of <0.05 was considered statistically significant. $P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results

3.1. Constitutive low-level expression of IL-17A in DC-IL-17A$^{ind/+}$ mice results in spontaneous development of skin lesions

IL-17A has been associated with autoimmune and autoinflammatory diseases such as psoriasis and arthritis [17,18]. To address the effects of low levels of IL-17A on immune homeostasis and, in particular, its ability to trigger skin inflammation *in vivo*, we targeted IL-17A expression to CD11c$^+$ DC, a cell type present in low numbers throughout the body [19]. This was accomplished by crossing CD11c-Cre mice [16] to an inducible IL-17A allele [14], which allows conditional constitutive expression of IL-17A. Moreover, in this novel DC-IL-17A^{ind} mouse model cells expressing IL-17A can be tracked by co-expression of the fluorescent reporter EGFP [14]. First, we determined IL-17A and EGFP expression by bone marrow-derived DC (BM-DC), *in vitro*-generated from DC-IL-17A$^{ind/+}$ transgenic (TG) or Cre-negative IL-17A$^{ind/+}$ non-transgenic (nTG) animals. Analysis of day 8 culture supernatants by cytometric bead array (CBA) revealed that DC-IL-17A$^{ind/+}$ BM-DC secreted IL-17A in the absence of exogenous stimuli (Supplementary Fig. 1A). Other pro-inflammatory mediators, namely IL-6, TNFα and CXCL1/2 were not detectable (data not shown). In accordance with efficient Cre activity, TG (46 ± 2.0%), but not nTG MHC-II^{high}CD11c^{high} BM-DC exhibited robust expression of EGFP (Supplementary Fig. 1B). Further flow cytometric analysis of *ex vivo* isolated DC demonstrated effective expression of EGFP in TG lymphoid as well as non-lymphoid tissue CD11c$^+$MHC-II$^+$ cells (Supplementary Fig. 1C–E). Since DC are also present at epithelial borders [20], we analyzed IL-17A mRNA expression in the skin by quantitative RT-PCR. As depicted in Fig. 1A, TG animals expressed significantly increased levels of IL-17A mRNA as compared to nTG controls, similar to lesional versus non-lesional skin of psoriasis patients [2]. Moreover, one third of the DC-IL-17A$^{ind/+}$ animals developed scaly skin lesions, which preferentially occurred at sites of mechanical stress (grooming), that is the ears, chin, head, neck and lower back (Fig. 1B). Small skin lesions in DC-IL-17A$^{ind/+}$ mice started to appear at the age of 18 weeks and gradually aggravated over time (Fig. 1C).

Taken together, these results establish that moderately elevated levels of IL-17A in DC-IL-17A$^{ind/+}$ mice can result in the gradual development of skin lesions in adult animals.

3.2. Lesions of DC-IL-17A$^{ind/+}$ mice display a typical psoriasiform histology and transcriptional signature

To further characterize the skin phenotype we performed histological analysis of uninvolved and lesional skin of TG mice in comparison to healthy skin of age-matched nTG littermates. H&E staining of TG lesional ear skin indicated epidermal hyperplasia and thickening of the stratum corneum (hyperkeratosis) (Fig. 2A). Lesional back skin revealed massive epidermal changes including hyperkeratosis, thickening of the epidermis (acanthosis), elongated rete ridges projecting into the dermis, loss of the granular layer, and a retention of nuclei, indicating disturbed KC differentiation (Fig. 2B). Increased cellularity in the dermis demonstrated the presence of a prominent leukocyte infiltrate (Fig. 2B). To confirm disturbed KC differentiation, we stained for Keratin-14 (K14) and K10, which are expressed respectively in basal and suprabasal KC

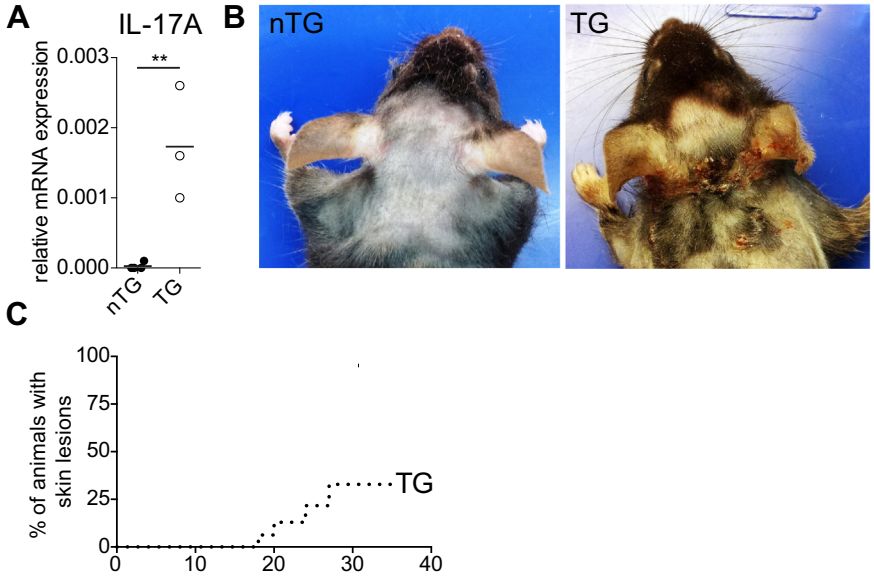

Fig. 1. Constitutive low-level expression of IL-17A in DC-IL-17A^{ind} mice results in gradual development of psoriasiform skin lesions. (A) Expression of IL-17A mRNA relative to GAPDH in back skin of IL-17A$^{ind/+}$ (nTG) and DC-IL-17A$^{ind/+}$ (TG) mice was analyzed by quantitative RT-PCR (one out of 3 representative experiments is depicted, $n \geqslant 3$ animals per group). (B) Representative images of 24 week-old shaved IL-17A$^{ind/+}$ (nTG) and DC-IL-17A$^{ind/+}$ (TG) mice, respectively, without and with skin lesions. (C) Development of skin lesions in TG animals over a period of 40 weeks ($n = 23$).

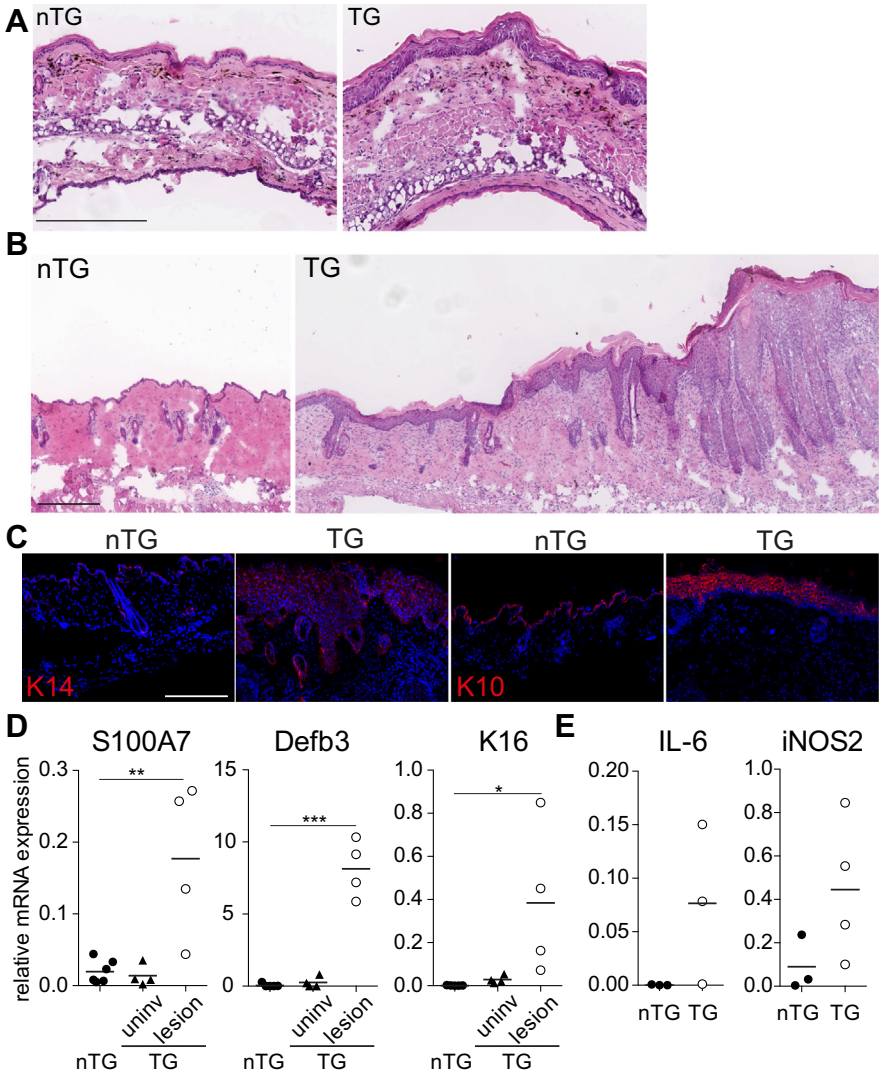

Fig. 2. Cutaneous lesions of DC-IL-17A$^{ind/+}$ mice exhibit a psoriasiform histology and transcriptional signature. Representative (A) ear and (B and C) neck skin sections of IL-17A$^{ind/+}$ (nTG) or DC-IL-17A$^{ind/+}$ (TG) mice (scale bar 250 μm; magnification ears 100× and back skin 50×). Skin sections were stained with (A and B) H/E or (C) fluorescently labeled antibodies for K10 and K14. Relative expression of (D) psoriasis-related genes S100A7, β-defensin-3 (Defb3) and Keratin-16 (K16) and (E) pro-inflammatory mediators IL-6 and iNOS2. mRNA expression was measured by quantitative RT-PCR in nTG control skin and uninvolved (only D) or lesional (D and E) skin of TG mice. One out of ⩾2 representative experiments with $n \geqslant 3$ animals per group is depicted.

in healthy skin of nTG animals (Fig. 2C). In contrast, lesional skin of TG mice displayed strong expression of K14 throughout all epidermal layers (Fig. 2C). Together with reduced expression of K10 in the epidermis of DC-IL-17A$^{ind/+}$ mice, this indicates abnormal differentiation of KC. To further evaluate the molecular nature of the epidermal hyperplasia, we tested lesional skin of DC-IL-17A$^{ind/+}$ mice for a transcriptional psoriatic signature [21]. Whole skin gene expression analysis established increased mRNA expression of S100A7, β-defensin 3 (Defb3), K16, IL-6 as well as iNOS2 in lesional TG skin, but not in uninvolved areas or skin of nTG littermates (Fig. 2D and E).

In summary, the histo-pathological features and genetic alterations of lesional skin of DC-IL-17A$^{ind/+}$ mice demonstrate a close resemblance to human psoriatic plaques.

3.3. Lesional skin of DC-IL-17A$^{ind/+}$ mice is infiltrated by neutrophils, myeloid cells and CD4$^+$ T cells

The inflammatory infiltrate in human plaque-type psoriasis is typically composed of neutrophils accumulating in epidermal Munro-abscesses, as well as myeloid cells, TCRγδ$^+$ and TCRαβ$^+$ CD4$^+$ T cells residing in the elongated dermal papillae [1]. To further demonstrate the psoriatic nature of the hyperplastic skin phenotype observed in DC-IL-17A$^{ind/+}$ mice, we dissected the cellular infiltrate in lesional TG skin. Immunohistochemical staining of skin sections revealed an accumulation of Gr-1$^+$ neutrophils and monocytes in lesional epidermis and dermis (Fig. 3A). Flow cytometric analysis of whole skin cell suspensions (for gating strategies see Supplementary Fig. 2) confirmed the influx of Ly-6G$^+$MHC-II^{neg} neutrophils (Fig. 3B) and immunohistochemistry indicated an increase of MHC-II$^+$ cells located in the dermis of the lesions (Fig. 3C). While lesional skin of DC-IL-17A$^{ind/+}$ mice contained similar numbers of MHC-II$^+$CD11c$^+$ DC (Fig. 3D), including only a minor fraction of CD64$^+$ or Ly-6C$^+$ inflammatory cells (Fig. 3E), the number of MHC-II$^+$CD11c^{neg} cells was significantly increased in inflamed TG skin (Fig. 3F). The majority of these cells expressed CD11b (Supplementary Fig. 2C) and markers defining activated inflammatory monocytes and macrophages (MHC-II$^+$, CD64$^+$, Ly-6C$^{int/high}$, F4/80$^{int/+}$) (Fig. 3G).

Under steady-state conditions, murine skin contains predominantly CD3$^+$γδ^{high} dendritic epidermal T cells (DETC) and few CD3$^+$CD4$^+$ and dermal γδ T cells, characterized by

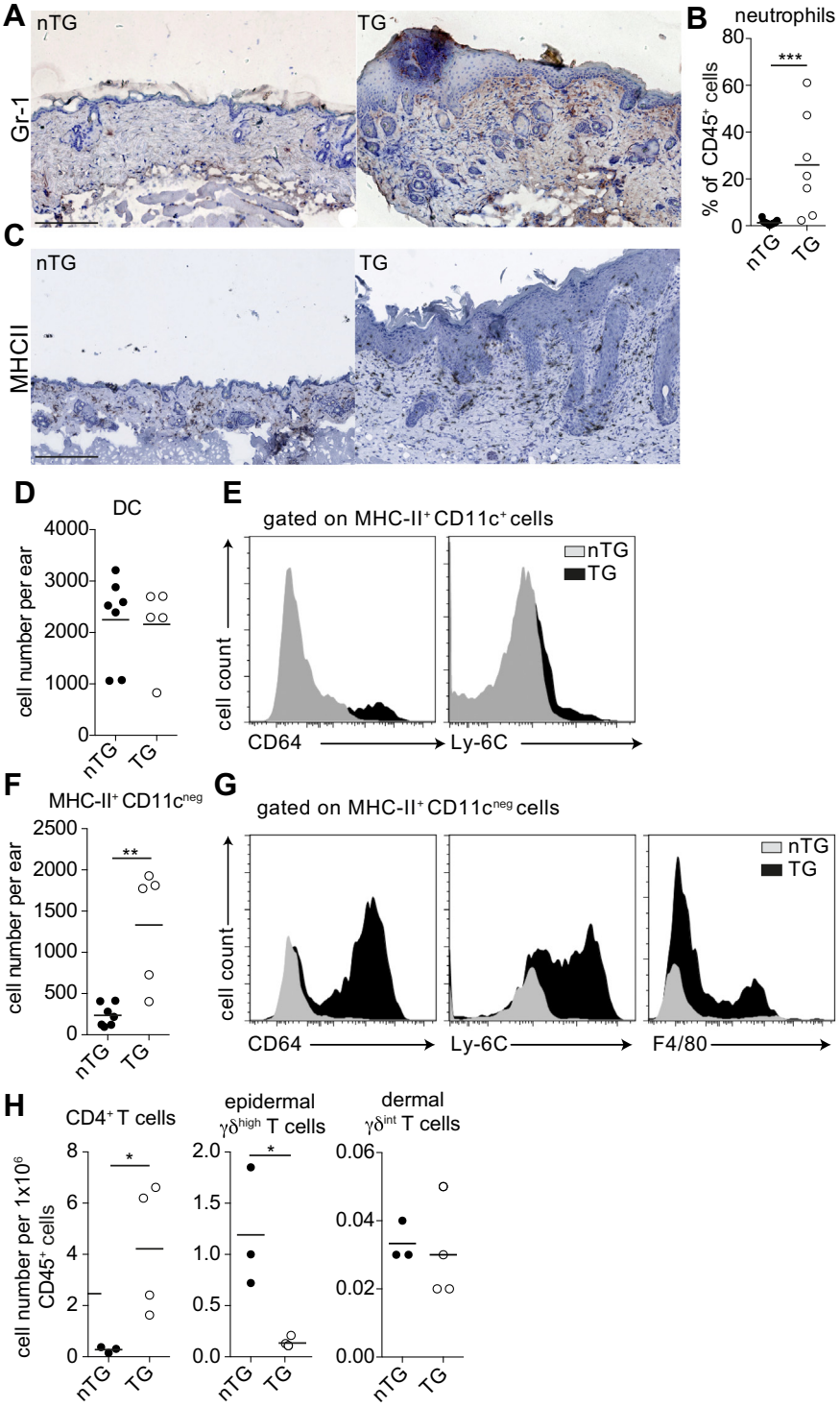

Fig. 3. Lesional skin of DC-IL-17A$^{ind/+}$ mice is infiltrated by neutrophils, myeloid cells and CD4$^+$ T cells. The inflammatory infiltrate was analyzed by (A, C) immunohistochemistry (IHC) of back skin sections and (B, D–H) flow cytometry of ear skin single-cell suspensions. (A) Gr-1$^+$ neutrophils/monocytes in nTG and TG skin (scale bar 250 μm; magnification 50×). (B) Quantification of skin-infiltrating neutrophils (CD45$^+$Ly-6G$^+$MHC-II^{neg}). (C) MHC-II$^+$ cells in nTG and lesional TG back skin. Absolute numbers of (D) MHC-II$^+$CD11c$^+$ and (F) MHC-II$^+$CD11c^{neg} cells per ear. Analysis of CD64, Ly-6C (and F4/80) expression by (E) MHC-II$^+$CD11c$^+$ and (G) MHC-II$^+$CD11c^{neg} cells. (H) γδ^{high} DETC, dermal γδ^{int} and CD4$^+$ T cells in nTG and lesional TG ear skin. Cells were pre-gated on CD45$^+$CD3$^+$ cells. Data show one out of ⩾2 representative experiments, $n \geqslant 3$ animals per group.

intermediate γδTCR expression (Fig. 3H and Supplementary Fig. 2C) [10]. Despite subtle epidermal changes in non-involved TG skin (Fig. 2B), we could not detect any inflammatory infiltrate (data not shown). In contrast, CD4$^+$ T cells were significantly increased in lesional skin of TG animals, whereas the frequency of epidermal γδ^{high} and dermal γδ^{int} T cells was, respectively, decreased and not significantly altered as compared to nTG controls (Fig. 3H).

