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Exogenous and endogenous triggers of distinct patterns of skin immunity in inflammatory skin diseases

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Preliminary remark

The format of this dissertation is publication-based according to §6 (2) “Regulations for the Award of Doctoral Degrees” of the Technical University of Munich and §14 (2) – (3) of the examination and study regulations (Studien- und Prüfungsordnung) for the Ph.D.-Program “Medical Life Science and Technology” School of Medicine, Technical University of Munich as it meets the following criteria:

(1) The dissertation is based on three publications in international peer-reviewed journals, which are accepted for publication or published. The doctoral candidate is first author of two publications.

(2) The publication-based dissertation provides a brief description of the scientific problem, problem-solving solutions, results and conclusions achieved and related literature.

(3) The dissertation contains a brief summary of each publication and the doctoral candidate’s individual contribution.

(4) The format of publication-based dissertation is supported by the Mentor and was approved by the Ph.D. program committee (Studienausschuss).

List of abbreviations

ACD: acute contact dermatitis

AE: atopic eczema

CD: cluster of differentiation

ICD: imiquimod induced contact dermatitis

IFN: interferon

Ig: immunoglobulin

IL: interleukin

ILC: innate-lymphoid-cells

ISD: inflammatory skin disease

LE: lupus erythematosus

LP: lichen planus

NK: natural-killer

PAMP: pathogen associated molecular pattern

pDC: plasmacytoid dendritic cell

RORC: retinoic-acid-orphan-receptor-C

sh: small hairpin

SNP: single-nucleotide-polymorphism

TGF: transforming-growth-factor

Th: T-helper cell

TLR: toll-like-receptor

TNF: tumor-necrosis-factor

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Introduction

Inflammatory skin diseases (ISD) are frequent in western countries and severely impact patient's quality of life. While increasing understanding about the two most common ISD, psoriasis and atopic eczema (AE), led to the development of targeted therapies, the vast majority of ISD is poorly understood. Diagnosis is primarily based on morphology and unspecific treatments are widely used in clinical practice. Research is hampered by disease heterogeneity and an unprecise, historically grown nomenclature. Novel scientific approaches are needed to better understand mechanisms of skin inflammation in humans. This thesis describes three different strategies to investigate disease specific immune regulations of ISD. Exogenous triggers are tested for their use to develop a human disease model of psoriasis. Epithelial derived cytokines are examined for their potential as new therapeutic targets, whereas molecular analysis of shared morphological patterns allows insight into characteristic reactions of the skin in response to endogenous immune stimuli.

