Aus der Klinik für Dermatologie, Venerologie und Allergologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Skin barrier and danger-signatures analysis in patients with atopic dermatitis upon skin irritation

zur Erlangung des akademischen Grades
Doctor medicinae (*Dr. med.*)

vorgelegt der Medizinischen Fakultät
Charité - Universitätsmedizin Berlin

von
Maria Michaelidou
aus Zypern (Strovolos)

Datum der Promotion: 4. Juni 2021

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AD	Atopic dermatitis
$CD4^+$ T cells	Helper T lymphocytes
$CD8^+$ T cells	Cytotoxic T lymphocytes
CE	Cornified envelope
Cldn-1	Claudin-1
FLG	Filaggrin
HC	Healthy controls
IgE	Immunoglobulin E
IL-1	lnterleukin-1
IL-1RAP	Interleukin-1 receptor accessory protein
lLCs	Innate lymphoid cells
IFN	Interferon
NF-κB	Nuclear factor kappa B
NLRs	Nod-like receptors
NMFs	Natural moisturizing factors
SCORAD	SCORing Atopic Dermatitis
SNPs	Single Nucleotide Polymorphisms
ST2	Suppression of tumorigenicity 2
TCI	Topical calcineurin inhibitors
TCS	Topical corticosteroids
TEWL	Transepidermal water loss
TIS score	Three Item Severity score
TJ	Tight junctions
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor alpha
TSLP	Thymic stromal lymphopoietin
ZO	Zonula occludens

ABSTRACT

Atopic dermatitis is one of the most common chronic dermatological diseases with a prevalence of 10–20% in children and of 1–3% in adults. Previous studies have demonstrated a correlation between AD and an abnormal expression of skin barrier genes in both lesional and non-lesional skin. Various studies have also highlighted the pivotal role of the cytokines of IL-1 family in the pathogenesis of AD.

Furthermore, it has been reported that an acute barrier disruption leads to an increase of TEWL and alters the expression of structural skin barrier proteins. Additionally, skin irritation caused by tape stripping promotes skin immunoreactivity by inducing the expression of inflammatory mediators including members of IL-1 family.

In this thesis, the expression of skin barrier genes and cytokines of the IL-1 family in lesional, non-lesional AD skin and healthy skin was studied. The main aim was to investigate the role of acute skin barrier disruption (caused by repeated tape stripping) on the expression of barrier genes and IL-1 cytokines in the human epidermis. The mRNA and protein expression of skin barrier genes (such as claudin-1, filaggrin, loricrin) as well as of IL-1 cytokines (e.g. IL-1α, IL-1β, IL-18, IL-33) were evaluated in skin samples by using qRT-PCR and immunohistochemistry.

Our results show that the tight junction protein Cldn-1 (at mRNA and protein levels) was down-regulated in lesional and non-lesional AD skin in comparison with the healthy skin. The mRNA expression of IL-18 was not altered in lesional and non-lesional skin of AD patients versus HC, but the IL-18 protein levels were higher in lesional and non-lesional AD skin than in healthy skin. Skin barrier disruption by tape stripping led to an increase of the mRNA expression of Cldn-1 (not statistically significant) and to a decrease of mRNA expression of filaggrin and loricrin in AD patients. In addition, after skin barrier disruption the mRNA levels of IL-18 remained unchanged, but the IL-18 protein levels were higher (not statistically significant) in AD patients.

In conclusion, expression of skin barrier genes and members of the IL-1 family are altered in lesional and non-lesional skin from AD patients and upon skin irritation. The challenge for the future is to better understand the molecular pathways that regulate epidermal TJ in atopic skin and to define better the IL-1-mediated signalling cascades involved in AD. This approach may provide novel therapeutic approaches in AD.

ZUSAMMENFASSUNG

Die atopische Dermatitis ist eine der häufigsten chronisch entzündlichen Hauterkrankungen (Prävalenz in Industrieländern: 10–20% aller Kinder und 1–3% der Erwachsene). Frühere Studien zeigen eine Assoziation zwischen der AD und einer veränderten Genexpression von Strukturproteinen in läsionaler and nicht-läsionaler atopischer Haut. Verschiedene Studien deuten auch auf eine zentrale Rolle von Zytokinen der IL-1-Familie in der Pathogenese der AD hin.

In klinischen Studien konnte gezeigt werden, dass die akute Störung der Hautbarriere zu einem Anstieg TEWL führt und die Expression der Strukturproteine beeinflusst. Die Hautirritation nach Tesafilm-Abriss (Tape stripping) fördert die Immunreaktivität und induziert die Expression von Entzündungsmediatoren, einschließlich der Zytokine der IL-1-Familie.

Im Rahmen dieser Promotionsarbeit wurde die Expression von epidermalen Strukturproteinen und Botenstoffen der IL-1 Zytokin-Familie vergleichend zwischen betroffener und nicht betroffener Haut von AD Patienten sowie gesunder Haut untersucht. Ein weiterer Schwerpunkt war die Untersuchung des Einflusses einer akuten Störung der Hautbarriere (verursacht durch wiederholten Tesafilm-Abriss) auf die Expression der Strukturproteine und die IL-1-Zytokine in der Epidermis. In Hautproben von rekrutierten Patienten wurde die mRNA- und Proteinexpression von Strukturproteinen (wie Claudin-1, Filaggrin, Loricrin) sowie der IL-1-Zytokine (z. B. IL-1α, IL-1β, IL-18, IL-33) mittels qRT-PCR und Immunhistochemie bestimmt. Zusätzlich wurde der TEWL vor dem Tesafilm-Abriss, direkt nach dem Tesafilm-Abriss und drei Stunden nach dem Tesafilm-Abriss bei den AD Patienten und der Kontrollgruppe gemessen.

Die Ergebnisse der vorliegenden Promotionsarbeit zeigten, dass die mRNA- und Proteinexpression des Tight Junction-Proteins Claudin-1 in läsionaler und nicht läsionaler atopischer Haut im Vergleich zu gesunder Haut herunterreguliert war. Die mRNA-Expression von IL-18 zeigte keine Veränderung in läsionaler und nicht-läsionaler atopischer Haut im Vergleich zu der gesunden Haut. Jedoch wurde eine erhöhte IL-18 Proteinexpression in läsionaler und nicht-läsionaler atopischer Haut verglichen mit der gesunden Haut festgestellt. Weiterhin zeigte sich bei AD-Patienten eine Zunahme (nicht statistisch signifikant) der Genexpression von Claudin-1 und eine Abnahme der Genexpression von Filaggrin und Loricrin nach Tape stripping. Interessanterweise war die Genexpression von IL-18 stabil, jedoch wurde eine erhöhte Proteinexpression von IL-18 (nicht statistisch signifikant) bei AD Patienten vor versus nach Tape stripping festgestellt.

Zusammenfassend findet sich eine veränderte Expression von epidermalen Strukturproteinen und der Zytokine der IL-1-Familie als Alarmsignatur nach einer Hautirritation in atopischer Haut. Die Regulation der Strukturproteine nach Hautirritation und die IL-1-vermittelten Signalkaskaden sollten zukünftig genauer untersucht werden. Ein besseres Verständnis der pathophysiologischen Grundlagen der AD stellt die Basis für die Entwicklung neuer therapeutischer Ansätze dar.

1. INTRODUCTION

1.1. Objectives

Over the years, various studies have highlighted the major function of the skin as an effective barrier between the external environment and the internal milieu. In response to danger signals (e.g. physical injury, allergens or microorganisms) epidermal cells regulate innate and adaptive immune reactions by producing a variety of proinflammatory mediators. The members of the IL-1 family (such as IL-1α, IL-1β, IL-18 and IL-33) have been characterized as crucial proinflammatory cytokines and have been implicated in the pathogenesis of AD. Moreover, it has been reported that skin irritation caused by tape stripping has an impact on the inflammatory epidermal barrier of healthy individuals. However, there have been no reports about the expression of IL-1α, IL-1β and IL-33 upon skin irritation in the epidermis of AD patients as well as of the IL-18 epidermal expression in AD patients and HC after tape stripping.

The following questions were addressed in this thesis:
a) How can the acute permeability barrier disruption (caused by repeated tape stripping) affect the expression of skin barrier genes (A) and of the members of the IL-1 family (B) in human epidermis?
b) Do the expression patterns of skin barrier genes and IL-1 cytokines differ between the lesional and non-lesional skin of AD patients and healthy skin?
c) What is the impact of an acute barrier disruption by tape stripping in AD patients as compared to HC?

By answering these questions, we sought to support a novel view on the inflammatory processes which are involved in orchestrating the initiation and maintenance of AD.

1.2. Atopic dermatitis (AD)

1.2.1. Definition, Epidemiology and Classification

Atopic dermatitis (AD) is a frequent and chronic/relapsing inflammatory skin disorder characterized by intense pruritus, xerosis and eczematous lesions marked by erythema, infiltration/papulation, oozing with crusting, excoriations and lichenification (Bieber, 2008; Leung, Boguniewicz, Howell, Nomura and Hamid, 2004). AD is frequently associated with other atopic diseases such as allergic rhinitis and asthma (Akdis, Akdis, Bieber, Bindslev-Jensen, Boguniewicz, Eigenmann, Hamid, Kapp, Leung, Lipozencic, Luger, Muraro, Novak, Platts-Mills,

Rosenwasser, Scheynius, Simons, Spergel, Turjanmaa, Wahn, Weidinger, Werfel, Zuberbier, European Academy of Clinical Immunology/American Academy of Allergy and Immunology, 2006). AD is a major public health problem worldwide with a prevalence of 10–20% in children and of 1–3% in adults. Epidemiological studies have reported that the prevalence of AD has increased over the last few decades in industrialized countries (Larsen and Hanifin, 2002).

The clinical manifestations of atopic dermatitis vary with age. In infancy, the initial eczematous lesions of early-onset atopic dermatitis are mostly located on the cheeks, forehead and scalp. Scratching is intense and causes crusted erosions. During childhood, lesions emerge on flexures, the nape and the dorsal sides of the limbs. In adolescence and adulthood, the predominant areas of involvement are the flexural folds, head and neck, upper arms and back, and the dorsal part of the hands, feet and toes. Dry, scaling erythematous papules as well as the configuration of lichenified plaques (because of the lesional chronicity) characterize the eruption. In each phase, pruritus that continues throughout the day and aggravates at night causes significant sleeplessness. Additionally, severe disease can provoke distress in patients, due to several factors including major psychological problems and impaired quality of life (Akdis, Akdis, Bieber, Bindslev-Jensen, Boguniewicz, Eigenmann, Hamid, Kapp, Leung, Lipozencic, Luger, Muraro, Novak, Platts-Mills, Rosenwasser, Scheynius, Simons, Spergel, Turjanmaa, Wahn, Weidinger, Werfel, Zuberbier, European Academy of Clinical Immunology/American Academy of Allergy and Immunology, 2006; Bieber, 2008).

Two subtypes of atopic dermatitis have been identified: an extrinsic subtype (with IgE-sensitization) affecting 70–80% of AD patients and an intrinsic subtype (without IgE-sensitization) involving 20–30% of AD patients (Leung, Boguniewicz, Howell, Nomura and Hamid, 2004). The patients with the extrinsic type show high concentrations of total and allergen-specific IgE, multiple type-I sensitizations to various aero- and food allergens, and a $CD4^+$ dominated cellular infiltrate in the skin. The intrinsic type has the following characteristics: clinical phenotype of AD, absence of other atopic disorders, negative type I skin hypersensitivity to inhalant and food allergens, normal total IgE levels in serum, and no detectable specific IgE antibodies to inhalant- and food allergens. Additionally, in patients with the intrinsic type of AD the cellular infiltrates harbour CD4- and CD8-positive cells (Akdis and Akdis, 2003; Worm, Forschner, Lee, Roehr, Edenharter, Niggemann and Zuberbier, 2006).

1.2.2. Pathophysiology

The pathophysiology of AD is characterised by a complex interplay between inflammatory processes, environmental factors, genetics and skin barrier dysfunction (Maintz and Novak, 2007; Wuthrich, Cozzio, Roll, Senti, Kundig and Schmid-Grendelmeier, 2007).

Various studies have highlighted the important role of skin-infiltrating lymphocytes during the initiation and progression of inflammation in atopic skin. The pivotal cells involved in the pathogenesis of AD fall into four categories. The dendritic cells (e.g. Langerhans cells and inflammatory dendritic epithelial cells) form the first group. These cells present allergens to lymphocytes, leading to a type 2 helper T cell (Th2) polarization via Immunoglobulin E (IgE) and non-IgE-mediated mechanisms (Ong, 2014). The Th2 cells are the second group with a pathogenic role in AD. Acute AD skin lesions reveal a Th2-dominant inflammation characterized by dermal infiltration of $CD4^+$ T cells and eosinophils, along with an elevated expression of Th2 pro-inflammatory cytokines (including IL-4, IL-5, and IL-13) (Hamid, Boguniewicz and Leung, 1994; Oyoshi, He, Kumar, Yoon and Geha, 2009). Activated eosinophils are the third cell type associated with the pathophysiology of AD and they are responsible for the local inflammation in the lesional areas (Kagi, Joller-Jemelka and Wuthrich, 1992). Accumulating evidence shows that keratinocytes are the fourth group of cells playing a crucial role in the pathophysiology of the disease (Ong, 2014; Oyoshi, He, Kumar, Yoon and Geha, 2009). In response to multiple stimulating factors, keratinocytes participate in the skin immune response through the release of pro-inflammatory cytokines and chemokines. For example, a mechanical injury inflicted by scratching leads to the production of a variety of pro-inflammatory mediators from epidermal keratinocytes. IL-1 and IL-18 are stored as biologically inactive precursors in keratinocytes, and after stimulation by danger signals, these pro-inflammatory cytokines are activated by CASP1 enzyme (Bieber, 2008; Oyoshi, He, Kumar, Yoon and Geha, 2009). Several studies have suggested a potential role for IL-18 in AD, and Single Nucleotide Polymorphisms (SNPs) in the IL-18 gene have been shown to be associated with the disease (Cheng, Hao, Zhou and Ma, 2014; Kim, Lee, Namkung, Park, Kim, Shin, Cho and Yang, 2007). In addition, IL-1 and IL-6 mRNAs are up-regulated in the skin after physical injury. Furthermore, there is an increased expression of thymic stromal lymphopoietin (TSLP) in keratinocytes of AD lesions. TSLP is an IL-7-like cytokine which promotes Th2 immune responses and a subsequent allergic inflammation. Numerous studies have shown that the TSLP is a master switch molecule that triggers the initiation and maintenance of AD (Oyoshi, He, Kumar, Yoon and Geha, 2009; Soumelis, Reche, Kanzler, Yuan, Edward, Homey, Gilliet, Ho,

Antonenko, Lauerma, Smith, Gorman, Zurawski, Abrams, Menon, McClanahan, de Waal-Malefyt Rd, Bazan, Kastelein and Liu, 2002).

AD patients exhibit epidermal barrier dysfunction and abnormal structure of the stratum corneum in both involved and uninvolved skin. Accumulating evidence has shown that a loss of skin barrier integrity and cohesion is a major factor for the initiation and/or exacerbation of AD. Alteration of the skin barrier leads to an increase of transepidermal water loss (TEWL) and to pruritus, which promotes the penetration of allergens, microbes and other irritants into the subepidermal layer (Mu, Zhao, Liu, Chang and Zhang, 2014; Seidenari and Giusti, 1995b; Thestrup-Pedersen, 1997). Matsumoto *et al.* reported that a mechanical injury (an itch-induced scratching) and the inflammation in lesional atopic skin are likely to worsen skin barrier dysfunction (Matsumoto, Sugiura and Uehara, 2000).

1.3. The skin

1.3.1. Anatomical skin structure

One of the major skin functions is to act as an effective barrier between the external environment and the internal milieu. The skin barrier provides protective functions in two directions: a) it minimizes excessive water loss from the organism to the outer atmosphere (known as the inside-outside barrier), and b) it prevents the penetration of environmental hazards including allergens and microorganisms (known as the outside-inside barrier) (Proksch, Folster-Holst, Brautigam, Sepehrmanesh, Pfeiffer and Jensen, 2009).

The skin is composed of three layers: the epidermis, dermis and subcutis. The epidermis is the uppermost layer of the skin (Figure 1)**.** It is a multi-layered, stratified epithelium and is formed by several layers of keratinocytes. The epidermal layers are the stratum basale, the stratum spinosum, the stratum granulosum and the stratum corneum. In the stratum basale, the keratinocytes are undifferentiated and have the ability to proliferate (Strachan and Ghadially, 2008). The stratum spinosum forms on top of basal cell layer and is characterized by numerous desmosomes, which contribute to the appearance of spindle-shaped cells. These cells express an early differentiation marker known as cytokeratin 10. The epidermal differentiation proceeds inside out, and this is obvious by the presence of involucrin (an intermediate differentiation marker) in the upper spinous cell layers but not in the lower ones. The stratum granulosum consists of 3–5 cell layers. These cells are characterized by lamellar bodies and keratohyalin granules, as well as filaggrin and loricrin, which are late differentiation markers. The non-nucleated stratum corneum is the

uppermost layer of the epidermis and consists of corneocytes and intercellular lipids. Each phase of differentiation is characterized by the presence of specific proteins (Kirschner and Brandner, 2012).

The epidermis is very important for skin barrier integrity and cohesion. It forms the physical, chemical/biochemical and adaptive immunological barrier. The physical barrier is principally located in the stratum corneum. The chemical (antimicrobial, innate immunity) barrier is composed of lipids, acids, antimicrobial peptides and macrophages. The immunological barrier consists of humoral and cellular components of the immune system (Proksch, Brandner and Jensen, 2008).

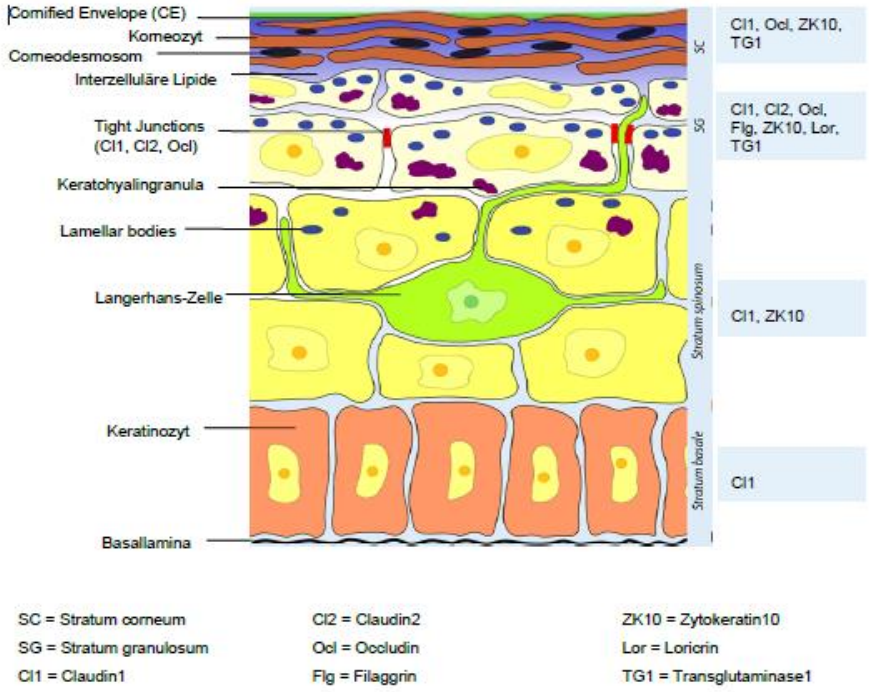

Figure 1: Anatomical structure of the epidermis

Taken from "The barrier function of normal skin. Morphologic and functional aspects of the skin barrier, Gruber R, Schmuth M, Hautarzt, (2014)"

1.3.2. Skin barrier proteins

1.3.2.1. Tight junctions

Tight junctions (TJ) are cell–cell junctions. Ultra-structurally, they are identified as very small subapical regions of direct contact between the plasma membranes of two adjacent cells without any intramembranous material or extracellular gaps ("kissing points") (Brandner, McIntyre, Kief, Wladykowski and Moll, 2003; Farquhar and Palade, 1963).

TJ consist of numerous transmembrane proteins, including members of the large claudin family, occludin and junctional adhesion molecules (JAMs). These proteins interact with cytoplasmic proteins, known as TJ-plaque proteins, including the zonula occludens proteins (ZO-1, ZO-2, ZO-3), cingulin and symplekin through a link to the cytoskeleton (actin filaments) (Aijaz, Balda and Matter, 2006; Brandner, McIntyre, Kief, Wladykowski and Moll, 2003; Kirschner, Bohner, Rachow and Brandner, 2010).

Several studies have indicated the critical functions of TJ in simple epithelia and endothelia: a) TJ form a paracellular barrier and regulate the paracellular permeability for ions, water, larger molecules and inflammatory cells ("barrier function"). In particular, claudins play a major role regarding barrier function. b) TJ proteins develop a physical barrier between the apical and basolateral plasma membrane which prevents the intramembrane diffusion of lipids and proteins ("fence function"). This function is also important for cell polarity. c) TJ play a role in the regulation of gene expression at different cellular levels. They can interact with transcription factors and are involved in translation, cell proliferation and cell differentiation. d) TJ proteins are suggested to have a role in directed vesicular transport (Kirschner, Bohner, Rachow and Brandner, 2010).

More than 24 claudins have been identified in the annotated mammalian genomes (Van Itallie and Anderson, 2006). The name claudin is derived from the Latin word claudere, which means "to close" (Findley and Koval, 2009). Claudins consist of four transmembrane domains: two extracellular loops, a short cytosolic N-terminus and a longer cytosolic C-terminus (Overgaard, Daugherty, Mitchell and Koval, 2011). The first loop affects paracellular charge selectivity, the second extracellular is the receptor for a bacterial toxin, and the C- terminus binds cytoplasmic proteins (Van Itallie and Anderson, 2006).

Claudins are the key structural components of the TJ present on the membranes of epithelial and endothelial cells (Heiskala, Peterson and Yang, 2001). Claudin family proteins interact with each other, as well as with other transmembrane TJ proteins (e.g. occludin) and cytosolic scaffolding proteins (e.g. zonula occludens-1). A key function of claudin family members is the formation of the equivalent of paracellular ion selective channels with specific permeability. Therefore, claudins are essential for the diffusion barrier (Overgaard, Daugherty, Mitchell and Koval, 2011). The most widely expressed TJ protein is claudin-1 (Cldn-1), which is recognized in all epidermal layers (Brandner, Kief, Grund, Rendl, Houdek, Kuhn, Tschachler, Franke and Moll, 2002; Brandner, McIntyre, Kief, Wladykowski and Moll, 2003).

The function of specific claudins can be illustrated by mutations leading to human disorders, many which have been mimicked in mouse knockout models (Findley and Koval, 2009). Pivotal evidence reporting the involvement of claudin in mammalian barrier function was gathered with Cldn-1-deficient mice. Furuse *et al.* demonstrated that the absence of Cldn-1 results in the death of the animals at the first day of birth with wrinkled skin, severe dehydration and increased epidermal permeability, as assessed by transepidermal water loss (TEWL). Interestingly, these mice had no abnormalities in the expression of structural proteins of the stratum corneum (e.g. loricrin, involucrin, transglutaminase-1) or lipids that may explain the severe skin phenotype (Furuse, Hata, Furuse, Yoshida, Haratake, Sugitani, Noda, Kubo and Tsukita, 2002). In humans, the loss of Cldn-1 is not lethal but leads to the NISCH syndrome (neonatal ichthyosis sclerosing cholangitis), which is associated with an impaired barrier function (Hadj-Rabia, Baala, Vabres, Hamel-Teillac, Jacquemin, Fabre, Lyonnet, De Prost, Munnich, Hadchouel and Smahi, 2004). De Benedetto *et al.* demonstrated a reduced expression of Cldn-1 in the non-lesional skin of patients with AD compared with non-atopic subjects. The down-regulation of Cldn-1 expression enhanced paracellular permeability, decreased resistance and increased proliferation, indicating a wound repair response. The decreased expression of Cldn-1 in AD might also enhance the penetration of environmental antigens and thus the triggering of atopic dermatitis lesions (De Benedetto, Rafaels, McGirt, Ivanov, Georas, Cheadle, Berger, Zhang, Vidyasagar, Yoshida, Boguniewicz, Hata, Schneider, Hanifin, Gallo, Novak, Weidinger, Beaty, Leung, Barnes and Beck, 2011; Kirschner and Brandner, 2012).

1.3.2.2. Intermediate filaments of cytoskeleton

Filaggrin is an intermediate filament-associated protein and its main function is to aggregate keratin filaments of the cellular cytoskeleton in the stratum corneum of mammalian epidermis. It is synthesized initially as profilaggrin, a highly phosphorylated polypeptide of approximately 500 kDa. Human profilaggrin consists of an N-terminal S100 calcium-binding domain, a B-domain, and two imperfect filaggrin-repeat domains flanking 10-12 perfect tandem repeats as well as a C-terminal domain. This large precursor protein is the major component of the keratohyalin granules that are found within the granular layer of the epidermis (Gan, McBride, Idler, Markova and Steinert, 1990; McGrath and Uitto, 2008; Presland, Haydock, Fleckman, Nirunsuksiri and Dale, 1992).