Taken together these data illustrate that constitutive expression of IL-17A targeted to CD11c$^+$ cells leads to the development of psoriasiform skin lesions accompanied by massive infiltration of myeloid cells and to a lesser extent CD4$^+$ T cells.

3.4. IL-17A overexpression enhances the number of circulating neutrophils and their migration to inflammatory sites

In response to the chemokines CXCL1 and CXCL2, neutrophils extravasate from the bloodstream and migrate to sites of inflammation, including Munro-abscesses, which represents a hallmark of human psoriasis [22]. Since neutrophils also accumulate in lesions of DC-IL-17A$^{ind/+}$ animals (Fig. 3A and B), we tested whether their influx was due to a local increase of CXCL1/2. Gene expression quantified by RT-PCR showed that constitutive IL-17A expression lead to elevated CXCL1 but not CXCL2 mRNA in the skin (Fig. 4A). Analysis of the serum of skin lesion-bearing DC-IL-17A$^{ind/+}$ mice also revealed significantly increased systemic levels of CXCL1 (Fig. 4B), which may lead to enhanced numbers of neutrophils and monocytes in the blood. As determined by flow cytometry, the frequency of Ly-6G^{+}MHC-II^{neg} neutrophils was increased in TG animals (Fig. 4C), and we detected more CD11b^{+}SSC^{low} blood monocytes that were slightly skewed towards immature Ly-6C^{high}CD62L^{+} classical monocytes (Fig. 4D).

These results establish that elevated IL-17A levels in DC-IL-17A$^{ind/+}$ mice affect both local and systemic expression of neutrophil-recruiting/-activating chemokines that lead to increased numbers of neutrophils and monocytes in the skin and bloodstream.

3.5. IL-17A dose-dependent onset of the psoriatic skin phenotype in DC-IL-17A^{ind} mice

The relatively late onset and gradual progression of the skin phenotype in DC-IL-17$^{ind/+}$ animals suggests that psoriatic lesion development is a function of rising IL-17A levels. To address this hypothesis, we monitored IL-17A serum levels during the life of DC-IL-17$^{ind/+}$ mice. Interestingly, IL-17A serum concentrations were already elevated in 9 week-old animals, i.e. prior to the first appearance of skin lesions, and continued to increase with age (Fig. 5A). In contrast, other pro-inflammatory cytokines including IL-6 (Fig. 5B), TNFα and IL-22 (data not shown) were not elevated in the sera of 20 week-old lesion-free DC-IL-17$^{ind/+}$ mice.

To further evaluate whether development of the skin phenotype is IL-17A dose-dependent, we generated DC-IL-17$^{ind/ind}$ mice (TG/TG) harboring two copies of the inducible IL-17A allele. First, we analyzed IL-17A, IL-6, CXCL1 and CCL2 levels in the serum of age-matched littermates. As expected, the concentration of IL-17A was doubled in DC-IL-17A$^{ind/ind}$ as compared to DC-IL-17A$^{ind/+}$ mice (Fig. 5B). In addition, levels of IL-6, CCL2 but not CXCL1 were significantly increased (Fig. 5B). Examination of the penetrance and course of the skin disease revealed that 100% of the DC-IL-17A$^{ind/ind}$ mice developed skin lesions starting at 8 weeks of age, which is significantly earlier as compared to only 30% of the DC-IL-17A$^{ind/+}$ littermates with a delayed onset starting at 18 weeks (Fig. 5C). These data indicate that both the incidence and onset of the inflammatory skin phenotype correlate with the expression levels of IL-17A. Furthermore TG/TG animals with skin lesions retained a significantly reduced body weight (17.4 ± 3 g) as compared to TG (25.8 ± 2 g) and nTG (27.3 ± 3 g) animals (Fig. 5D). Side-by-side comparison of the psoriatic gene expression signature in established plaques of DC-IL-17A$^{ind/+}$ and DC-IL-17A$^{ind/ind}$ mice by quantitative RT-PCR revealed that S100A7, K16 and Defb3 transcripts were significantly increased in lesional skin of both transgenic mice, as compared to nTG control skin (Fig. 5E). In line with a comparable course of disease following its onset, the expression levels of the psoriasis-associated genes were similar in skin lesions of DC-IL-17A$^{ind/+}$ and DC-IL-17A$^{ind/ind}$ mice.

Together these results establish that the incidence and time (age) of onset of psoriasiform skin lesions in DC-IL-17A^{ind} mice are IL-17A dose-dependent. On the other hand, once lesion development is triggered, the severity of the skin disease phenotype is independent of the amount of IL-17A.

3.6. Acute skin trauma triggers chronic psoriasiform disease in DC-IL-17A^{ind} mice

Human psoriatic plaques mainly occur at anatomic locations that are exposed to mechanical stress, such as elbows and knees [1,23]. In line, psoriasiform lesions in TG mice preferentially

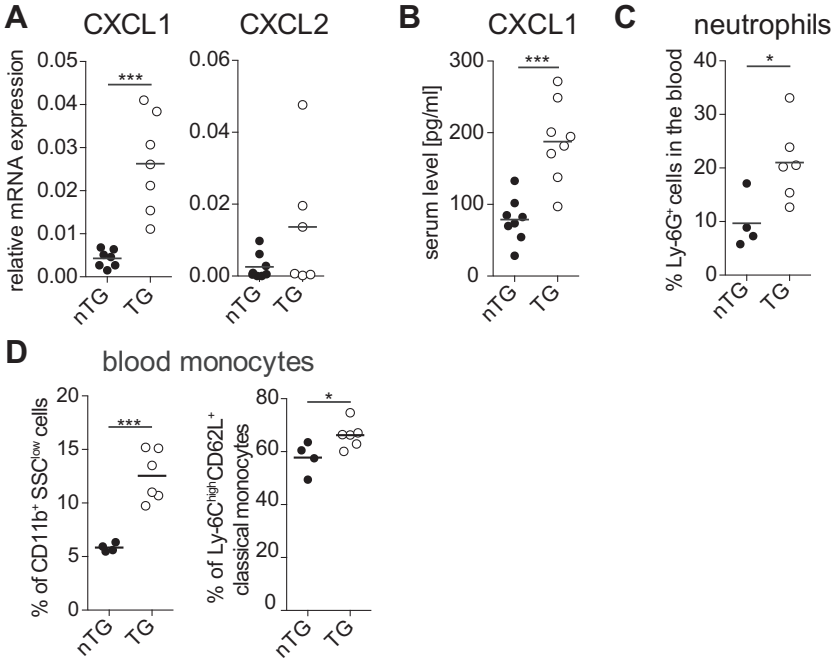

Fig. 4. Constitutive low-level IL-17A induces local as well as systemic expression of neutrophil chemoattractants and increase of blood neutrophils. (A) Relative expression of CXCL1/2 mRNA was measured by quantitative RT-PCR in nTG and lesional skin of TG mice. (B) Serum levels of CXCL1 in nTG and TG mice were determined by CBA. (C and D) FACS analysis to determine the frequency of (C) Ly-6G^{+} MHC-II^{neg} neutrophils and (D) CD11b^{+} SSC^{low} monocytes and Ly-6C^{high} CD62L^{+} immature monocytes. Data depict one out of 2 representative experiments with $n \geqslant 4$ animals per group.

developed at sites of mechanical stress due to scratching and grooming (Fig. 1A). Hence, we hypothesized that disruption of the epidermal barrier triggers lesion development in DC-IL-17A$^{ind/+}$ mice. To this aim, we used tape-stripping (TS), which is a consistent experimental approach to mimic mechanical injury [24,25]. Specifically, TS induces a transient skin inflammation and immune cell infiltration resolving after 4 days in wild-type animals [26]. The right ear and shaved back skin of DC-IL-17A$^{ind/+}$ and nTG littermates were tape-stripped 15 times and examined 96 h later. Histological analysis of tape-stripped ear (Fig. 6A) and back skin (Fig. 6B) revealed thickening of the epidermis and leukocyte infiltration only in DC-IL-17A$^{ind/+}$ mice, whereas skin of nTG littermates remained healthy. The inflammatory infiltrate in the ears of TG animals consisted of neutrophils (Ly6G^{+}MHC-II^{neg}), macrophages/monocytes (MHC-II^{+}CD11c^{neg}), DC (MHC-II^{+}CD11c^{+}) and CD4^{+} T cells (Fig. 6C).

In summary, these results indicate that acute disruption of the epidermal barrier by TS is able to trigger psoriatic skin lesions in the DC-IL-17A$^{ind/+}$ model.

4. Discussion

Given that psoriasis is only observed in humans, the lack of a suitable mouse model has hampered our understanding of its pathogenesis. In this regard different approaches to model the disease using transplantation and genetic mouse models have been undertaken to dissect the complex molecular and cellular pathways in psoriasis [7]. Humanized xenotransplantation models have proven useful to establish the pathogenic role of T cells and characterize other important mediators of plaque initiation and progression such as type-I IFN, plasmacytoid DC and IL-23 [27–30]. Although they probably most closely resemble psoriasis, their major disadvantage is that they are technically difficult and do not allow to investigate systemic effects of the disease. To dissect the signaling networks important for psoriasis initiation different knockout and transgenic mice have been generated that revealed the importance of NF-κB, TNFα, IL-1 and IL-12/IL-23 signaling pathways for psoriasis pathogenesis [31–33]. However, due to the complexity of the disease they often mimic only selective aspects of psoriasis.

More recently, repetitive skin painting of Aldara cream containing 5% of imiquimod has emerged as a powerful mouse model of psoriatic plaque formation [13]. Initially, it highlighted the general importance of the IL-23/IL-17 axis [8,34] and later on the crucial role of conventional DC and innate lymphocyte populations for the initiation of disease [9–12]. Imiquimod-induced psoriasis closely mirrors the complex cellular interactions, cytokines and inflammatory pathways driving psoriatic plaque formation, but its major drawback is the lack of chronicity, which limits, in particular, testing novel therapeutic interventions [13].

Based on the key role of IL-17, we sought to develop a more physiologic, that is chronic psoriasis mouse model by targeting constitutive IL-17A expression to CD11c^{+} DC in DC-IL-17A^{ind} mice. DC are present in low numbers – no more than 2–3% of all leukocytes – in all tissues, including the skin, which is similar to the

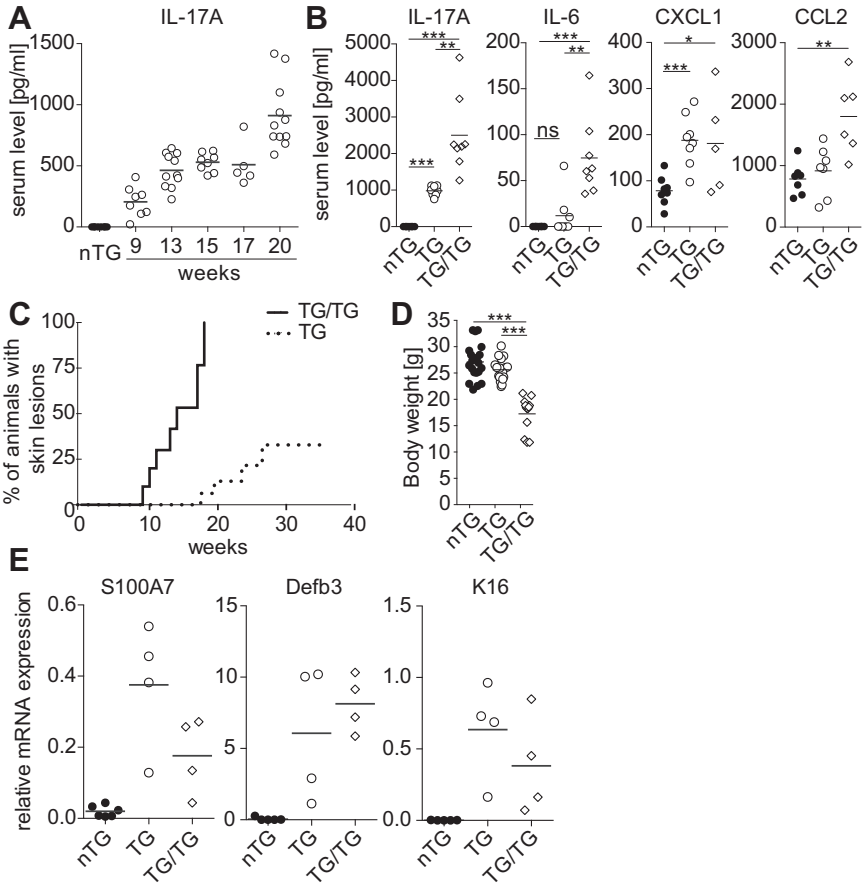

Fig. 5. Onset of the psoriasiform skin phenotype is dependent on the amount of IL-17A. (A) Serum of IL-17A$^{ind/+}$ (nTG) and DC-IL-17A$^{ind/+}$ (TG) mice was collected at different time points and IL-17A was measured by CBA. (B) Serum levels of IL-17A, IL-6 CXCL1 and CCL2 were determined in age-matched (20 week-old) nTG, TG and TG/TG (DC-IL-17A$^{ind/ind}$) mice, harboring one or two copies of the inducible IL-17A allele. (C) Percentage of TG/TG and TG animals with skin lesions over a period of 40 weeks. 100% of TG/TG animals develop skin lesions between the age of 8 and 18 weeks ($n = 18$), while 30% of TG animals exhibit lesions starting at the age of 18 weeks ($n = 23$). Body weight of 14 week-old mice ($n \geqslant 13$). (E) Relative expression of the psoriasis-related genes S100A7, β-defensin-3 (Defb3) and Keratin-16 (K16) was measured by quantitative RT-PCR in nTG and lesional skin of 24 week-old TG and 12 week-old TG/TG mice. One out of ⩾2 representative experiments with $n \geqslant 4$ animals per group is depicted.