Definitions

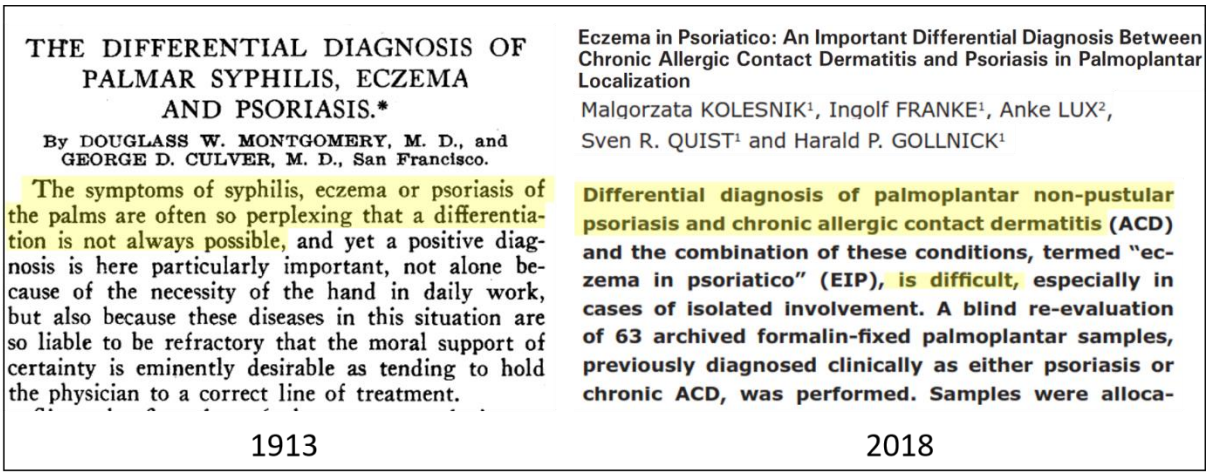
Pathologies of the skin can be divided into four groups: Malignant, infectious, hereditary and (chronic) inflammatory. Below this level of definition, all skin diseases can be influenced by additional factors such as genetic susceptibility, environmental changes, metabolic disorders, psychosocial influences and interplay with other organisms. While malignancy is clearly defined by uncontrolled growth, invasiveness and metastasis formation, infectious diseases are caused by the invasion of at least one exogeneous microbe. Hereditary diseases are based on mutations leading to loss or gain of structure, function or metabolism. ISD, however, are a heterogeneous group of skin diseases and therefore more difficult to define. In general, ISD are regarded as inflamed conditions of the skin which do not fulfil the criteria of malignancy and infections. Within the group ISD, some authors discriminate between autoimmune and chronic inflammatory skin diseases. However, chronicity and autoimmunity are inconsistently defined and the pathogenesis of many ISD is still poorly understood. Therefore, hereafter only the term "ISD" will be used. Other sub classifications of ISD which are based on morphology ("exanthematic"), analogies ("psoriasis-like") or suspected disease pathology ("extrinsic", "intrinsic") will also be avoided as these names developed historically and might be misleading.

Inflammatory skin diseases – problems faced in clinical practice and research

ISDs are frequent. The prevalence of the most common entities is estimated between 1 and 8.5% of the general population (psoriasis) (Griffiths *et al.*, 2017) and up to 20% in specific cohorts (AE in children) in western countries (Illi *et al.*, 2004). Patients not only suffer from skin related symptoms like pruritus, pain or disabling lesions, but also from social stigmata, psychiatric disorders and systemic comorbidities like arthritis, asthma or an increased cardiovascular risk (Schmitt *et al.*, 2016; Thyssen *et al.*, 2018). As for laypersons it is impossible to distinguish contagious skin diseases from non communicable ISD, affected people are confronted with discrimination and social marginalization. Isolation and learned helplessness hamper contacts between physicians and patients (Dalgard *et al.*, 2015; Misery *et al.*, 2018). The health care situation is still insufficient and increased efforts in patient care and research are requested by the World-Health-Organization (Langenbruch *et al.*, 2016; World-Health-Organization., 2016).

Research in the field of ISD is impeded by the heterogeneity of skin diseases and a historical nomenclature. Dermatology developed as a discipline of clinical description, which led to a multitude of disease names, disease variants and sub-diagnosis (Ghosh and Jain, 2013). For instance the term “eczema”, which is itself inconsistently defined, comprises the terms “atopic eczema”, “dyshidrotic eczema”, “contact eczema”, “nummular eczema”, “hyperkeratotic palmar eczema” and many more. However, it is unclear, if all these diseases build a group in terms of common disease mechanisms. For decades this nomenclature has been accepted as for the majority of skin diseases there was no understanding of disease pathogenesis and scientific knowledge and techniques to gain more insight into underlying mechanisms had to develop first.

Diagnosis is mostly based on morphology, either by description of a skin lesion or by histological examination of a skin biopsy. Although this procedure comprises a high error risk, it is still routinely used in clinical practice today. Differential diagnosis is impeded by overlapping clinical pictures especially at particular sites of the body, such as the palmoplantar region (Figure 1) (Kolesnik *et al.*, 2018; Montgomery and Culver, 1913). Molecular diagnostic tools are mostly used to rule out infections or malignancy, but only exist for few ISD (Garzorz-Stark *et al.*, 2016).