The proprotein itself has no keratin-binding activity, but during the terminal differentiation of keratinocytes, keratohyalin granules degranulate in response to increased Ca^{2+} levels, and

profilaggrin is dephosphorylated and proteolysed into multiple filaggrin monomers at the border between the stratum granulosum and stratum corneum. The filaggrin monomers bind to keratin intermediate filaments, thereby leading to their aggregation into macrofibrils (hence the name, filaggrin: filament aggregation protein) (Nishifuji and Yoon, 2013; Sandilands, Terron-Kwiatkowski, Hull, O'Regan, Clayton, Watson, Carrick, Evans, Liao, Zhao, Campbell, Schmuth, Gruber, Janecke, Elias, van Steensel, Nagtzaam, van Geel, Steijlen, Munro, Bradley, Palmer, Smith, McLean and Irvine, 2007).

In the upper stratum corneum, filaggrin is degraded into amino acids (e.g. pyrrolidone carboxylic acid and urocanic acid) by numerous proteases, including caspase 14. The degradation products form as natural moisturizing factors (NMFs) that contribute to the retention of water within corneocytes. This is very important for the osmolarity, flexibility and barrier function of the stratum corneum (Mildner, Jin, Eckhart, Kezic, Gruber, Barresi, Stremnitzer, Buchberger, Mlitz, Ballaun, Sterniczky, Fodinger and Tschachler, 2010; Nishifuji and Yoon, 2013).

Several reports have highlighted the critical role of filaggrin in maintaining a protective skin barrier against the external environment. Loss-of-function mutations in the filaggrin gene (FLG) are found in up to 50% of patients with moderate to severe AD, whereas they are present in up to 10% of the normal population. R501X and 2282del4 are the two most common mutations in populations of European ancestry. Clinically, these mutations are associated with the autosomal dominant skin-scaling disorder ichthyosis vulgaris and represent a strong genetic predisposing factor for the development of atopic dermatitis (Irvine and McLean, 2006; McGrath and Uitto, 2008; Mildner, Jin, Eckhart, Kezic, Gruber, Barresi, Stremnitzer, Buchberger, Mlitz, Ballaun, Sterniczky, Fodinger and Tschachler, 2010; Palmer, Irvine, Terron-Kwiatkowski, Zhao, Liao, Lee, Goudie, Sandilands, Campbell, Smith, O'Regan, Watson, Cecil, Bale, Compton, DiGiovanna, Fleckman, Lewis-Jones, Arseculeratne, Sergeant, Munro, El Houate, McElreavey, Halkjaer, Bisgaard, Mukhopadhyay and McLean, 2006).

However, the exact mechanisms through which FLG mutations contribute to an increased AD risk are not yet entirely understood. Previous studies have shown that individuals with FLG mutations have reduced levels of NMFs, a higher TEWL and an increased risk for persistent dry skin, when compared with non-carriers (Bohme, Soderhall, Kull, Bergstrom, van Hage and Wahlgren, 2012; Jungersted, Scheer, Mempel, Baurecht, Cifuentes, Hogh, Hellgren, Jemec, Agner and Weidinger, 2010; Kezic, Kemperman, Koster, de Jongh, Thio, Campbell, Irvine, McLean, Puppels and Caspers, 2008; Winge, Hoppe, Berne, Vahlquist, Nordenskjold, Bradley and Torma, 2011).

Additionally, if there is a reduction or complete absence of filaggrin expression, the epidermal barrier is impaired and might be penetrated by external allergens and microbes, which are presented to antigen-presenting cells. Consequently, this might initiate the development of chronic skin inflammation and systemic Th2-polarized responses, including atopic asthma (Irvine and McLean, 2006; Kawasaki, Kubo, Sasaki and Amagai, 2011; McGrath and Uitto, 2008; Sandilands, Terron-Kwiatkowski, Hull, O'Regan, Clayton, Watson, Carrick, Evans, Liao, Zhao, Campbell, Schmuth, Gruber, Janecke, Elias, van Steensel, Nagtzaam, van Geel, Steijlen, Munro, Bradley, Palmer, Smith, McLean and Irvine, 2007).

1.3.2.3. Cornified Envelope Proteins

During the final phases of keratinocyte differentiation, a protein/lipid polymer structure, the cornified envelope (CE), is formed just below the plasma membrane of terminally differentiated keratinocytes. It is composed of two parts: a protein envelope and a lipid envelope (McGrath and Uitto, 2008; Proksch, Brandner and Jensen, 2008).

The CE consists of a 10 nm thick layer of several precursor proteins, including loricrin, involucrin, cystatin α, desmoplakin, envoplakin, filaggrin, several keratins, and members of a family of small proline-rich proteins (SPRRPs). These proteins are cross-linked extensively by calcium-dependent transglutaminases (TGases). This cross-linking results in a protein complex that is significantly insoluble and resistant to typical biochemical extraction procedures. In the specific case of the epidermis, a 5 nm thick layer of ceramide lipids is attached to the extracellular surface of the protein layer. This organizes extracellular lipids, leading to the structure of ordered lamellae (Ishida-Yamamoto and Iizuka, 1998; Kalinin, Marekov and Steinert, 2001; Koch, de Viragh, Scharer, Bundman, Longley, Bickenbach, Kawachi, Suga, Zhou, Huber, Hohl, Kartasova, Jarnik, Steven and Roop, 2000; Marekov and Steinert, 1998; Steinert and Marekov, 1995; Wertz, Madison and Downing, 1989).

The lamellar structure of the cornified envelope is essential for the effective physical and water barrier function of the skin as it has the ability to protect from environmental hazards and prevent the loss of water and electrolytes (Kalinin, Marekov and Steinert, 2001; Roop, 1995).

The protein composition of the CE varies between different tissues and body sites. Loricrin is the dominant component of the CE. It comprises up to 70% of the interfollicular epidermis (Hohl, Lichti, Breitkreutz, Steinert and Roop, 1991; Steven and Steinert, 1994). Also, in human foreskin

epidermis, the cytoplasmic two-thirds of the CE is composed of > 85% of loricrin (Steinert and Marekov, 1995).

Loricrin is a glycine-serine-cysteine-rich protein synthesized in the granular layer of the epidermis, where it accumulates in granules before it is integrated into the developing CE (Bickenbach, Greer, Bundman, Rothnagel and Roop, 1995; Steven, Bisher, Roop and Steinert, 1990). The principal function of loricrin is the reinforcement of the CE and the enhancement of its defensive barrier function (Hohl, Lichti, Breitkreutz, Steinert and Roop, 1991). Loricrin also provides flexibility to the CE by its interaction with the keratin intermediate filaments (Yoneda and Steinert, 1993). The association of loricrin with nectin is also important, which provides protection against mechanical stress (Wakamatsu, Ogita, Okabe, Irie, Tanaka-Okamoto, Ishizaki, Ishida-Yamamoto, Iizuka, Miyoshi and Takai, 2007).

Keratinocytes modify their gene expression profile during terminal differentiation. An accurate balance between proliferation and differentiation of keratinocytes is pivotal. Alterations in these coordinated processes, as well as genetic abnormalities in the genes encoding the structure proteins of the CE, can lead to an irregular stratification and keratinization (McGrath and Uitto, 2008). Mutations in the human loricrin gene contribute to a hyperkeratotic, dominantly inherited skin disease known as loricrin keratoderma (also known as Vohwinkel syndrome with ichthyosis), or to a progressive symmetric erythrokeratoderma, or to a congenital ichthyosiform erythroderma (Ishida-Yamamoto and Iizuka, 1998; Schmuth, Fluhr, Crumrine, Uchida, Hachem, Behne, Moskowitz, Christiano, Feingold and Elias, 2004). Loricrin-deficient mice show skin abnormalities, such as congenital erythroderma with a shiny, translucent skin at birth. In addition, the neonatal mutant mice show a higher susceptibility to mechanical stress, which may secondarily lead to the alteration of the barrier function (Koch, de Viragh, Scharer, Bundman, Longley, Bickenbach, Kawachi, Suga, Zhou, Huber, Hohl, Kartasova, Jarnik, Steven and Roop, 2000).

1.3.3. Skin barrier and its disruption upon skin irritation

An acute skin barrier disruption (e.g. after a mechanical injury) leads to the activation of keratinocytes. The stimulated keratinocytes release IL-1α, which triggers the production of other alarm signals and pro-inflammatory mediators. The keratinocyte activation cycle is characterised by alterations in the expression of skin barrier genes, enabling them to repair the damage and maintain skin homeostasis.

The most widely used method to assess the barrier function of the skin is the transepidermal water loss (TEWL) measurement. A skin barrier disruption results in an increased TEWL (Fluhr, Feingold and Elias, 2006).

Previous studies have investigated the impact of skin barrier disruption (caused by tape stripping) on the expression of skin barrier genes (such as Cldn-1, FLG and loricrin). Baek *et al.* showed that the TJ barrier was disrupted directly after stripping and then normalized within one hour after stripping. An up-regulation of mRNA and protein levels of Cldn-1 started one hour after injury (Baek, Lee, Choi, Choi and Lee, 2013). This fast recovery kinetic of the TJ barrier might be a compensation mechanism of the skin to enhance the inside-out permeability function and to maintain homeostasis. Other groups demonstrated a decrease of mRNA expression of FLG (Gerritsen, van Pelt and van de Kerkhof, 1996; Rinaldi, Morita, Wawrzyniak, Dreher, Grant, Svedenhag and Akdis, 2019; Thyssen and Kezic, 2014) and loricrin (Rinaldi, Morita, Wawrzyniak, Dreher, Grant, Svedenhag and Akdis, 2019) after tape stripping. A possible explanation for the down-regulation of FLG expression is the disruption by stripping of the calcium gradient and the reduced Ca^{2+} levels in stratum granulosum (Thyssen and Kezic, 2014). Increased Ca^{2+} levels are essential for the proteolytic conversion of profilaggrin into FLG monomers (Nishifuji and Yoon, 2013; Sandilands, Terron-Kwiatkowski, Hull, O'Regan, Clayton, Watson, Carrick, Evans, Liao, Zhao, Campbell, Schmuth, Gruber, Janecke, Elias, van Steensel, Nagtzaam, van Geel, Steijlen, Munro, Bradley, Palmer, Smith, McLean and Irvine, 2007)

1.4. Interleukin-1 (IL-1) family of cytokines

1.4.1. An overview of IL-1α and IL-1β

lnterleukin (IL)-1 cytokines are members of the interleukin (IL)-1 super family. Two isoforms are identified: interleukin (IL) 1 alpha (IL-1α) and 1 beta (IL-1β).

The receptors for IL-1 cytokines, interleukin-1 receptor type I (IL-1R-I) and interleukin-1 receptor type II (IL-1RII) are structurally related to Toll-like receptors (TLRs). Upon ligand binding, IL-1R accessory protein (known as IL-1RAcP or IL-1R3) is involved in signal transduction pathways (Greenfeder, Nunes, Kwee, Labow, Chizzonite and Ju, 1995). The signal transduction is initiated by IL-1 receptor-associated kinase (IRAK) activation. It is followed by the activation of transcription factors, e.g. nuclear factor kappa B (NF-κB), and signaling by c-Jun N-terminal kinase and p38 mitogen-activated protein kinase (JNK-MAPK) pathway. This results in the subsequent regulation of inflammatory gene expression (Bou-Dargham, Khamis, Cognetta and Sang, 2017). The signal transduction occurs exclusively through the type I IL-1R receptor. The

type II IL-1R is a decoy receptor and does not recruit the proteins which are essential for the signal transduction (Colotta, Re, Muzio, Bertini, Polentarutti, Sironi, Giri, Dower, Sims and Mantovani, 1993). IL-1α and IL-1β are the two principal IL-1 receptor (IL-1R) agonists, while IL-1Ra, the third member of the IL-1 family, represents the natural antagonist. IL-1Ra acts as a physiological inhibitor for the function of both IL-1α and IL-1β and has an anti-inflammatory activity. IL-1 cytokine transcription is induced by several proinflammatory mediators including pathogen-associated molecular patterns (PAMPs) and cytokines, e.g. TNF-α, IFN-α and IFN-β and IL-1β itself (Barksby, Lea, Preshaw and Taylor, 2007).

Like other cytokines of the IL-1 family, IL-1α has the barrel-like single β-trefoil structure, lacks a signal peptide and binds to immunoglobulin-like receptors (Driscoll, Gronenborn, Wingfield and Clore, 1990). The IL1α gene is located on chromosome 2. IL-1α is translated as 31 kDa precursor protein (pro-IL-1α) and is already biologically active in this form. It locates to the cytoplasm, nucleus, or membrane of cells including keratinocytes, monocytes, and B-lymphocytes. A calcium-dependent protease known as calpain processes the pro-IL-1α to its secreted mature 17-kDa form (Dinarello, 1996). The secretion of the mature form is mediated by caspase-1 and inflammasome (Netea, van de Veerdonk, van der Meer, Dinarello and Joosten, 2015) and is dependent on IL-1β (Fettelschoss, Kistowska, LeibundGut-Landmann, Beer, Johansen, Senti, Contassot, Bachmann, French, Oxenius and Kundig, 2011).

According to its location, pro-IL-1α performs different functions. In the nucleus, this pro-form associates with the chromatin and alters the expression of genes involved in cell proliferation, differentiation and inflammation (Dinarello, 2009; Werman, Werman-Venkert, White, Lee, Werman, Krelin, Voronov, Dinarello and Apte, 2004; Yano, Banno, Walsh and Blumenberg, 2008). In keratinocytes, pro-IL-1α mediates processes for hyperproliferation and the formation of inflammatory lesions in skin diseases (Dinarello, 2009; Yano, Banno, Walsh and Blumenberg, 2008). Pro-IL-1α that resides in the membrane of monocytes and B-lymphocytes is responsible for the immunostimulation (Dinarello, 2009). The pro-IL-1α also acts extracellulary as an alarm signal (alarmin). It is released from necrotic cells under stress conditions and leads to the initiation and maintenance of inflammation (Bou-Dargham, Khamis, Cognetta and Sang, 2017).

The isoform IL-1β is encoded by a gene located on chromosome 2. It lacks a signal sequence and is synthesized as 31 kDa precursor protein (pro-IL-1β). This pro-cytokine is biologically inactive (Netea, van de Veerdonk, van der Meer, Dinarello and Joosten, 2015). Pro-IL-1β is cleaved either by an intracellular caspase-1 (also known as IL-1β-converting enzyme) or by an extracellular

neutrophilic protease into the 17 kDa active form. The activation of pro-IL-1β occurs in inflammasomes (Dinarello, 2011; Thornberry, Bull, Calaycay, Chapman, Howard, Kostura, Miller, Molineaux, Weidner, Aunins, Elliston, Ayala, Casano, Chin, Ding, Egger, Gaffney, Limjuco, Palyha, Raju, Rolando, Salley, Yamin, Lee, Shively, Maccross, Mumford, Schmidt and Tocci, 1992). The extracellular IL-1β is the primary isoform that mediates the activation of IL-1R-I on adjacent cells and the alteration of tight junctions (Dinarello, 1996; Dinarello, 2009). It plays an important role in both innate and adaptive immune response.

1.4.2. Role of IL-1α and IL-1β in AD

The production of the first-line IL-1 cytokines by epithelial cells has been described as an initiatory switch which promotes allergic immune responses at epithelial interfaces (Bernard, Carrasco, Laoubi, Guiraud, Rozieres, Goujon, Duplan, Bessou-Touya, Nicolas, Vocanson and Galliano, 2017). Using a model of experimental asthma provoked by house dust mite, Willart *et al.* reported that the production of IL-1α by bronchial epithelial cells induced IL-33 (but not TSLP) secretion and provided Th2 responses that are similar to those in AD-related models (Willart, Deswarte, Pouliot, Braun, Beyaert, Lambrecht and Hammad, 2012).

Several reports have shown that IL-1α and IL-1β regulate the induction and enhancement of inflammatory responses and contribute to the development of skin inflammation in AD. IL-1α initiates the inflammatory processes, while both isoforms propagate and maintain the inflammation by inducing expression of various cytokines, proinflammatory mediators and adhesion molecules (Rider, Carmi, Guttman, Braiman, Cohen, Voronov, White, Dinarello and Apte, 2011). The aeroallergen house dust mite and the hemolysins/lipoproteins of Staphylococcus aureus have been recognized as two major AD triggers. These environmental stimuli interact with TLRs/NLRs (NLRs: Nod-like receptors) and promote the activation of the NLRP3 (NoD-like receptor family, pyrin domain containing 3) inflammasome in keratinocytes, as well as the production of IL-1β by keratinocytes (Craven, Gao, Allen, Gris, Wardenburg, McElvania-TeKippe, Ting and Duncan, 2009; Dai, Sayama, Tohyama, Shirakata, Hanakawa, Tokumaru, Yang, Hirakawa and Hashimoto, 2011; Munoz-Planillo, Franchi, Miller and Nunez, 2009).

Skin barrier disruption can be an additional factor for the IL-1β release from keratinocytes in AD. Elias and Wakefield demonstrated that the reduction of filaggrin levels as well as its acidic derivative in AD results in an increase of pH of the stratum corneum and in the activation of serine proteases. These proteases have an impact on the epidermal remodeling by cleaving the inactive precursor proteins IL-1α and IL-1β into their active forms (Elias and Wakefield, 2011). Along this

line, Kezic *et al.* suggested a connection between FLG mutations and elevated IL-1α and IL-1β levels in the uninvolved skin of AD patients (Kezic, O'Regan, Lutter, Jakasa, Koster, Saunders, Caspers, Kemperman, Puppels, Sandilands, Chen, Campbell, Kroboth, Watson, Fallon, McLean and Irvine, 2012).

Bernard *et al.* reported that IL-1β contributes to the development and/or maintenance of the AD phenotype. Thus, the AD-like epidermal inflammation was illustrated by two observations: i) IL-1β affects the epidermal homeostasis by altering the keratinocyte differentiation, and ii) IL-1β induces the production of key AD mediators including TSLP. First, these authors investigated the impact of IL-1β on the integrity of the epidermal skin barrier and identified a reduced expression of filaggrin, loricrin and Cldn-1. The reduced expression of these proteins within the epidermal differentiation complex (EDC) may contribute to the skin barrier disruption. Second, they investigated the role of IL-1β in the induction of other inflammatory mediators and showed that mainly alone IL-1β activates a robust TSLP production via a NF-κB-dependent pathway. They also suggested that IL-1β may synergize with environmental danger signals to promote the production of TSLP by keratinocytes from lesional AD skin (Bernard, Carrasco, Laoubi, Guiraud, Rozieres, Goujon, Duplan, Bessou-Touya, Nicolas, Vocanson and Galliano, 2017). Because TSLP is the master switch molecule of the Th2 immune response that characterizes AD (Soumelis, Reche, Kanzler, Yuan, Edward, Homey, Gilliet, Ho, Antonenko, Lauerma, Smith, Gorman, Zurawski, Abrams, Menon, McClanahan, de Waal-Malefyt Rd, Bazan, Kastelein and Liu, 2002; Ziegler and Artis, 2010), this observation indicates that IL-1β is involved in Th2 immune responses in AD. Recent evidence in models of Cryopyrin-Associated Periodic Syndromes (CAPS) syndromes have confirmed the direct link between IL-1β and Th2 bias, by demonstrating that the disruption of IL-1R signaling pathway leads to an abrogation of Th2 differentiation (Brydges, Mueller, McGeough, Pena, Misaghi, Gandhi, Putnam, Boyle, Firestein, Horner, Soroosh, Watford, O'Shea, Kastner and Hoffman, 2009). Additionally, in a mouse model with corneodesmosin knockout, the blockade of the IL-1 signaling pathway results in the suppression of TSLP production and in an improvement of the AD-like phenotype (Li, Leyva-Castillo, Hener, Eisenmann, Zaafouri, Jonca, Serre, Birling and Li, 2016).

Moreover, the IL-1α and IL-1β mRNA expression has been shown to be significantly increased in acute and chronic lesions of AD in both extrinsic and intrinsic sub-forms (Bianchi, Ribet, Casas, Lejeune, Schmitt and Redoules, 2012; Gittler, Shemer, Suarez-Farinas, Fuentes-Duculan, Gulewicz, Wang, Mitsui, Cardinale, Strong, Krueger and Guttman-Yassky, 2012; Suarez-Farinas, Dhingra, Gittler, Shemer, Cardinale, de Guzman Strong, Krueger and Guttman-Yassky, 2013). In

addition, the serum levels of IL-1β were higher in AD patients and IL-1β serum levels were reported to positively correlate with AD severity (Nutan, Kanwar and Parsad, 2012). An increased endogenous secretion of IL-1α and an enhanced stimulation of IL-2 receptors in children with food-sensitive AD have also shown that these cytokines are involved in the inflammatory processes in AD (Greally, Hussain, Price and Coleman, 1992).

All these reports point to the pivotal role of IL-1α and IL-1β in the pathogenesis of AD and suggest potential therapeutic approaches in the treatment of AD by targeting the IL-1 pathway.

1.4.3. An overview of IL-18

Interleukin (IL)-18 is a member of the IL-1 superfamily of cytokines and is also known as interferon (IFN)-γ-inducing factor (Okamura, Tsutsui, Komatsu, Yutsudo, Hakura, Tanimoto, Torigoe, Okura, Nukada, Hattori, Akita, Namba, Tanabe, Konishi, Fukuda and Kurimoto, 1995). IL-18 was initially identified as an "IFN-γ-inducing factor" from the murine liver (Okamura, Nagata, Komatsu, Tanimoto, Nukata, Tanabe, Akita, Torigoe, Okura, Fukuda and Kurimoto, 1995). The IL-18 gene is located on chromosome 9 in mice and on chromosome 11 in humans. IL-18 is produced by keratinocytes and inflammatory dendritic epidermal cells (Novak, Valenta, Bohle, Laffer, Haberstok, Kraft and Bieber, 2004). This cytokine shares similarities with members of the IL-1 family, especially IL-1β and IL-33, in terms of structure, receptor utilization and cytokine processing. Like other cytokines of the IL-1 family, IL-18 lacks a signal peptide and is therefore produced and stored in the intracellular space as an inactive precursor protein, the pro-IL-18. This pro-form is cleaved to produce the biologically active IL-18 by canonical protease caspase-1 as well as by non-canonical proteases including mast cell chymase, granzyme B and neutrophil-derived proteinase-3 (Lee, Cho and Park, 2015; Sedimbi, Hagglof and Karlsson, 2013; Zedan, Rasheed, Farouk, Alzolibani, Bin Saif, Ismail and Al Robaee, 2015).

1.4.4. Role of IL-18 in AD

IL-18 is involved in a variety of inflammatory skin diseases, including AD. A Th2 response producing IL-4 and IL-13, followed by a Th1 response, is thought to play a major role in the pathogenesis of AD (Lee, Cho and Park, 2015; Sedimbi, Hagglof and Karlsson, 2013). Numerous reports have highlighted a pleiotropic role for IL-18. This cytokine seems to be a main mediator in both innate and acquired immune responses (Okamura, Tsutsui, Kashiwamura, Yoshimoto and Nakanishi, 1998). The effect of IL-18 depends on the surrounding environment of cytokines (Figure 2). IL-18, in the presence of IL-12 or IL-15, stimulates the Th1 response that induces IFN-

γ production and inhibits the production of IgE from B cells. However, in the absence of IL-12, IL-18 increases IgE production and promotes the Th2 response that enhances the production of Th2 cytokines such as IL-4, IL-5, IL-9 and IL-13 by basophils, mast cells and helper T cells ($CD4^+$ T cells) (Yoshimoto, Tsutsui, Tominaga, Hoshino, Okamura, Akira, Paul and Nakanishi, 1999).

Tsutsui *et al.* reported that IL-18 directly stimulates mast cells, resulting in the release of chymase. This mast cell chymase cleaves the pro-IL-18 and accelerates the inflammatory responses in AD skin lesions. These authors also reported that cytotoxic T lymphocytes ($CD8^+$ T cells) promote the production of granzyme B, leading to the activation of keratinocyte-derived pro-IL-18. Additionally, IL-18 stimulates CD1d-dependent natural killer T (NKT) cells. These cells are the principal source of the IL-4 that induces the production of IgE in B cells (Tsutsui, Yoshimoto, Hayashi, Mizutani and Nakanishi, 2004).

Furthermore, the yeast Malassezia furfur (a known trigger of AD) stimulates peripheral blood monocyte-derived dendritic cells, resulting in an increased production of IL-18 along with TNF-α and IL-1β (Buentke, Heffler, Wallin, Lofman, Ljunggren and Scheynius, 2001). Previous studies demonstrated that the expression of IL-18 increases after an exposure to allergens (e.g. house dust mite) and to lesional infections. Therefore, IL-18 contributes to the development of Staphylococcus aureus-associated AD in mouse models and humans (Inoue, Aihara, Kirino, Harada, Komori-Yamaguchi, Yamaguchi, Nagashima and Ikezawa, 2011b; Terada, Tsutsui, Imai, Yasuda, Mizutani, Yamanishi, Kubo, Matsui, Sano and Nakanishi, 2006). Moreover, several studies reported elevated levels of IL-18 in serum from AD patients and in AD mouse models, as well as a positive correlation of serum IL-18 with AD severity (Tanaka, Tsutsui, Yoshimoto, Kotani, Matsumoto, Fujita and Wang, 2001; Zedan, Rasheed, Farouk, Alzolibani, Bin Saif, Ismail and Al Robaee, 2015). All these observations and reports have highlighted the role of IL-18 in the pathogenesis of AD.