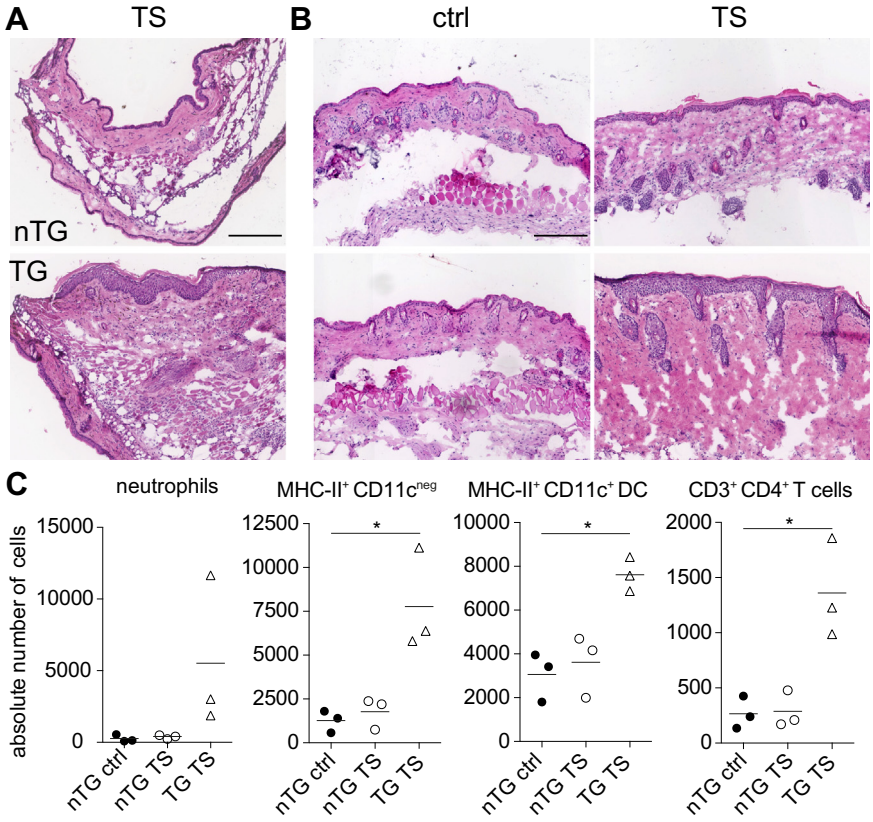

Fig. 6. Tape-stripping triggers chronic psoriasiform skin inflammation in DC-IL-17A$^{ind/+}$ mice. IL-17A$^{ind/+}$ (nTG) and phenotype-less DC-IL-17A$^{ind/+}$ (TG) mice were tape-stripped (TS) and skin histology and inflammatory infiltrate were analyzed 96 h post TS. H/E stained representative (A) ear and (B) back skin sections (scale bar 200 μm, magnification 50×). (C) FACS analysis of Ly-6G^{+} MHC-II^{neg} neutrophils, MHC-II^{+} CD11c^{neg} macrophages and monocytes and CD3^{+} CD4^{+} T cells. One out of ⩾2 representative experiments with $n \geqslant 3$ animals per group is depicted.

frequency of IL-17A producing innate lymphocytes and Th17 cells in active psoriatic plaques [2,9]. Indeed, 9 week-old DC-IL-17A^{ind} mice exhibited moderately elevated serum IL-17 levels that gradually increased with age and led to the development of skin lesions in 30% of TG animals starting at 18 weeks. This delayed and slowly progressing skin phenotype is strikingly different from the rapid and severe disease erupting in K14-IL-17A^{ind} or K5-IL-17C mice at 3 and 8 weeks of age, respectively [15,35]. Moreover, whereas mice with KC-specific overexpression of IL-17 display dry and flaky skin involving the whole body, DC-IL-17A^{ind} mice develop demarcated lesions similar to the human disease.

Lesional skin of DC-IL-17A^{ind} mice recapitulated several hallmarks of psoriatic plaques, including KC hyper-proliferation and disturbed differentiation as well as leukocyte infiltration. In addition, the expression of molecular markers defining psoriatic skin was enhanced. These include S100A7 and Defb3, which exert antimicrobial activity but also serve as neutrophil chemoattractants [1,36,37]. Moreover, we detected a significant local and systemic increase of the chemokines CXCL1 and CCL2. This in turn led to elevated frequencies of neutrophils and immature monocytes in the blood, and their infiltration into skin lesions of DC-IL-17A^{ind} mice. While abundant infiltrates can be found in both human psoriasis and atopic dermatitis that share epidermal hyperplasia as a common feature, psoriasis is characterized as a disease rich in neutrophils [1,38]. Hence, the prominent infiltration of neutrophils into lesional skin of DC-IL-17A^{ind} mice, clearly distinguishes the DC-IL-17A^{ind} phenotype from atopic dermatitis and probably reflects the critical role of the IL-23/IL-17 axis in psoriasis [38]. The concurrent accumulation of myeloid cells in lesional skin of DC-IL-17A^{ind} is likely mediated by IL-17RA signaling events, which promote granulopoiesis as well as recruitment of neutrophils and monocytes to inflammatory sites via CXC- and CCL-chemokine expression [3,39]. In accordance, stimulation of human KC with IL-17A induces the expression of neutrophil chemoattractants [3] and ubiquitous overexpression of IL-17A in mice leads to granulopoiesis, severe skin inflammation and a failure to thrive [14], similar to the severe phenotype of K14-IL-17A^{ind} animals [15].

The gradual and moderate skin disease in DC-IL-17A^{ind} as compared to K14-IL-17A^{ind} mice raised the hypothesis of an IL-17A dose-dependent onset and severity of the skin phenotype. In agreement, doubling IL-17A levels in homozygous DC-IL-17A$^{ind/ind}$ animals led to an accelerated development of skin lesions between 8 and 18 weeks of age and with 100% penetrance. Conversely, there may be a threshold of IL-17A *in situ* beyond which skin homeostasis is irrevocably disturbed and chronic inflammation starts to develop.

The close resemblance of the DC-IL-17A^{ind} mouse model to human psoriasis is further supported by the intriguing observation that the lesions occur at anatomical locations experiencing skin trauma, e.g. due to scratching and grooming. Moreover, applying controlled mechanical stress to the skin surface by TS was able to trigger psoriatic plaque formation in DC-IL-17A^{ind} mice. Similarly, psoriasis patients often harbor plaques at elbows and knees and persisting lesions can be induced on non-involved skin by local injury [1,23]. Disruption of the barrier by mechanical stress leads to the release of inflammatory mediators by epithelial and immune cells, after which homeostasis is easily restored in healthy but not psoriatic individuals. Hence, IL-17A may synergize *in vivo* with other cytokines, including TNFα and IL-1β, leading to an excessive

release of inflammatory mediators [40]. Taken together these findings indicate that KC require signaling via other pathways than exclusively IL-17A for the development of full-blown skin inflammation. They also strongly suggest that tissue damage caused by local injury is able to trigger psoriatic lesion formation in the presence of moderate amounts of constitutive IL-17A *in vivo*.

In conclusion, the skin phenotype developing in DC-IL-17A^{ind} mice closely resembles human plaque-type psoriasis in terms of histologic characteristics, composition of the cellular infiltrate and molecular signature. Due to the gradual development and chronicity of disease and, in particular, the ability to induce skin lesions by epidermal trauma, the DC-IL-17A^{ind} mouse model represents a valuable tool to unravel disease pathogenesis and may provide a unique platform to test novel therapeutics for the treatment of human psoriasis.

Disclosures

The authors state no conflicting interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cellimm.2015.11.006.

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3.4 Antagonization of IL-17A attenuates skin inflammation and vascular dysfunction in mouse models of psoriasis

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Antagonization of IL-17A Attenuates Skin Inflammation and Vascular Dysfunction in Mouse Models of Psoriasis

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Besides skin inflammation, patients with severe psoriasis suffer from an increased risk of cardiovascular mortality. IL-17A plays a central role in the development of psoriasis and might connect skin and vascular disease. The aim of this study was to clarify whether anti-IL-17A therapy could also ameliorate the vascular dysfunction associated with severe psoriasis. We analyzed three murine models with varying severities of psoriasis-like skin disease concerning their vascular function and inflammation: (i) *K14-IL-17A*$^{ind/+}$ mice with keratinocyte-specific IL-17A overexpression and an early-onset severe psoriasis-like phenotype; (ii) homozygous *CD11c-IL-17A*$^{ind/ind}$ and heterozygous *CD11c-IL-17A*$^{ind/+}$ mice overexpressing IL-17A in CD11c^{+} cells, leading to a delayed onset of moderate psoriasis-like skin disease; and (iii) the acute model of imiquimod-induced psoriasis-like skin inflammation. Similar to the severity of skin disease, vascular dysfunction correlated with peripheral IL-17A levels and neutrophil infiltration into the aortic vessel wall. Successful anti-IL-17A treatment of psoriatic skin lesions diminished peripheral oxidative stress levels, proinflammatory cytokines, and vascular inflammation. These data highlight the pivotal role of IL-17A linking the development of skin lesions and vascular disease in psoriasis. Anti-IL-17A therapy might thus represent a useful approach to attenuate and prevent vascular disease in psoriasis patients.

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INTRODUCTION

Psoriasis is the most common chronic skin disease worldwide, affecting up to 6.5% of the European population (Papp et al., 2012). The IL-23/IL-17A axis forms the major immune pathway in the pathogenesis of psoriasis (Di Cesare et al., 2009; Girolomoni et al., 2017). IL-23 secreted by conventional dendritic cells (Singh et al., 2016; Wohn et al., 2013) activates IL-17A-producing γδ T cells and T helper type 17 cells, driving the development and perpetuation of the psoriatic skin lesions (Cai et al., 2011; Pantelyushin et al., 2012). During psoriatic plaque formation, IL-17A and associated proinflammatory cytokines trigger epidermal hyperplasia, excessive keratinocyte proliferation, dermal thickening, and hypervascularity, as well as the recruitment and activation of neutrophils, monocytes, dendritic cells, and T cells into the skin (Nakajima et al., 2011; Nickoloff, 2007; Wohn et al., 2016).

A severe manifestation of psoriasis often requires immunosuppressive therapy (Nast et al., 2015). Monoclonal antibodies targeting IL-17A (secukinumab [Langley et al., 2014] and ixekizumab [Leonardi et al., 2012]) or its receptor (brodalumab [Lebwohl et al., 2015]) represent novel highly selective and efficient treatment options for patients with severe psoriasis (Kurschus and Moos, 2017; Leonardi et al., 2012; Papp et al., 2012; Waisman, 2012). Psoriasis is more than a skin disease and, in particular, patients suffering from severe psoriasis carry an increased risk for cardiovascular mortality, independent of the traditional cardiovascular risk factors such as smoking or hypercholesteremia (Gelfand et al., 2010; Mehta et al., 2010). IL-17A has already been identified as a key factor in both disease patterns: it is not only important in the pathogenesis of psoriasis (Di Cesare et al., 2009) but also contributes to the development of vascular dysfunction and hypertension (Madhur et al., 2010) and has been suggested to connect

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Abbreviations: AngII, angiotensin II; ACh, acetylcholine; IMQ, imiquimod; PASI, Psoriasis Area and Severity Index; ROS/RNS, reactive oxygen and nitrogen species

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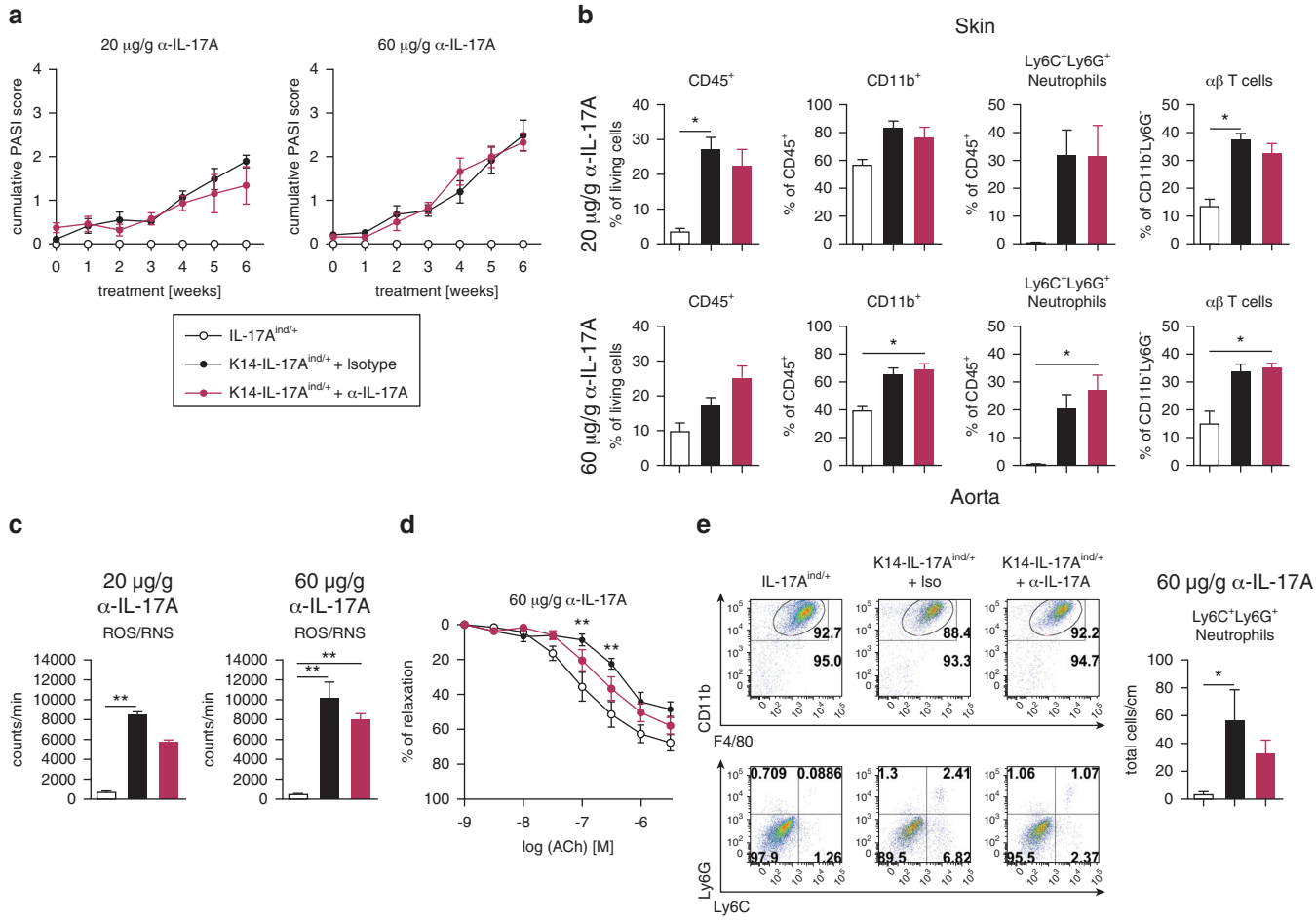

Figure 1. Psoriasis-like skin disease in K14-IL-17A$^{ind/+}$ mice is not attenuated by anti-IL-17A treatment. K14-IL-17A$^{ind/+}$ and IL-17A$^{ind/+}$ control mice were treated for 6 weeks with 20 μg/g or 60 μg/g body weight anti-IL-17A antibody (BZN035) or isotype control antibody (YB-91-QE90) starting at the age between 3 and 5 weeks. (**a**) Erythema, scaling, skin thickness, and affected area were determined for calculating the cumulative PASI score. Two-way analysis of variance with Bonferroni multiple comparisons test. (**b**) Flow cytometric analysis of ear skin in IL-17A$^{ind/+}$ and K14-IL-17A$^{ind/+}$ ± 20 μg/g or 60 μg/g anti-IL-17A antibody. To determine αβ T cells and CD11b$^+$ myeloid cells, cells were pre-gated on living CD45.2$^+$CD11b$^-$Ly6G$^-$ and CD45.2$^+$ cells, respectively. (**c**) ROS/RNS measurement in whole blood was performed after stimulation with Phorbol 12,13-dibutyrate (PDBu) for 20 minutes. Repeated measurements of pooled samples, Kruskal-Wallis with Dunn post hoc tests (no normal distribution). n = 3–13 mice per group. (**d**) Isometric tension studies of isolated aortic rings of 60 μg/g body weight treated K14-IL-17A$^{ind/+}$ mice in response to ACh. Two-way analysis of variance with Bonferroni post hoc test, n = 5–9 mice. (**e**) Flow cytometric analysis of aortas of IL-17A$^{ind/+}$ and K14-IL-17A$^{ind/+}$ mice ± 60 μg/g anti-IL-17A antibody, n = 4–10. Kruskal-Wallis with Dunn multiple comparison tests. *$P < 0.05$, **$P < 0.01$. ACh, acetylcholine; Iso, isotype control; M, mol/L; PASI, Psoriasis Area and Severity Index; ROS/RNS, reactive oxygen and nitrogen species.

psoriasis and cardiovascular comorbidity (Golden et al., 2013; Vena et al., 2010b). In the vasculature, IL-17A is an essential cytokine contributing to angiotensin II (AngII)-induced vascular dysfunction and hypertension (Madhur et al., 2010; Saleh et al., 2016). Hence, mice lacking IL-17A display reduced vascular inflammation and attenuated vascular dysfunction in response to AngII treatment (Madhur et al., 2010). Conversely, mice overexpressing IL-17A in keratinocytes (K14-IL-17A$^{ind/+}$ mice) exhibit severe psoriasiform skin inflammation and vascular dysfunction, in conjunction with infiltration of the vasculature by inflammatory myeloid cells. Nonetheless, anti-inflammatory intervention targeting IL-6 or tumor necrosis factor-α showed only limited efficacy in improving vascular dysfunction in K14-IL-17A$^{ind/+}$ mice (Karbach et al., 2014).