THE DIFFERENTIAL DIAGNOSIS OF PALMAR SYPHILIS, ECZEMA AND PSORIASIS.*

By DOUGLASS W. MONTGOMERY, M. D., and GEORGE D. CULVER, M. D., San Francisco.

The symptoms of syphilis, eczema or psoriasis of the palms are often so perplexing that a differentiation is not always possible, and yet a positive diagnosis is here particularly important, not alone because of the necessity of the hand in daily work, but also because these diseases in this situation are so liable to be refractory that the moral support of certainty is eminently desirable as tending to hold the physician to a correct line of treatment.

1913

Eczema in Psoriatico: An Important Differential Diagnosis Between Chronic Allergic Contact Dermatitis and Psoriasis in Palmoplantar Localization

Malgorzata KOLESNIK[1], Ingolf FRANKE[1], Anke LUX[2], Sven R. QUIST[1] and Harald P. GOLLNICK[1]

Differential diagnosis of palmoplantar non-pustular psoriasis and chronic allergic contact dermatitis (ACD) and the combination of these conditions, termed "eczema in psoriatico" (EIP), is difficult, especially in cases of isolated involvement. A blind re-evaluation of 63 archived formalin-fixed palmoplantar samples, previously diagnosed clinically as either psoriasis or chronic ACD, was performed. Samples were alloca-

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Figure 1
Two exemplary publications demonstrating that difficulties in differential diagnosis based on morphology have stayed unchanged for more than a century.

The skin immune system – interplay between skin resident and infiltrating immune cells

The basis for the scientific development in ISD was a better understanding of the skin immune system. Barrier organs like the skin, lung and gut are the first contact between a pathogen and the organism and thus play a pivotal role in differentiating between adequate local reaction, activation of systemic inflammation or tolerogenic interaction with harmless microbiota (Lauffer and Ring, 2015). Inflammation of the skin is not only mediated by infiltrating immune cells but also by skin resident cells, such as keratinocytes, Langerhans cells and mast cells. Keratinocytes build the outermost layer of the skin, the epidermis. Besides a function in maintaining a physical and chemical barrier, keratinocytes are equipped with different toll-like-receptors (TLR) to sense pathogen associated molecular patterns (PAMP) and release antimicrobial peptides and chemotactic mediators (Nestle *et al.*, 2009). Rapid proliferation of keratinocytes leads to increased desquamation, a mechanism which can be regarded as a physical elimination of pathogens or degenerated host cells. Langerhans cells are skin specific antigen presenting cells, which are located in the epidermis and migrate to lymph nodes for antigen presentation once they have contact with foreign structures (Heath and Carbone, 2013). Mast cells are located around blood vessels and are activated by PAMPs, cross-linked immunoglobulin (IG)-E molecules and unspecific triggers. Once stimulated, mast cells release proteases and inflammatory mediators leading to vasodilatation, local edema and pruritus (Otsuka and Kabashima, 2015). Thus, skin resident cells are involved in every inflammatory condition of the skin and

act as local accelerators of immune responses. The direction of an immune response, however, is determined by adaptive immune cells, in particular T cells.

Specific T cell subsets play a central role in orchestrating immune reactions. While cluster of differentiation (CD)-8 positive T cells mediate cytotoxicity in anti-viral and anti-tumor immune responses, CD4-positive T cells develop into distinct subtypes, which are defined by expression of transcription factors and production of cytokines. Proinflammatory T cell subsets are T helper (Th)-1 cells with the key cytokines interferon (IFN)-γ and tumor-necrosis-factor (TNF)-α, Th2 cells with the key cytokines interleukin (IL)-4, IL-5 and IL-13 and Th17 cells, which produce IL-17 and IL-22 and are dependent on the presence of IL-23. Regulatory T cells, which are characterized by the production of transforming-growth-factor (TGF)-β and IL-10 mediate anti-inflammatory effects and are involved in wound repair. T cells orchestrate distinct immune polarizations, which involve other immune cells, soluble immune mediators and epithelial reactions (Figure 2) (Eyerich and Zielinski, 2014).