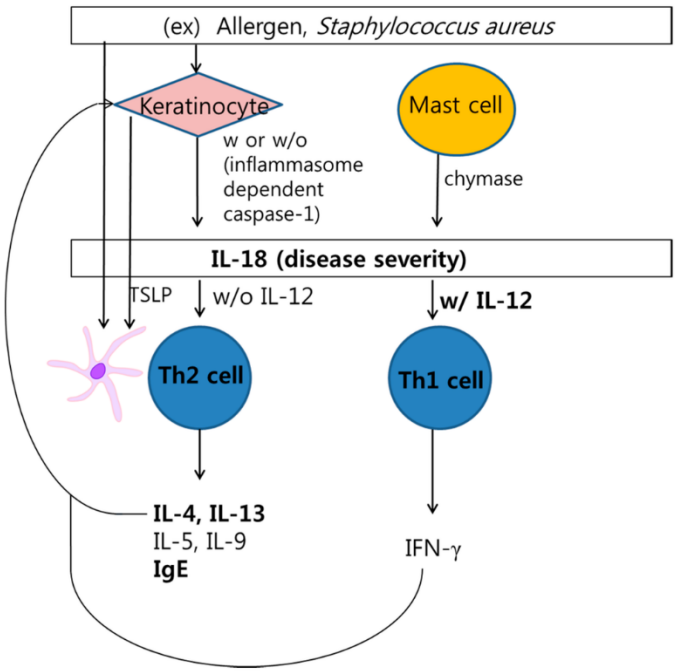

Figure 2: The role of IL-18 in the pathogenesis of AD

Taken from "IL-18 and Cutaneous Inflammatory Diseases, Ji hyun Lee, Dae Ho Cho and Hyun Jeong Park, International journal of molecular sciences (2015)"

1.4.5. An overview of IL-33

The cytokine IL-33 is a member of the IL-1-family and found to be expressed in various tissues and cells, including keratinocytes, mast cells, purified dendritic cells, endothelial cells and immune cells. IL-33 is also classified as a member of the "alarmin" defense system due to its release upon cellular stress and stimulation of the immune system (Gadina and Jefferies, 2007; Haraldsen, Balogh, Pollheimer, Sponheim and Kuchler, 2009; Pushparaj, Tay, H'Ng S, Pitman, Xu, McKenzie, Liew and Melendez, 2009). The caspase-1-dependent cleavage of pro-IL-33 results in the release of IL-33 as an active cytokine. The IL-33 receptor complex (membrane-type) is comprised of ST2 (suppression of tumorigenicity 2) and IL-1RAP (interleukin-1 receptor accessory protein). Besides the transmembrane isoform of ST2 (ST2L), there is also a shedded one. This second type is known as soluble ST2 (sST2) and acts as "decoy" receptor (Savinko, Matikainen, Saarialho-Kere, Lehto, Wang, Lehtimaki, Karisola, Reunala, Wolff, Lauerma and Alenius, 2012).

1.4.6. Role of IL-33 in AD

Mechanical disruption/scratching, allergens, bacteria, exogenous proteases and other trigger factors induce the production of IL-33, IL-25 and TSLP from keratinocytes. IL-33 acts alone or

together with other cytokines and activates mast cells, dendritic cells and eventually lymphocytes in skin inflammation. The activation of mast cells results in the modulation of the function of dendritic cells, eosinophils and T cells. This modulation occurs via the secretion of different cytokines, chemokines, growth factors, proteases and leukotrienes. Dendritic cells regulate the function of Th2 cells and B cells (at an early stage) as well as the function of Th1 cells (probably at a later stage). These regulations occur via the IL-33-induced production of IL-25 and IL-18. Moreover, the production of Th2-associated mediators after IL-33 stimulation indicates the pivotal role of IL-33 in Th2-associated diseases such as AD. Another cell type verified to regulate Th2-driven immune responses in many organs are innate lymphoid cells (ILCs). They are a key link between the interaction among innate and adaptive immune responses. Although the potential role of ILCs in Th2 regulation within the skin is not completely understood, these cells may promote the enhancement of the Th2 immune response pathway in the skin. The interplay between the immune cells and their cell-specific secretion products demonstrates the potential role of IL-33 in inflammatory skin diseases and the pathophysiology of atopic dermatitis (Figure 3) (Carmi-Levy, Homey and Soumelis, 2011; Cevikbas and Steinhoff, 2012).

IL-33 exerts its effects by binding and activating its receptor complex (ST2L/IL-1RAP) on various types of cells. The expression of the transmembrane ST2 form of the IL-33 receptor is highest on mast cells, Th2 cells and eosinophils that are crucial cells in the pathogenesis of AD (Savinko, Matikainen, Saarialho-Kere, Lehto, Wang, Lehtimaki, Karisola, Reunala, Wolff, Lauerma and Alenius, 2012). Previous reports demonstrated a critical role of this IL-33/ST2 pathway in epithelial integrity, inflammation and allergic immune responses. The activation of the transmembrane ST2 receptor on keratinocytes, mast cells or other immune cells leads to the activation of several signal proteins including NF-κB (Ali, Mohs, Thomas, Klare, Ross, Schmitz and Martin, 2011; Funakoshi-Tago, Tago, Sato, Tominaga and Kasahara, 2011). These signaling pathways regulate the expression of factors that are involved in skin inflammatory diseases (Liew, Pitman and McInnes, 2010; Pushparaj, Tay, H'Ng S, Pitman, Xu, McKenzie, Liew and Melendez, 2009). The IL-33/ST2-induced immune regulation is involved in both innate and adaptive immunity in skin and has an important role in the pathogenesis of AD. However, recent reports pointed to the inhibitory abilities of the other form of IL-33 receptor, soluble ST2, in inflammatory diseases and therefore the anti-inflammatory role of the IL-33/ST2 pathway. Thus, IL-33 is a unique cytokine which is involved in pro- as well as anti-inflammatory processes during different stages of immune response (Cevikbas and Steinhoff, 2012).

Additionally, recent evidence has shown that IL-33 impairs the transcription of various skin barrier genes such as filaggrin and down-regulates the expression of some structural proteins in the epidermis. Furthermore, this cytokine reduces the epidermal growth, leading to an impaired formation and barrier function of the stratum corneum. These findings have demonstrated a key role of IL-33 in the intraepidermal pathophysiology of AD (Nygaard, Van Den Bogaard, Niehues, Hvid, Deleuran, Johansen and Vestergaard, 2017).

Moreover, several studies have shown that IL-33 expression is up-regulated in the epidermis of AD patients (Oboki, Ohno, Kajiwara, Arae, Morita, Ishii, Nambu, Abe, Kiyonari, Matsumoto, Sudo, Okumura, Saito and Nakae, 2010; Savinko, Matikainen, Saarialho-Kere, Lehto, Wang, Lehtimaki, Karisola, Reunala, Wolff, Lauerma and Alenius, 2012). Nygaard *et al.* reported that IL-33 serum levels were significantly increased in AD patients in comparison to HC. In addition, serum levels of IL-33 were higher in children than in adult AD patients (Nygaard, Hvid, Johansen, Buchner, Folster-Holst, Deleuran and Vestergaard, 2016).

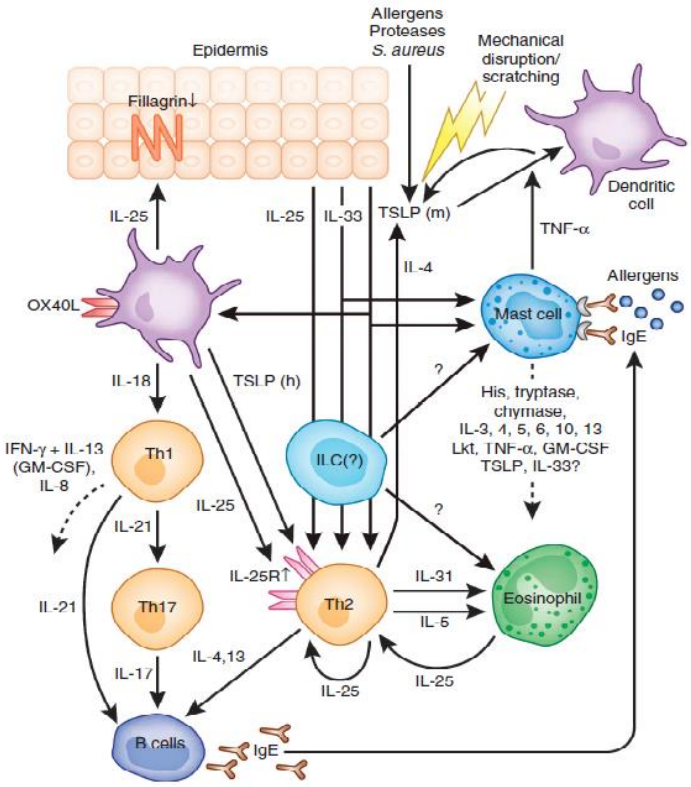

Figure 3: Potential role of IL-33 in skin inflammation and pathophysiology of AD

Taken from "IL-33: A Novel Danger Signal System in Atopic Dermatitis, Ferda Cevikbas and Martin Steinhoff, J Invest Dermatol (2012)"

1.4.7. Role of IL-1 family of cytokines upon skin irritation

The physical epidermal barrier and inflammatory epidermal barrier are both influenced by tape stripping. Skin irritation using the method of tape stripping leads to an increased penetration of exogenous stimuli. In addition, it results in an enhancement of skin immunoreactivity by inducing mRNA expression of various cytokines including members of IL-1 family (Dickel, Gambichler, Kamphowe, Altmeyer and Skrygan, 2010). The production of IL-1α by stimulated keratinocytes upon skin irritation is regarded to be one of the primary alarm signals and further stimulates the release of other pro-inflammatory mediators such as IL-1β, TNF-α, IL-6 and IL-8 by other cells. Additionally, Redhu *et al.* reported that the physical skin irritation (by tape stripping) results in TSLP production by simultaneous activation of the IL-1 pathway (Redhu, Franke, Kumari, Francuzik, Babina and Worm, 2020).

In mice, several studies demonstrated the role of the IL-1 family of cytokines upon skin irritation. Wood *et al.* investigated the epidermal expression of IL-1 after barrier disruption in a mouse model, reporting that the mRNA levels of IL-1α and IL-1β increased at 2.5 and 7 h after tape stripping and returned to normal levels after 18 h. They also investigated the expression levels of these cytokines in the dermis. In contrast to IL-1α, the dermal IL-1β mRNA was increased at 2.5, 7 and 18 h following barrier disruption (Wood, Stalder, Liou, Campbell, Grunfeld, Elias and Feingold, 1997). In addition, Ye *et al.* demonstrated that the acute disruption of the permeability barrier by tape stripping in a mouse model enhanced the production of several cytokines, including IL-1α (Ye, Garg, Calhoun, Feingold, Elias and Ghadially, 2002). Additionally, Wood *et al.* reported that the acute barrier disruption by tape stripping stimulates IL-1α expression and induces the release from a pre-formed pool in murine skin. They show that IL-1α increased in murine epidermis and dermis within 10 min, continued to be elevated at 2 and 4 h, and returned to basal levels after 24 h (Wood, Elias, Calhoun, Tsai, Grunfeld and Feingold, 1996). A recent study in mice indicated that the disruption of the epidermal barrier induces an up-regulation of IL-33 transcription, as well as an increase of IL-33 protein expression in the epidermis (Bruhs, Proksch, Schwarz and Schwarz, 2017).

In humans, Nickoloff and Naidu analyzed the mRNA expression of various mediators that are involved in the regulation of inflammation after acute barrier disruption. The test site was buttock skin of healthy individuals. They found an increase in several epidermal mRNAs (e.g. TNF-α, IL-8, IL-10) 6 h after repeated tape stripping (80–100 times). IL-1β mRNA was present at time 0 and at 1 h, but was not detectable 6 h after tape stripping (Nickoloff and Naidu, 1994). In one other *in vivo* human study, Dickel *et al.* reported the elevated expression of epidermal mRNAs including

IL-33 mRNA expression, 6 h after tape stripping with calculated numbers of tape strips on the upper back skin of healthy volunteers. In addition, this study demonstrated an up-regulation of IL-1β mRNA expression 6 h after tape stripping down to the stratum lucidum. The appearance of the stratum lucidum indicates the almost complete removal of the stratum corneum. This finding suggests that the sensitization by tape stripping procedure might be increased by the over-intense epidermal barrier disruption. However, the mRNA expression of IL-1α was not up-regulated at the investigated time point, regardless of the number of tape strips (Dickel, Gambichler, Kamphowe, Altmeyer and Skrygan, 2010). Reilly and Green studied the effects of physical irritants on the profile of acute inflammatory mediators in healthy human skin. They demonstrated that the levels of IL-1α protein were significantly increased in tape-stripped human skin (Reilly and Green, 1999). More recently, Doge *et al.* investigated the biochemical changes of *ex vivo* human skin in response to physical barrier disruption, reporting that IL-1α protein levels were increased directly after 50 times tape stripping and decreased over time (Doge, Avetisyan, Hadam, Pfannes, Rancan, Blume-Peytavi and Vogt, 2017).

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Antibodies

Table 1: List of antibodies

Antibody	Species	Clonality	Supplier	Catalog Number
Anti-human Claudin1	Rabbit	Polyclonal	ThermoScientific	PA5-32350
Anti-human Filaggrin	Mouse	Monoclonal	Abcam	ab17808
Anti-human Loricrin	Rabbit	Polyclonal	Abcam	ab85679
Anti-human IL-1α	Mouse	Monoclonal	LSBio	LS-B1581
Anti-human IL-1β	Rabbit	Polyclonal	Bioss	bs-0812R
Anti-human IL-18	Rabbit	Polyclonal	LSBio	LS-C313397
Anti-human IL-33	Goat	Monoclonal	R&D Systems	MAB32653

2.1.2 Reagents and Chemicals

Table 2: List of reagents and chemicals

Reagent	Supplier	Catalog Number
Antibody diluent (Dako REALTM)	DAKO Diagnostika	S0809
Avidin/Biotin Blocking Kit	Vector Laboratories, Inc.	SP-2001
β-Mercaptoethanol	Sigma-Aldrich	M6250
Bovine serum albumin (BSA)	PAA	K45-001
Chloroform	J.T. Baker	7386
DNAse	Macherey-Nagel	740.955250
dNTP-Mix-10	Applied Biosystems	N8080235
En Vision+ System-HRP(AEC)	Dako	K-4005
Ethanol	J.T. Baker	8025
GenTherm DNA Polymerase	Rapidozym GmbH	GC-002-0250
Glycoblue	Ambion	#AM9515
Hydrogen peroxide (H2O2)	Sigma-Aldrich	216763
Isopropanol	Merck	4022536533339
Linola® Sept	Dr. Wolff	4076.00.00
Rotor-Gene SYBR Green PCR Master Mix	Qiagen	204074
LSAB2 System-HRP	Dako	K0675
NucleoSpin® RNA II	Macherey-Nagel	740955.250
PBS	GE Healthcare	H15-002
Peroxidase block	Dako	S2001

Proteinase K	Macherey-Nagel	740506
REPLI-g WTA Single Cell Kit	Qiagen	150065
TaqMan® Reverse transcription reagents	Applied Biosystems	N8080239
Transcriptor High Fidelity cDNA Synthesis Kit	Roche	05081963001
Trizol	Invitrogen	15596-026
Tween 20	Sigma-Aldrich	P1379-500ML
Xylol	Roth	9713.3
Water	Braun	2351744

2.1.3. Materials

Table 3: List of materials

Material	Supplier	Catalog Number
Biosphere® Filter Tips 0.5-20 µL 2-100 µL 100-1000 Ml	Sarstedt	 70.1116.210 70.760.212 70.762.211
Conical tube,15 mL	BD FalconTM	352096
Conical tube, 50 mL	BD FalconTM	352070
Curette Stiefel, 4mm and 7mm	SmithKline Beecham Ltd, UK	05.SF004.11/18
Micro tube, 0.5 mL	Sarstedt	72.699
Micro tube, 1.5 mL	Sarstedt	72.690.001
Micro tube, 2 mL	Sarstedt	72.691
Lancets	ALK-Abelló	760-14-J17E, CE 0297
Plastic wrap	Toppits	4008871200846
Precellys Steel Kit 2.8 mm	Peqlab	91-PCS-MK28
Quality Tips without filter 10 µL 200 µL 1000 µL	Sarstedt	 70.1130 70.760.002 70.762
Serological Pipet 5 mL 10 mL 25 mL	BD FalconTM	 357543 357551 357525
Strip Tubes/Caps for qPCR 0.1 ml	Qiagen, Hilden	154050616
3M™ Blenderm™ surgical tape	Neuss, Germany	1525-1

2.1.4. Instruments

Table 4: List of instruments

Instrument	Supplier	Type
Centrifuge	Thermo Scientific, Schwerte	Megafuge 1.0R
Freezer (−80°C)	Heraeus Holding, Hanau	Hera Freeze
Freezer (−20°C) Refrigerator (4°C)	EUREKA, Bonn	TKF380
Hard rubber roller	SIGA company	SKU-5052
Inverted Reflected-Light Microscope	Zeiss, Jena	Zeiss Axiovert 10
Microplate reader	Dynex Technologies, Chantilly	Dynatech MRX
Multipipette	Eppendorf, Hamburg	Multipipette® plus
Pipette	Eppendorf, Hamburg	Eppendorf Reference®/ Research®
Pipettor	Hirschmann Laborgeräte, Eberstadt	Pipetus standard
PCR machine	Thermo Electron Corporation	Px2 Thermal Cycler
Power Supply	BioRad, Munich	POWER PAC 300
Thermocycler for PCR	Qiagen, Hilden	Rotor Gene Q
Spectrophotometer	DeNovix, USA	DS-11
Tabletop centrifuge with refrigeration	Eppendorf, Hamburg	Centrifuge 5417C
Tabletop centrifuge	Eppendorf, Hamburg	Centrifuge 5417R
Tewameter® TM 300	Courage and Khazaka electronic, Cologne	TM 300
Thermomixer	Eppendorf, Hamburg	Thermomixer comfort
Tissue homogenizer	Bertin Technologies, Montigny-le-Bretonneux	Precellys 24
Vortexer	Heidolph, Schwabach	REAX 2000

2.1.5. Software

Table 5: List of software

Software	Developer	Version
Axio Vision	Carl Zeiss AG, Oberkochen	4.6.3
Endnote® Basic	Thomson Reuters Corporation, New York City, USA	X7.0
Microsoft Excel	Microsoft Corporation, Redmond/Washington, USA	2013
Microsoft Word	Microsoft Corporation, Redmond/Washington, USA	2013
Prism	GraphPad, La Jolla/ California, USA	6.0
Rotor Gene Q Software	Qiagen, Hilden	2.2.3.11

2.2. Methods

2.2.1. Subjects

2.2.1.1. Recruitment, groups and medical history of subjects

This study was conducted between May 2016 and July 2017 according to the principles of the Declaration of Helsinki, and was approved by the Charité's Ethics Committee (application number: EA1/194/14). Written informed consent was obtained from all participants prior to the study. The study included 36 AD patients and 19 non-atopic, healthy controls (HC) from the Comprehensive Allergy Centre of the Department of Dermatology, Venerology and Allergology of Charité-Universitätsmedizin Berlin. All the participants were interviewed for their personal and family history of atopy and a clinical examination was performed by a dermatologist (myself). The 36 patients showed atopic eczema/dermatitis syndrome (AEDS) as defined by the criteria of Hanifin and Rajka.

Non-atopic, healthy subjects were defined as having no personal or family history of allergic diseases, no personal history of chronic systemic or skin diseases, and a serum total IgE that was ≤ 2 SD of age-dependent norms. Some healthy subjects also presented some allergic symptoms of less clinical relevance. In 6 HC out of 19 subjects with positive skin prick test an atopy score was assessed, as inaugurated and validated by Diepgen *et al.* (Diepgen and Fartasch, 1992; Diepgen, Fartasch and Hornstein, 1989; Diepgen, Sauerbrei and Fartasch, 1996). It became generally

accepted that an individual with a diagnostic atopy score less than 10 is considered to have no "atopic skin diathesis" (Berndt, Hinnen, Iliev and Elsner, 1999) and thereby participated in our study as HC. This atopy scoring system is based on anamnestic and clinical criteria of atopic symptoms and skin signs that are summarized as follows: family history of atopic march (first-degree relatives), personal history of allergic rhinitis or asthma, cradle cap in the newborn period, pruritus when sweating, wool or metal intolerance (e.g. nickel sensitivity), photophobia, pityriasis alba, xerosis, ear rhagades, dyshidrosis/pompholyx, hyperlinearity in the palms, atopic winter feet, nipple eczema, perlèche, Hertoghe's sign, dirty neck, keratosis pilaris, acrocyanosis, white dermographism, total serum IgE>150 U per ml, and positive test for IgE-mediated allergy (Diepgen, Fartasch and Hornstein, 1989; Diepgen, Sauerbrei and Fartasch, 1996).

All subjects who were recruited in the study fulfilled the inclusion and exclusion criteria. Exclusion criteria were as follows: age ≤ 18 years and ≥ 60 years, coagulation disorders and/or the use of anticoagulant medication within 7 days before the biopsies, severe concomitant diseases (e.g. patients with severe cardiovascular conditions or severe endocrinological and metabolic disorders), treatment with immunosuppressive/immunomodulating drugs (e.g. systemic steroids, cyclosporine, azathioprine, methotrexate, mycophenolate-mofetil, Janus kinase inhibitors, IFN-γ, etc.) as well as with phototherapy for AD within 28 days before the biopsies, use of topical corticosteroids (TCS) or topical calcineurin inhibitors (TCI) within 14 days or with moisturizers containing urea at the site of biopsy within 7 days before the biopsies, pregnant or breastfeeding women, and participation in another interventional clinical study at the same time.

2.2.1.2. Assessment of AD severity (SCORAD and TIS Score)

The SCORAD (SCORing Atopic Dermatitis) is one of the best clinical tools that has been developed to standardize the evaluation of the extent and severity of AD. This severity index was created and validated by the European Task Force on atopic dermatitis in 1993. The SCORAD is widely used in clinical practice and in clinical research, and the assessment is composed of 3 parts: A=extent or affected Body Surface Area (BSA), B=severity, and C=subjective symptoms. To determine the extent of AD, the rule of nines is applied to evaluate the affected area as a percentage of each defined body area. The extent (A) is reported as the sum of all affected areas and has a maximum score of 100%. The intensity part of the SCORAD system includes 6 parameters: erythema, oedema/papulation, excoriations, lichenification, oozing/crusting and dryness (dryness was evaluated on the skin not affected by eczema). The severity of each parameter can be graded on a scale from 0 to 3 as follows: none (0), mild (1), moderate (2), or severe (3). The intensity is

assigned as “B” in the SCORAD calculation and has a maximum of 18 total points. The two most representative symptoms affecting the quality of life of AD patients (throughout the 3 previous days and nights) are itch and sleeplessness. Subjective assessment (C) of the intensity of pruritus and insomnia is recorded by the patient on a visual analogue scale, where 0 is no pruritus or insomnia and 10 is the worst imaginable pruritus or insomnia. This parameter has a maximum possible score of 20. The SCORAD index formula is: A/5 + 7B/2 + C, where the maximum score is 103. A SCORAD index below 25 indicates a mild form of AD, between 25 and 50 is classified as moderate AD, and above 50 is evaluated as a severe form of AD.

The TIS score (Three Item Severity score) is a simple system and is based on the evaluation of only 3 intensity parameters (erythema, oedema and excoriation) on a scale from 0 to 3. The range of the TIS score lies between 0 and 9. Typically, a representative area of atopic lesion is selected. In our study, we evaluated the TIS score of the lesional area where we subsequently performed the skin biopsy. In this area, the intensity of each of these three parameters was assessed as none (0), mild (1), moderate (2) or severe (3) (Oranje, Glazenburg, Wolkerstorfer and Spek, 2007; Wolkerstorfer, Van der Spek, Glazenburg, Mulder and Oranje, 1999).

2.2.1.3. Functional skin barrier assessment (TEWL)

A determined evaporation of water from the skin surface occurs as part of the normal skin metabolism. Even a slight damage in the skin barrier function can increase the water loss. The measurement of transepidermal water loss (TEWL) is an indispensable parameter for the evaluation of the epidermal permeability barrier function. Tape stripping is a convenient irritation method and is widely used for the induction of barrier disruption, which leads to an increase of TEWL. Numerous studies indicate the development of impaired barrier function and subsequently of an elevated TEWL in both lesional and non-lesional skin in atopic dermatitis (Alexander, Brown, Danby and Flohr, 2018; Fluhr, Feingold and Elias, 2006).

For the assessment of the TEWL in our study, we used the Tewameter® TM 300 measuring device, which is an open chamber TEWL machine. The density gradient of the water evaporation was measured indirectly by two pairs of sensors (i.e. sensors of temperature and relative humidity inside the hollow cylinder). The values were analysed by a microprocessor and expressed in g/h/m².

After approximately 20 minutes of acclimatization time, the Tewameter® probe was applied with moderate pressure on the skin of the subject. The process lasted until the TEWL value achieved a

stable plateau and took place in a room with well-maintained air-conditioning. We took TEWL measurements from lesional skin as well as from non-lesional skin before tape stripping, directly after tape stripping (0h), and three hours after tape stripping, in order to monitor the changes in TEWL. The participants were advised not to apply emollients to skin for 12 hours before the reading was taken.

2.2.1.4. Skin Prick Testing and blood samples

To determine the allergy status of the patients, skin prick testing (SPT) was performed and total IgE levels in serum were measured.