Here, we sought to study an alternative anti-inflammatory interventional approach by targeting IL-17A in psoriasis with a particular focus on vascular dysfunction, using three established translational models of psoriasis-like skin disease: (i) *K14-IL-17A$^{ind/+}$* mice overexpressing IL-17A in K14$^+$ keratinocytes and developing an early-onset severe psoriatic skin disease correlated with vascular inflammation and hypertension (Croxford et al., 2014; Karbach et al., 2014), (ii) homozygous *CD11c-IL-17A$^{ind/ind}$* and heterozygous *CD11c-IL-17A$^{ind/+}$* mice expressing IL-17A in CD11c$^+$ cells and displaying moderate to severe psoriasis-like skin lesions with a delayed onset (Wohn et al., 2016); and (iii) besides these chronic models mice with imiquimod-induced psoriatic plaque formation, representing an acute short-term model of psoriasis-like skin involvement (van der Fits et al., 2009).

RESULTS

Anti-IL-17A treatment fails to improve skin and vascular disease in mice with severe early-onset psoriasis-like skin disease

To determine whether anti-IL-17A therapy is effective for attenuating vascular disease in a model of severe psoriasis-

Table 1. Skin and Vascular Involvement in the Different Experimental Models of Psoriasis-like Disease Analyzed in This Study

Model of Psoriasis-like Skin Disease	Severity of Skin Involvement	Onset of Skin Phenotype (Incidence)	Skin IL-17A Levels	Systemic IL-17A Levels	Vascular Dysfunction	Anti-IL-17A Treatment Improves	
						Skin Disease	Vascular Dysfunction and Inflammation
K14-IL-17A$^{ind/+}$	Severe and chronic	3 weeks (100%)	1,814.0 ± 300.5 pg/mg protein	6,730 ± 515.9 pg/ml	Yes	No	No
CD11c-IL-17A$^{ind/ind}$	Moderate to severe and chronic	9–18 weeks (100%)	582.8 ± 67.0 pg/mg protein	1,440 ± 200.4 pg/ml	Yes (in older mice), partially (in young mice)	Yes	Yes
CD11c-IL-17A$^{ind/+}$	Moderate and chronic	18–26 weeks (30%)	385.7 ± 43.77 pg/mg protein	320.4 ± 31.4 pg/ml	By trend	ND	ND
IMQ-induced *(C57BL/6J)*	Moderate to severe, acute (short-term)	Induced in adult mice (7 weeks old) over 5–7 consecutive days (100%)	271.3 ± 52.5 pg/mg protein	3.16 ± 1.42 pg/ml	No (only signs of beginning inflammation)	Yes	Yes

Abbreviation: ND, not determined.

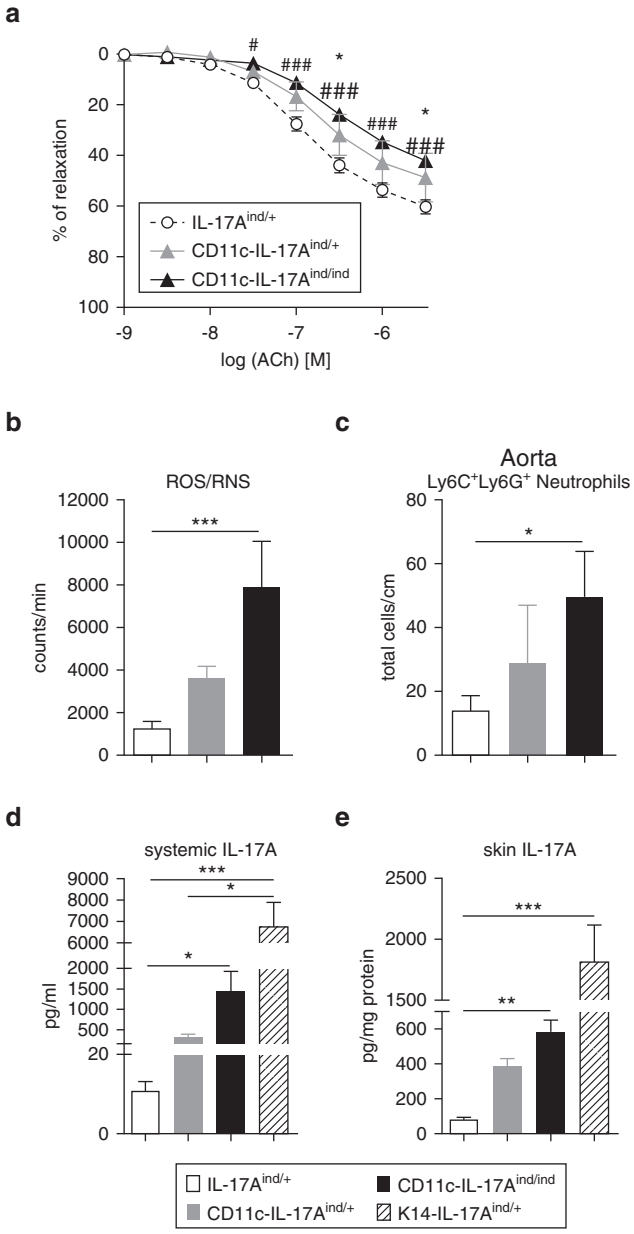

Figure 2. Constitutive expression of IL-17A in CD11c$^+$ cells leads to psoriasis-like skin lesions and vascular dysfunction in a dose-dependent manner. (**a**) Isometric tension studies of aortic rings of 10- to 19-week-old CD11c-IL-17A$^{ind/+}$, CD11c-IL-17A$^{ind/ind}$, and control mice in response to ACh. Two-way analysis of variance test and Bonferroni post hoc test, n = 3–14. (**b**) ROS/RNS measurement of whole blood (20 minutes PDBu stimulation), repeated measurements of pooled samples. Kruskal-Wallis with Dunn multiple comparisons tests, n = 5–11. (**c**) Flow-cytometric analysis of aortas. Total cell number of Ly6G$^+$Ly6C$^+$ neutrophils per 1 cm of aorta is shown. Cells pre-gated on living, CD45.2$^+$, and CD11b$^+$ cells, n = 3–9. Kruskal-Wallis with Dunn multiple comparisons tests. (**d**) Systemic (plasma) and (**e**) local (skin) IL-17A levels in 10- to 19-week-old IL-17A$^{ind/+}$, CD11c-IL-17A$^{ind/+}$, CD11c-IL-17A$^{ind/ind}$, and K14-IL-17A$^{ind/+}$ mice; n = 4–6 in **d**, n = 5–16 in **e**. Kruskal-Wallis with Dunn multiple comparisons tests. IL-17A$^{ind/+}$ control compared with CD11c-IL-17A$^{ind/+}$mice: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; IL-17A$^{ind/+}$ control compared with CD11c-IL-17A$^{ind/ind}$ mice: $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$. ACh, acetylcholine; M, mol/L; ROS/RNS, reactive oxygen and nitrogen species.

like skin disease, we treated K14-IL-17A$^{ind/+}$ mice with an IL-17A-neutralizing antibody (20 μg/g body weight) versus isotype control starting between 3 and 5 weeks of age over a period of 6 weeks. Under anti-IL-17A treatment, the severity of skin disease was not significantly lessened, as determined by cumulative Psoriasis Area and Severity Index (PASI) score (Figure 1a, and see Supplementary Figure S1a online for single PASI scores). Skin-invading CD45$^+$ leukocytes including CD11b$^+$ myeloid cells, neutrophils (CD11b$^+$Ly6C$^+$Ly6G$^+$), and T cells were not reduced by anti-IL-17A treatment (Figure 1b, and see Supplementary Figure S1c). Reactive oxygen and nitrogen species (ROS/RNS) levels in the blood were slightly but not significantly reduced in the anti-IL-17A antibody-treated mice (Figure 1c).

Therefore, in a second experimental approach, we applied the anti-IL-17A antibody at a higher concentration (60 μg/g body weight) in an attempt to more effectively antagonize the high peripheral IL-17A levels in the K14-IL-17A$^{ind/+}$ mice (Croxford et al., 2014; Karbach et al., 2014). Even this high-dose treatment was not able to attenuate skin disease in K14-IL-17A$^{ind/+}$ mice (Figure 1a, and see Supplementary Figure S1b for single PASI scores.) Accordingly, there was no reduction in skin-infiltrating inflammatory cells (total leukocytes, myeloid cells, neutrophils, or T cells) in anti-IL-17A compared to isotype-treated K14-IL-17A$^{ind/+}$ mice (Figure 1b, and see Supplementary Figure S1c). Neither did peripheral oxidative stress levels decrease upon anti-IL-17A treatment (Figure 1c). As previously described, vascular dysfunction in K14-IL-17A$^{ind/+}$ mice was correlated with vascular inflammation and infiltration of Ly6C$^+$Ly6G$^+$ neutrophils into the aortic vessel wall (Karbach et al., 2014). High-dose anti-IL-17A treatment caused a small but statistically nonsignificant improvement of endothelial dysfunction (Figure 1d). In agreement, the number of Ly6C$^+$Ly6G$^+$ neutrophils accumulating in the aortic wall was slightly but not significantly reduced in K14-IL-17A$^{ind/+}$ mice under anti-IL-17A treatment (Figure 1e). Taken together, the K14-IL-17A$^{ind/+}$ mouse strain exhibits a harsh, psoriasis-like skin phenotype together with severe vascular dysfunction, against which anti-IL-17A treatment in conventional dosages was not effective.

Mice with gradual development of moderate to severe psoriasis-like skin disease also suffer from vascular dysfunction

CD11c-IL-17A$^{ind/ind}$ and CD11c-IL-17A$^{ind/+}$ mice express IL-17A in CD11c$^+$ cells, which leads to steadily rising systemic IL-17A levels with increasing age (Wohn et al., 2016). The animals acquire a moderate to severe form of psoriasis-like skin disease with a more delayed onset than in the K14-IL-17A$^{ind/+}$ mice. The kinetics of skin lesion development is IL-17A-dose dependent. In homozygous CD11c-IL-17A$^{ind/ind}$ mice, lesions appear between 9 and 18 weeks of age with 100% incidence, whereas only about 30% of heterozygous CD11c-IL-17A$^{ind/+}$ mice acquire lesions, starting at 18 weeks age of (Wohn et al., 2016) (Table 1).

Homozygous CD11c-IL-17A$^{ind/ind}$ and heterozygous CD11c-IL-17A$^{ind/+}$ mice (10–19 weeks old) both showed vascular dysfunction typically associated with psoriasis (Figure 2a). Moreover, peripheral ROS/RNS levels were significantly elevated in the homozygous CD11c-IL-17A$^{ind/ind}$ mice compared with IL-17A$^{ind/+}$ controls and slightly but not significantly increased in heterozygous CD11c-IL-17A$^{ind/+}$ animals (Figure 2b). Neutrophil infiltration into the aortic vessel wall was significantly augmented in homozygous CD11c-IL-17A$^{ind/ind}$ and by trend also in heterozygous CD11c-IL-17A$^{ind/+}$ mice (Figure 2c). In parallel, systemic IL-17A levels were significantly amplified in CD11c-IL-17A$^{ind/ind}$ mice (1,440 ± 200.4 pg/ml) and by trend also in CD11c-IL-17A$^{ind/+}$ mice (320.4 ± 31.4 pg/ml) compared with IL-17A$^{ind/+}$ control animals (10.9 ± 1.03 pg/ml) (Figure 2d). Even in homozygous CD11c-IL-17A$^{ind/ind}$ mice, IL-17A cytokine levels were 4.7-fold lower than in K14-IL-17A$^{ind/+}$ mice (6,730 ± 1,151 pg/ml). Systemic IL-17A levels showed a parallel increase to local IL-17A levels in the skin (CD11c-IL-17A$^{ind/+}$ = 385.7 ± 43.77 pg/mg protein, CD11c-IL-17A$^{ind/ind}$ = 582.8 ± 67.0 pg/mg protein, K14-IL-17A$^{ind/+}$ = 1,814.0 ± 300.5 pg/mg protein) (Figure 2e). Taken together, these data indicate that the severity of both the psoriatic skin lesions and the associated vascular dysfunction represent an IL-17A dose-dependent effect.

Successful anti-IL-17A therapy of late-onset moderate to severe psoriasis-like skin lesions and vascular dysfunction

CD11c-IL-17A$^{ind/ind}$ mice were treated over 4 weeks starting at an age of 12 weeks (i.e., after the appearance of psoriatic skin lesions), with 60 μg/g body weight of anti-IL-17A or isotype control antibody. Antagonizing IL-17A in CD11c-IL-17A$^{ind/ind}$ mice eradicated cutaneous lesions and prevented their reappearance for the duration of the treatment (Figure 3a). Microabscess formation and epidermal thickening combined with hyperkeratosis were absent in CD11c-IL-17A$^{ind/ind}$ mice after 4 weeks of anti-IL-17A injection (Figure 3b). Invasion of neutrophils, a hallmark of psoriatic skin inflammation, and CD11b$^+$ myeloid cells into the ear skin were, respectively, brought back to baseline and attenuated in anti-IL-17A-treated CD11c-IL-17A$^{ind/ind}$ mice (Figure 3c and d). Moreover, vascular function in CD11c-IL-17A$^{ind/ind}$ mice was completely restored by the anti-IL-17A treatment (Figure 3e), and there was a decrease in oxidative stress formation in peripheral blood of anti-IL-17A- but not isotype-treated CD11c-IL-17A$^{ind/ind}$ mice (Figure 3f). Consequently, fewer neutrophils infiltrated the aortic vessel wall of CD11c-IL-17A$^{ind/ind}$ mice under anti-IL-17A treatment (Figure 3g). In peripheral blood, there was a tendency towards reduced levels of the proinflammatory cytokines IL-6, tumor necrosis factor-α (TNF-α), and IL-22, whereas IL-1β levels were not lower after anti-IL-17A antibody treatment (see Supplementary Figure S2a online).