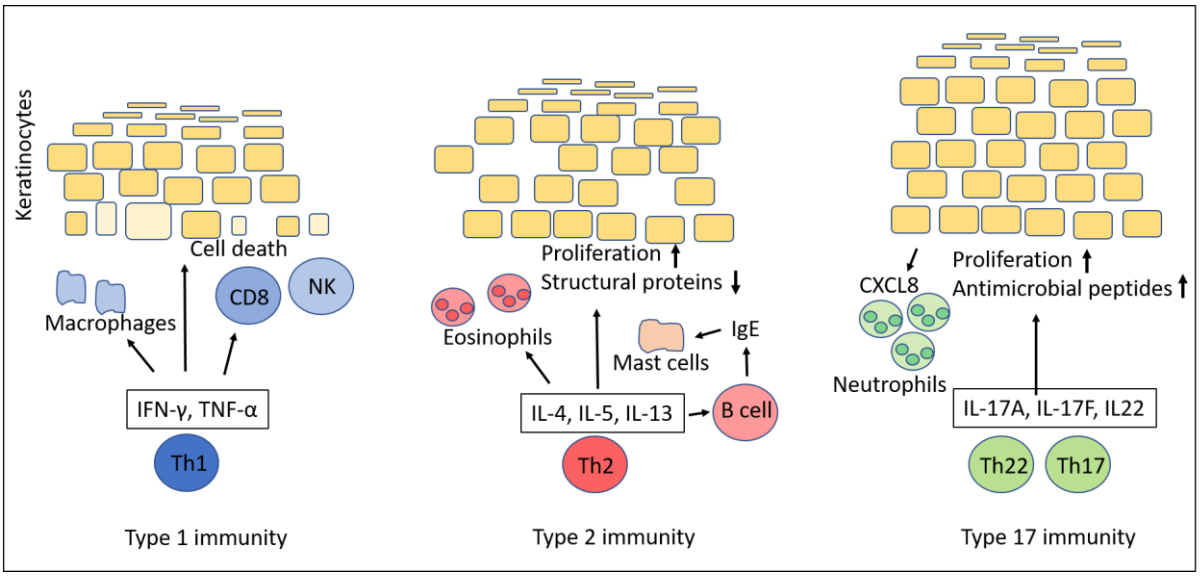

Figure 2

Schematic representation of effects of type 1, type 2 and type 17 immunity in the skin.

Each immune reaction ought to defend the organism against different pathogens, control malignant proliferations or ensure tissue homeostasis. Sensing and antigen-presenting cells, like dendritic cells, Langerhans cells and macrophages, steer the constantly regrowing pool of naïve T cells towards the direction of maturation required for the respective situation (Heath and Carbone, 2013). According to the T cell subsets,

three pro-inflammatory immune polarizations can be defined (Eyerich and Eyerich, 2017).

Type 1 immunity is mediated by IFN-γ and TNF-α. IFN-γ is produced by Th1 cells, innate-lymphoid-cells (ILC)-1, CD8-positive T cells, natural-killer (NK) cells and dendritic cells (Jankovic and Feng, 2015), while TNF-α is mainly produced by macrophages and to a lesser extent by T cells (Arango Duque and Descoteaux, 2014). The natural role is the control of pathogenic processes which mainly take place within a host cell. This can be viral infections, intracellular microbes (e.g. mycobacteria tuberculosis) or malignant cell changes. Th1 cells are induced by IL-12 and IFN-γ, while IFN-γ inhibits the generation of Th2 cells. IFN-γ is one of the strongest epithelial stimuli, which induces the production of chemokines (CXCL10, CXCL11) and pro-inflammatory cytokines (IL-1β, TNF-α) as well as apoptosis in keratinocytes (Albanesi *et al.*, 2001).