SPT is an essential test procedure which can help to prove the diagnosis of a suspected type I sensitization. This reliable method was used in our study to confirm or rule out an IgE sensitization in subjects with atopic dermatitis. SPTs were carried out on the inner part of the forearm of the participants (36 AD patients and 19 HC). First, a numbered tape was placed vertically on the forearm. Each number corresponds to a different allergen which was going to be tested. The distance between two skin prick tests should be at least 2 cm to avoid false-positive reactions due to direct contamination of an adjacent allergen extract. One small droplet of each allergen solution, including the positive and negative controls, was placed adjacent to the corresponding numbers on the tape. SPT was performed using the following allergens: birch pollen, grass pollen, house dust mite (Dermatophagoides pteronyssinus), cat dander and mould (Alternaria alternata). A histamine solution was used as a positive and a saline solution as a negative control. A sterile prick lancet was used to prick the allergen into the skin at each droplet. A new lancet was used for each allergen to avoid cross-contamination from the previous allergen tested. When all the drops had been pricked, the solutions were carefully blotted off with a clean tissue. The test results were assessed 15–20 minutes after the application. We first measured positive and negative controls. The histamine control should be positive and become itchy, red and swollen with a wheal in the centre. The negative control should reveal no response to exclude the presence of urticarial dermographism. Afterwards, the size of the wheal of each particular test was measured with a scale. A wheal diameter of greater than 3mm was considered a positive response. The wheal which is a raised, red, itchy bump with a surrounding “flare”, indicates the presence of the IgE antibody when the person is exposed to specific allergens. We kept also a chart and recorded the wheal size in numerical form (millimetres) next to each allergen name (Heinzerling, Mari, Bergmann, Bresciani, Burbach, Darsow, Durham, Fokkens, Gjomarkaj, Haahtela, Bom, Wohrl, Maibach and Lockey, 2013).

On the day the study was carried out, we took blood from each patient (1 serum tube and 1 EDTA tube). Serum was stored at -80 °C and ETDA was stored at -20 °C until further analysis. Total serum IgE levels were measured by ELISA.

2.2.1.5. Physical irritation of the skin

Tape stripping is a widely accepted method for the induction of a controlled barrier disruption of the stratum corneum. This technique is minimally invasive and *in vivo* is used to sequentially remove the layers of the stratum corneum by a repeated application of adhesive tapes. Several studies suggest that at the flexor surface of the forearm, about 30 times tape stripping is required to strip off most of the horny layer. The impairment of the skin barrier results in a 20- to 25-fold increase of the TEWL and induces the production of different inflammatory mediators.

In our study, tape stripping was carried out on the forearm of all subjects and the selected skin area was eczema free. A transparent, latex free, hypoallergenic tape (3M™ Blenderm™ surgical tape, Neuss, Germany; metric 25 mm × 4.5 m) was used. The location of skin irritation was marked with a skin marker pen, and the adhesive tape was sequentially applied and removed on the test skin area. Before the removal of the tape, a small piece of clean white paper was placed above the tape and the area was rolled 20 times with moderate strength using a roller. With this method, a standardized trauma of the skin was provided. The tape stripping was performed until the appearance of stratum lucidum (break-off criterion: three small glistening spots). This indicates the almost complete removal of the stratum corneum. All the patients underwent tape stripping 30 times, except 5 AD patients and 2 HC who were tape stripped 40 times. The application of 40 times tape stripping was based on clinical signs (i.e. inadequate erythema or papulation) and/or on an insufficient increase of TEWL. To evaluate the damage of the epidermal skin barrier, TEWL was measured before tape stripping, directly after tape stripping and three hours after tape stripping.

2.2.1.6. Skin biopsies (curettage and punch biopsies)

Two methods were used for this study. In the first, punch biopsies were obtained from the subjects; in the second, the curettage method was performed. The first method was used in 6 AD patients and 6 HC. The second method was used in 32 AD patients and 17 HC. In 2 AD patients and in 4 HC both methods were performed, but at different time points (days).

Two punch biopsies (one from non-lesional skin before skin irritation and one from non-lesional skin three hours after the irritation) of 4 mm were taken from each subject after local anaesthetic

injection and standard aseptic technique from the flexor surface of the forearm. One additional punch biopsy was carried out on the lesional skin of atopic dermatitis patients. Typically, this third biopsy was also obtained from the skin of the forearm. When this was unattainable, an AD lesion in another area was chosen. The punch biopsies were performed by using a sterile 4 mm skin punch. Each 4 mm punch biopsy was divided vertically in two pieces. One half was placed in neutral buffered formalin (10%) for at least 6 hours, embedded in paraffin and prepared for the immunohistochemistry. The other half was immediately snap-frozen in liquid nitrogen and stored at −80°C before RNA isolation was performed.

The curettage method is the procedure of scraping skin with a spoon-shaped instrument (curette) to remove skin tissues. A local anaesthetic cream was applied to each biopsy site (non-lesional skin, non-lesional skin three hours after tape stripping and lesional skin) prior to curettage. Subjects with a known allergy to local anaesthetics were not allowed to participate. The topical anaesthetic formulation we used for the local anaesthesia was EMLA cream, which contains 25 mg lidocaine and 25 mg prilocaine. A thick layer of EMLA (approximately 1 g) was applied to the skin of each biopsy site under an occlusive dressing. The intended application time of the cream was at least one hour to achieve a reliable anaesthesia and reduce the pain for the subsequent procedure. In patients with atopic dermatitis, the application of EMLA may result in an increased incidence of local vascular reactions with erythema, and in some cases petechia and purpura. Therefore, a shorter application time was performed on the atopic skin of patients. The curettage method was started immediately after the removal of the cream and the standard aseptic technique by using a 4 mm or 7 mm round curette blade. From each biopsy site, the upper layer of epidermis of two small areas (with a diameter of approximately 4 mm) were scratched off until bleeding occurred. Finally, the wounds were covered with a dressing for a better healing process and for reducing the risk of infection. Each sample was placed directly in trizol and stored at -80°C before RNA isolation.

2.2.2. Enzyme-linked Immunosorbent Assay (ELISA)

Serum samples were obtained from clotted blood after centrifugation at 14,000 rpm for 10 min. Next, serum samples were stored at -80°C until further analysis. ELISA is an enzyme-linked immunosorbent assay for the quantitative detection of antigens in serum. In this study, human ELISA kits were used according to the manufacturer's protocol, to determine serum levels of total IgE.

2.2.3. RNA isolation from the curettage samples

After performing the curettage, the samples were directly placed in 1.5 ml tubes containing 1 ml trizol reagent and stored at -80°C. For the RNA isolation, 200 µl of chloroform were added to each sample, vortexed vigorously for 15 s and incubated for five minutes at room temperature. The samples were centrifuged at 20,000 x *g* for 15 minutes at room temperature. After the centrifugation, 3 phases are detectable: the aqueous phase (upper layer) which includes RNA, the interphase (middle layer) containing the DNA, and the organic phase (lower layer) containing the protein. The aqueous phase (300 µl) was transferred into a new tube and 2 µl of GlycoBlue (30 ng) were added. Next, 500 µl of isopropanol were added to each sample, mixed for precipitation, incubated for 10 minutes at room temperature and centrifuged again at 12,000 x *g* for 10 minutes at 4°C. The supernatant was completely removed and the remaining pellet was washed with 500 µl ice cold 70% EtOH by centrifugation at 12,000 x *g* for 5 minutes at 4°C. The EtOH was removed and the pellet was dried at room temperature. The precipitated RNA was resolved in 20 µl of DEPC water and dissolved at 37°C for 5 minutes. The RNA concentration was measured via UV-spectroscopy at 260 nm.

2.2.4. RNA isolation from punch biopsies

Isolation of total RNA from skin biopsies was performed using a NucleoSpin® RNA isolation kit according to the manufacturer's protocol. The skin samples were placed into a bead tube containing 500 µl of RA1 buffer and 5 µl β-mercaptoethanol (β-ME). The samples were homogenized by precellys homogenizer at 5,500 rpm for 2x 30 seconds in a 5 second interval. The homogenates were transferred to NucleoSpin Filters and centrifuged at 11,000 x *g* for 2 minutes at room temperature. The supernatant was removed and transferred into a new collection tube. For tissue digestion, 500 µl of RNase-free water (Braun) and 10% proteinase K were added to each tube and mixed. The lysate was incubated for 15 minutes at 55 °C and afterwards the lysate was centrifuged at 10,000 x *g* for 3 min. RNA isolation was performed again, following the manufacturer's protocol along with a DNase digestion step for 15 min at room temperature. The RNA was eluted in 30 µl of RNase-free water. Finally, the RNA concentration was measured using DeNovix DS-11 spectrophotometer at 260 nm.

2.2.5. Reverse transcription

The total RNA was reverse transcribed into cDNA using TaqMan® reverse transcription reagent according to the manufacturer's experimental protocol (Table 6). In each case, 1 µg of total RNA was transcribed into cDNA in a thermocycler in a 20 µl reaction containing the components shown

in Table 7. Also, sterile H_20 was added to bring the volume up to 20 µl. Finally, all cDNA samples were stored at -20 °C.

Table 6: Reverse transcription

Steps	Temperature	Time
Incubation	25 °C	10 min
Reverse transcription (RT)	48 °C	40 min
RT inactivation	95 °C	5 min

Table 7: Reaction mix for cDNA synthesis

Component	Final concentration	Volume
10x TaqMan RT buffer	1x	2 µl
Magnesium chloride (25 mM)	5,5 mM	4,4 µl
deoxyNTPs (10 mM)	2 mM	4 µl
Random hexamer primer (50 µM)	1,25 µM	0,5 µl
Oligo $d(T)_{16}$ primer (50 µM)	1,25 µM	0,5 µl
RNase Inhibitor (20 U/µl	0,4 U/µl	0,4 µl
MultiScribe Reverse Transkriptase (50 U/µl)	1,25 U/µl	0,5 µl

2.2.6. Amplification of RNA

The amplification of purified RNA was carried out using the REPLI-g WTA Single Cell Kit according to the manufacturer's protocol. First, 100 ng of purified RNA was placed into a microcentrifuge tube and H_20 sc was added to bring the volume up to 8 µl. Afterwards, 3 µl of NA Denaturation Buffer was added, mixed by vortexing, centrifuged briefly and incubated for 3 minutes at 95°C. Then, 2 µl of gDNA Wipeout Buffer was also added, vortexed and another centrifugation step was briefly performed, followed by an incubation for 10 minutes at 42°C. The Quantiscript RT mix was prepared fresh (see Table 8) and 7 µl of this mix was added to the sample, mixed by vortexing, centrifuged briefly and incubated for 60 minutes at 42°C. The reaction was stopped by incubating for 3 minutes at 95°C and the mixture was cooled on ice. After the fresh preparation of the ligation mix (see Table 9), 10 µl of it was added to the previous Quantiscript RT mix reaction and once again mixed by vortexing, centrifuged briefly and incubated for 30 minutes at 24°C. The reaction was stopped by incubating for 5 minutes at 95°C. Subsequently, the REPLI-g SensiPhi amplification mix was prepared (see Table 10) and 30 µl of this mix was added to the ligation reaction from the previous process, vortexed, centrifuged briefly and incubated for 2 hours

at 30°C. The reaction was stopped by incubating for 5 minutes at 65°C. The concentrations of amplified cDNA typically ranged between 150-350 ng/µl. The amplified cDNA was stored at –15°C to –30°C until required for downstream applications.

Table 8: Preparing Quantiscript RT mix

Component	Volume/reaction
RT/Polymerase Buffer	4 µl
Random Primer	1 µl
Oligo dT Primer	1 µl
Quantiscript RT Enzyme Mix	1 µl
Total volume	7 µl

Table 9: Preparing ligation mix

Component	Volume/reaction
Ligase Buffer	8 µl
Ligase Mix	2 µl
Total volume	10 µl

Table 10: Preparing REPLI-g SensiPhi amplification mix

Component	Volume/reaction
REPLI-g sc Reaction Buffer	29 µl
REPLI-g SensiPhi DNA Polymerase	1 µl
Total volume	30 µl

2.2.7. Real-time quantitative polymerase chain reaction (qRT-PCR)

For the quantification of gene expression in skin samples, a fluorescence based real-time quantitative polymerase chain reaction (qRT-PCR) was performed. After RNA was amplified and reverse transcribed into cDNA as described previously, qPCR was carried out with Rotor-Gene SYBR Green PCR Master Mix according to the protocol shown in Table 11. The cDNA was pre-diluted at 1:3 (punch biopsies) and 1:200 (curettage samples). The primers used were designed by Rotor Gene Q Software and are listed in Table 12. For comparisons between samples, the relative expression of all target genes in test samples were normalized to the corresponding housekeeping gene (i.e. Cyclophilin B) levels, using the 2-ΔΔCT method (Schmittgen and Livak, 2008).

Table 11: Reaction setup

Component	Volume/reaction	Final concentration
2x Rotor-Gene SYBR Green PCR Master Mix	5 µl	1x
Primer forward	1 µl	1 µM
Primer reverse	1 µl	1 µM
Template cDNA	2 µl	≤ 100 ng/reaction
RNase-free water	1 µl	
Total reaction volume	10 µl	

Table 12: List of primers

Genes	Sequence (from 5´to 3´, fw= forward, rv= reverse)	Product size (bp)
Claudin1	GGCAGATCCAGTGCAAAGTC (fw) TTCATACACTTCATGCCAACG (rv)	139
Profilaggrin	TGAGGCATACCCAGAGGACT (fw) CTGTATCGCGGTGAGAGGAT (rv)	122
Loricrin	GGAGTTGGAGGTGTTTTCCA (fw) ACTGGGGTTGGGAGGTAGTT (rv)	177
human IL-1α	TGATCAGTACCTCACGGCTG (fw) TGGTCTTCATCTTGGGCAGT (rv)	156
human IL-1β	CGAGGGAGAAACTGGCAGAT (fw) AAGCCATCATTTCACTGGCG (rv)	147
human IL-18	TCGGGAAGAGGAAAGGAACC (fw) TCATTGCCACAAAGTTGATGC (rv)	124
human IL-33	AACACAGCAAGCAAAGCCTT (fw) TCTCCTAAAGTAACAGGCCTCC (rv)	132
GAPDH	ATCgACCACTACCTgggCAA (fw) TTCTgCATCACgTCCCggA (rv)	unknown
Cyclophilin B	AAgATgTCCCTgTgCCCTAC (fw) ATggCAAgCATgTggTgTTT (rv)	unknown

2.2.8. Immunohistochemistry

Immunohistochemistry was performed in cooperation with Dr. rer. medic. Anja Kühl, Charité-Campus Benjamin Franklin, Medizinische Klinik 1, Berlin.

Paraffin sections were dewaxed and stained histochemically with hematoxylin and eosin (H&E) for overview. For immunohistochemistry, the paraffin sections were deparaffinized and subjected to a heat-induced epitope retrieval step followed by incubation with the respective primary

antibodies. Anti-human claudin-1, IL-18, and loricrin were directly detected by EnVision+ System- HRP Labelled Polymer Anti-Rabbit (Agilent Technologies, Santa Clara, CA, USA). Anti-human IL-33 was indirectly detected by EnVision™+ System- HRP Labelled Polymer Anti-Rabbit (Agilent Technologies, Santa Clara, CA, USA) using rabbit anti-goat secondary antibody (Dianova). HRP was visualized with diaminobenzidine (Agilent Technologies, Santa Clara, CA, USA) as chromogen. Anti-human Filaggrin and IL-1 alpha were directly detected using the LSAB method employing the Dako REAL™ Detection System, Alkaline Phosphatase/RED, Rabbit/Mouse (Agilent Technologies, Santa Clara, CA, USA). Anti-human IL-1 beta was indirectly detected using the Dako REAL™ Detection System, Alkaline Phosphatase/RED, rabbit/mouse (Agilent Technologies, Santa Clara, CA, USA) and biotinylated donkey anti-rabbit secondary antibody (Dianova). Nuclei were counterstained with hematoxylin and slides mounted with glycerol gelatin (both Merck KGaA, Darmstadt, Germany). Negative controls were performed by omitting the primary antibody. Images were acquired using the AxioImager Z1 microscope (Carl Zeiss MicroImaging, Inc.). Positive cells were quantified in 5 high power fields (field of vision in x400 original magnification). All evaluations were performed in a blinded manner.

2.3. Statistical analysis

Results are reported as mean ± standard error of the mean (SEM). Data with only two groups were statistically analyzed using *t*-test (paired or unpaired) if values were normally distributed. When the data from the two groups were not normally distributed, Wilcoxon test for matched pairs or Mann-Whitney unpaired test was applied. Statistical analysis for three-group comparisons were performed with ordinary one-way ANOVA or with the Kruskal-Wallis test, depending on the data distribution. To determine if the data were normally distributed, the Kolmogorov-Smirnov test, the D'Agostino-Pearson test and the Shapiro-Wilk test were used. Correlations between different parameters were assessed using Spearman's rank correlation. *P*-values less than 0.05 were considered statistically significant. Data were analyzed with GraphPad Prism Version 6.0 software.

3. RESULTS

3.1. SCORAD, TIS Score and Skin Prick Testing

The participants ranged in age from 23 to 58 years (mean AD: 34 and HC: 37 years) and the majority were females in both groups (AD females/males=25/11 and HC females/males=13/6). Seventeen out of the 36 AD patients had allergic asthma. Moreover, 29 out of the 36 patients had allergic rhino/conjunctivitis as indicated by the clinical history and symptoms. The clinical history was confirmed by either *in vivo* (Skin Prick Test) or *in vitro* (measurement of specific IgE) tests. Therefore, 29 of the AD patients were considered to have an extrinsic form of AD and 7 of the AD patients were classified as having an intrinsic form.

To evaluate the severity of AD, we used two clinical scores: SCORAD and TIS Score. Regarding the SCORAD evaluation, 12 of the 36 patients (33.33%) had mild AD, 17 patients (47.22%) moderate AD, and 7 patients (19.44%) severe AD at the day of the examination. Additionally, our 36 AD patients had an average SCORAD of 33.88 points.

We also evaluated the TIS score of the lesional area, where we subsequently performed the skin biopsy. Three patients (8.33%) had TIS < 3, 14 patients (38.89%) had TIS = 3-5, and 13 patients (36,11%) had TIS ≥ 6. TIS score evaluation (and subsequently a skin biopsy) was not performed in 6 patients (16.67%) with lesional AD areas, because these areas were not suitable for skin biopsies.

Both AD patients and HC underwent a skin prick test; 26 out of 36 AD patients and 6 out of 19 HC had positive prick test for at least one of the testing allergens (Table 13).

Table 13: Baseline characteristics for the atopic dermatitis (AD) and control group

	AD group (*n* = 36)	Control group (*n* = 19)
Age, years	34 (min: 23, max: 58)	37 (min: 25, max: 53)
Gender		
Males, *n* (%)	11 (30.56 %)	6 (31.58 %)
Females, *n* (%)	25 (69.44 %)	13 (68.42 %)
SCORAD score, mean	33.88 (min: 0, max: 76.2)	NA
AD severity[a]		
Mild, *n* (%)	12 (33.33 %)	NA
Moderate, *n* (%)	17 (47.22 %)	NA
Severe, *n* (%)	7 (19.44 %)	NA
TIS score[b]		
NA, *n* (%)	6 (16.67 %)	NA
Mild, *n* (%)	3 (8.33 %)	NA
Moderate, *n* (%)	14 (38.89 %)	NA
Severe, *n* (%)	13 (36.11 %)	NA
Total IgE (LU/ml), mean	229 (min: 0, max: 10300)	8 (min: 0, max: 213.77)
Allergic Rhinitis, n	29	6
Allergic Asthma, n	17	1
Prick test positive, n	26	6

NA: not applicable

min: minimum, max: maximum

[a]AD severity was categorized as mild (SCORAD < 25), moderate (SCORAD = 25–50) and severe (SCORAD ≥ 50)

[b]TIS score was categorized as mild (TIS < 3), moderate (TIS = 3-5) and severe (TIS ≥ 6). We evaluated the TIS score of the lesional area, where we subsequently performed the skin biopsy. Two patients did not have any lesional areas on the day of the examination and were considered to have TIS=0. TIS score evaluation (and subsequently a skin biopsy) was not performed in 6 patients with lesional AD areas, because these areas were not suitable for skin biopsies.

3.2. Transepidermal Water Loss (TEWL) of lesional and non-lesional skin (before and after irritation)

TEWL was measured before tape stripping, directly after tape stripping (0h), and three hours after tape stripping in AD patients and HC. TEWL showed a significant increase directly as well as three hours after tape stripping in both AD patients and HC (Figure 4a). We also compared TEWL between non-lesional and lesional skin of AD patients, and TEWL was significantly increased in the lesional skin in comparison with the non-lesional skin of AD patients (Figure 4b).

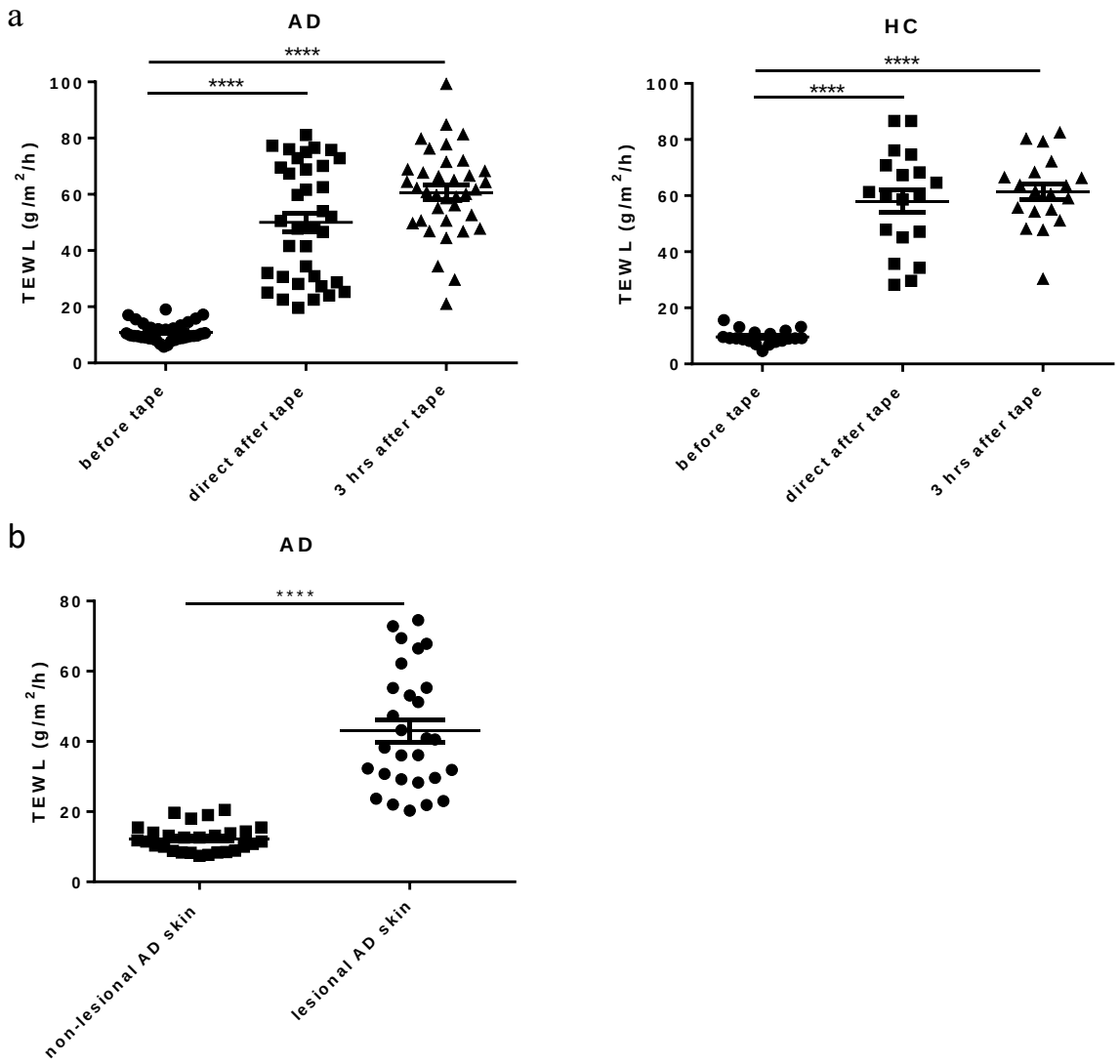

Figure 4: Evaluation of TEWL

(a) TEWL before, direct after and three hours after tape stripping in AD (n=36) and HC (n=19).

(b) Comparison of TEWL between non-lesional and lesional skin of AD patients (n=28).

AD patient number varies due to clinical limitations.

Dot plots are represented with mean ± SEM. (****$p < 0.0001$)

3.3. The expression of skin barrier genes in AD patients and HC

To determine the skin barrier in more detail the mRNA and protein levels of skin barrier genes were studied in comparison. Claudin-1, filaggrin and loricrin were assessed in non-lesional and lesional skin of AD patients and in HC.

Figure 5 shows the mRNA and protein levels of Cldn-1 among HC, and the non-lesional and lesional skin of AD patients. The skin samples were taken by punch biopsies. We observed a significant down-regulation of Cldn-1 mRNA expression in lesional AD skin compared with controls, as well as in non-lesional AD skin compared with controls.

Next, the protein expression of Cldn-1 was analysed between lesional AD skin and HC, as well as between non-lesional AD skin and HC. In the first case (in the comparison between AD lesional skin and healthy skin) this down-regulation was significant. However, there was no significant alteration in the protein expression levels of Cldn-1 in non-lesional AD skin compared with healthy skin (but there was a tendency of down-regulation).