Beyond this remarkable efficacy in a therapeutic setting, applying the anti-IL-17A antibody to younger CD11c-IL-17A$^{ind/ind}$ mice starting at the age of 8 weeks (i.e., before the onset of the skin phenotype), completely prevented skin lesion development (Figure 3h). Epidermal thickness was only slightly increased in younger CD11c-IL-17A$^{ind/ind}$ animals and was brought back to baseline under anti-IL-17A antibody treatment (see Supplementary Figure S3a). Similarly, the elevated frequencies of CD11b$^+$ myeloid cells, Ly6C$^+$Ly6G$^+$ neutrophils, Ly6C$^+$ monocytes, and F4/80$^+$ macrophages all were normalized (see Supplementary

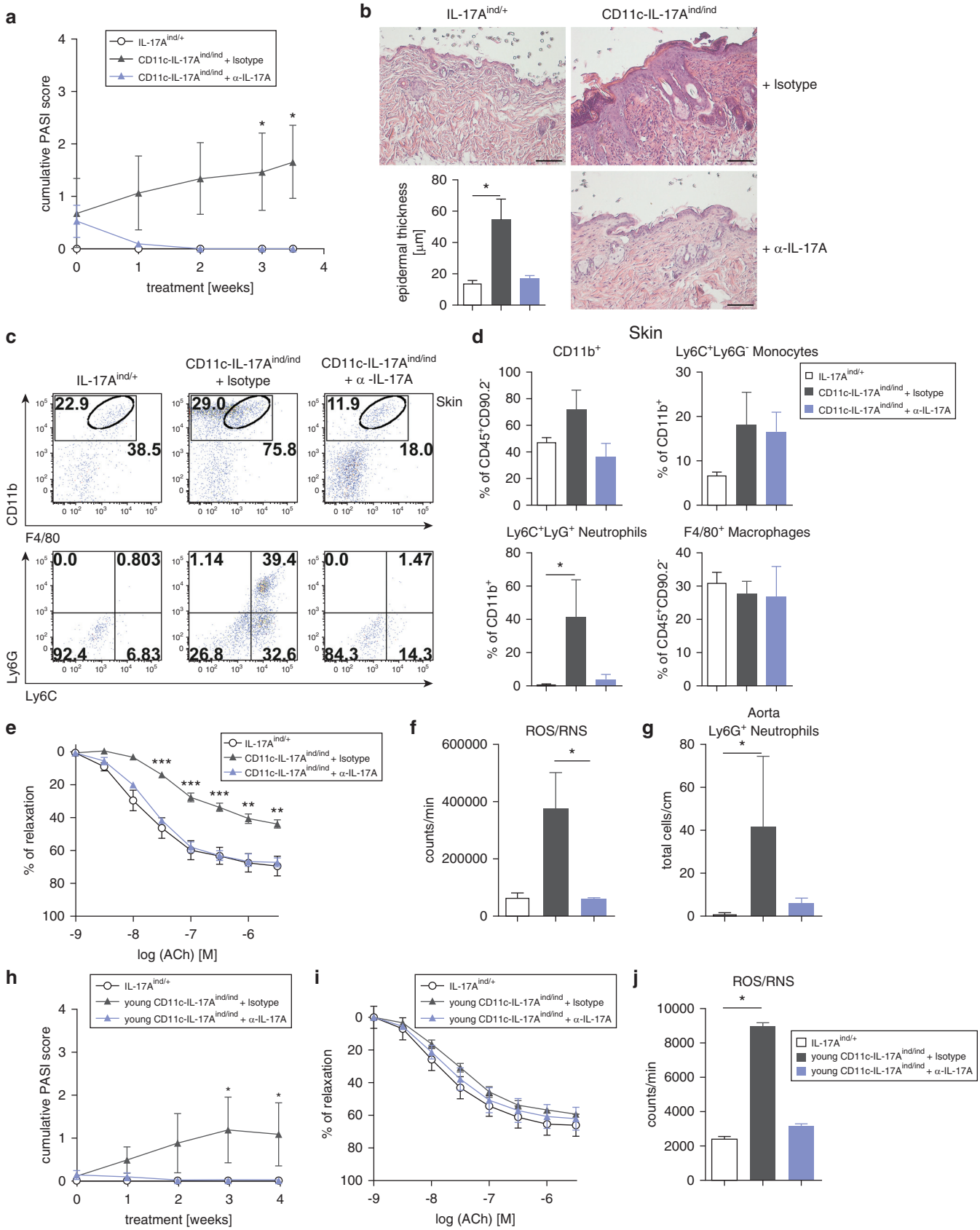

Figure 3. Anti-IL-17A treatment of CD11c-IL-17A$^{ind/ind}$ mice cures skin and vascular disease in older mice and prevents the development of disease in young animals. IL-17A$^{ind/+}$ control mice and CD11c-IL-17A$^{ind/ind}$ mice were treated for 4 weeks (60 µg/g body weight anti-IL-17A antibody [BZN035] or isotype control antibody [YB-91-QE90] starting at the age of either (**a**–**g**) 12 weeks or (**h**–**j**) 8 weeks (young CD11c-IL-17A$^{ind/ind}$ mice without skin lesions). (**a**) Weekly cumulative PASI score of treated CD11c-IL-17A$^{ind/ind}$ or control mice. Two-way analysis of variance and Bonferroni post hoc test (antibody-treated vs. isotype-

Figure S3b). At this young age, the CD11c-IL-17A$^{ind/ind}$ mice did not yet exhibit any significant vascular dysfunction (Figure 3i), although oxidative stress levels in the peripheral blood were already augmented and could be attenuated by anti-IL-17A antibody (Figure 3j). In conclusion, these data indicate that in this late-onset, moderate to severe murine psoriasis-like skin disease model, cutaneous and vascular inflammation could be inhibited by anti-IL-17A and dissolved in both a therapeutic and prophylactic treatment protocol.

Limited vascular inflammation without vascular dysfunction in murine acute, short-term, psoriasis-like skin disease

To evaluate murine psoriasis-like skin disease independent of genetic IL-17A overproduction, we selected the acute model of imiquimod (IMQ)-induced psoriasis-like skin disease triggered via toll-like receptor 7/8 activation of dendritic cells (Wohn et al., 2013), which is largely dependent on the IL-23/IL-17A axis (van der Fits et al., 2009). Topical application of IMQ over 5 or 7 consecutive days led to the occurrence of erythema, scaling, and thickening of the skin, as previously described (El Malki et al., 2013; van der Fits et al., 2009; Wohn et al., 2013) (Figure 4a and b, and see Supplementary Figure S4 online). Simultaneous injection of anti-IL-17A significantly delayed the onset and attenuated the severity of IMQ-induced skin disease, confirming the relevance of IL-17A in this model (Figure 4a and b, and see Supplementary Figure S4).

While systemic IL-17A levels in the serum were not elevated upon IMQ treatment (Figure 4c), local IL-17A in the skin was significantly increased (Figure 4d), although to a lesser extent than in the different genetic models (Table 1). Accordingly, IMQ-induced infiltration of CD45$^+$ leukocytes, including CD11b$^+$ myeloid cells, Ly6C$^+$Ly6G$^+$ neutrophils, Ly6C$^+$ monocytes, and F4/80$^+$ macrophages into the ear skin was impaired by anti-IL-17A treatment (Figure 4e). Oxidative stress levels in the peripheral blood were increased in mice with IMQ-induced skin disease and slightly but not significantly curtailed by anti-IL-17A (Figure 4f). Proinflammatory IL-6, IL-22, and IL-1β cytokine levels were elevated by trend in IMQ-treated mice and attenuated under anti-IL-17A treatment (see Supplementary Figure S2b). In contrast to the chronic models, vascular function as analyzed by acetylcholine (ACh)-triggered aortic relaxation was not impaired in short-term IMQ-treated mice (data not shown). There was a small increase in inflammatory CD45$^+$ leukocytes and CD11b$^+$ myeloid cells infiltrating the aortic vessel wall under IMQ treatment, which was partially resolved by anti-IL-17A, and—in line with the unaltered vascular function—the number of Ly6C$^+$Ly6G$^+$ neutrophils in the aorta was not affected (Figure 4g).

In summary, short-term skin treatment with IMQ raised oxidative stress levels and CD45$^+$ inflammatory cells in the aortic vessel wall without mediating vascular dysfunction. The ability of anti-IL-17A to attenuate acute psoriatic skin disease, peripheral oxidative stress levels, and beginning vascular inflammation further supports its feasibility as a prophylactic treatment option.

DISCUSSION

In this study we demonstrate that vascular inflammation and dysfunction in three established models of psoriasis-like skin disease are correlated with the severity of skin lesion manifestation and cutaneous as well as peripheral IL-17A levels (Table 1). The K14-IL-17A$^{ind/+}$ mice acquire the highest local and systemic IL-17A levels and exhibit a particularly severe psoriasis-like skin phenotype. In addition, they show highly elevated peripheral oxidative stress levels and suffer from vascular dysfunction based on aortic neutrophil infiltration. Homozygous CD11c-IL-17A$^{ind/ind}$ mice and heterozygous CD11c-IL-17A$^{ind/+}$ mice show a delayed onset of moderate to severe psoriasis-like skin disease associated with reduced amounts of cutaneous IL-17A. In agreement with elevated skin and a stepwise increase in systemic IL-17A (Wohn et al., 2016), homozygous CD11c-IL-17A$^{ind/ind}$ mice develop earlier and more severe skin lesions, as well as more pronounced vascular inflammation and dysfunction, than heterozygous CD11c-IL-17A$^{ind/+}$ mice. These findings are in line with the correlation between the severity of skin disease and systemic IL-17A levels in psoriasis patients (Arican et al., 2005). Moreover, our data establish a direct link between systemic IL-17A levels and the severity of skin disease and vascular inflammation/dysfunction. In fact, the observation that 16-week-old but not in 12-week-old CD11c-IL-17A$^{ind/ind}$ mice exhibit vascular dysfunction suggests that, similar to the formation of skin lesions (Wohn et al., 2016), a certain threshold of systemic IL-17A must be exceeded for vascular dysfunction to develop. Consistent with the central role of IL-17A in the development of vascular dysfunction and inflammation (Madhur et al., 2010; Saleh et al., 2016), our results link the severity of skin disease and the amount of cutaneous and systemic IL-17A with peripheral oxidative stress levels and vascular dysfunction. On the other hand, in the IMQ model of acute moderate to severe psoriatic skin lesion development, we detect only beginning

treated CD11c-IL-17A$^{ind/ind}$ mice). (**b**) Hematoxylin and eosin staining of skin sections of anti-IL-17A- or isotype-treated CD11c-IL-17A$^{ind/ind}$ and control mice. Epidermal thickness was determined using ImageJ software (National Institutes of Health, Bethesda, MD). n = 3–4. Scale bar = 100 μm. (**c, d**) Flow cytometric analysis of ear skin of treated CD11c-IL-17A$^{ind/ind}$ and control mice. For statistical analysis, the anti-IL-17A- and isotype-treated CD11c-IL-17A$^{ind/ind}$ mice were compared with control mice. (**e**) Relaxation curves of isolated aortic rings in response to ACh of anti-IL-17A- or isotype-treated CD11c-IL-17A$^{ind/ind}$ and control mice. Two-way analysis of variance and Bonferroni post hoc test (antibody- and isotype-treated CD11c-IL-17A$^{ind/ind}$ mice, n = 3–4). (**f**) ROS/RNS measurement in whole blood (10 minutes of PDBu stimulation), repeated measurements of pooled samples; n = 3–4. (**g**) Flow cytometric analysis of aortas of anti-IL-17A- or isotype-treated CD11c-IL-17A$^{ind/ind}$ and control mice for neutrophils; n =3–4. (**h**) Cumulative PASI score, anti-IL-17A-/isotype-treated young CD11c-IL-17A$^{ind/ind}$ and control mice. Two-way analysis of variance and Bonferroni post hoc test (antibody-treated vs. isotype-treated young CD11c-IL-17A$^{ind/ind}$ mice), n = 3–4. (**i**) Relaxation curves of aortic rings of anti-IL-17A- or isotype-treated young CD11c-IL-17A$^{ind/ind}$ and control mice in response to ACh. Two-way analysis of variance, Bonferroni post hoc test. (**j**) ROS/RNS measurement in whole blood of anti-IL-17A- or isotype-treated young CD11c-IL-17A$^{ind/ind}$ or control mice (20 minutes of stimulation with PDBu), repeated measurements of pooled samples; n = 3–4. If not indicated otherwise, statistical analysis was performed with Kruskal-Wallis and Dunn multiple comparison tests. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. ACh, acetylcholine; M, mol/L; PASI, Psoriasis Area and Severity Index; ROS/RNS, reactive oxygen and nitrogen species.

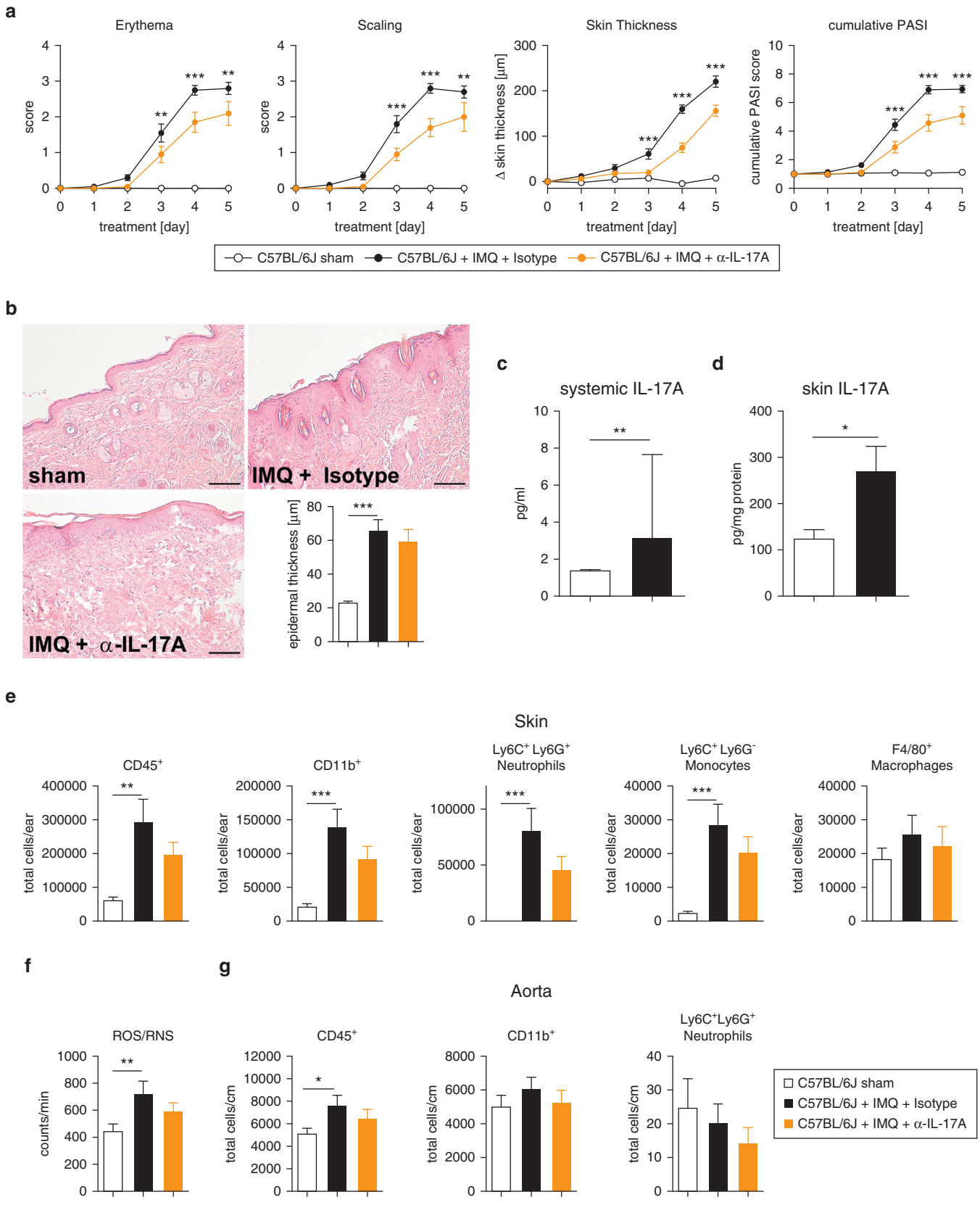

Figure 4. Anti-IL-17A treatment in IMQ-induced psoriasis-like skin disease. C57BL/6J mice were treated 5 days with Aldara (MEDA Pharma, Solna, Sweden) cream (5% IMQ) and injected with 0.6 mg anti-IL-17A (BZN035)/isotype control (YB-91-QE90) on days 0 and 3. (**a**) Single (erythema, scaling, skin thickness) and cumulative PASI scores of IMQ- and sham-treated mice and control mice; n = 13–15, two-way analysis of variance. (**b**) Hematoxylin and eosin staining of skin sections of IMQ- and sham-treated mice and control mice. One-way analysis of variance and Bonferroni post hoc test. Representative images of n = 10 mice. Scale bar = 100 µm. (**c**) Plasma and (**d**) skin IL-17A levels of IMQ-treated mice and sham-treated control mice; n = 10, Mann-Whitney *t* test in **c**; n = 5,

vascular inflammation, together with elevated peripheral oxidative stress levels. This is in accordance with the short-term nature of psoriatic skin disease, which leads to only moderately up-regulated IL-17A levels in the skin and peripheral blood (Table 1). Thus, it may be useful to study vascular inflammation/dysfunction in a prolonged IMQ-induced disease model (Terhorst et al., 2015). Aortic neutrophil infiltration in the presented genetic psoriatic skin disease models is lower than in mice with AngII-induced vascular dysfunction (Wenzel et al., 2011); nevertheless, they exhibit comparable vascular dysfunction. The aortic inflammation in the genetic IL-17A^{ind} psoriasis mouse models occurs over a longer period of time than in mice treated with AngII. It is tempting to speculate that additional effects of IL-17A other than neutrophil infiltration may contribute to impaired vascular function.