Type 2 immunity ought to control extracellular pathogens, like parasitic infections. The key cytokines IL-4, IL-5 and IL-13 are mainly produced by Th2 cells and ILC2 (Jankovic and Feng, 2015). IL-4 and IL-13 induce the maturation of B cells and the production of IgE and IgG4 antibodies. Furthermore, IL-4 inhibits the generation of Th1 cells and IL-13 decreases the activation of macrophages (Varin *et al.*, 2010). IL-5 stimulates eosinophil granulocytes and mediates maturation and effector functions of B cells (Kouro and Takatsu, 2009). The IL-4 receptor subunit α can be bound by both cytokines, IL-4 and IL-13 (Grunig *et al.*, 1998). Stimulation with IL-4 induces proliferation of keratinocytes, but downregulates structural proteins and surface lipids of the skin (e.g. filaggrin) and leads to enhanced bacterial colonization (Brunner *et al.*, 2017a).

Type 17 immunity is dominated by Th17 cells, which are characterized by the transcription factor retinoic-acid-orphan-receptor-C (RORC) controlling the production of IL-17A and IL-22. Th17 cells arise under the influence of TGF-β, IL-1β and IL-6 (Korn *et al.*, 2009). TGF-β has broad anti-inflammatory effects and maintains a non-inflamed environment in the absence of pathogens. IL-1β and IL-6, however, are early and potent pro-inflammatory stimuli which are released by sentinels like epithelial cells and dendritic cells. Thus, the cytokine milieu inducing Th17 cells is typical for an early state of inflammation. Th17 cells produce IL-17A and IL-17F, which induce proliferation of keratinocytes as well as the release of antimicrobial peptides and chemokines (Eyerich

et al., 2017). As a result, neutrophil granulocytes are recruited to the site of infection, which consecutively eliminate pathogens by phagocytosis, release of proteases and reactive oxygen species as well as by building neutrophil extracellular traps (NETosis) (Kaplan and Radic, 2012). Besides Th17 cells, Th22 cells, which are characterized by their capability to produce IL-22 and not IL-17, contribute to the local inflammation of the skin as well. IL-22 mediates keratinocyte proliferation and release of antimicrobial peptides (Eyerich *et al.*, 2009). Under physiological conditions type 17 immunity is essential for anti-fungal immune responses (Eyerich *et al.*, 2008).

Besides the physiologic role, each type of immunity can be attributed to certain inflammatory conditions. Th1 and Th17 were detected in autoimmune diseases, such as multiple sclerosis (Sie *et al.*, 2014). Th2 cells, however, are linked to allergic diseases like allergic rhino conjunctivitis or asthma (Licona-Limon *et al.*, 2013).

Targeting specific immune polarizations of inflammatory skin diseases

This concept was transferred to dermatological research and skin diseases were investigated for their cytokine milieu and immune system polarizations. Due to technical progress in molecular biology and bioinformatic analysis, gene sequencing studies in large cohorts identified risk genes for certain skin diseases. In particular, single-nucleotide-polymorphism (SNP) of IL-12B and IL-23R were detected as risk factors for psoriasis (Cargill *et al.*, 2007). While IL-12 is involved in the generation of Th1 cells, IL-23 is a survival factor for Th17 cells. Hence, these findings pointed towards an involvement of type 1 and type 17 immunity in psoriasis. Further studies confirmed that IL-23 subunits p19 and p40 are strongly expressed in psoriatic skin (Lee *et al.*, 2004). Direct injection of IL-23 into the skin of mice leads to the induction of TNF-α and hyperplasia of the epidermis, both typical features of human psoriasis (Chan *et al.*, 2006). Only a few years later, this knowledge was used to investigate new targeted therapies. Ustekinumab, an antibody directed against IL-12p40, the common subunit of IL-12 and IL-23 receptor, showed high efficacy in the treatment of psoriasis (Krueger *et al.*, 2007; Papp *et al.*, 2008). As the importance of type 17 immunity became more and more clear, further biologics against key cytokines or receptors of type 17 immunity proved to be highly effective in the treatment of psoriasis (Noda *et al.*, 2015). In parallel research about AE, another frequent ISD which is characterized by dry skin, itching skin lesions and a disturbed microbial colonization of the skin, revealed the pivotal role of type 2 immunity for disease pathogenesis (Lauffer and Ring, 2016). Here,