Additionally, we compared the mRNA and protein levels of FLG and loricrin in punch biopsy samples among HC, non-lesional and lesional skin of AD patients (Figure 6, Table 14). The mRNA levels of FLG and loricrin were also decreased in the non-lesional skin of AD patients in comparison with HC (but the difference did not reach statistical significance). However, there was no significant alteration in the protein levels of FLG and loricrin in non-lesional AD skin compared with healthy skin. Furthermore, the mRNA and protein levels of FLG and loricrin were lower in lesional AD skin compared with healthy skin (at mRNA levels this down-regulation was statistically significant).

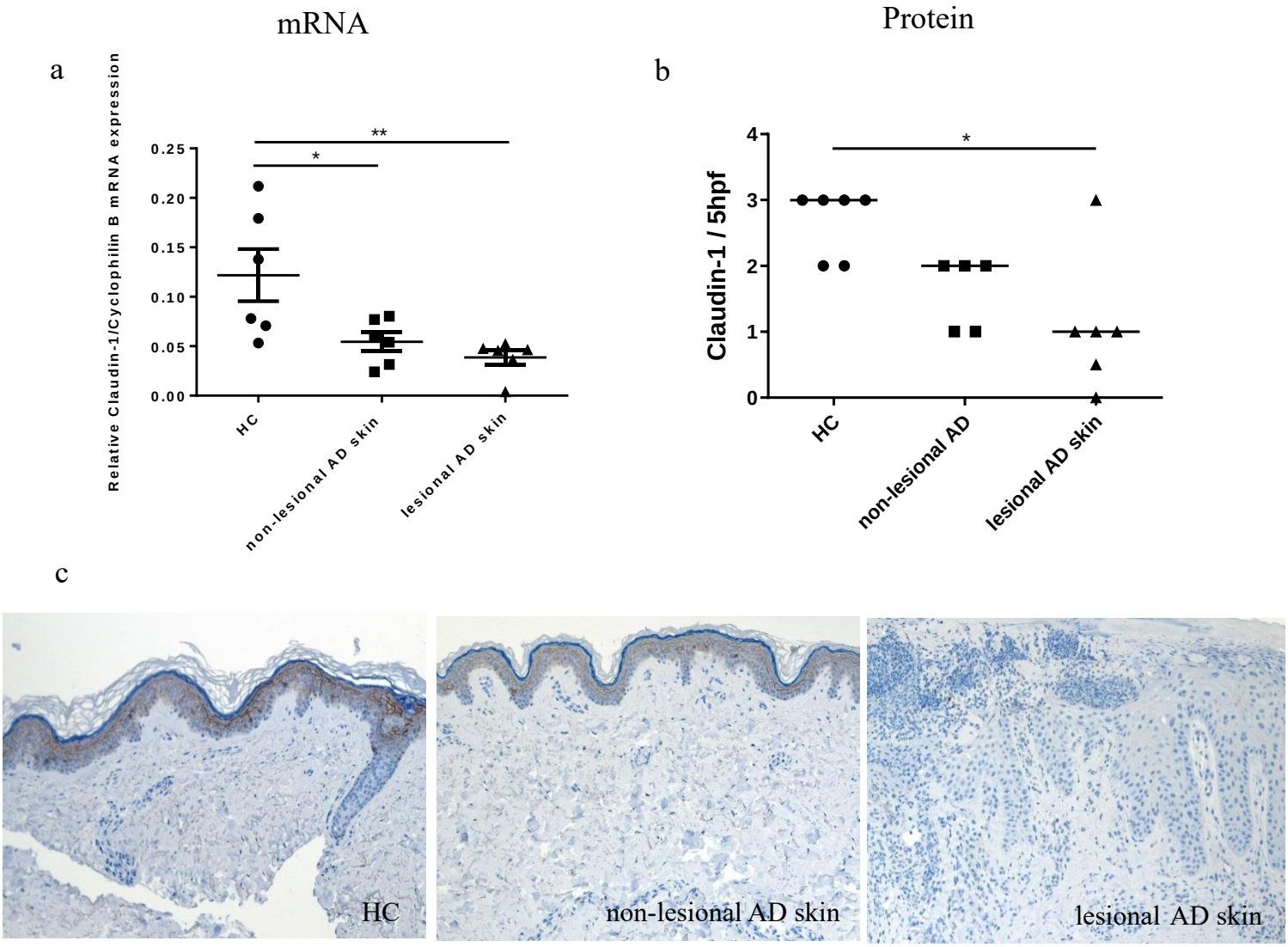

Figure 5: Comparison of mRNA and protein levels of Cldn-1 between lesional and non-lesional skin of AD patients and HC (punch biopsy samples)

(a) The mRNA levels of Cldn-1 in HC (n=6), and in non-lesional (n=6) and lesional AD skin (n=6). Skin samples were taken by punch biopsies. The mRNA expression of Cldn-1 was measured by quantitative qRT-PCR and normalized to the housekeeping gene Cyclophilin B.

Immunohistochemistry:

(b) protein expression of Cldn-1 in punch biopsies from HC (n=6) as well as from non-lesional (n=5) and lesional AD skin (n=6).

(c) Representative staining images for skin punch biopsies were taken from HC and AD patients (non-lesional and lesional skin).

Dot plots are represented with mean ± SEM (a) and the median (b). (*$p < 0.05$, **$p < 0.01$)

Patient numbers vary due to technical limitations.

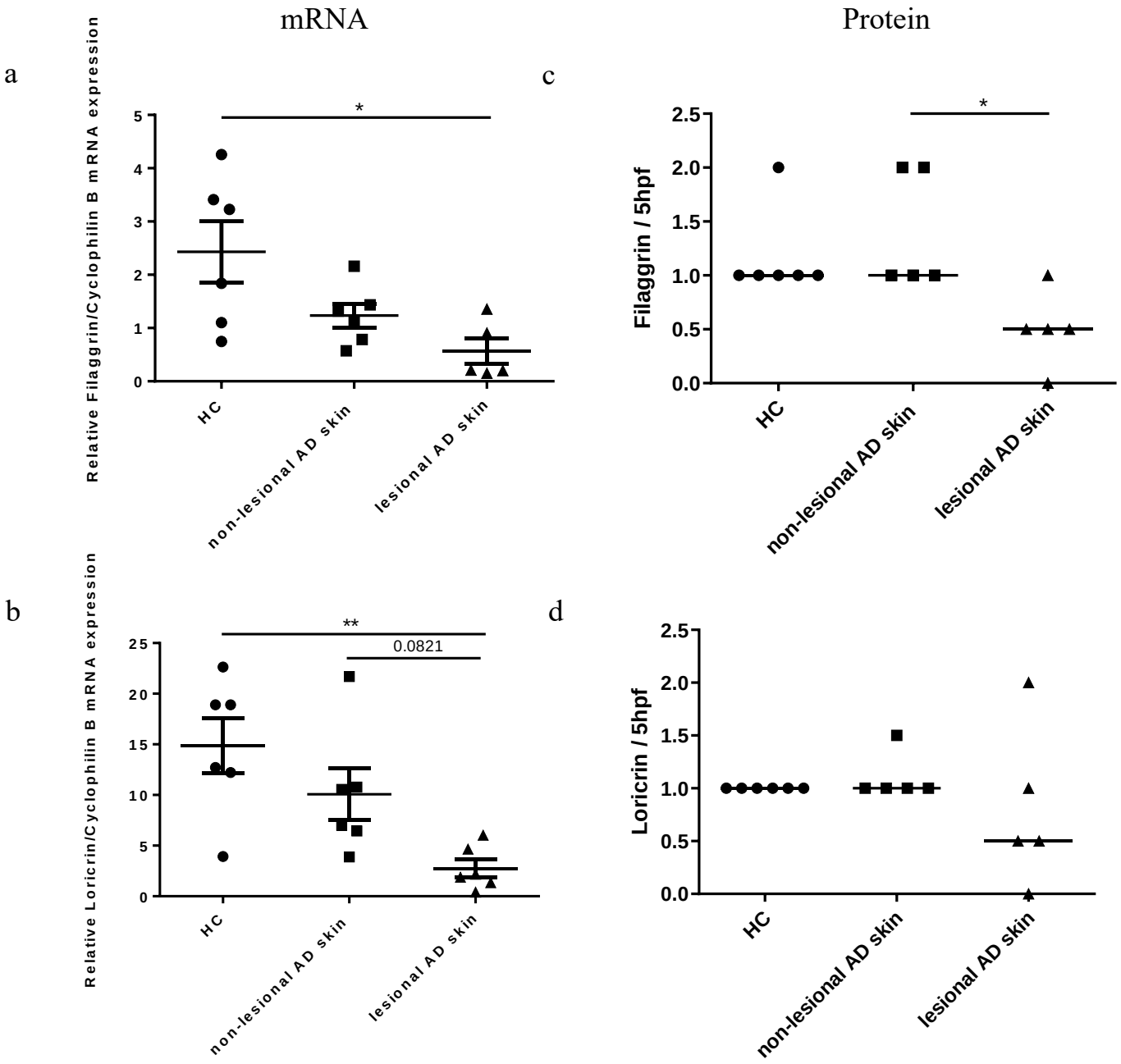

Figure 6: Comparison of mRNA and protein levels of filaggrin and loricrin between lesional and non-lesional skin of AD patients and HC (punch biopsy samples)

(a) The mRNA levels of filaggrin in HC (n=6), and in non-lesional (n=6) and lesional AD skin (n=5). (b) The mRNA levels of loricrin in HC (n=6), and in non-lesional (n=6) and lesional AD skin (n=6).

Skin samples were taken by punch biopsies. The mRNA expression of filaggrin and loricrin was measured by quantitative qRT-PCR and normalized to the housekeeping gene Cyclophilin B.

Immunohistochemistry:

(c) and (d): Protein expression of filaggrin and loricrin in punch biopsies from HC (n=6) as well as from non-lesional (n=5) and lesional AD skin (n=5).

Dot plots are represented with mean ± SEM (a and b) and the median (c and d). (*$p < 0.05$, **$p < 0.01$)

Patient numbers vary due to technical limitations.

Table 14: Comparison of mRNA and protein levels of skin barrier genes between non-lesional and lesional skin of AD patients and HC

(Skin samples were taken by punch biopsies)

	non-lesional AD skin vs. HC		lesional AD skin vs. HC	
	mRNA	**Protein**	**mRNA**	**Protein**
Claudin-1	↓ (*)	↓	↓ (**)	↓ (*)
Filaggrin	↓	=	↓ (*)	↓
Loricrin	↓	=	↓ (**)	↓

*p < 0.05, **p < 0.01

3.4. The expression of IL-1 cytokines in AD patients and HC

To investigate the IL-1 cytokine family members in more detail we studied IL-1α, IL-1β, IL-18 and IL-33 in lesional and non-lesional AD skin in comparison to HC. IL-1β and IL-33 mRNA expression were comparable between HC and non-lesional skin versus lesional skin from AD patients. However, IL-1α mRNA was expressed higher not only in non-lesional, but also in lesional atopic skin. Moreover, IL-1β protein expression was significantly up-regulated in lesional AD skin versus non-lesional AD skin and healthy skin. IL-1α and IL-33 protein levels of both the unaffected and affected areas of AD patients were higher than those of HC (not statistically significant) (Table 15).

Figure 7a-c shows the data regarding IL-18 expression among HC, and in the non-lesional and lesional skin of AD patients (in the punch biopsy samples), wherein mRNA IL-18 expression was comparable between the groups. The protein levels of IL-18 were significantly higher in lesional AD skin as compared with those of HC. The cytokine levels of non-lesional AD skin were higher than those of non-atopic subjects, but the difference did not reach statistical significance. Additionally, the levels of IL-18 were higher in the lesional AD skin versus the non-lesional AD skin, but the difference was not statistically significant.

Table 15: Comparison of mRNA and protein levels of cytokines between non-lesional and lesional skin of AD patients and HC

(Skin samples were taken by punch biopsies)

	mRNA		
	non-lesional AD skin vs. HC	**lesional AD skin vs. HC**	**lesional vs. non-lesional AD skin**
IL-1beta	=	=	=
IL-33	=	=	=
IL-1alpha	↑	↑	↑

	Protein		
	non-lesional AD skin vs. HC	**lesional AD skin vs. HC**	**lesional vs. non-lesional AD skin**
IL-1beta	=	↑ (****)	↑ (****)
IL-33	↑	↑	=
IL-1alpha	↑	↑	=

****$p < 0.0001$

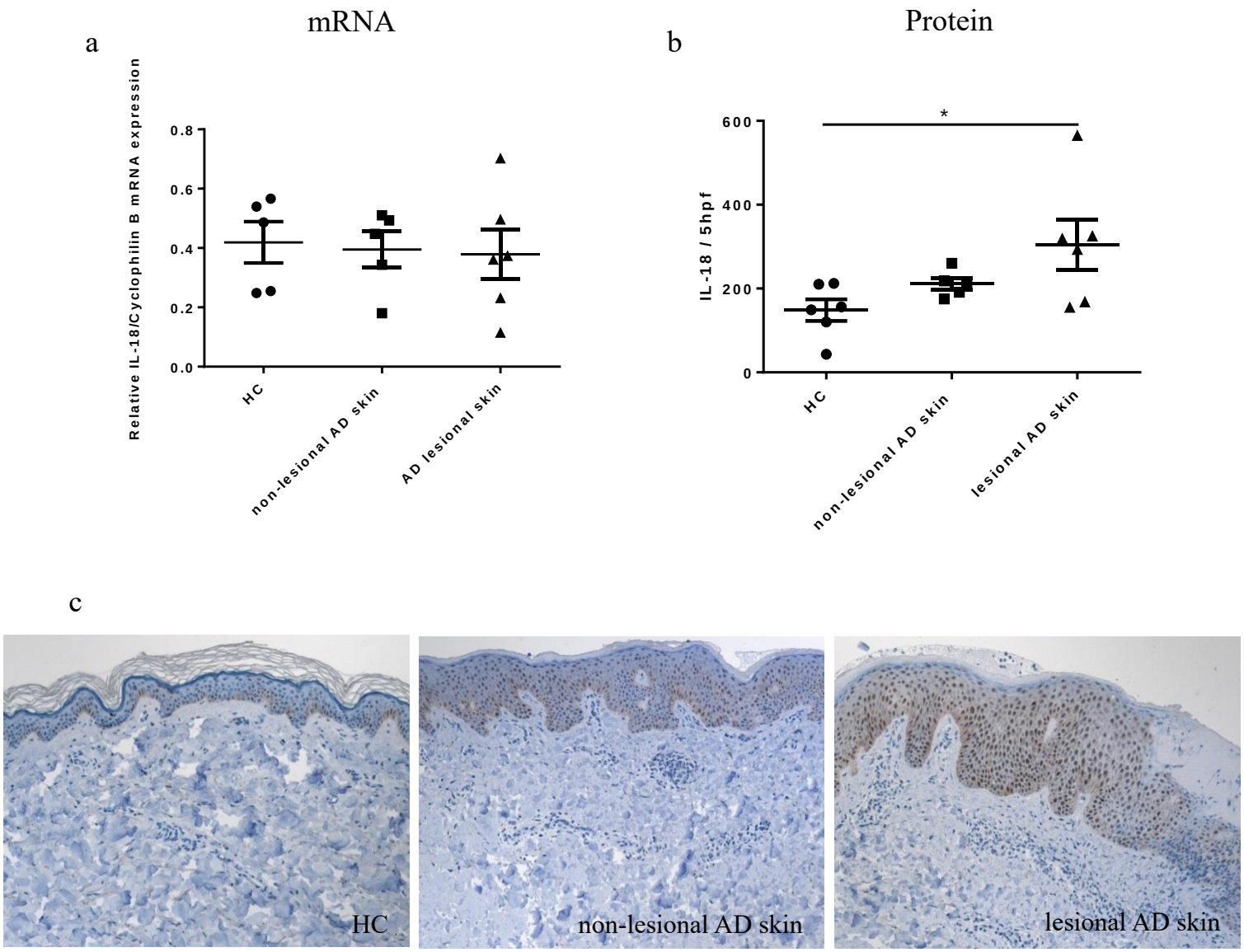

Figure 7: Comparison of the mRNA and protein levels of IL-18 between lesional and non-lesional skin of AD patients and HC (punch biopsy samples)

(a) The mRNA levels of IL-18 in HC (n=5), and in non-lesional (n=5) and lesional skin (n=6) of AD patients. Skin samples were taken by punch biopsies. The mRNA expression of IL-18 was measured by qRT-PCR and normalized to the housekeeping gene Cyclophilin B.

Immunohistochemistry: (b) protein expression of IL-18 in punch biopsies from HC (n=6) as well as from non-lesional (n=5) and lesional AD skin (n=6).

(c) Representative staining images for skin biopsies were taken from HC and AD patients (non-lesional and lesional skin).

Dot plots are represented with mean ± SEM. (*$p < 0.05$)

Patient numbers vary due to technical limitations.

3.5. Regulation of skin barrier genes before and after irritation

To investigate the role of an acute permeability barrier disruption on the expression of the barrier protein, samples before and after irritation were analysed. Here, we additionally studied punch biopsy samples before and after curettage. In AD patients, the relative mRNA expression of Cldn-1 tended to increase three hours after tape stripping, but the difference did not reach statistical significance. In HC there was no significant alteration in the mRNA expression of Cldn-1 in the tape-stripped skin in comparison with the untreated skin (Figure 8a and Table 16).

To investigate the Cldn-1 at protein expression before and after tape stripping, immunohistochemistry was performed on the samples of the subgroup of punch biopsies. There was neither a difference of protein expression of Cldn-1 in AD patients nor in HC after tape stripping (Figure 8b).

We also evaluated the mRNA expression of FLG before and after tape stripping in the epidermis of healthy individuals and patients with AD (in the curettage samples). Non-lesional, tape-stripped skin from AD patients revealed a significant lower FLG gene expression in comparison with the untreated skin. In contrast, in healthy individuals the mRNA expression of FLG was not altered after tape stripping. We also investigated the effect of barrier disruption on the expression of loricrin in the epidermis. In both AD patients and healthy individuals, the mRNA expression of this skin barrier gene was significantly decreased after tape stripping (Table 16).

Finally, we determined the protein levels of FLG and loricrin before and after tape stripping. In both AD patients and HC, the FLG and loricrin protein levels in the epidermis did not differ significantly after barrier disruption by tape stripping (Table 16).

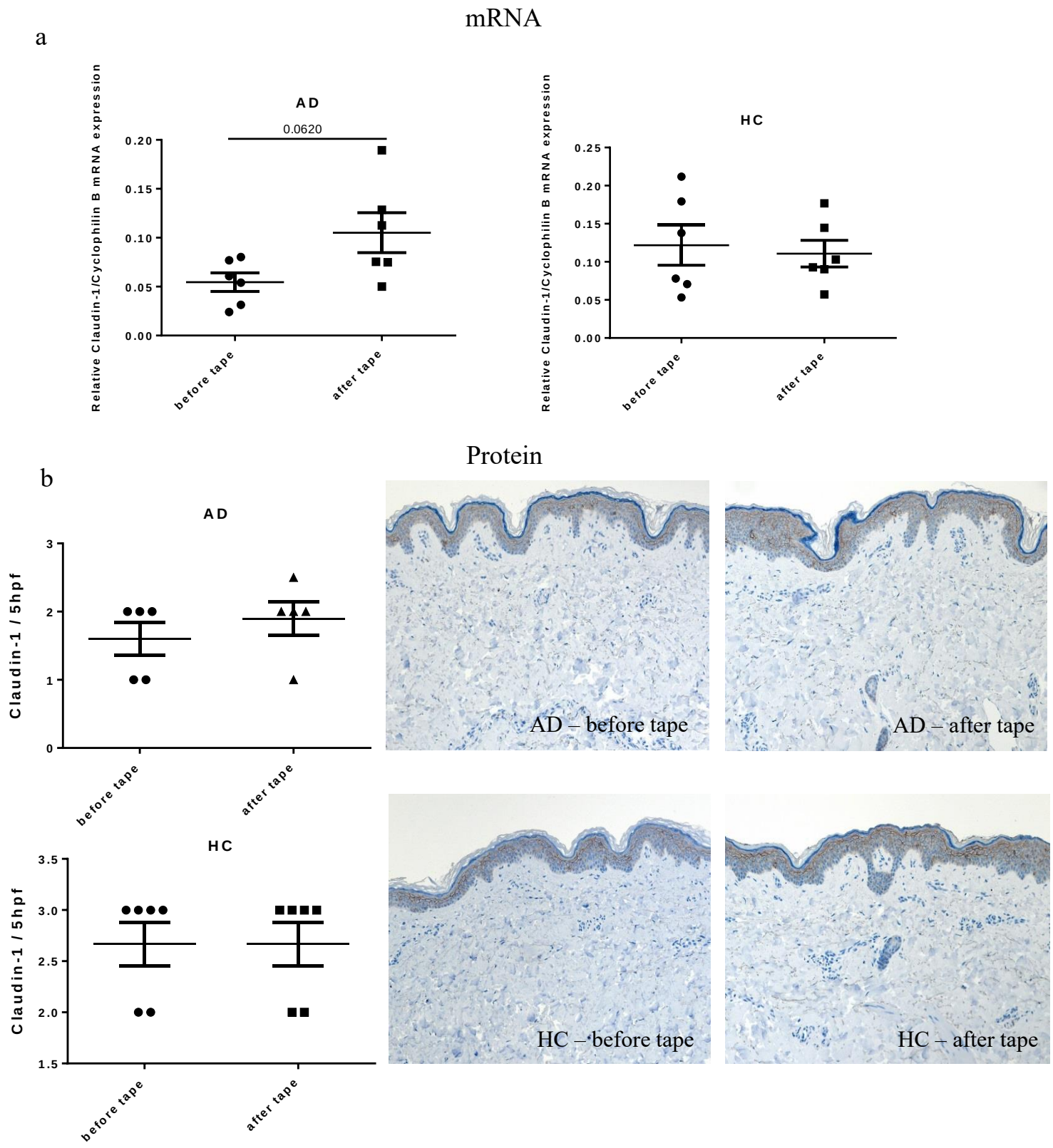

Figure 8: mRNA and protein expression of Cldn-1 in the skin before and three hours after tape stripping (punch biopsy samples)

(a) The mRNA levels of Cldn-1 in AD patients (n=6) and in HC (n=6) before and three hours after tape stripping. Skin samples were taken by punch biopsies.

(b) Immunohistochemistry: protein expression of Cldn-1 in skin biopsies from AD patients (n=5) and HC (n=6) before and after tape stripping. Representative staining images of Cldn-1 protein expression in both AD patients and HC before and after tape stripping.

Dot plots are represented with the mean ± SEM. Patient numbers vary due to technical limitations.

Table 16: Summary of mRNA and protein expression of skin barrier genes after tape stripping

	AD		HC	
	mRNA (curettage)	**Protein (punch biopsies)**	**mRNA (curettage)**	**Protein (punch biopsies)**
Claudin-1	↑	=	=	=
Filaggrin	↓ (*)	=	=	=
Loricrin	↓ (*)	=	↓ (**)	=

*p < 0.05, **p < 0.01

3.6. Regulation of expression of IL-1 family before and after irritation

Finally, we studied the expression of the IL-1 family members in the context of irritation and again used punch biopsies and also curettage material for analysis.

The mRNA levels of IL-1α, which were evaluated by qRT-PCR, increased significantly at three hours following acute barrier disruption by tape stripping in healthy individuals (Table 17). In contrast, mRNA levels of IL-1α showed no change after tape stripping in patients with AD. To investigate the IL-1α at protein level before and after tape stripping, immunohistochemistry was performed (on the samples of the subgroup of punch biopsies). In AD patients, IL-1α did not differ significantly after tape stripping. In HC, IL-1α protein expression showed a significant up-regulation after tape stripping (Table 17).

Next, we assessed the mRNA expression of IL-1β after acute barrier disruption in the epidermis of healthy individuals and patients with AD. Non-lesional skin from AD patients showed a significant increase in IL-1β expression when tape stripped in comparison with the untreated skin. By contrast, in healthy individuals the mRNA expression of IL-1β revealed no significant difference after tape stripping. Immunohistochemistry was performed for the determination of IL-1β protein levels before and after tape stripping. In HC, the protein levels of IL-1β showed no significant difference after barrier disruption by tape stripping. However, in AD patients the protein expression of IL-1β showed a significant decrease in tape-stripped skin in comparison with the untreated skin (Table 17).

Furthermore, in healthy individuals (in the curettage samples), quantitative qRT-PCR analysis revealed an up-regulation in the mRNA expression of IL-33 after tape stripping. Conversely, in AD patients the mRNA expression of IL-33 did not differ significantly after tape stripping. By contrast, in both HC and AD patients, the protein levels of IL-33 showed no significant difference after barrier disruption by tape stripping (Table 17).

Finally, we assessed the expression of IL-18 after acute barrier disruption by tape stripping in the epidermis of healthy individuals and AD patients. IL-18 mRNA expression was not altered by tape stripping in AD or HC. However, we observed an up-regulation of IL-18 protein expression in AD (even though this was not significant) but not in HC (Figure 9).