Our results are consistent with reports of human studies indicating that patients with severe psoriasis have an increased risk of cardiovascular mortality that is independent of the traditional cardiovascular risk factors (Mehta et al., 2010; Vena et al., 2010a; Vena et al., 2010b). Specifically, severe psoriasis confers an additional 6.2% absolute risk of a 10-year rate of major adverse cardiac events compared with the general population (Mehta et al., 2011a). The mechanisms of this association remain elusive, as does the possible impact that current treatment protocols of psoriasis may have on the cardiovascular risk and disease. Likewise, the role of systemic immunotherapy on cardiovascular disease development in autoimmune disease patients remains to be elucidated in further studies (Takata et al., 2011; Zhang et al., 2016). Recently, evidence for beneficial effects of anti-inflammatory treatment with a monoclonal antibody targeting IL-1β in patients with known coronary artery disease reinforced the importance of inflammation and possible anti-inflammatory antibody treatment in cardiovascular disease (Ridker et al., 2017a, 2017b). The cytokine IL-17A, which can be directly antagonized for effective treatment of psoriasis, forms one possible connection between skin and vessel disease (Golden et al., 2013; Vena et al., 2010b). IL-17A does not only promote neutrophil infiltration into the skin (Di Cesare et al., 2009) but also into the aortic vessel wall (Karbach et al., 2014) and thereby leads to vascular dysfunction and hypertension (Madhur et al., 2010). In line with this, psoriasis patients exhibit increased vascular inflammation in multiple segments of the aorta compared with healthy controls (Mehta et al., 2011b).

In this study, anti-IL-17A treatment was not effective in the very severe K14-IL-17A$^{ind/+}$ psoriatic skin disease model to ameliorate skin nor vascular disease. This may be due to the very early (data not shown) and particularly high peripheral IL-17A levels. In CD11c-IL-17A$^{ind/ind}$ mice that produce about 4.7-fold lower amounts of IL-17A, anti-IL-17A was highly effective for treating cutaneous and associated vascular inflammation/dysfunction. Both skin and vascular disease were, respectively, completely cured and prevented when anti-IL-17A was applied therapeutically and prophylactically. These findings may have vital therapeutic implications for cardiovascular risk prevention and management in psoriasis patients. In fact, anti-IL-17A therapy may not only lower the risk of cardiovascular complications in patients with severe psoriasis as is currently done, but it may also be warranted to attenuate vascular inflammation and prevent cardiovascular disease when applied earlier, that is, in moderate psoriasis. Further supporting this therapeutic strategy, antibodies to IL-17A or the IL-17 receptor A subunit lowered blood pressure by 30 mm Hg and ameliorated vascular inflammation in a model of AngII-induced arterial hypertension (Saleh et al., 2016). Moreover, Li et al. (2018) recently described that arterial thrombus formation was attenuated in a mouse model of psoriasis under IL-17A inhibition, indicating that targeting cytokines that mediate psoriatic inflammation may indeed improve cardiovascular comorbidities. Our data demonstrate that psoriasis-associated vascular inflammation/dysfunction can potentially be cured and prevented by anti-IL-17A treatment.

From a clinical perspective, our study underlines the need to keep vascular disease and dysfunction in mind when treating psoriasis patients (Gelfand et al., 2011; Mehta et al., 2010). In different psoriasis-like mouse models, we could establish a direct link between the severity of skin involvement, local and circulating IL-17A cytokine levels, and vascular inflammation/dysfunction. Hence, our findings suggest that in psoriasis, the vasculature reacts in a similar way as the skin. IL-17A–mediated growing skin inflammation and plaque formation correlate with increasing inflammation of the vessel wall, leading to impaired vascular function, and most likely the development of hypertension and, eventually, increased cardiovascular mortality. Thus, psoriasis patients need to be educated properly about their increased risk of cardiovascular disease. Moreover, a combined dermatological-cardiological treatment may be warranted, including regular blood pressure controls and adequate therapy in the case of hypertension, to limit this life-limiting comorbidity of psoriasis (No et al., 2017).

In conclusion, our mouse data indicate that anti-IL-17A treatment of psoriasis may have a similar beneficial effect on the coexisting vascular inflammation. In addition, our psoriasis mouse models based on transgenic overexpression of IL-17A represent valuable tools for investigating the effects of biological therapies on cardiovascular disease in psoriasis. Our findings warrant future long-term studies in psoriasis patients to better understand the effects of biologicals, in particular IL-17A therapies, on cardiovascular comorbidity.

MATERIALS AND METHODS

Mouse models of psoriasis-like skin disease

IL-17A$^{ind/ind}$ mice were either crossed to K14-Cre or CD11c-Cre to obtain K14-IL-17A$^{ind/+}$ (Croxford et al., 2014), CD11c-IL-17A$^{ind/+}$,

unpaired Student *t* test in **d**. (**e**) Flow cytometric analysis of ear skin. One-way analysis of variance and Bonferroni post hoc test; n = 14–15. (**f**) ROS/RNS measurement in whole blood (20 minutes of stimulation with PDBu), repeated measurements of pooled samples. One-way analysis of variance and Bonferroni post hoc test; n = 8–9. (**g**) Flow cytometric analysis of aortas of IMQ-treated mice plus anti-IL-17A/isotype- and sham-treated control mice. Kruskal-Wallis and Dunn multiple comparison tests, n = 15. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. IMQ, imiquimod; PASI, Psoriasis Area and Severity Index; ROS/RNS, reactive oxygen and nitrogen species.

or CD11c-IL-17A$^{ind/ind}$ mice (Wohn et al., 2016). Besides these genetic models, psoriasis-like skin disease was drug induced by IMQ treatment of 7-week-old C57BL/6J mice over 5 consecutive days, as described previously (El Malki et al., 2013; van der Fits et al., 2009). All animals were housed and treated in accordance with relevant laws and institutional guidelines of the Central Animal Facility of the University Medical Center Mainz, Germany. Experiments were approved by the Animal Care and Use Committee from the Land of Rhineland-Palatine, approval numbers G13-1-099 and G 15-1-051.

Psoriasis Area and Severity Index

The severity of psoriasis-like skin disease was determined by modified PASI scoring, as described previously (El Malki et al., 2013; Karbach et al., 2014). Briefly, erythema and scaling were scored (score range = 0–4), skin thickness of the ears and the back skin were measured using a caliper (μm), and the percentage of affected skin was determined. The cumulative PASI score was calculated for the K14-IL-17A$^{ind/+}$ and CD11c-IL-17A$^{ind/ind}$ mice as follows: (erythema score + scaling score + skin thickness change [%]) × affected area [%]. For IMQ experiments, the percentage of affected skin is not included in the scoring because it depends on the treated area. Hence, the modified PASI is calculated as erythema score + scaling score + skin thickness change (%). In the figures, the cumulative PASI score and/or the single scores for skin thickness, scaling, and erythema are depicted.

Anti-IL-17A treatment

We received the anti-IL-17A antibody from Novartis (clone, BZN035; isotype control, YB-91-QE90) and used the following treatment regimens: Both K14-IL-17A$^{ind/+}$ and CD11c-IL-17A$^{ind/ind}$ mice were injected twice a week with a dosage of 20 μg/g body weight or 60 μg/g body weight of anti-IL-17A over a period of 4–6 weeks. In the acute IMQ-induced psoriasis-like skin disease model, 0.6 mg anti-IL-17A/injection was applied two times (days 0 and 3) during the 5-day and three times (days 0, 3, and 6) during the 7-day IMQ treatment protocols, respectively. Anti-IL-17A treatment did not affect the weight of the mice (data not shown).

Detection of ROS/RNS formation with L012-enhanced chemiluminescence

Oxidative burst of white blood cells in the whole blood was determined by 8-amino-5-chloro-7-phenylpyridol[3,4-d]pyridazine-1,4-(2H,3H)dione sodium salt (L012) -enhanced chemiluminescence. After injection of 200 IU of heparin into the beating heart of the anesthetized mouse, venous blood was drawn from the right ventricle. Enhanced chemiluminescence was counted in a volume of 200 μl per well of blood samples diluted 1:50 in phosphate buffered saline containing Ca^{2+}/Mg^{2+} (1 mmol/L) with L012 (100 μmol/L) in the presence of Phorbol 12,13-dibutyrate (PDBu) (10 μmol/L) at intervals of 5 minutes using a Centro plate reader (Berthold Technology, Bad Wildbad, Germany). Enhanced chemiluminescence was expressed as counts per minute or percentage of increase related to the control group after incubation for 10–20 minutes.

Vascular tone experiments

The vascular responsiveness to vasodilators (increasing doses of ACh in a range from 10^{-9}–$10^{-5.5}$ mol/L) of isolated aortic rings was studied. Isolated aortas were cut into 4-mm segments and put on force transducers (from Kent Scientific Corp., Torrington, CT, and from PowerLab, AD Instruments, Spechbach, Germany) in organ chambers filled with Krebs-Henseleit solution (98.93 mmol/L of NaCl, 4.69 mmol/L of KCl, 2.49 mmol/L of $CaCl_2$, 1.2 mmol/L of $MgSO_4$, 0.613 mmol/L of K_2HPO_4, 25 mmol/L of $NaHCO_3$, 11.1 mmol/L of D-glucose, 37 °C, pH 7.35) bubbled with carbogen gas (95% O_2/5% CO_2). Indomethacin 10 μmol/L was added to prevent endogenous synthesis of prostaglandins. Aortic segments were stretched gradually over 30 minutes to reach a resting tension of 1.0 g. After the preconstriction with prostaglandin $F_2\alpha$ (3.3 μmol/L) or phenylephrine (10^{-8}–$10^{-5.5}$ mol/L) to reach 50%–70% of the maximum tone induced by KCl, cumulative concentration-relaxation curves were recorded in response to increasing concentrations of ACh.

Flow cytometry

Spleens, lymph nodes, and ears were mechanically disrupted and digested with 400 U/ml Collagenase IV (Worthington Biochemical Corp., Lakewood, NJ) and for the ears additionally with 100 U/ml hyaluronidase (Sigma-Aldrich, St. Louis, MO) and 0.5 U/ml DNase (Promega, Madison, WI) or with 0.25 mg/ml Liberase (Roche, Basel, Switzerland) in RPMI for 30–60 minutes at 37 °C. The aorta was digested by using collagenase II (1 mg/ml) and DNase I (50 μg/ml) or Liberase (1 mg/ml) for 30 minutes at 37 °C. Erythrocytes of the spleen and the blood were lysed by BD FACS lysing solution (BD Biosciences, San Jose, CA). Alternatively, erythrocytes of the spleen were lysed with Tris-ammonium chloride (150 mmol/L NH_4Cl, 100 mmol/L KHCO 3, 10 mmol/L EDTA-2Na, pH 7.2). Subsequently, cells were filtered through 40-μm or 70-μm cell strainers (Falcon, BD Biosciences) to obtain single-cell suspensions, which were pre-incubated in FACS buffer containing Fc-Block for at least 10 minutes. Cells were surface-stained with various combinations of fluorescence-conjugated antibodies at 4 °C for 30 minutes to discriminate B220$^+$ B cells (clone: RA3-6B2), TCRβ$^+$ T cells (clone: H57-597), CD11b$^+$ myeloid cells (clone: M1/70), GR-1$^+$ (Ly6C$^+$Ly6G$^+$) neutrophils (Ly6C, clone: AL-21; Ly6G, clone: 1A8), and F4/80$^+$ monocytes/macrophages (clone: BM8). Flow cytometric acquisition was performed on a FACS Canto II (BD Biosciences) and analyzed using FlowJo software (FlowJo, Ashland, OR).

Cytokine detection

Plasma cytokine concentrations of IL-17A, IL-6, IL-22, IL-23, tumor necrosis factor-α, and IL-1β were determined by Luminex Multiplex Assay according to the manufacturer's instructions (Thermo Fisher Scientific). For skin IL-17A detection, back skin of mice was isolated and triturated with a homogenizer (Kinematica, Bohemia, NY). Cell debris was precipitated via centrifugation (10,000*g*, 4 °C, 10 minutes), and IL-17A was measured in the supernatant using the IL-17A DuoSet ELISA kit (R&D Systems) and normalized to the total protein concentration, which was determined via Bradford assay.

Statistical analysis

Statistical analysis was performed with GraphPad Prism software, version 7 (GraphPad Software, La Jolla, CA). First, the data were analyzed for normal distribution (Kolmogorow-Smirnow test). When normal distribution was given, we applied the one-way analysis of variance test with Bonferroni post hoc test. If no normal distribution was given, Kruskal-Wallis test with Dunn multiple comparison or comparison of selected columns was used as appropriate and indicated in the figure legends. PASI scores and aortic relaxation curves were analyzed by two-way analysis of variance with Bonferroni post hoc test. *P* values of less than 0.001, 0.01, and 0.05 were considered statistically significant. Data are presented as mean ± standard error of the mean.

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CONFLICT OF INTEREST

FK is an employee of Novartis. AW is a consultant to Novartis. The other authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.09.021.

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4 Discussion and Outlook

4.1 The enigmatic role of E-cadherin on LC

Contrary to general assumption, in this thesis we demonstrate that E-cadherin is dispensable to maintain LC in the epidermis and does not influence LC maturation and migration. Moreover, we show that LC lacking E-cadherin display a dramatically altered morphology characterized by more rounded cell bodies and fewer dendrites. However, these morphological changes had no impact on their function when we analyzed mice with a CD11c-specific deficiency of E-cadherin (CD11c-Ecad^{del} mice) in an allergic contact dermatitis and psoriasis model.

4.1.1 The role of E-cadherin in LC maturation

During the last years it became increasingly clear that E-cadherin is not only expressed on epithelial cells, but also on a variety of leukocytes including LC, macrophages, and γδ T cells (Van den Bossche and Van Ginderachter, 2013).

A direct functional involvement of E-cadherin in regulating maturation of epidermal LC has been demonstrated by the observation that ligation of E-cadherin on immature LC inhibits their maturation *in vitro* (Riedl et al., 2000). In contrast, maturation of LC in the skin of CD11c-Ecad^{del} and control mice was similar (Brand et al., 2019). This might be due to the fact that two different systems were used. In this earlier study immature LC were generated from human CD34^{+} hematopoietic progenitors *in vitro* in the presence of transforming growth factor β1 (TGF-β1). Antibody ligation of E-cadherin on the surface of this immature LC, after mechanical cluster disaggregation, inhibits their maturation (Riedl et al., 2000). This *in vitro* differentiation model mimics only parts of the complex *in vivo* situation. For example, other cell types in the skin, which potentially interact with E-cadherin on LC, are absent and a contribution of these cells on LC maturation cannot be excluded.

Next to LC, E-cadherin is expressed on BMDC and it has been shown that disruption of homophilic E-cadherin binding leads to their phenotypic maturation, including upregulation of MHCII, costimulatory molecules, and CCR7. These phenotypically matured BMDC fail to produce proinflammatory cytokines (Jiang et al., 2007). In contrast, our data reveal that maturation and cytokine production of BMDC from CD11c-Ecad^{del} mice are not altered (Brand et al., 2019) suggesting that mechanical disruption of E-cadherin contacts induces a maturation program that is not activated in cells lacking

E-cadherin. Notably, the observed phenotypic maturation upon cluster disruption is shown to be mediated via β-catenin. Disengagement from E-cadherin resulted in a sudden release of membrane-bound β-catenin and the translocation into the nucleus where it regulates gene transcription. We demonstrated that this membrane-bound pool of β-catenin is absent in E-cadherin–deficient BMDC and therefore not available to induce spontaneous maturation.

In mouse skin it was assumed that LC are the only DC that express E-cadherin (Borkowski et al., 1994, Tang et al., 1993). Our study demonstrates that, next to LC, also the small subset of Langerin$^+$ dDC expresses E-cadherin. During the time E-cadherin on DC was first studied, this subset of Langerin$^+$ dDC was not identified yet (Bursch et al., 2007, Ginhoux et al., 2007, Poulin et al., 2007). LC in the epidermis were characterized by their expression of CD11c and MHCII. The very low level of E-cadherin detected on Langerin$^+$ dDC compared to LC (Brand et al., 2019) could be assumed as background staining. However, this is unlikely because β-catenin expression of Langerin$^+$ dDC and LC in CD11c-Ecad^{del} mice was significantly decreased. In contrast to E-cadherin, β-catenin is expressed on all DC subsets in the skin of control mice indicating that the significant reduction on Langerin$^+$ dDC and LC in CD11c-Ecad^{del} mice is due to E-cadherin deficiency.