dupilumab, an antibody directed against the shared subunit of the IL-4 and IL-13 receptor, was approved in 2017 (Beck *et al.*, 2014). Strikingly, these therapies not only target one single ISD, but also other diseases with a similar immune polarization like disease related comorbidities. In particular, dupilumab is also effective in the treatment of allergic asthma (Wenzel *et al.*, 2016), while biologics against IL-17A have demonstrated efficacy in the treatment of psoriasis arthritis (McInnes *et al.*, 2015). Thus, psoriasis and AE are two role models demonstrating how specific immune triggers elicit specific inflammatory conditions of the skin, which can be treated by neutralizing key cytokines. This progress is based on three factors:

1. Psoriasis and AE are clearly defined by typical morphology, histopathology and clinical symptoms.

2. Psoriasis and AE are frequent. New targets are a potential market for pharmaceutical companies.

3. Psoriasis and AE are chronic but not life-threatening diseases. Clinical trials with new compounds can be performed in a relatively healthy study population.

Open questions – how to deal with rare diseases and discover new targets?

The scientific development in psoriasis and AE is a great success. However, the majority of ISD remains poorly understood and does not fulfil the criteria mentioned for psoriasis and AE. Meanwhile, there is an unmet medical need for new targeted therapies, as unspecific treatments, such as local or systemic immunosuppression, are still widely used in clinical practice. Progress is complicated by rarity and heterogeneity of ISD, lack of standardized disease scores and definitions. For rare diseases, it appears infeasible to collect a sufficient number of patient's demographics, patient's tissue samples and participants for clinical trials. Historically grown nomenclature categorizes ISD in a multitude of different diagnoses and sub diagnosis (Figure 3). Therefore, new scientific approaches are needed to deal with heterogeneity of skin diseases and discover new targets for rare skin diseases.