Table 17: mRNA and protein expression of cytokines after tape stripping

	AD		HC	
	mRNA (curettage)	**Protein (punch biopsies)**	**mRNA (curettage)**	**Protein (punch biopsies)**
IL-1alpha	=	=	↑ (**)	↑ (*)
IL-1beta	↑ (*)	↓ (*)	=	=
IL-33	=	=	↑ (***)	=

*p < 0.05, **p < 0.01, ***p < 0.001

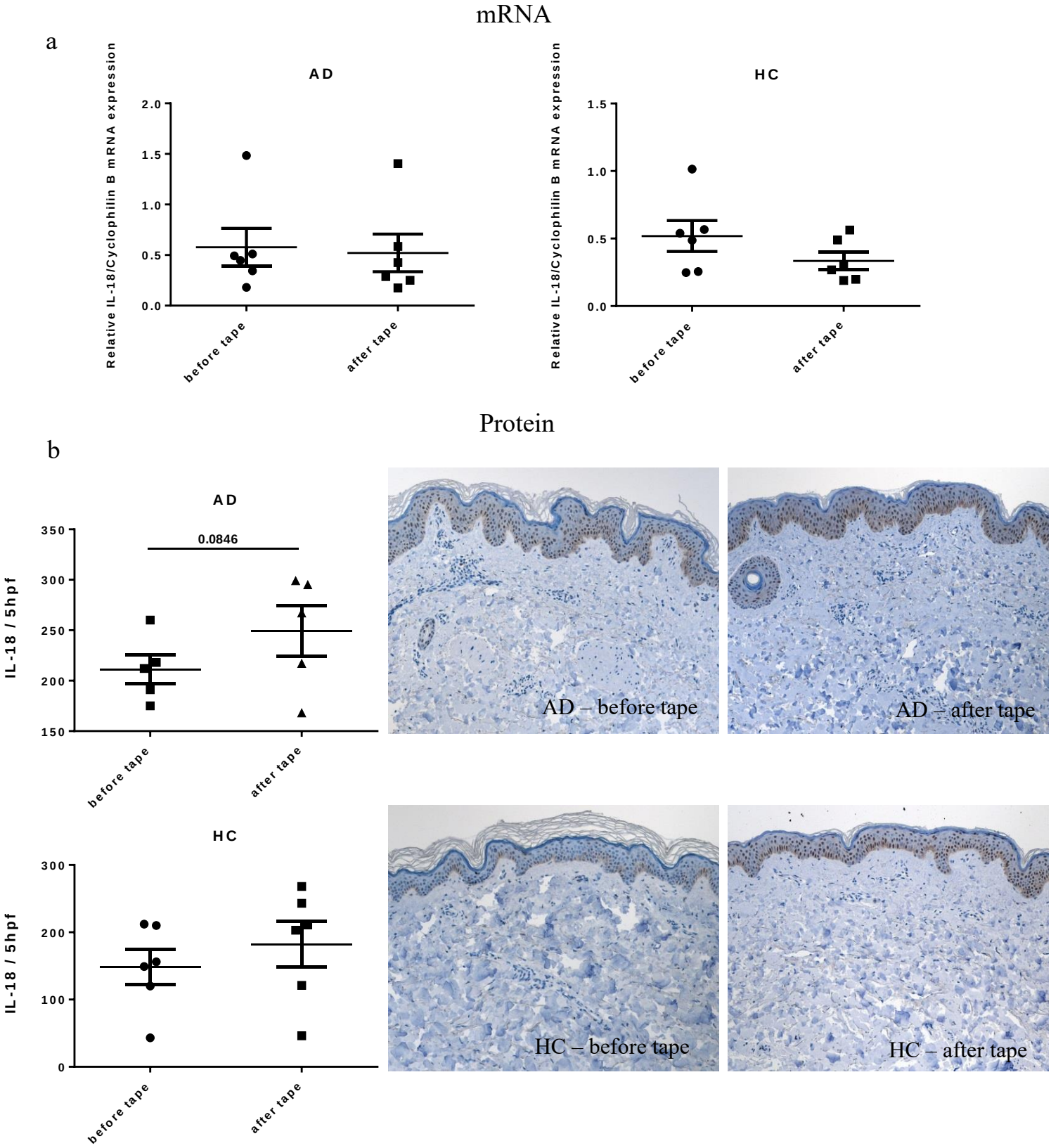

Figure 9: mRNA and protein expression of IL-18 in the skin before and three hours after tape stripping (punch biopsy samples)

IL-18 expression at (a) mRNA levels in the skin before and after tape stripping in AD patients (n=6) and in HC (n=6). (b) Protein levels by immunohistochemistry in the skin of AD patients (n=5) and HC (n=6). Representative staining images for IL-18 protein expression in AD patients and HC before and after tape stripping.

Data are presented as the mean ± SEM. Patient numbers vary due to technical limitations.

4. DISCUSSION

The expression of barrier proteins and early inflammatory cytokines play a crucial role in the initiation of skin inflammation in AD. This study evaluated these key elements in detail by: (a) considering lesional versus non-lesional AD skin in comparison to HC; and (b) by considering the impact of irritation on the expression of these molecules.

4.1. Cldn-1 is down-regulated in lesional and non-lesional AD skin

Tight junctions have been demonstrated to play a key role in skin barrier function. Cldn-1 is the most widely expressed TJ protein and remarkably important for a functional epidermal TJ. Atopic dermatitis is a common human inflammatory skin disease and has been correlated with an abnormal expression of TJ components in both involved and uninvolved skin. An epidermal barrier dysfunction leads to an increase of transepidermal water loss, to xerosis cutis and promotes the penetration of environmental allergens into the subepidermal layer, playing a pivotal role in the pathophysiology of AD (Mu, Zhao, Liu, Chang and Zhang, 2014; Seidenari and Giusti, 1995a; Thestrup-Pedersen, 1997).

Our results show that the tight junction protein Cldn-1 was significantly lower expressed in non-lesional AD skin in comparison with the skin of HC. We observed a reduced expression of Cldn-1, which was measured at both mRNA and protein levels. This is in concordance with published data demonstrating Cldn-1 down-regulation in the non-lesional skin of Northern American AD patients. De Benedetto *et al.* demonstrated a reduced expression of Cldn-1 in the non-lesional skin of AD patients compared with non-atopic subjects. The decreased expression of Cldn-1 in non-lesional AD skin might also enhance the penetration of environmental antigens and thus the triggering of atopic dermatitis lesions. They also reported that single-nucleotide polymorphisms in the region of Cldn-1 gene were associated with AD (De Benedetto, Rafaels, McGirt, Ivanov, Georas, Cheadle, Berger, Zhang, Vidyasagar, Yoshida, Boguniewicz, Hata, Schneider, Hanifin, Gallo, Novak, Weidinger, Beaty, Leung, Barnes and Beck, 2011; Kirschner and Brandner, 2012).

Our results were further supported by a report by Hadj-Rabia *et al.* These authors described an uncommon human syndrome called neonatal ichthyosis with sclerosing cholangitis (NISCH). This syndrome is caused by mutation in Cldn-1 gene and is associated with impaired barrier function. The skin of NISCH patients is erythematous and xerotic. The loss of Cldn-1 induces a dry appearance of the skin, which is similar with the non-lesional skin of AD patients (Hadj-Rabia,

Baala, Vabres, Hamel-Teillac, Jacquemin, Fabre, Lyonnet, De Prost, Munnich, Hadchouel and Smahi, 2004).

In contrast to the findings from De Benedetto *et al.* obtained from a Northern American cohort, Gruber *et al.* did not observe a consistent decreased expression of Cldn-1 in non-lesional skin compared with healthy skin in a central European cohort (De Benedetto, Rafaels, McGirt, Ivanov, Georas, Cheadle, Berger, Zhang, Vidyasagar, Yoshida, Boguniewicz, Hata, Schneider, Hanifin, Gallo, Novak, Weidinger, Beaty, Leung, Barnes and Beck, 2011; Gruber, Bornchen, Rose, Daubmann, Volksdorf, Wladykowski, Vidal, Peters, Danso, Bouwstra, Hennies, Moll, Schmuth and Brandner, 2015).

One possible explanation for this variation may be different genetic backgrounds of the AD cohorts examined in these studies. This hypothesis is supported by the data in the De Benedetto *et al.* study, whose authors suggested genetic differences between the Caucasian and African American AD patients (De Benedetto, Rafaels, McGirt, Ivanov, Georas, Cheadle, Berger, Zhang, Vidyasagar, Yoshida, Boguniewicz, Hata, Schneider, Hanifin, Gallo, Novak, Weidinger, Beaty, Leung, Barnes and Beck, 2011). Additionally, Ross-Hansen *et al.* did not confirm the association of Cldn-1 single-nucleotide polymorphisms with AD in a Danish cohort (Ross-Hansen, Linneberg, Johansen, Hersoug, Brasch-Andersen, Menne and Thyssen, 2013).

Another explanation is that the skin biopsy was obtained from the lower forearms in De Benedetto *et al.* study, as it was in our study (De Benedetto, Rafaels, McGirt, Ivanov, Georas, Cheadle, Berger, Zhang, Vidyasagar, Yoshida, Boguniewicz, Hata, Schneider, Hanifin, Gallo, Novak, Weidinger, Beaty, Leung, Barnes and Beck, 2011). By contrast, Gruber *et al.* took the skin biopsy specimens from untanned lower back skin. The lower forearms are more likely to be exposed to the sun compared with the lower back (Gruber, Bornchen, Rose, Daubmann, Volksdorf, Wladykowski, Vidal, Peters, Danso, Bouwstra, Hennies, Moll, Schmuth and Brandner, 2015). A previous study indicated the down-regulation of the Cldn-1 expression in sun-exposed skin (Rachow, Zorn-Kruppa, Ohnemus, Kirschner, Vidal-y-Sy, von den Driesch, Bornchen, Eberle, Mildner, Vettorazzi, Rosenthal, Moll and Brandner, 2013). Further data analysing the expression of Cldn-1 at different anatomical sites would be beneficial to better understand the role of the anatomical site regarding Cldn-1 expression.

A third minor explanation for this discrepancy may also be the different materials and technical methods used in the studies. Furthermore, other factors regulating Cldn-1 skin expression cannot be ruled out.

Furthermore, our data demonstrate a down-regulation of Cldn-1 in lesional AD skin compared with the skin of HC. This data is in line with studies performed in a central European cohort (Gruber, Bornchen, Rose, Daubmann, Volksdorf, Wladykowski, Vidal, Peters, Danso, Bouwstra, Hennies, Moll, Schmuth and Brandner, 2015) as well as in a South American cohort (Batista, Perez, Orfali, Zaniboni, Samorano, Pereira, Sotto, Ishizaki, Oliveira, Sato and Aoki, 2015).

Our findings are also in accordance with the data reported by studies that used genetically altered mice. Gruber *et al*. used an AD-like allergic dermatitis (AID) mouse model (characterized by absence of barrier impairment and extrinsically induced inflammation) to investigate in more detail what causes Cldn-1 down-regulation in lesional AD skin. They observed a down-regulation of Cldn-1 levels in eczematic mouse skin. Moreover, they found a lack of Cldn-1 at the cell-cell borders, as well as decreased Cldn-1 immunoreactivity in the epidermal layers. These results indicated the inflammation as a cause for Cldn-1 down-regulation in AD (Gruber, Bornchen, Rose, Daubmann, Volksdorf, Wladykowski, Vidal, Peters, Danso, Bouwstra, Hennies, Moll, Schmuth and Brandner, 2015).

Also, Furuse *et al.* used Cldn-1-deficient mice to show the key role of claudin in mammalian barrier function. They reported that the lack of Cldn-1 results in the death of the animals at the first day of birth with severe dehydration, wrinkled skin and increased epidermal permeability, as assessed by transepidermal water loss. Interestingly, these mice had no change in the expression of structural proteins of the stratum corneum (e.g. loricrin) that may explain the skin phenotype that was described above (Furuse, Hata, Furuse, Yoshida, Haratake, Sugitani, Noda, Kubo and Tsukita, 2002).

4.2. Regulation of skin barrier genes upon skin irritation

Tight junctions form a barrier and regulate the paracellular permeability for small and large molecules in response to external stimuli e.g. pathogens, UV irradiation and wounding. Among TJ proteins, Cldn-1 has been shown to be a key determinant in skin barrier function. Furthermore, the influence of the stratum corneum barrier status on the TJ barrier function and their physiological relationship remains to be investigated.

In the present study, we investigated the effect of an acute permeability barrier disruption (caused by repeated tape stripping) on the expression of Cldn-1 in human epidermis. In AD patients, the mRNA expression of Cldn-1 tended to increase three hours after tape stripping, but the difference did not reach statistical significance. By contrast, in healthy individuals the mRNA expression of

Cldn-1 showed no significant difference three hours after tape stripping. To further support our previous observations, we validated the Cldn-1 protein levels before and after tape stripping. In both AD patients and healthy individuals, the Cldn-1 protein expression was not significantly altered when comparing the tape-stripped and untreated skin.

A previous study by Baek *et al.* also investigated the impact of stratum corneum barrier disruption on the expression of Cldn-1. This group used female mice, the disruption of the stratum corneum was also caused by repeated tape stripping, and the skin samples were taken from the tape-stripped murine skin at 15 minutes, 30 minutes, 1 hour, 3 hours and 6 hours after each treatment. They examined the mRNA and protein levels of Cldn-1, observing no significant difference in mRNA expression of Cldn-1 and a down-regulation of Cldn-1 protein level at 15 and 30 minutes after tape stripping. By contrast, they found a significant up-regulation of mRNA and protein levels of Cldn-1 between the untreated and tape-stripped group at 1, 3, and 6 hours after tape stripping. These observations show that the TJ barrier was disrupted following the injury of the stratum corneum by tape stripping and then normalized within one hour after treatment. Furthermore, an up-regulation of mRNA and protein levels of Cldn-1 started one hour after injury. These data indicate a fast recovery kinetic of the TJ barrier in response to acute SC barrier disruption (Baek, Lee, Choi, Choi and Lee, 2013).

Our data for the mRNA expression of Cldn-1 upon physical irritation are in accordance with the data of the above-mentioned study. By contrast, with respect to Cldn-1 protein levels, we observed a discrepancy between our observations and the findings of that study. A possible explanation for this discrepancy can be the different intensity and end point of tape stripping, as well as the different materials and technical methods used in the studies. A second explanation is that we performed the skin biopsies on the lower forearms of humans. By contrast, the skin samples were taken from the flank of mice in Baek *et al.* study (Baek, Lee, Choi, Choi and Lee, 2013). Another hypothesis could also be the late recovery/up-regulation point of Cldn-1 protein levels in our cohort. We measured the Cldn-1 protein levels at only one time point after tape stripping (three hours). Further studies assessing the protein levels at different time points would be interesting.

Filaggrin is an intermediate filament-associated protein and is synthesized initially as profilaggrin (Gan, McBride, Idler, Markova and Steinert, 1990; McGrath and Uitto, 2008; Presland, Haydock, Fleckman, Nirunsuksiri and Dale, 1992). Previous reports have highlighted the important role of FLG in osmolarity, flexibility and maintaining the barrier function of the stratum corneum (Mildner, Jin, Eckhart, Kezic, Gruber, Barresi, Stremnitzer, Buchberger, Mlitz, Ballaun,

Sterniczky, Fodinger and Tschachler, 2010; Nishifuji and Yoon, 2013). Loricrin is the key component of the cornified envelope. The CE is a lamellar structure with the ability to provide protection from environmental hazards and to prevent the loss of water and electrolytes. Therefore, the CE is regarded as having a central role for an effective physical and water barrier function of the skin (Kalinin, Marekov and Steinert, 2001; Roop, 1995).

In our study, we evaluated the mRNA expression of FLG and loricrin upon skin irritation in epidermis of AD patients and HC. The tape-stripped skin revealed a significant decrease of FLG and loricrin gene expression compared with the untreated skin (exception: mRNA expression of FLG in healthy individuals showed no significant alteration after tape stripping).

Our findings are highly consistent with published data from previous studies. These groups demonstrated a decrease of mRNA levels of FLG (Gerritsen, van Pelt and van de Kerkhof, 1996; Rinaldi, Morita, Wawrzyniak, Dreher, Grant, Svedenhag and Akdis, 2019; Thyssen and Kezic, 2014) and loricrin (Rinaldi, Morita, Wawrzyniak, Dreher, Grant, Svedenhag and Akdis, 2019) after tape stripping.

A possible scenario for the down-regulation of FLG expression upon acute permeability barrier perturbation is the disruption of the calcium gradient and the reduced Ca^{2+} levels in stratum granulosum (Thyssen and Kezic, 2014). Increased Ca^{2+} levels are essential for the dephosphorylation and proteolysis of profilaggrin into FLG monomers at the border between the stratum granulosum and stratum corneum (Nishifuji and Yoon, 2013; Sandilands, Terron-Kwiatkowski, Hull, O'Regan, Clayton, Watson, Carrick, Evans, Liao, Zhao, Campbell, Schmuth, Gruber, Janecke, Elias, van Steensel, Nagtzaam, van Geel, Steijlen, Munro, Bradley, Palmer, Smith, McLean and Irvine, 2007).

4.3. IL-18 protein expression is up-regulated in lesional and non-lesional AD skin

Interleukin (IL)-18 is a member of the IL-1 superfamily of cytokines (Okamura, Tsutsui, Komatsu, Yutsudo, Hakura, Tanimoto, Torigoe, Okura, Nukada, Hattori, Akita, Namba, Tanabe, Konishi, Fukuda and Kurimoto, 1995) and has been closely associated with the pathogenesis of AD. Numerous studies have uncovered the pleiotropic role of this cytokine. On one hand, in the presence of IL-12, IL-18 triggers the Th1 response that provokes IFN-γ production. On the other, in the absence of IL-12, IL-18 increases IgE production and induces the Th2 response that reinforces the production of Th2 cytokines such as IL-4, IL-5, IL-9 and IL-13 (Yoshimoto, Tsutsui, Tominaga, Hoshino, Okamura, Akira, Paul and Nakanishi, 1999). A Th2 response

producing IL-4 and IL-13 (both have been recognized as major inflammatory cytokines in AD) along with a Th1 response seem to play a pivotal role in the pathogenesis of AD (Lee, Cho and Park, 2015; Sedimbi, Hagglof and Karlsson, 2013).

In the present study, we investigated the mRNA expression of IL-18 in lesional and non-lesional skin of AD patients and healthy individuals. The mRNA IL-18 expression was comparable between the groups. Next, we used immunohistochemistry to investigate IL-18 protein expression among HC, and in non-lesional and lesional AD skin. The cytokine levels were significantly higher in AD lesional skin than in healthy skin. The IL-18 levels of the non-lesional skin of AD patients were higher than those of HC, but the alteration did not reach statistical significance. Additionally, the immunohistochemical staining revealed an up-regulation (not statistically significant) of IL-18 protein levels in AD lesional versus the non-lesional AD skin.

In agreement with our data, Inoue *et al.* demonstrated that IL-18 levels measured by ELISA were significantly higher in atopic skin than in healthy skin. Additionally, they suggested a correlation between the level of IL-18 in the horny layer and the severity of AD, as well as a significant decrease of IL-18 levels after treatment of AD (Inoue, Aihara, Kirino, Harada, Komori-Yamaguchi, Yamaguchi, Nagashima and Ikezawa, 2011a). Our data are also in line with a study where the IL-18 protein expression in the epidermis of patients with AD (non-lesional and lesional skin) and healthy controls was investigated. The IL-18 protein levels evaluated by ELISA were significantly increased in lesional AD skin in comparison with non-lesional AD skin and HC. In addition, the protein expression of IL-18 was significantly higher in non-lesional AD skin in comparison with HC (Amarbayasgalan, Takahashi, Dekio and Morita, 2013).

Our findings further support the key role of IL-18 in the pathogenesis of AD. Previous reports have indicated that the IL-18 expression increases after the exposure to allergens (e.g. house dust mite) and infections. Therefore IL-18 contributes to the development of *Staphylococcus aureus*-associated AD in mouse models and humans (Inoue, Aihara, Kirino, Harada, Komori-Yamaguchi, Yamaguchi, Nagashima and Ikezawa, 2011b; Terada, Tsutsui, Imai, Yasuda, Mizutani, Yamanishi, Kubo, Matsui, Sano and Nakanishi, 2006). Furthermore, various studies have shown elevated levels of IL-18 in serum from AD patients and in AD mouse models, as well as a positive correlation of serum IL-18 with AD severity (Tanaka, Tsutsui, Yoshimoto, Kotani, Matsumoto, Fujita and Wang, 2001; Zedan, Rasheed, Farouk, Alzolibani, Bin Saif, Ismail and Al Robaee, 2015).

A detailed analysis of the literature revealed more evidence about the importance of IL-18 in the development of AD. In mouse models in which transgenic mice specifically overexpress IL-18, they spontaneously develop AD-like dermatitis and later show increased IgE levels in their serum (Konishi, Tsutsui, Murakami, Yumikura-Futatsugi, Yamanaka, Tanaka, Iwakura, Suzuki, Takeda, Akira, Nakanishi and Mizutani, 2002; Nakano, Tsutsui, Terada, Yasuda, Matsui, Yumikura-Futatsugi, Yamanaka, Mizutani, Yamamura and Nakanishi, 2003; Yamanaka, Tanaka, Tsutsui, Kupper, Asahi, Okamura, Nakanishi, Suzuki, Kayagaki, Black, Miller, Nakashima, Shimizu and Mizutani, 2000). Moreover, Nakano *et al.* pointed out that IL-18 is the pathological inducer of dermatitis, as a blockade of IL-18 inhibited the development of dermatitis in these transgenic mice (Nakano, Tsutsui, Terada, Yasuda, Matsui, Yumikura-Futatsugi, Yamanaka, Mizutani, Yamamura and Nakanishi, 2003).

4.4. Regulation of IL-1 family expression upon skin irritation

The inflammatory epidermal barrier is influenced by tape stripping. Skin irritation caused by the method of tape stripping promotes an enhancement of skin immunoreactivity by inducing the expression of various cytokines including members of IL-1 family (Dickel, Gambichler, Kamphowe, Altmeyer and Skrygan, 2010).

It has been reported that repeated tape stripping induced an up-regulation of IL-1α, IL-1β and IL-33 expression in healthy individuals. However, there have been no reports about the expression of these cytokines in the epidermis of patients with AD, as well as none concerning IL-18 expression in AD patients and HC. Therefore, we evaluated the expression of IL-1α, IL-1β, IL-18 and IL-33 upon skin irritation in epidermis of the AD patients and HC.

We found that the mRNA and protein levels of IL-1α were significantly higher three hours after tape stripping in healthy individuals, but not in AD patients. In non-atopic subjects the expression of IL-1β and IL-18 (at mRNA and protein levels) revealed no significant differences after the acute barrier disruption in the epidermis. By contrast, in AD patients the mRNA expression of IL-1β showed a significant up-regulation and the IL-1β protein expression showed a significant down-regulation in tape-stripped skin compared with the untreated skin. Furthermore, IL-18 mRNA expression was not altered by tape stripping in AD patients. However, we observed an up-regulation of IL-18 protein expression in AD (even though this was not significant) three hours after skin barrier disruption. Additionally, we evaluated the mRNA and protein expression of IL-33. In healthy individuals, the mRNA expression of IL-33 was significantly increased after tape

stripping, but the IL-33 protein expression showed no significant alteration. In AD patients, both mRNA and protein levels of IL-33 showed no significant difference after barrier disruption by tape stripping.

Some of our findings are in accordance with previous studies, while some of our data are somewhat contradictory with the literature. We hypothesize that possible factors for these discrepancies might be the different number of tape strips, the variations regarding analysed time points and the differences of the anatomical areas where the skin samples were obtained. Further studies evaluating the expression of the IL-1 family members at different time points in AD patients and HC are required and may deliver additional novel insights.

In humans, Nickoloff and Naidu analyzed the mRNA expression of various mediators in skin derived from the buttock of healthy volunteers after acute barrier disruption. They found an increase in several mRNAs within the epidermis 6 h after repeated tape stripping (80–100 times). IL-1β mRNA was present at time 0 and at 1 h, but was not detectable 6 h after tape stripping (Nickoloff and Naidu, 1994). In another *in vivo* human study, Dickel *et al.* demonstrated the up-regulated expression of epidermal mRNAs, including IL-1β and IL-33, 6 h after tape stripping with calculated numbers of tape strips on the upper back skin of HC. However, the mRNA expression of IL-1α was not elevated at the investigated time point (Dickel, Gambichler, Kamphowe, Altmeyer and Skrygan, 2010). Reilly and Green reported that the levels of IL-1α protein were significantly increased in tape-stripped healthy human skin (Reilly and Green, 1999). More recently, Doge *et al.* showed that IL-1α protein levels were increased directly after 50 times tape stripping and decreased over time. The test site was the abdominal skin of healthy individuals (Doge, Avetisyan, Hadam, Pfannes, Rancan, Blume-Peytavi and Vogt, 2017).

In mice, various studies have indicated the regulation of cytokines of the IL-1 family upon skin irritation. Wood *et al.* reported that acute disruption of the permeability barrier by tape stripping stimulates IL-1α expression in a mouse model. They showed that IL-1α increased in the murine epidermis within 10 min, remained elevated at 2 and 4 h, and returned to normal after 24 h (Wood, Elias, Calhoun, Tsai, Grunfeld and Feingold, 1996). In another mouse study, Wood *et al.* reported that the epidermal mRNA levels of IL-1α and IL-1β increased at 2.5 and 7 h after tape stripping and returned to basal levels after 18 h. (Wood, Stalder, Liou, Campbell, Grunfeld, Elias and Feingold, 1997). Additionally, Ye *et al*. reported that acute barrier disruption by tape stripping reinforced the production of several cytokines including IL-1α (Ye, Garg, Calhoun, Feingold, Elias and Ghadially, 2002). Kumari *et al.* also demonstrated that the physical disruption of skin barrier

leads to the release of IL-1α which causes the induction of TSLP (Kumari, Babina, Hazzan and Worm, 2015). A recent study reported that physical skin irritation (by tape stripping) results in TSLP production by simultaneous activation of the IL-1 pathway (Redhu, Franke, Kumari, Francuzik, Babina and Worm, 2020). Another study has demonstrated that the disruption of the epidermal barrier induces an up-regulation in the transcription of IL-33 along with an increase of IL-33 protein expression in the epidermis of mice (Bruhs, Proksch, Schwarz and Schwarz, 2017).