4.1.2 Impact of E-cadherin on LC migration

E-cadherin has primarily been described as a cell adhesion molecule and central component of adherens junctions (van Roy and Berx, 2008). LC and KC are linked via homophilic E-cadherin binding (Tang et al., 1993) and during migration of LC to LN, E-cadherin is downregulated (Jakob and Udey, 1998). Therefore, it has been assumed that E-cadherin is important to maintain LC in the epidermis and that its downregulation may even control LC mobilization. When we analyzed the LC numbers in mice lacking E-cadherin, we found similar numbers in the skin and skin-draining LN, indicating that lack of E-cadherin by itself does not automatically induce LC migration to LN (Brand et al., 2019). Also mild inflammation, as induced by FITC painting, does not trigger enhanced LC migration to LN in the absence of E-cadherin binding. This finding is further supported by the fact that EMT, a genetic program involved in LC migration (Hieronymus et al., 2015, Konradi et al., 2014), is not induced in E-cadherin-deficient LC. Specifically, LC maturation and migration results in a switch from an adhesive to a motile state which is accompanied by the downregulation of epithelial genes, including E-cadherin and

EpCam, together with the upregulation of mesenchymal genes like N-cadherin (Hieronymus et al., 2015). CD11c-Ecad^{del} and control mice displayed similar expression of both EMT markers, EpCam and N-cadherin, indicating that downregulation of E-cadherin alone does not induce EMT in LC. The molecular mechanism by which EMT triggers downregulation of E-cadherin on LC needs to be further investigated.

4.1.3 E-cadherin-mediated LC adhesion and morphology

In addition to LC maintenance, it is the general dogma that E-cadherin is essential for LC seeding of the epidermis (Jakob and Udey, 1998). This concept is further supported by the pivotal role of TGF-β1 and TGF-β receptor signaling for LC development and differentiation, including induction of E-cadherin expression, and LC positioning in the epidermis (Bobr et al., 2012, Kel et al., 2010). However, due to the relatively late expression of the *CD11c* promoter during LC development (Kel et al., 2010), we do not know whether E-cadherin is important during early seeding of the epidermis during ontogeny (Chorro et al., 2009, Hoeffel et al., 2012).

Surprisingly, we observed that LC lacking E-cadherin fail to acquire their typical dendritic morphology. Instead, these cells exhibit a more rounded cell body with very few remaining dendrites (Brand et al., 2019), leading to our hypothesis that LC lacking E-cadherin are less tightly anchored to surrounding KC anymore via homophilic bindings. This was confirmed by the observation that different digestion-enzymes greatly affect the number of LC released from the tissue. Specifically, a milder digestion with Collagenase IV, which lacks any additional Trypsin activity, releases more LC from the skin of CD11c-Ecad^{del} than from control mice. On the other hand, digestion with Liberase, containing Trypsin activity, results in the release of more LC, but with similar LC numbers from CD11c-Ecad^{del} and control skin. It has already been demonstrated for muscle stem cells that digestion with Trypsin in combination with Collagen significantly increases the cell-yield from the tissue as compared to trypsin digestion alone (Miersch et al., 2018). Furthermore, Liberase has been shown to be the most effective enzyme for the isolation of macrophages from the lung as compared to Collagenase D or elastase (Atif et al., 2018). This indicates that the choice of tissue digestion greatly influences LC yields and this observation is of general importance when assessing absolute LC numbers in the skin. The easier release of LC from the skin of CD11c-Ecad^{del} mice compared to controls suggests that E-cadherin-deficient LC adhere less tightly to KC and require E-cadherin to form their typical dendrites.

Interestingly, wild type LC display a unique behavior characterized by rhythmic extension and retraction of their dendrites into the intercellular spaces between KC to the stratum corneum and can capture protein Ag without disturbing barrier integrity (Nishibu et al., 2006, Ouchi et al., 2011). Since E-cadherin-deficient LC lack their dendrites, the ability to acquire Ag from the surface of the skin could be altered, which may affect the interaction with the skin microbiome, i.e. commensals and pathogens. *Staphylococcus aureus (S. aureus)* is an important cause of skin and soft tissue infections and is strongly associated with the inflammatory skin disease atopic dermatitis. While LC are essential to induce Th17 responses upon epicutaneous exposure to *S. aureus*, the molecular mechanisms underlying the interaction between *S. aureus* and LC are not completely understood (van Dalen et al., 2019). To test any role for E-cadherin, *S. aureus* could be applied on the skin of CD11c-Ecad^{del} and control mice. Next to skin infection models with bacteria, the interaction of E-cadherin-deficient LC and commensal fungi could also be impaired. The commensal yeast *Malassezia* is a prominent part of the skin microbiota, and its dysbiosis is associated with atopic dermatitis and other common inflammatory skin disorders (Sparber and LeibundGut-Landmann, 2017). LC seem to play an important role in producing early on high levels of IL-23 which then activates T cells and innate cells to produce IL-17. This *Malassezia*-induced Th17 response is pivotal in orchestrating antifungal immunity and in actively promoting skin inflammation (Sparber et al., 2019, Sparber and LeibundGut-Landmann, 2019). Whether and how E-cadherin on LC and the lack of their dendrites affect the interaction with the cutaneous microbiota and their surveillance of invading pathogens remains to be investigated.

4.1.4 Role of LC E-cadherin during skin inflammation

β-catenin has been suggested to promote a tolerogenic DC phenotype *in vivo* via the induction of IL-10–producing T cells (Jiang et al., 2007). In line with this, Manicassamy *et al.* demonstrated that a CD11c-specific deletion of β-catenin in mice results in an imbalance between regulatory versus inflammatory T cell subsets and an increased susceptibility to dextran sodium sulfate (DSS)-induced colitis (Manicassamy et al., 2010). In contrast, an inflammatory E-cadherin^{+} DC subset that accumulates during colitis has been described and adoptive transfer of these cells into T cell-restored immunodeficient hosts resulted in the exacerbation of colitis (Siddiqui et al., 2010). Moreover, E-cadherin deletion in CD11c^{+} cells attenuated DSS-induced colitis (Ihara et al., 2018), indicating an inflammatory role of E-cadherin^{+} DC. Whether and how E-cadherin on these DC contributes to the inflammatory response is not yet known.

LC and T cells can interact via E-cadherin−KLRG1/ CD103 binding and could therefore influence the ability of E-cadherin-deficient LC to govern T cell-mediated immunity.

The role of LC in CHS, a T cell-mediated contact hypersensitivity reaction remains controversial. Using huLangerin-DTA mice, which constitutively lack LC from birth, it has been shown that LC play an active role in CHS by suppressing the immune response (Kaplan et al., 2005). In contrast, depletion of LC shortly prior CHS induction (muLangerin-DTR mice), resulted in similar (Kissenpfennig et al., 2005) or diminished (Bennett et al., 2005) CHS reaction. Using mice with E-cadherin deficient LC we detected a similar CHS-induced ear swelling reaction compared to control mice. Furthermore, the percentage and absolute cell numbers of CD4, CD8 and γδ T cells from the skin of CD11c-Ecad^{del} and control mice are similar (Brand et al., 2019). This is an unexpected finding regarding the multiple E-cadherin-dependent pathways that are potentially affected by the lack of E-cadherin on LC (Van den Bossche and Van Ginderachter, 2013). For example, E-cadherin engagement leads to phosphoinositide 3-kinase (PI3K) activation in KC and epithelial cells (De Santis et al., 2009). A similar mechanism in LC could strongly affect immunogenicity versus tolerogenicity, since PI3K activity instructs an anti-inflammatory phenotype (Fukao et al., 2002). Moreover, E-cadherin is known to suppress NF-κB activity, which is a strong driver of DC maturation and activation (Kuphal et al., 2004). Binding to KLRG1, an inhibitory receptor (Banh et al., 2009), might influence the interaction of LC with different immune cells. KLRG1 is expressed by mature NK cells (Robbins et al., 2002) and subsets of activated CD4$^+$ and CD8$^+$ T cells, as well as, on subtypes of CD4$^+$ Treg. KLRG1 is used as a marker to distinguish short-lived effector CD8$^+$ T cells from memory precursors and for example in activated CD8$^+$ T cells, KLRG1 signaling results in dysfunction of proliferation (Beyersdorf et al., 2001). Another binding-partner of E-cadherin is CD103 which is abundantly expressed on intraepithelial αβ and γδ T cells (IEL and DETC) as well as on different subpopulations of DC (del Rio et al., 2010). It has been shown that CD103$^+$ DC, that are found in lymphoid and various nonlymphoid organs, display distinctive functions, such as cross-presentation of Ag in LN, instruction of tissue-homing capacities on the activated T cells, as well as induction of Treg (del Rio et al., 2010, Suffia et al., 2005). Given these data, the lack of E-cadherin on LC could alter the interaction with a variety of CD103-expressing cells, all of which play an important role in immune defense (Van den Bossche et al., 2012). Nevertheless, ear swelling reactions and the cellular infiltrate from the skin of CD11c-Ecad^{del} and control mice are similar, indicating that LC E-cadherin does not govern CHS responses.

Skin DC have also been implicated in the pathogenesis of psoriasis, but the roles for specific DC subsets, in particular LC, remain elusive. In human psoriatic skin lesions LC are diminished (Glitzner et al., 2014). In mice, depletion of LC before the onset of disease has no effect, demonstrating that LC are dispensable to initiate and drive psoriatic plaque formation in mice (Glitzner et al., 2014, Wohn et al., 2013). Wohn *et al*. reported that directly upon IMQ treatment Langerin^{neg} dDC are responsible for IL-23 production and subsequent dermatitis (Wohn et al., 2013). While others described LC as major source of IL-23 which is essential to trigger the inflammatory cascade (Yoshiki et al., 2014), Singh *et al.* identified moDC and moLC as the major IL-23 producers (Singh et al., 2016). In contrast, depletion of LC during active psoriasis aggravates symptoms in mice, suggesting that LC are responsible for keeping a suppressive environment due to balancing the anti-inflammatory IL-10 and proinflammatory IL-23 axis (Glitzner et al., 2014, Terhorst et al., 2015). Besides these controversial findings of LC in psoriatic skin inflammation, about the role of E-cadherin on LC and whether it contributes to psoriasis development is nothing known yet.
DETC, which express E-cadherin, are elevated in psoriatic skin but their role in disease progression remains elusive (Laggner et al., 2011, Lee et al., 1994). E-cadherin on DETC controls their activation by acting as an inhibitory receptor (Uchida et al., 2011), suggesting that E-cadherin binding between LC and DETC may limit psoriatic skin inflammation. Application of IMQ revealed no differences in ear swelling, redness and scaling, which is in line with similar cellular infiltrates in the skin of CD11c-Ecad^{del} and control mice. These findings indicate that E-cadherin interactions of LC and other E-cadherin expressing cells, in particular, DETC are not involved in IMQ-induced skin disease.

Taken together, despite many convincing indications and contrary to common knowledge, our data establish that E-cadherin is dispensable to maintain LC in the epidermis and does not regulate LC maturation and migration during steady-state and inflammation. Furthermore E-cadherin on LC does not contribute to LC function in CHS and psoriasis.

4.2 IL-17A links psoriatic skin lesion development to cardiovascular disease

In the second part of this thesis we developed a new chronic mouse model for psoriasis by targeting IL-17A expression to $CD11c^+$ cells. We showed that mice with constitutive low-level expression of this cytokine develop a moderate to severe psoriasis-like skin disease. Furthermore, these mice suffer from vascular dysfunction, a known comorbidity of psoriasis patients. Successful anti-IL-17A treatment of psoriatic skin lesions in different mouse models of psoriasis, diminished also peripheral oxidative stress levels and vascular inflammation and should therefore be considered as therapy to attenuate and prevent vascular disease in patients with moderate psoriasis.

4.2.1 CD11c-IL17A^{ind} mouse model for psoriasis and cardiovascular disease

Psoriasis is a chronic inflammatory skin disease of unknown etiology affecting millions of people worldwide. To dissect the complex cellular and molecular pathways that drive the pathogenesis of the disease, appropriate mouse models are needed. During the last years several xenograft, genetically engineered, and drug-induced models have been developed. However, they only resemble some aspects of the human disease. Xenotransplantation, in which uninvolved non-lesional psoriatic skin or plaque-type psoriasis skin is transplanted onto severely immunodeficient mice, probably most closely resemble psoriasis in terms of genetic, phenotypic, and immunopathogenic processes (Boyman et al., 2004, Wrone-Smith and Nickoloff, 1996). The major drawback is that the model is technically difficult, lacks systemic effects and can therefore be not used to study comorbidities resulting from psoriasis. A technically easier mouse model of psoriasis has been developed by van der Fits *et al*., based on the observation that daily topical application of Aldara cream, containing 5% of the TLR7-ligand IMQ, induces skin inflammation and pathology in mice closely resembling plaque-type psoriasis in humans (van der Fits et al., 2009). Initially, it highlighted the importance of the IL-23/IL-17 axis (Di Cesare et al., 2009) and later on the crucial role of cDC and innate lymphocytes for the initiation of psoriatic skin lesions (Cai et al., 2011, Pantelyushin et al., 2012, Wohn et al., 2013). The unique power of this acute model lies in dissecting the early cellular and molecular events during psoriatic plaque formation, which is difficult to achieve in patients. However, its major drawback is the lack of chronicity, which makes it impossible to study psoriasis related comorbidities.

Based on the key role of IL-17A, Croxford *et al.* developed a genetic mouse model by overexpressing IL-17A in KC (K14-IL-17A^{ind}). These mice develop a severe psoriasiform skin disease shortly after birth, which is associated with increased reactive oxygen species formation resulting in endothelial dysfunction (Croxford et al., 2014, Karbach et al., 2014). K14-IL-17A^{ind} mice suffer from a severe systemic inflammation leading to their early death by the age of 10-16 weeks. Considering this drastic phenotype, we sought to develop a more physiologic psoriasis mouse model by targeting constitutive IL-17A expression to CD11c^{+} cells (CD11c-IL-17A^{ind} mice) (Wohn et al., 2016). Although CD11c^{+} cells do not express IL-17A, they are present in low numbers in the skin, similar to the frequency of IL-17A producing innate lymphocytes and Th17 cells in active psoriatic plaques, which are the important initiators during disease development (Cai et al., 2011, Lowes et al., 2008).

Indeed, constitutive low-level expression of IL-17A by CD11c^{+} cells results in the gradual development of chronic skin lesions (Wohn et al., 2016). Lesional skin of CD11c-IL-17A^{ind} mice displays massive epidermal changes including thickening of the epidermis (acanthosis), hyperkeratosis, and elongated rete ridges, resembling the hallmarks of psoriatic plaques in humans (Lowes et al., 2014, Nestle et al., 2009b).

Psoriasis patients often harbor plaques at anatomical locations experiencing skin trauma like the elbows and knees (Malakou et al., 2018). This feature, known as the Koebner phenomenon, explains the development of psoriatic plaques in apparently healthy skin following trauma and/or mechanical stress (scratches, abrasion, pressure from tight shoes, shaving) (Malakou et al., 2018, Sagi and Trau, 2011). Psoriasiform lesions in CD11c-IL-17A^{ind} mice also preferentially occur at sites of mechanical stress (ears and neck) due to scratching and grooming. Moreover, acute disruption of the epidermal barrier by tape stripping triggers psoriatic skin lesions in CD11c-IL-17A^{ind} mice confirming the close resemblance to the human disease (Wohn et al., 2016).

Another characteristic feature of psoriatic lesions is that many AMP are highly expressed, and especially the association between psoriasis and β-defensin (Defb) or S100 proteins has been well established (Morizane and Gallo, 2012). Accordingly, lesional skin of CD11c-IL-17A^{ind} mice displays a higher expression of the AMP S100A7 and Defb3, which also serve as neutrophil chemo-attractants (Bals et al., 1999, Glaser et al., 2011). Indeed, the increased cellularity in the dermis reveals the presence of leukocytes infiltrating the skin. In line with this, significant local and systemic increase of the chemokines CXCL1 and CCL2 were detected, mediating the infiltration of neutrophils and monocytes, respectively. In psoriasis LL-37 induces CXCL1 through IL-36R

signaling in KC, which in turn, facilitates the recruitment of neutrophils in lesional skin (Li et al., 2014). This prominent infiltration of neutrophils into the skin, clearly distinguishes the CD11c-IL-17A^{ind} phenotype from atopic dermatitis in which neutrophils are rarely present (Bieber, 2010).

Psoriasis is not only a skin disease, but also associated with an increased risk for CVD, which is the most common cause of morbidity and mortality in patients with psoriasis (Gelfand et al., 2010, Lockshin et al., 2018). IL-17A has already been identified as a key factor in both diseases: it is not only important in the pathogenesis of psoriasis (Blauvelt and Chiricozzi, 2018, Martin et al., 2013) but also contributes to the development of vascular dysfunction (Karbach et al., 2014, Madhur et al., 2010). Therefore, it has been hypothesized that IL-17A is the key cytokine connecting the two diseases (Golden et al., 2013, Lockshin et al., 2018).
Based on this hypothesis, we analyzed CD11c-IL-17A^{ind} mice regarding their endothelial function. Indeed, mice with gradual development of moderate to severe psoriasis-like skin disease also suffer from vascular dysfunction (Schüler et al., 2019). Moreover, peripheral ROS levels were significantly elevated in the homozygous CD11c-IL-17A$^{ind/ind}$ mice compared with controls and also slightly increased in heterozygous CD11c-IL-17A$^{ind/+}$ animals (Wohn et al., 2016). In the vasculature, IL-17 induces hypertension by decreasing endothelial production of nitric oxide, and furthermore, endothelial dysfunction can be normalized by antagonization of IL-17 (Nguyen et al., 2013). Another study from Madhur *et al.* identified IL-17 as an essential cytokine contributing to angiotensin II (AngII)-induced vascular dysfunction since mice lacking IL-17A display reduced vascular inflammation and attenuated vascular dysfunction in this model (Madhur et al., 2010). IL-17A has been shown to recruit neutrophils to sites of inflammation and in the AngII-induced hypertension model, the developing vascular dysfunction results due to the immune cell infiltration into the vessel wall (Wenzel et al., 2011). In agreement, CD11c-IL-17A$^{ind/ind}$ mice display a massive neutrophil infiltration into the aortic vessel wall what could be one of the reasons for the vascular dysfunction in this model. Psoriasis and atherosclerosis, which is the major cause of CVD, have similar underlying immunologic mechanisms (Boehncke et al., 2011). In psoriasis, DC secrete IL-12 and IL-23, resulting in T cell differentiation into Th1 and Th17 subtypes. Th17 cells secrete IL-17 and IL-22 which promote KC proliferation (Nestle et al., 2009b). In atherosclerosis, endothelial activation at sites of arterial plaques promotes monocyte and lymphocyte extravasation and induces IL-12 and IL-23 production by macrophages and DC. Th1 cells promote further atherosclerotic plaque growth, whereas Th17 cells

induce intraplaque neoangiogenesis (Armstrong et al., 2012, Lockshin et al., 2018). These data combined with our findings, that development of psoriatic skin lesions by overexpression of IL-17A also results in the development of vascular dysfunction, confirm the pivotal role of this cytokine in linking the two diseases.

We further demonstrate that the incidence and time of onset of psoriasiform skin lesions and vascular dysfunction are IL-17A dose-dependent. K14-IL-17A^{ind} mice exhibit severe disease with dry and flaky skin involving the whole body, immediately after birth (Croxford et al., 2014). Additionally, these mice suffer from severe vascular dysfunction (Karbach et al., 2014). In contrast, CD11c-IL-17A^{ind} mice display a delayed and slowly progressing skin phenotype with demarcated lesions. Whereas 100% of homozygous (CD11c-IL17A$^{ind/ind}$) mice developed skin lesions starting at 8 weeks of age, only 30% of the heterozygous (CD11c-IL-17A$^{ind/+)}$ littermates developed skin lesions with a delayed onset starting at 18 weeks. Similar to the skin phenotype, homozygous CD11c-IL-17A$^{ind/ind}$ mice develop a more pronounced vascular inflammation and dysfunction than heterozygous CD11c-IL-17A$^{ind/+}$ mice. Interestingly, when we monitored IL-17A serum levels of heterozygous CD11c-IL-17A$^{ind/+}$ mice, cytokine concentrations were already elevated in 9 week-old animals, that is before the first appearance of skin lesions, and continued to increase with age. Compared to heterozygous K14-IL-17A$^{ind/+}$ mice, IL-17A cytokine levels were 4.7-fold lower in homozygous CD11c-IL-17A$^{ind/ind}$ mice. This indicates that there might be a threshold of IL-17A beyond which skin homeostasis is irrevocably disturbed and chronic inflammation and CVD start to develop. These findings are in line with the correlation between the severity of skin disease and systemic IL-17A levels in psoriasis patients (Arican et al., 2005). The hypothesis, that a certain threshold of IL-17A has to be reached, is further supported by the fact that in the IMQ-induced psoriasis model vascular dysfunction could not be observed. We detected only starting vascular inflammation, together with elevated peripheral oxidative stress levels and systemic IL-17A levels even lower than in heterozygous CD11c-IL-17A$^{ind/+}$ mice (Schüler et al., 2019). Whereas in this genetic model a constitutive expression of IL-17A is present throughout life, the IMQ model represents an acute short-term model. To test whether vascular dysfunction could also be provoked in this model, the long term IMQ-induced psoriasis model by Terhorst *et al.* could be applied, in which IMQ is painted onto the skin for 14 days to mimic also the later chronic phase of disease (Terhorst et al., 2015). Since we established that psoriatic plaque development in CD11c-IL-17A^{ind} mice could be triggered by mechanical stress, it would also be interesting to investigate whether the development of vascular inflammation could be accelerated.

Taken together, these data indicate that the severity of both the psoriatic skin lesions and the associated vascular dysfunction are IL-17A dose-dependent.

In conclusion, the skin phenotype developing in CD11c-IL-17A^{ind} mice closely resembles human plaque-type psoriasis in terms of histologic characteristics, composition of the cellular infiltrate and molecular signature. Moreover, CD11c-IL-17A^{ind} mice display elevated oxidative stress levels and develop vascular dysfunction, a comorbidity of psoriasis in humans. Therefore, CD11c-IL-17A^{ind} mice represent a valuable model to unravel the pathogenesis of psoriasis and cardiovascular disease and may provide a unique tool to test novel therapeutics for the treatment of both diseases in humans.

4.2.2 IL-17A as therapeutic target for psoriasis and cardiovascular disease

The lack of awareness of the link between skin and cardiovascular disease results in an undertreatment of cardiovascular risk factors in psoriasis patients (Ahlehoff et al., 2012, Wild et al., 2019). Common psoriasis treatment only targets the skin disease although a combined treatment approach should be considered. In humans, improvement in psoriasis skin disease severity was associated with the improvement in aortic vascular inflammation (Dey et al., 2017).

IL-17A plays a striking role in linking psoriasis and CVD, hence, targeting this cytokine could be warranted to treat both diseases simultaneously or prophylactically in moderate psoriasis disease patients. Indeed, different monoclonal antibodies which target IL-17A (secukinumab (Langley et al., 2014) and ixekizumab (Leonardi et al., 2012)) have been shown to be successful in the treatment of psoriasis patients. In K14-IL-17A$^{ind/+}$ mice, anti-IL-17A treatment was not efficient to reduce skin nor vascular disease indicated by similar systemic oxidative stress levels and aortic inflammation in K14-IL-17A$^{ind/+}$ compared to untreated mice (Schüler et al., 2019). An explanation why the treatment failed could be the very early and high peripheral IL-17A levels. In this severe model antibody concentration and the frequency of the injections could be increased to neutralize IL-17A more efficiently. The assumption that IL-17A levels are too high for efficient treatment is strengthened by the observation that IL-17A antagonization in CD11c-IL-17A$^{ind/ind}$ mice was able to cure skin, as well as, vascular disease. Systemic oxidative stress levels were normalized and little aortic infiltration was observed. Furthermore, when anti-IL-17A was applied before onset of psoriasis, both skin and vascular disease could be prevented suggesting a prophylactic function of anti-IL-17A

(Schüler et al., 2019). Injection of anti-IL-17A simultaneously to the IMQ-treatment, resulted in a significantly delayed onset and attenuated severity of the skin disease. Oxidative stress levels in the peripheral blood were increased after IMQ treatment and slightly but not significantly reduced by anti-IL-17A.

Human studies already demonstrated that systemic anti-inflammatory treatment with biological agents resulted in lower CVD events compared to patients treated with other anti-psoriatic therapies (Ahlehoff et al., 2013). Evidence for beneficial effects of treatment with anti-IL-1β in patients with known coronary artery disease reinforced the importance of anti-inflammatory treatment in cardiovascular disease (Ridker et al., 2017). The observation, that anti-IL17A treatment is efficient in psoriasis and the resulting vascular dysfunction is further supported by the study of Saleh *et al.,* showing that antibodies to IL-17A or the IL-17R lowered blood pressure and ameliorated vascular inflammation in a model of AngII-induced arterial hypertension (Saleh et al., 2016). In a similar context, inhibition of IL-17A attenuated arterial thrombus formation in a mouse model of psoriasis (Li et al., 2018).

In conclusion, our findings highlight the importance of a combined dermatological-cardiological treatment of psoriasis patients. Patients need to be educated properly about their increased risk of CVD and regular screenings for CVD risk factors should be performed. Furthermore, to lower the risk of cardiovascular complications a treatment with biologics should be considered not only for patients with severe but also with mild to moderate psoriasis. Our findings warrant future long-term studies in psoriasis patients to better understand the effects of biologicals, in particular IL- 17A therapies, on cardiovascular comorbidity.

5 Summary

In the first part of this thesis we analyzed the cell adhesion molecule E-cadherin, which is a major component of adherens junctions and marker for LC in the epidermis. Here, LC attach to the surrounding KC via homophilic E-cadherin binding and downregulation of E-cadherin coincides with LC migration. To date, it is unknown whether E-cadherin is just a marker identifying LC or whether it regulates LC homeostasis and function. Therefore, we investigated the role of E-cadherin cell adhesion and signaling in DC *in vivo*, in mice with conditional knockout of E-cadherin in all $CD11c^+$ cells (CD11c-$Ecad^{del}$). In the absence of E-cadherin, LC numbers in CD11c-$Ecad^{del}$ and control mice were comparable and stable, even in aged animals. Intriguingly, E-cadherin deficient LC displayed a more rounded cell body and fewer dendrites compared to controls. Nevertheless, maturation and migration of E-cadherin-deficient LC were not altered, neither under steady state nor inflammatory conditions. In conclusion, these data demonstrate that E-cadherin is dispensable to maintain LC in the epidermis and does not regulate LC maturation, migration nor function.

In the second part of this thesis we developed a chronic psoriasis mouse model by targeting IL-17A expression to $CD11c^+$ cells (CD11c-$IL17A^{ind}$). Patients with severe psoriasis suffer from an increased risk of cardiovascular mortality. Indeed, we demonstrated that overexpression of IL-17A in $CD11c^+$ cells leads to psoriasis-like skin disease and vascular dysfunction. Interestingly, systemic IL-17A serum level correlated with the severity of skin and vascular phenotype. In addition, we showed that neutralization of IL-17A can protect CD11c-$IL17A^{ind}$ mice from the concurrent development of skin lesions and cardiovascular disease. This was manifested by abolished psoriatic plaque formation and an improved vascular function. Our data highlight the pivotal role of IL-17A linking the development of skin lesions and vascular disease in psoriasis. Therefore, CD11c-$IL17A^{ind}$ mice represent a novel mouse model to investigate the correlation between IL-17A mediated chronic skin disease and vascular dysfunction as well as to validate new therapeutic targets.

6 Zusammenfassung

Im ersten Teil dieser Arbeit haben wir das Zelladhäsionsmolekül E-cadherin in Langerhans-Zellen (LC) analysiert. Dieses ist ein Hauptbestandteil von Adherens Junctions und bildet über homophile E-cadherin-Bindungen ein dichtes Netzwerk zwischen epidermalen LC und Keratinozyten. Bisher ist nicht bekannt ob E-cadherin eine Rolle bei der Regulierung der LC-Homöostase spielt. Daher untersuchten wir den Einfluss von E-cadherin in der Zelladhäsion und -signalübertragung in LC *in vivo.* Dafür generierten wir Mäuse in denen die Expression von E-cadherin spezifisch in $CD11c^+$ Zellen ausgeschaltet wurde. Die Abwesenheit von E-cadherin beeinflusste die LC-Dichte in CD11c-$Ecad^{del}$ Mäusen selbst bei älteren Tieren nicht. Interessanterweise zeigten E-cadherin-defiziente LC eine komplett veränderte Morphologie, was sich in runderen Zellkörpern und einer geringeren Anzahl an Dendriten wiederspiegelte. Dennoch waren Reifung und Wanderung weder im Steady-State noch unter entzündlichen Bedingungen verändert. Zusammenfassend zeigen unsere Daten, dass E-cadherin nicht notwendig ist, um LC in der Epidermis zu verankern und dass es weder die Reifung, Wanderung noch die Funktion von LC reguliert.

Im zweiten Teil dieser Arbeit entwickelten wir ein chronisches Psoriasis-Mausmodell (CD11c-$IL17A^{ind}$), indem wir das Zytokin IL-17A in $CD11c^+$ Zellen überexprimierten. Patienten mit schwerer Psoriasis leiden neben Hautentzündungen unter einem erhöhten Risiko für kardiovaskuläre Erkrankungen. In der Tat haben wir gezeigt, dass eine Überexpression von IL-17A in Mäusen nicht nur zu einer Psoriasis-ähnlichen Hautkrankheit, sondern auch zu einer Gefäßfunktionsstörung führt. Interessanterweise korrelierte der systemische IL-17A-Serumspiegel mit dem Schweregrad des Haut- und vaskulären Phänotyps. Darüber hinaus haben wir gezeigt, dass die Neutralisation von IL-17A in CD11c-$IL17A^{ind}$-Mäusen sowohl vor der Entwicklung von Hautläsionen als auch Herz-Kreislauf-Erkrankungen schützen kann. Dies äußerte sich in einer verminderten Bildung von Psoriasis-Plaques und einer verbesserten Gefäßfunktion. Unsere Daten unterstreichen die zentrale Rolle von IL-17A bei der Entstehung von Hautläsionen und Gefäßerkrankungen bei Psoriasis. Daher stellen CD11c-$IL17A^{ind}$-Mäuse ein neues Mausmodell dar, um die Korrelation zwischen IL-17A-vermittelter chronischer Hautkrankheit und vaskulärer Dysfunktion zu untersuchen und neue therapeutische Ansätze zu etablieren.

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8 List of figures

9 Own contribution to the respective publications

Publication 1:

E-cadherin is dispensable to maintain Langerhans cells in the epidermis

- Design, performance and evaluation of all experiments
- Preparation of graphs and figures
- Preparation of the manuscript together with Prof. Dr. Björn E. Clausen

Publication 2:

Monitoring skin dendritic cells in steady state and inflammation by immunofluorescence microscopy and flow cytometry

- Performance of the FITC and tape stripping experiment
- Preparation of epidermal sheets
- Proofreading of the manuscript

Publication 3:

Gradual development of psoriatic skin lesions by constitutive low-level expression of IL-17A

- Analysis of the skin lesion development over a period of 40 weeks
- Cytokine analysis in the serum of the mice
- Proofreading of the manuscript

Publication 4:

Antagonization of IL-17A attenuates skin inflammation and vascular dysfunction in mouse models of psoriasis

- Performance and evaluation of all skin experiments
- Assistance with vascular dysfunction experiments and analysis
- Preparation of graphs and figures
- Preparation of the manuscript together with Rebecca Schüler, Dr. Susanne Karbach and Prof. Dr. Björn E. Clausen

10 Eidesstattliche Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit selbstständig und nur mit zur Hilfenahme der angegebenen Quellen angefertigt habe. Weiterhin wurde die Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegt. Teile der Arbeit wurden bereits für Veröffentlichungen verwendet und sind entsprechend gekennzeichnet.

Mainz den, 24.10.2019

Anna Brand