5. CONCLUSIONS AND RECOMMENDATIONS

The epidermis is very important for the formation of skin barrier integrity and cohesion, and builds the physical, chemical and immunological barrier against the environment. The physical barrier of the epidermis is primarily located in the stratum corneum. It has been reported that a barrier dysfunction extends beyond the stratum corneum to tight junctions, which are the second physical barrier structure. An impaired TJ barrier initiates inflammatory processes as well as an immune dysregulation and may promote the development of AD.

This thesis has demonstrated that the expression of skin barrier genes (Cldn-1, FLG and loricrin) is down-regulated in lesional and non-lesional AD skin (exception: no alteration of FLG and loricrin protein levels in non-lesional AD skin versus healthy skin). Collectively, our data suggest that an alteration of the epidermal skin barrier (especially an alteration of Cldn-1 expression) is a key determinant of skin barrier dysfunction in AD and that Cldn-1 may be a susceptibility gene in the pathogenesis of AD.

In this thesis, the regulation of skin barrier genes upon skin irritation by tape stripping was also investigated. All in all, the faster recovery kinetic of TJ in comparison with the SC barrier in response to acute permeability barrier disruption may be a compensation mechanism of the skin implicating the protective homeostasis of skin barrier.

The future challenge in this field is to better understand the molecular pathways that regulate epidermal TJ (function and composition) in healthy and atopic skin. Hopefully, in the future we will be able to use novel therapeutics to regulate epidermal TJ and restore TJ integrity. This approach may provide an innovative therapeutic opportunity in AD and other atopic diseases.

Numerous studies have suggested that cytokines of IL-1 family act as a master switch that triggers both the initiation and maintenance of AD. IL-1α initiates the inflammatory processes, while both IL-1α and IL-1β propagate and maintain skin inflammation in AD by inducing the expression of various cytokines and proinflammatory mediators. IL-18 has also been recognized to fulfill a pivotal role in the pathogenesis of AD either by promoting Th1- or Th2-related responses. Additionally, the production of Th2-associated mediators after IL-33 stimulation indicated the role of IL-33 in orchestrating inflammatory responses at the onset and the perpetuation of Th2-associated diseases such as AD.

In this study, we investigated the expression of cytokines of the IL-1 family in lesional and non-lesional AD skin in comparison with healthy skin, as well as their regulation upon skin irritation.

In summary, we suggest that these members of IL-1 family may be one of the alarm signals in the skin of AD patients upon skin irritation. Further studies in the future could help to better define the IL-1-mediated signalling cascades involved in AD and to identify the possible triggers for activation of these pathways. In conclusion, we suggest that the regulation of IL-18 in inflammatory conditions may open new avenues for potential therapeutic approaches in the treatment of AD by targeting the IL-1 pathway.

REFERENCES

AIJAZ, S., BALDA, M. S. & MATTER, K. 2006. Tight junctions: molecular architecture and function. *Int Rev Cytol,* 248**,** 261-98.

AKDIS, C. A. & AKDIS, M. 2003. Immunological differences between intrinsic and extrinsic types of atopic dermatitis. *Clinical and Experimental Allergy,* 33**,** 1618-1621.

AKDIS, C. A., AKDIS, M., BIEBER, T., BINDSLEV-JENSEN, C., BOGUNIEWICZ, M., EIGENMANN, P., HAMID, Q., KAPP, A., LEUNG, D. Y., LIPOZENCIC, J., LUGER, T. A., MURARO, A., NOVAK, N., PLATTS-MILLS, T. A., ROSENWASSER, L., SCHEYNIUS, A., SIMONS, F. E., SPERGEL, J., TURJANMAA, K., WAHN, U., WEIDINGER, S., WERFEL, T., ZUBERBIER, T., EUROPEAN ACADEMY OF, A., CLINICAL IMMUNOLOGY/AMERICAN ACADEMY OF ALLERGY, A. & IMMUNOLOGY 2006. Diagnosis and treatment of atopic dermatitis in children and adults: European Academy of Allergology and Clinical Immunology/American Academy of Allergy, Asthma and Immunology/PRACTALL Consensus Report. *J Allergy Clin Immunol,* 118**,** 152-69.

ALEXANDER, H., BROWN, S., DANBY, S. & FLOHR, C. 2018. Research Techniques Made Simple: Transepidermal Water Loss Measurement as a Research Tool. *J Invest Dermatol,* 138**,** 2295-2300 e1.

ALI, S., MOHS, A., THOMAS, M., KLARE, J., ROSS, R., SCHMITZ, M. L. & MARTIN, M. U. 2011. The dual function cytokine IL-33 interacts with the transcription factor NF-kappaB to dampen NF-kappaB-stimulated gene transcription. *J Immunol,* 187**,** 1609-16.

AMARBAYASGALAN, T., TAKAHASHI, H., DEKIO, I. & MORITA, E. 2013. Interleukin-8 content in the stratum corneum as an indicator of the severity of inflammation in the lesions of atopic dermatitis. *Int Arch Allergy Immunol,* 160**,** 63-74.

BAEK, J. H., LEE, S. E., CHOI, K. J., CHOI, E. H. & LEE, S. H. 2013. Acute modulations in stratum corneum permeability barrier function affect claudin expression and epidermal tight junction function via changes of epidermal calcium gradient. *Yonsei Med J,* 54**,** 523-8.

BARKSBY, H. E., LEA, S. R., PRESHAW, P. M. & TAYLOR, J. J. 2007. The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders. *Clinical and Experimental Immunology,* 149**,** 217-225.

BATISTA, D. I., PEREZ, L., ORFALI, R. L., ZANIBONI, M. C., SAMORANO, L. P., PEREIRA, N. V., SOTTO, M. N., ISHIZAKI, A. S., OLIVEIRA, L. M., SATO, M. N. & AOKI, V. 2015. Profile of skin barrier proteins (filaggrin, claudins 1 and 4) and Th1/Th2/Th17 cytokines in adults with atopic dermatitis. *J Eur Acad Dermatol Venereol,* 29**,** 1091-5.

BERNARD, M., CARRASCO, C., LAOUBI, L., GUIRAUD, B., ROZIERES, A., GOUJON, C., DUPLAN, H., BESSOU-TOUYA, S., NICOLAS, J. F., VOCANSON, M. & GALLIANO, M. F. 2017. IL-1beta induces thymic stromal lymphopoietin and an atopic dermatitis-like phenotype in reconstructed healthy human epidermis. *J Pathol,* 242**,** 234-245.

BERNDT, U., HINNEN, U., ILIEV, D. & ELSNER, P. 1999. Role of the atopy score and of single atopic features as risk factors for the development of hand eczema in trainee metal workers. *Br J Dermatol,* 140**,** 922-4.

BIANCHI, P., RIBET, V., CASAS, C., LEJEUNE, O., SCHMITT, A. M. & REDOULES, D. 2012. Analysis of Gene Expression in Atopic Dermatitis Using a Microabrasive Method. *Journal of Investigative Dermatology,* 132**,** 469-472.

BICKENBACH, J. R., GREER, J. M., BUNDMAN, D. S., ROTHNAGEL, J. A. & ROOP, D. R. 1995. Loricrin expression is coordinated with other epidermal proteins and the appearance of lipid lamellar granules in development. *J Invest Dermatol,* 104**,** 405-10.

BIEBER, T. 2008. Atopic dermatitis. *N Engl J Med,* 358**,** 1483-94.

BOHME, M., SODERHALL, C., KULL, I., BERGSTROM, A., VAN HAGE, M. & WAHLGREN, C. F. 2012. Filaggrin mutations increase the risk for persistent dry skin and eczema independent of sensitization. *J Allergy Clin Immunol,* 129**,** 1153-5.

BOU-DARGHAM, M. J., KHAMIS, Z. I., COGNETTA, A. B. & SANG, Q. A. 2017. The Role of Interleukin-1 in Inflammatory and Malignant Human Skin Diseases and the Rationale for Targeting Interleukin-1 Alpha. *Med Res Rev,* 37**,** 180-216.

BRANDNER, J. M., KIEF, S., GRUND, C., RENDL, M., HOUDEK, P., KUHN, C., TSCHACHLER, E., FRANKE, W. W. & MOLL, I. 2002. Organization and formation of the tight junction system in human epidermis and cultured keratinocytes. *Eur J Cell Biol,* 81**,** 253-63.

BRANDNER, J. M., MCINTYRE, M., KIEF, S., WLADYKOWSKI, E. & MOLL, I. 2003. Expression and localization of tight junction-associated proteins in human hair follicles. *Arch Dermatol Res,* 295**,** 211-21.

BRUHS, A., PROKSCH, E., SCHWARZ, T. & SCHWARZ, A. 2017. Disruption of the epidermal barrier induces regulatory T cells via IL-33 in mice. *J Invest Dermatol*.

BRYDGES, S. D., MUELLER, J. L., MCGEOUGH, M. D., PENA, C. A., MISAGHI, A., GANDHI, C., PUTNAM, C. D., BOYLE, D. L., FIRESTEIN, G. S., HORNER, A. A., SOROOSH, P., WATFORD, W. T., O'SHEA, J. J., KASTNER, D. L. & HOFFMAN, H. M. 2009. Inflammasome-Mediated Disease Animal Models Reveal Roles for Innate but Not Adaptive Immunity. *Immunity,* 30**,** 875-887.

BUENTKE, E., HEFFLER, L. C., WALLIN, R. P., LOFMAN, C., LJUNGGREN, H. G. & SCHEYNIUS, A. 2001. The allergenic yeast Malassezia furfur induces maturation of human dendritic cells. *Clin Exp Allergy,* 31**,** 1583-93.

CARMI-LEVY, I., HOMEY, B. & SOUMELIS, V. 2011. A modular view of cytokine networks in atopic dermatitis. *Clin Rev Allergy Immunol,* 41**,** 245-53.

CEVIKBAS, F. & STEINHOFF, M. 2012. IL-33: a novel danger signal system in atopic dermatitis. *J Invest Dermatol,* 132**,** 1326-9.

CHENG, D. Y., HAO, Y. W., ZHOU, W. L. & MA, Y. R. 2014. The Relationship between Interleukin-18 Polymorphisms and Allergic Disease: A Meta-Analysis. *Biomed Research International*.

COLOTTA, F., RE, F., MUZIO, M., BERTINI, R., POLENTARUTTI, N., SIRONI, M., GIRI, J. G., DOWER, S. K., SIMS, J. E. & MANTOVANI, A. 1993. Interleukin-1 Type-Ii Receptor - a Decoy Target for Il-1 That Is Regulated by Il-4. *Science,* 261**,** 472-475.

CRAVEN, R. R., GAO, X., ALLEN, I. C., GRIS, D., WARDENBURG, J. B., MCELVANIA-TEKIPPE, E., TING, J. P. & DUNCAN, J. A. 2009. Staphylococcus aureus alpha-Hemolysin Activates the NLRP3-Inflammasome in Human and Mouse Monocytic Cells. *Plos One,* 4.

DAI, X. J., SAYAMA, K., TOHYAMA, M., SHIRAKATA, Y., HANAKAWA, Y., TOKUMARU, S., YANG, L. J., HIRAKAWA, S. & HASHIMOTO, K. 2011. Mite allergen is a danger signal for the skin via activation of inflammasome in keratinocytes. *Journal of Allergy and Clinical Immunology,* 127**,** 806-U486.

DE BENEDETTO, A., RAFAELS, N. M., MCGIRT, L. Y., IVANOV, A. I., GEORAS, S. N., CHEADLE, C., BERGER, A. E., ZHANG, K., VIDYASAGAR, S., YOSHIDA, T., BOGUNIEWICZ, M., HATA, T., SCHNEIDER, L. C., HANIFIN, J. M., GALLO, R. L., NOVAK, N., WEIDINGER, S., BEATY, T. H., LEUNG, D. Y., BARNES, K. C. & BECK, L. A. 2011. Tight junction defects in patients with atopic dermatitis. *J Allergy Clin Immunol,* 127**,** 773-86 e1-7.

DICKEL, H., GAMBICHLER, T., KAMPHOWE, J., ALTMEYER, P. & SKRYGAN, M. 2010. Standardized tape stripping prior to patch testing induces upregulation of Hsp90, Hsp70, IL-33, TNF-alpha and IL-8/CXCL8 mRNA: new insights into the involvement of 'alarmins'. *Contact Dermatitis,* 63**,** 215-22.

DIEPGEN, T. L. & FARTASCH, M. 1992. Recent epidemiological and genetic studies in atopic dermatitis. *Acta Derm Venereol Suppl (Stockh),* 176**,** 13-8.

DIEPGEN, T. L., FARTASCH, M. & HORNSTEIN, O. P. 1989. Evaluation and relevance of atopic basic and minor features in patients with atopic dermatitis and in the general population. *Acta Derm Venereol Suppl (Stockh),* 144**,** 50-4.

DIEPGEN, T. L., SAUERBREI, W. & FARTASCH, M. 1996. Development and validation of diagnostic scores for atopic dermatitis incorporating criteria of data quality and practical usefulness. *J Clin Epidemiol,* 49**,** 1031-8.

DINARELLO, C. A. 1996. Biologic basis for interleukin-1 in disease. *Blood,* 87**,** 2095-147.

DINARELLO, C. A. 2009. Immunological and inflammatory functions of the interleukin-1 family. *Annu Rev Immunol,* 27**,** 519-50.

DINARELLO, C. A. 2011. Interleukin-1 in the pathogenesis and treatment of inflammatory diseases. *Blood,* 117**,** 3720-3732.

DOGE, N., AVETISYAN, A., HADAM, S., PFANNES, E. K. B., RANCAN, F., BLUME-PEYTAVI, U. & VOGT, A. 2017. Assessment of skin barrier function and biochemical changes of ex vivo human skin in response to physical and chemical barrier disruption. *Eur J Pharm Biopharm,* 116**,** 138-148.

DRISCOLL, P. C., GRONENBORN, A. M., WINGFIELD, P. T. & CLORE, G. M. 1990. Determination of the Secondary Structure and Molecular Topology of Interleukin-1-Beta by Use of 2-Dimensional and 3-Dimensional Heteronuclear N-15-H-1 Nmr-Spectroscopy. *Biochemistry,* 29**,** 4668-4682.

ELIAS, P. M. & WAKEFIELD, J. S. 2011. Therapeutic implications of a barrier-based pathogenesis of atopic dermatitis. *Clin Rev Allergy Immunol,* 41**,** 282-95.

FARQUHAR, M. G. & PALADE, G. E. 1963. Junctional Complexes in Various Epithelia. *Journal of Cell Biology,* 17**,** 375-&.

FETTELSCHOSS, A., KISTOWSKA, M., LEIBUNDGUT-LANDMANN, S., BEER, H. D., JOHANSEN, P., SENTI, G., CONTASSOT, E., BACHMANN, M. F., FRENCH, L. E., OXENIUS, A. & KUNDIG, T. M. 2011. Inflammasome activation and IL-1beta target IL-1alpha for secretion as opposed to surface expression. *Proc Natl Acad Sci U S A,* 108**,** 18055-60.

FINDLEY, M. K. & KOVAL, M. 2009. Regulation and roles for claudin-family tight junction proteins. *IUBMB Life,* 61**,** 431-7.

FLUHR, J. W., FEINGOLD, K. R. & ELIAS, P. M. 2006. Transepidermal water loss reflects permeability barrier status: validation in human and rodent in vivo and ex vivo models. *Exp Dermatol,* 15**,** 483-92.

FUNAKOSHI-TAGO, M., TAGO, K., SATO, Y., TOMINAGA, S. & KASAHARA, T. 2011. JAK2 is an important signal transducer in IL-33-induced NF-kappa B activation. *Cellular Signalling,* 23**,** 363-370.

FURUSE, M., HATA, M., FURUSE, K., YOSHIDA, Y., HARATAKE, A., SUGITANI, Y., NODA, T., KUBO, A. & TSUKITA, S. 2002. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol,* 156**,** 1099-111.

GADINA, M. & JEFFERIES, C. A. 2007. IL-33: a sheep in wolf's clothing? *Sci STKE,* 2007**,** pe31.

GAN, S. Q., MCBRIDE, O. W., IDLER, W. W., MARKOVA, N. & STEINERT, P. M. 1990. Organization, structure, and polymorphisms of the human profilaggrin gene. *Biochemistry,* 29**,** 9432-40.

GERRITSEN, M. J., VAN PELT, J. P. & VAN DE KERKHOF, P. C. 1996. Response of the clinically uninvolved skin of psoriatic patients to tape stripping during acitretin treatment. *Acta Derm Venereol,* 76**,** 6-9.

GITTLER, J. K., SHEMER, A., SUAREZ-FARINAS, M., FUENTES-DUCULAN, J., GULEWICZ, K. J., WANG, C. Q. F., MITSUI, H., CARDINALE, I., STRONG, C. D., KRUEGER, J. G. & GUTTMAN-YASSKY, E. 2012. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *Journal of Allergy and Clinical Immunology,* 130**,** 1344-1354.

GREALLY, P., HUSSAIN, M. J., PRICE, J. F. & COLEMAN, R. 1992. Interleukin-1 alpha and soluble interleukin-2 receptor in atopic dermatitis. *Arch Dis Child,* 67**,** 1413.

GREENFEDER, S. A., NUNES, P., KWEE, L., LABOW, M., CHIZZONITE, R. A. & JU, G. 1995. Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. *J Biol Chem,* 270**,** 13757-65.

GRUBER, R., BORNCHEN, C., ROSE, K., DAUBMANN, A., VOLKSDORF, T., WLADYKOWSKI, E., VIDAL, Y. S. S., PETERS, E. M., DANSO, M., BOUWSTRA, J. A., HENNIES, H. C., MOLL, I., SCHMUTH, M. & BRANDNER, J. M. 2015. Diverse regulation of claudin-1 and claudin-4 in atopic dermatitis. *Am J Pathol,* 185**,** 2777-89.

HADJ-RABIA, S., BAALA, L., VABRES, P., HAMEL-TEILLAC, D., JACQUEMIN, E., FABRE, M., LYONNET, S., DE PROST, Y., MUNNICH, A., HADCHOUEL, M. & SMAHI, A. 2004. Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. *Gastroenterology,* 127**,** 1386-90.

HAMID, Q., BOGUNIEWICZ, M. & LEUNG, D. Y. 1994. Differential in situ cytokine gene expression in acute versus chronic atopic dermatitis. *J Clin Invest,* 94**,** 870-6.

HARALDSEN, G., BALOGH, J., POLLHEIMER, J., SPONHEIM, J. & KUCHLER, A. M. 2009. Interleukin-33-cytokine of dual function or novel alarmin? *Trends in Immunology,* 30**,** 227-233.

HEINZERLING, L., MARI, A., BERGMANN, K. C., BRESCIANI, M., BURBACH, G., DARSOW, U., DURHAM, S., FOKKENS, W., GJOMARKAJ, M., HAAHTELA, T.,

BOM, A. T., WOHRL, S., MAIBACH, H. & LOCKEY, R. 2013. The skin prick test - European standards. *Clin Transl Allergy,* 3**,** 3.

HEISKALA, M., PETERSON, P. A. & YANG, Y. 2001. The roles of claudin superfamily proteins in paracellular transport. *Traffic,* 2**,** 93-8.

HOHL, D., LICHTI, U., BREITKREUTZ, D., STEINERT, P. M. & ROOP, D. R. 1991. Transcription of the human loricrin gene in vitro is induced by calcium and cell density and suppressed by retinoic acid. *J Invest Dermatol,* 96**,** 414-8.

INOUE, Y., AIHARA, M., KIRINO, M., HARADA, I., KOMORI-YAMAGUCHI, J., YAMAGUCHI, Y., NAGASHIMA, Y. & IKEZAWA, Z. 2011a. Interleukin-18 is elevated in the horny layer in patients with atopic dermatitis and is associated with Staphylococcus aureus colonization. *Br J Dermatol,* 164**,** 560-7.

INOUE, Y., AIHARA, M., KIRINO, M., HARADA, I., KOMORI-YAMAGUCHI, J., YAMAGUCHI, Y., NAGASHIMA, Y. & IKEZAWA, Z. 2011b. Interleukin-18 is elevated in the horny layer in patients with atopic dermatitis and is associated with Staphylococcus aureus colonization. *British Journal of Dermatology,* 164**,** 560-567.

IRVINE, A. D. & MCLEAN, W. H. I. 2006. Breaking the (Un)sound barrier: Filaggrin is a major gene for atopic dermatitis. *Journal of Investigative Dermatology,* 126**,** 1200-1202.

ISHIDA-YAMAMOTO, A. & IIZUKA, H. 1998. Structural organization of cornified cell envelopes and alterations in inherited skin disorders. *Exp Dermatol,* 7**,** 1-10.

JUNGERSTED, J. M., SCHEER, H., MEMPEL, M., BAURECHT, H., CIFUENTES, L., HOGH, J. K., HELLGREN, L. I., JEMEC, G. B., AGNER, T. & WEIDINGER, S. 2010. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. *Allergy,* 65**,** 911-8.

KAGI, M. K., JOLLER-JEMELKA, H. & WUTHRICH, B. 1992. Correlation of eosinophils, eosinophil cationic protein and soluble interleukin-2 receptor with the clinical activity of atopic dermatitis. *Dermatology,* 185**,** 88-92.

KALININ, A., MAREKOV, L. N. & STEINERT, P. M. 2001. Assembly of the epidermal cornified cell envelope. *J Cell Sci,* 114**,** 3069-70.

KAWASAKI, H., KUBO, A., SASAKI, T. & AMAGAI, M. 2011. Loss-of-function mutations within the filaggrin gene and atopic dermatitis. *Curr Probl Dermatol,* 41**,** 35-46.

KEZIC, S., KEMPERMAN, P. M., KOSTER, E. S., DE JONGH, C. M., THIO, H. B., CAMPBELL, L. E., IRVINE, A. D., MCLEAN, W. H., PUPPELS, G. J. & CASPERS, P. J. 2008. Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. *J Invest Dermatol,* 128**,** 2117-9.

KEZIC, S., O'REGAN, G. M., LUTTER, R., JAKASA, I., KOSTER, E. S., SAUNDERS, S., CASPERS, P., KEMPERMAN, P. M., PUPPELS, G. J., SANDILANDS, A., CHEN, H., CAMPBELL, L. E., KROBOTH, K., WATSON, R., FALLON, P. G., MCLEAN, W. H. & IRVINE, A. D. 2012. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J Allergy Clin Immunol,* 129**,** 1031-9 e1.

KIM, E., LEE, J. E., NAMKUNG, J. H., PARK, J. H., KIM, S., SHIN, E. S., CHO, E. Y. & YANG, J. M. 2007. Association of the single-nucleotide polymorphism and haplotype of the interleukin 18 gene with atopic dermatitis in Koreans. *Clin Exp Allergy,* 37**,** 865-71.

KIRSCHNER, N., BOHNER, C., RACHOW, S. & BRANDNER, J. M. 2010. Tight junctions: is there a role in dermatology? *Arch Dermatol Res,* 302**,** 483-93.

KIRSCHNER, N. & BRANDNER, J. M. 2012. Barriers and more: functions of tight junction proteins in the skin. *Ann N Y Acad Sci,* 1257**,** 158-66.

KOCH, P. J., DE VIRAGH, P. A., SCHARER, E., BUNDMAN, D., LONGLEY, M. A., BICKENBACH, J., KAWACHI, Y., SUGA, Y., ZHOU, Z., HUBER, M., HOHL, D., KARTASOVA, T., JARNIK, M., STEVEN, A. C. & ROOP, D. R. 2000. Lessons from loricrin-deficient mice: compensatory mechanisms maintaining skin barrier function in the absence of a major cornified envelope protein. *J Cell Biol,* 151**,** 389-400.

KONISHI, H., TSUTSUI, H., MURAKAMI, T., YUMIKURA-FUTATSUGI, S., YAMANAKA, K., TANAKA, M., IWAKURA, Y., SUZUKI, N., TAKEDA, K., AKIRA, S., NAKANISHI, K. & MIZUTANI, H. 2002. IL-18 contributes to the spontaneous development of atopic dermatitis-like inflammatory skin lesion independently of IgE/stat6 under specific pathogen-free conditions. *Proc Natl Acad Sci U S A,* 99**,** 11340-5.

KUMARI, V., BABINA, M., HAZZAN, T. & WORM, M. 2015. Thymic stromal lymphopoietin induction by skin irritation is independent of tumour necrosis factor-alpha, but supported by interleukin-1. *Br J Dermatol,* 172**,** 951-60.

LARSEN, F. S. & HANIFIN, J. M. 2002. Epidemiology of atopic dermatitis. *Immunology and Allergy Clinics of North America,* 22**,** 1-+.

LEE, J. H., CHO, D. H. & PARK, H. J. 2015. IL-18 and Cutaneous Inflammatory Diseases. *Int J Mol Sci,* 16**,** 29357-69.

LEUNG, D. Y., BOGUNIEWICZ, M., HOWELL, M. D., NOMURA, I. & HAMID, Q. A. 2004. New insights into atopic dermatitis. *J Clin Invest,* 113**,** 651-7.

LI, J. G., LEYVA-CASTILLO, J. M., HENER, P., EISENMANN, A., ZAAFOURI, S., JONCA, N., SERRE, G., BIRLING, M. C. & LI, M. 2016. Counterregulation between thymic stromal lymphopoietin- and IL-23-driven immune axes shapes skin inflammation in mice with epidermal barrier defects. *Journal of Allergy and Clinical Immunology,* 138**,** 150-+.

LIEW, F. Y., PITMAN, N. I. & MCINNES, I. B. 2010. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nat Rev Immunol,* 10**,** 103-10.

MAINTZ, L. & NOVAK, N. 2007. Getting more and more complex: the pathophysiology of atopic eczema. *Eur J Dermatol,* 17**,** 267-83.

MAREKOV, L. N. & STEINERT, P. M. 1998. Ceramides are bound to structural proteins of the human foreskin epidermal cornified cell envelope. *J Biol Chem,* 273**,** 17763-70.

MATSUMOTO, M., SUGIURA, H. & UEHARA, M. 2000. Skin barrier function in patients with completely healed atopic dermatitis. *J Dermatol Sci,* 23**,** 178-82.

MCGRATH, J. A. & UITTO, J. 2008. The filaggrin story: novel insights into skin-barrier function and disease. *Trends Mol Med,* 14**,** 20-7.

MILDNER, M., JIN, J., ECKHART, L., KEZIC, S., GRUBER, F., BARRESI, C., STREMNITZER, C., BUCHBERGER, M., MLITZ, V., BALLAUN, C., STERNICZKY, B., FODINGER, D. & TSCHACHLER, E. 2010. Knockdown of filaggrin impairs diffusion barrier function and increases UV sensitivity in a human skin model. *J Invest Dermatol,* 130**,** 2286-94.

MU, Z., ZHAO, Y., LIU, X., CHANG, C. & ZHANG, J. 2014. Molecular biology of atopic dermatitis. *Clin Rev Allergy Immunol,* 47**,** 193-218.

MUNOZ-PLANILLO, R., FRANCHI, L., MILLER, L. S. & NUNEZ, G. 2009. A Critical Role for Hemolysins and Bacterial Lipoproteins in Staphylococcus aureus-Induced Activation of the Nlrp3 Inflammasome. *Journal of Immunology,* 183**,** 3942-3948.

NAKANO, H., TSUTSUI, H., TERADA, M., YASUDA, K., MATSUI, K., YUMIKURA-FUTATSUGI, S., YAMANAKA, K., MIZUTANI, H., YAMAMURA, T. & NAKANISHI, K. 2003. Persistent secretion of IL-18 in the skin contributes to IgE response in mice. *Int Immunol,* 15**,** 611-21.

NETEA, M. G., VAN DE VEERDONK, F. L., VAN DER MEER, J. W., DINARELLO, C. A. & JOOSTEN, L. A. 2015. Inflammasome-independent regulation of IL-1-family cytokines. *Annu Rev Immunol,* 33**,** 49-77.

NICKOLOFF, B. J. & NAIDU, Y. 1994. Perturbation of epidermal barrier function correlates with initiation of cytokine cascade in human skin. *J Am Acad Dermatol,* 30**,** 535-46.

NISHIFUJI, K. & YOON, J. S. 2013. The stratum corneum: the rampart of the mammalian body. *Vet Dermatol,* 24**,** 60-72 e15-6.

NOVAK, N., VALENTA, R., BOHLE, B., LAFFER, S., HABERSTOK, J., KRAFT, S. & BIEBER, T. 2004. Fc epsilon RI engagement of Langerhans cell-like dendritic cells and inflammatory dendritic epidermal cell-like dendritic cells induces chemotactic signals and different T-cell phenotypes in vitro. *Journal of Allergy and Clinical Immunology,* 113**,** 949-957.

NUTAN, F. N., KANWAR, A. J. & PARSAD, D. 2012. The effect of topically applied corticosteroids on interleukin 1beta levels in patients with atopic dermatitis. *J Eur Acad Dermatol Venereol,* 26**,** 1020-2.

NYGAARD, U., HVID, M., JOHANSEN, C., BUCHNER, M., FOLSTER-HOLST, R., DELEURAN, M. & VESTERGAARD, C. 2016. TSLP, IL-31, IL-33 and sST2 are new biomarkers in endophenotypic profiling of adult and childhood atopic dermatitis. *J Eur Acad Dermatol Venereol,* 30**,** 1930-1938.

NYGAARD, U., VAN DEN BOGAARD, E. H., NIEHUES, H., HVID, M., DELEURAN, M., JOHANSEN, C. & VESTERGAARD, C. 2017. The "Alarmins" HMBG1 and IL-33 Downregulate Structural Skin Barrier Proteins and Impair Epidermal Growth. *Acta Dermato-Venereologica,* 97**,** 305-312.

OBOKI, K., OHNO, T., KAJIWARA, N., ARAE, K., MORITA, H., ISHII, A., NAMBU, A., ABE, T., KIYONARI, H., MATSUMOTO, K., SUDO, K., OKUMURA, K., SAITO, H. & NAKAE, S. 2010. IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proceedings of the National Academy of Sciences of the United States of America,* 107**,** 18581-18586.

OKAMURA, H., NAGATA, K., KOMATSU, T., TANIMOTO, T., NUKATA, Y., TANABE, F., AKITA, K., TORIGOE, K., OKURA, T., FUKUDA, S. & KURIMOTO, M. 1995. A Novel Costimulatory Factor for Gamma-Interferon Induction Found in the Livers of Mice Causes Endotoxic-Shock. *Infection and Immunity,* 63**,** 3966-3972.

OKAMURA, H., TSUTSUI, H., KASHIWAMURA, S. I., YOSHIMOTO, T. & NAKANISHI, K. 1998. Interleukin-18: A novel cytokine that augments both innate and acquired immunity. *Advances in Immunology, Vol 70,* 70**,** 281-312.

OKAMURA, H., TSUTSUI, H., KOMATSU, T., YUTSUDO, M., HAKURA, A., TANIMOTO, T., TORIGOE, K., OKURA, T., NUKADA, Y., HATTORI, K., AKITA, K., NAMBA, M.,

TANABE, F., KONISHI, K., FUKUDA, S. & KURIMOTO, M. 1995. Cloning of a New Cytokine That Induces Ifn-Gamma Production by T-Cells. *Nature,* 378**,** 88-91.

ONG, P. Y. 2014. New insights in the pathogenesis of atopic dermatitis. *Pediatr Res,* 75**,** 171-5.

ORANJE, A. P., GLAZENBURG, E. J., WOLKERSTORFER, A. & SPEK, F. B. D. W. V. D. 2007. Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. *British Journal of Dermatology,* 157**,** 645-648.

OVERGAARD, C. E., DAUGHERTY, B. L., MITCHELL, L. A. & KOVAL, M. 2011. Claudins: control of barrier function and regulation in response to oxidant stress. *Antioxid Redox Signal,* 15**,** 1179-93.

OYOSHI, M. K., HE, R., KUMAR, L., YOON, J. & GEHA, R. S. 2009. Cellular and molecular mechanisms in atopic dermatitis. *Adv Immunol,* 102**,** 135-226.

PALMER, C. N. A., IRVINE, A. D., TERRON-KWIATKOWSKI, A., ZHAO, Y. W., LIAO, H. H., LEE, S. P., GOUDIE, D. R., SANDILANDS, A., CAMPBELL, L. E., SMITH, F. J. D., O'REGAN, G. M., WATSON, R. M., CECIL, J. E., BALE, S. J., COMPTON, J. G., DIGIOVANNA, J. J., FLECKMAN, P., LEWIS-JONES, S., ARSECULERATNE, G., SERGEANT, A., MUNRO, C. S., EL HOUATE, B., MCELREAVEY, K., HALKJAER, L. B., BISGAARD, H., MUKHOPADHYAY, S. & MCLEAN, W. H. I. 2006. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nature Genetics,* 38**,** 441-446.

PRESLAND, R. B., HAYDOCK, P. V., FLECKMAN, P., NIRUNSUKSIRI, W. & DALE, B. A. 1992. Characterization of the human epidermal profilaggrin gene. Genomic organization and identification of an S-100-like calcium binding domain at the amino terminus. *J Biol Chem,* 267**,** 23772-81.

PROKSCH, E., BRANDNER, J. M. & JENSEN, J. M. 2008. The skin: an indispensable barrier. *Exp Dermatol,* 17**,** 1063-72.

PROKSCH, E., FOLSTER-HOLST, R., BRAUTIGAM, M., SEPEHRMANESH, M., PFEIFFER, S. & JENSEN, J. M. 2009. Role of the epidermal barrier in atopic dermatitis. *J Dtsch Dermatol Ges,* 7**,** 899-910.

PUSHPARAJ, P. N., TAY, H. K., H'NG S, C., PITMAN, N., XU, D., MCKENZIE, A., LIEW, F. Y. & MELENDEZ, A. J. 2009. The cytokine interleukin-33 mediates anaphylactic shock. *Proc Natl Acad Sci U S A,* 106**,** 9773-8.

RACHOW, S., ZORN-KRUPPA, M., OHNEMUS, U., KIRSCHNER, N., VIDAL-Y-SY, S., VON DEN DRIESCH, P., BORNCHEN, C., EBERLE, J., MILDNER, M., VETTORAZZI, E., ROSENTHAL, R., MOLL, I. & BRANDNER, J. M. 2013. Occludin is involved in adhesion, apoptosis, differentiation and Ca2+-homeostasis of human keratinocytes: implications for tumorigenesis. *PLoS One,* 8**,** e55116.

REDHU, D., FRANKE, K., KUMARI, V., FRANCUZIK, W., BABINA, M. & WORM, M. 2020. Thymic stromal lymphopoietin production induced by skin irritation results from concomitant activation of protease-activated receptor 2 and interleukin 1 pathways. *Br J Dermatol,* 182**,** 119-129.

REILLY, D. M. & GREEN, M. R. 1999. Eicosanoid and cytokine levels in acute skin irritation in response to tape stripping and capsaicin. *Acta Derm Venereol,* 79**,** 187-90.

RIDER, P., CARMI, Y., GUTTMAN, O., BRAIMAN, A., COHEN, I., VORONOV, E., WHITE, M. R., DINARELLO, C. A. & APTE, R. N. 2011. IL-1 alpha and IL-1 beta Recruit Different Myeloid Cells and Promote Different Stages of Sterile Inflammation. *Journal of Immunology,* 187**,** 4835-4843.

RINALDI, A. O., MORITA, H., WAWRZYNIAK, P., DREHER, A., GRANT, S., SVEDENHAG, P. & AKDIS, C. A. 2019. Direct assessment of skin epithelial barrier by electrical impedance spectroscopy. *Allergy,* 74**,** 1934-1944.

ROOP, D. 1995. Defects in the barrier. *Science,* 267**,** 474-5.

ROSS-HANSEN, K., LINNEBERG, A., JOHANSEN, J. D., HERSOUG, L. G., BRASCH-ANDERSEN, C., MENNE, T. & THYSSEN, J. P. 2013. The role of glutathione S-transferase and claudin-1 gene polymorphisms in contact sensitization: a cross-sectional study. *Br J Dermatol,* 168**,** 762-70.

SANDILANDS, A., TERRON-KWIATKOWSKI, A., HULL, P. R., O'REGAN, G. M., CLAYTON, T. H., WATSON, R. M., CARRICK, T., EVANS, A. T., LIAO, H., ZHAO, Y., CAMPBELL, L. E., SCHMUTH, M., GRUBER, R., JANECKE, A. R., ELIAS, P. M., VAN STEENSEL, M. A., NAGTZAAM, I., VAN GEEL, M., STEIJLEN, P. M., MUNRO, C. S., BRADLEY, D. G., PALMER, C. N., SMITH, F. J., MCLEAN, W. H. & IRVINE, A. D. 2007. Comprehensive analysis of the gene encoding filaggrin uncovers prevalent and rare mutations in ichthyosis vulgaris and atopic eczema. *Nat Genet,* 39**,** 650-4.

SAVINKO, T., MATIKAINEN, S., SAARIALHO-KERE, U., LEHTO, M., WANG, G., LEHTIMAKI, S., KARISOLA, P., REUNALA, T., WOLFF, H., LAUERMA, A. & ALENIUS, H. 2012. IL-33 and ST2 in atopic dermatitis: expression profiles and modulation by triggering factors. *J Invest Dermatol,* 132**,** 1392-400.

SCHMITTGEN, T. D. & LIVAK, K. J. 2008. Analyzing real-time PCR data by the comparative C-T method. *Nature Protocols,* 3**,** 1101-1108.

SCHMUTH, M., FLUHR, J. W., CRUMRINE, D. C., UCHIDA, Y., HACHEM, J. P., BEHNE, M., MOSKOWITZ, D. G., CHRISTIANO, A. M., FEINGOLD, K. R. & ELIAS, P. M. 2004. Structural and functional consequences of loricrin mutations in human loricrin keratoderma (Vohwinkel syndrome with ichthyosis). *J Invest Dermatol,* 122**,** 909-22.

SEDIMBI, S. K., HAGGLOF, T. & KARLSSON, M. C. 2013. IL-18 in inflammatory and autoimmune disease. *Cell Mol Life Sci,* 70**,** 4795-808.

SEIDENARI, S. & GIUSTI, G. 1995a. Objective assessment of the skin of children affected by atopic dermatitis: A study of pH, capacitance and TEWL in eczematous and clinically uninvolved skin. *Acta Dermato-Venereologica,* 75**,** 429-433.

SEIDENARI, S. & GIUSTI, G. 1995b. Objective assessment of the skin of children affected by atopic dermatitis: a study of pH, capacitance and TEWL in eczematous and clinically uninvolved skin. *Acta Derm Venereol,* 75**,** 429-33.

SOUMELIS, V., RECHE, P. A., KANZLER, H., YUAN, W., EDWARD, G., HOMEY, B., GILLIET, M., HO, S., ANTONENKO, S., LAUERMA, A., SMITH, K., GORMAN, D., ZURAWSKI, S., ABRAMS, J., MENON, S., MCCLANAHAN, T., DE WAAL-MALEFYT RD, R., BAZAN, F., KASTELEIN, R. A. & LIU, Y. J. 2002. Human epithelial cells trigger dendritic cell mediated allergic inflammation by producing TSLP. *Nat Immunol,* 3**,** 673-80.

STEINERT, P. M. & MAREKOV, L. N. 1995. The proteins elafin, filaggrin, keratin intermediate filaments, loricrin, and small proline-rich proteins 1 and 2 are isodipeptide cross-linked components of the human epidermal cornified cell envelope. *J Biol Chem,* 270**,** 17702-11.

STEVEN, A. C., BISHER, M. E., ROOP, D. R. & STEINERT, P. M. 1990. Biosynthetic pathways of filaggrin and loricrin--two major proteins expressed by terminally differentiated epidermal keratinocytes. *J Struct Biol,* 104**,** 150-62.

STEVEN, A. C. & STEINERT, P. M. 1994. Protein composition of cornified cell envelopes of epidermal keratinocytes. *J Cell Sci,* 107 (Pt 2)**,** 693-700.

STRACHAN, L. R. & GHADIALLY, R. 2008. Tiers of clonal organization in the epidermis: the epidermal proliferation unit revisited. *Stem Cell Rev,* 4**,** 149-57.

SUAREZ-FARINAS, M., DHINGRA, N., GITTLER, J., SHEMER, A., CARDINALE, I., DE GUZMAN STRONG, C., KRUEGER, J. G. & GUTTMAN-YASSKY, E. 2013. Intrinsic atopic dermatitis shows similar TH2 and higher TH17 immune activation compared with extrinsic atopic dermatitis. *J Allergy Clin Immunol,* 132**,** 361-70.

TANAKA, T., TSUTSUI, H., YOSHIMOTO, T., KOTANI, M., MATSUMOTO, M., FUJITA, A. & WANG, W. 2001. Interleukin-18 is elevated in the sera from patients with atopic dermatitis and from atopic dermatitis model mice, NC/Nga. *International Archives of Allergy and Immunology,* 125**,** 236-240.

TERADA, M., TSUTSUI, H., IMAI, Y., YASUDA, K., MIZUTANI, H., YAMANISHI, K., KUBO, M., MATSUI, K., SANO, H. & NAKANISHI, K. 2006. Contribution of IL-18 to atopic-dermatitis-like skin inflammation induced by Staphylococcus aureus product in mice. *Proc Natl Acad Sci U S A,* 103**,** 8816-21.

THESTRUP-PEDERSEN, K. 1997. Which factors are of relevance in the pathogenesis of atopic dermatitis? *European Journal of Dermatology,* 7**,** 549-553.

THORNBERRY, N. A., BULL, H. G., CALAYCAY, J. R., CHAPMAN, K. T., HOWARD, A. D., KOSTURA, M. J., MILLER, D. K., MOLINEAUX, S. M., WEIDNER, J. R., AUNINS, J., ELLISTON, K. O., AYALA, J. M., CASANO, F. J., CHIN, J., DING, G. J. F., EGGER, L. A., GAFFNEY, E. P., LIMJUCO, G., PALYHA, O. C., RAJU, S. M., ROLANDO, A. M., SALLEY, J. P., YAMIN, T. T., LEE, T. D., SHIVELY, J. E., MACCROSS, M., MUMFORD, R. A., SCHMIDT, J. A. & TOCCI, M. J. 1992. A Novel Heterodimeric Cysteine Protease Is Required for Interleukin-1-Beta Processing in Monocytes. *Nature,* 356**,** 768-774.

THYSSEN, J. P. & KEZIC, S. 2014. Causes of epidermal filaggrin reduction and their role in the pathogenesis of atopic dermatitis. *J Allergy Clin Immunol,* 134**,** 792-9.

TSUTSUI, H., YOSHIMOTO, T., HAYASHI, N., MIZUTANI, H. & NAKANISHI, K. 2004. Induction of allergic inflammation by interleukin-18 in experimental animal models. *Immunol Rev,* 202**,** 115-38.

VAN ITALLIE, C. M. & ANDERSON, J. M. 2006. Claudins and epithelial paracellular transport. *Annu Rev Physiol,* 68**,** 403-29.

WAKAMATSU, K., OGITA, H., OKABE, N., IRIE, K., TANAKA-OKAMOTO, M., ISHIZAKI, H., ISHIDA-YAMAMOTO, A., IIZUKA, H., MIYOSHI, J. & TAKAI, Y. 2007. Up-regulation of loricrin expression by cell adhesion molecule nectin-1 through Rap1-ERK signaling in keratinocytes. *J Biol Chem,* 282**,** 18173-81.

WERMAN, A., WERMAN-VENKERT, R., WHITE, R., LEE, J. K., WERMAN, B., KRELIN, Y., VORONOV, E., DINARELLO, C. A. & APTE, R. N. 2004. The precursor form of IL-1alpha is an intracrine proinflammatory activator of transcription. *Proc Natl Acad Sci U S A,* 101**,** 2434-9.

WERTZ, P. W., MADISON, K. C. & DOWNING, D. T. 1989. Covalently Bound Lipids of Human Stratum-Corneum. *Journal of Investigative Dermatology,* 92**,** 109-111.

WILLART, M. A. M., DESWARTE, K., POULIOT, P., BRAUN, H., BEYAERT, R., LAMBRECHT, B. N. & HAMMAD, H. 2012. Interleukin-1 alpha controls allergic sensitization to inhaled house dust mite via the epithelial release of GM-CSF and IL-33. *Journal of Experimental Medicine,* 209**,** 1505-1517.

WINGE, M. C., HOPPE, T., BERNE, B., VAHLQUIST, A., NORDENSKJOLD, M., BRADLEY, M. & TORMA, H. 2011. Filaggrin genotype determines functional and molecular alterations in skin of patients with atopic dermatitis and ichthyosis vulgaris. *PLoS One,* 6**,** e28254.

WOLKERSTORFER, A., VAN DER SPEK, F. B. D., GLAZENBURG, E. J., MULDER, P. G. H. & ORANJE, A. P. 1999. Scoring the severity of atopic dermatitis: Three item severity score as a rough system for daily practice and as a pre-screening tool for studies. *Acta Dermato-Venereologica,* 79**,** 356-359.

WOOD, L. C., ELIAS, P. M., CALHOUN, C., TSAI, J. C., GRUNFELD, C. & FEINGOLD, K. R. 1996. Barrier disruption stimulates interleukin-1 alpha expression and release from a pre-formed pool in murine epidermis. *J Invest Dermatol,* 106**,** 397-403.

WOOD, L. C., STALDER, A. K., LIOU, A., CAMPBELL, I. L., GRUNFELD, C., ELIAS, P. M. & FEINGOLD, K. R. 1997. Barrier disruption increases gene expression of cytokines and the 55 kD TNF receptor in murine skin. *Experimental Dermatology,* 6**,** 98-104.

WORM, M., FORSCHNER, K., LEE, H. H., ROEHR, C. C., EDENHARTER, G., NIGGEMANN, B. & ZUBERBIER, T. 2006. Frequency of atopic dermatitis and relevance of food allergy in adults in Germany. *Acta Derm Venereol,* 86**,** 119-22.

WUTHRICH, B., COZZIO, A., ROLL, A., SENTI, G., KUNDIG, T. & SCHMID-GRENDELMEIER, P. 2007. Atopic eczema: genetics or environment? *Ann Agric Environ Med,* 14**,** 195-201.

YAMANAKA, K., TANAKA, M., TSUTSUI, H., KUPPER, T. S., ASAHI, K., OKAMURA, H., NAKANISHI, K., SUZUKI, M., KAYAGAKI, N., BLACK, R. A., MILLER, D. K., NAKASHIMA, K., SHIMIZU, M. & MIZUTANI, H. 2000. Skin-specific caspase-1-transgenic mice show cutaneous apoptosis and pre-endotoxin shock condition with a high serum level of IL-18. *J Immunol,* 165**,** 997-1003.

YANO, S., BANNO, T., WALSH, R. & BLUMENBERG, M. 2008. Transcriptional responses of human epidermal keratinocytes to cytokine interleukin-1. *J Cell Physiol,* 214**,** 1-13.

YE, J., GARG, A., CALHOUN, C., FEINGOLD, K. R., ELIAS, P. M. & GHADIALLY, R. 2002. Alterations in cytokine regulation in aged epidermis: implications for permeability barrier homeostasis and inflammation. I. IL-1 gene family. *Exp Dermatol,* 11**,** 209-16.

YONEDA, K. & STEINERT, P. M. 1993. Overexpression of human loricrin in transgenic mice produces a normal phenotype. *Proc Natl Acad Sci U S A,* 90**,** 10754-8.

YOSHIMOTO, T., TSUTSUI, H., TOMINAGA, K., HOSHINO, K., OKAMURA, H., AKIRA, S., PAUL, W. E. & NAKANISHI, K. 1999. IL-18, although antiallergic when administered

with IL-12, stimulates IL-4 and histamine release by basophils. *Proceedings of the National Academy of Sciences of the United States of America,* 96**,** 13962-13966.

ZEDAN, K., RASHEED, Z., FAROUK, Y., ALZOLIBANI, A. A., BIN SAIF, G., ISMAIL, H. A. & AL ROBAEE, A. A. 2015. Immunoglobulin e, interleukin-18 and interleukin-12 in patients with atopic dermatitis: correlation with disease activity. *J Clin Diagn Res,* 9**,** WC01-5.

ZIEGLER, S. F. & ARTIS, D. 2010. Sensing the outside world: TSLP regulates barrier immunity. *Nat Immunol,* 11**,** 289-93.

STATUTORY DECLARATION

"I, Maria Michaelidou, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic: Skin barrier and danger-signatures analysis in patients with atopic dermatitis upon skin irritation, independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me".

Date, Signature

CURRICULUM VITAE

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht

ACKNOWLEDGEMENTS

I would first like to express my deep and sincere gratitude to my thesis advisor Professor Dr. med. Margitta Worm for giving me the opportunity to perform my doctoral study in her laboratory at the Department of Dermatology in Charité - Berlin University of Medicine. It was a privilege and an honour to be her student. I am thankful for her constructive feedback, thoughtful suggestions and valuable guidance during these years.

I especially thank my second supervisor Dr. Vandana Kumari for her invaluable help with the experimental methods and for her continuous support and insightful comments about my thesis.

I am very grateful to Dr. rer. medic. Anja Kühl from Charité-Campus Benjamin Franklin, who helped me with the evaluation of immunohistochemistry as well as Dennis Ernst for his great support in the laboratory and in determining the IgE levels in the sera of the participants.

I sincerely thanked Dr. Magda Babina und Dr. Kristin Franke for their constant and constructive feedback and valuables remarks on my doctoral study.

I wish to thank the whole working group for the wonderful working atmosphere and for their constructive advice. Specifically, I would like to express my sincere thanks to Dr. Sabine Dölle, Dr. Tarek Hazzan, Dr. Maria Nassiri, Davender Redhu, Dr. Marina Aparicio Soto, Dr. Wojciech Francuzik, Dr. Magdalena Kraft, Shruti Rastogi, Sandra Treptow and Josephine Scholz.

Last but not least, I would like to thank my family and especially my parents for their unconditional love, mental support and constant encouragement throughout my whole career and in my life in general.