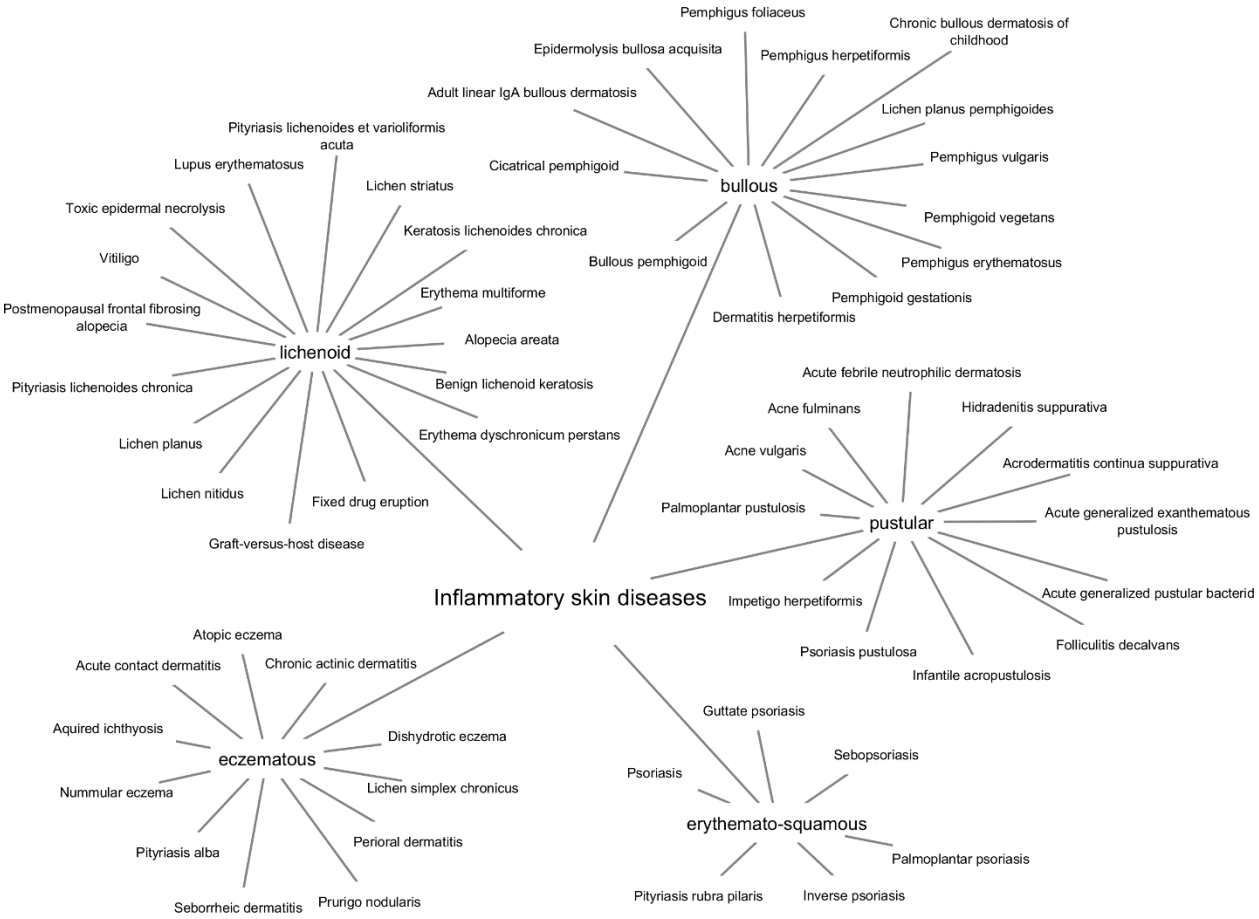

Figure 3

Overview of different inflammatory skin disease according to the current nomenclature categorized by morphological features.

Problem-solving solutions – disease models and studies on human tissue samples

In order to gain more insight into disease pathogenesis animal and human disease models are used. Several mouse models exist for psoriasis, AE, pemphigus vulgaris and lupus erythematosus. Some are based on the application of an agent on the skin, which leads to a specific kind of inflammation. Imiquimod, a TLR7 and TLR8 agonist, induces a murine skin reaction, which resembles human psoriasis in terms of histological architecture and the induction of the Th17 immune axis (van der Fits *et al.*, 2009). The application of a vitamin D derivate (calcipotriol), however, leads to a diverse reaction. Here, mice show a form of dermatitis which is accompanied by increased IgE serum levels and Th2 related cytokines and genes, thus representing the inflammation typical for AE (Li *et al.*, 2006). Mouse models are useful to study the impact of single genes, proteins or cell types, as they can be selectively neutralized or knocked-down.

However, they are limited by the number of existing disease models and differences in immune reactions compared to humans. Therefore, findings in murine models need to be validated in the human system.

The number of human skin disease models is limited. The application of allergens on the skin of sensitized subjects can be used as a model to study early immune reactions of AE (Darsow *et al.*, 2004). Provocation with ultraviolet-light can induce new skin lesions in patients with cutaneous lupus erythematosus (Kuhn *et al.*, 2001). However, the vast majority of human studies is performed on patient's tissue samples. Methods used are genome studies, gene expression analysis, determination of serum cytokines and in vitro studies with primary cells. However, they are limited by the availability of rare disease samples, the heterogeneity of human study populations and potential *in vitro* artefacts. Investigation of special patient cohorts can be useful to reduce complexity of immune regulations. Patients, who suffer from more than one ISD at the same time are a human model to study skin specific immune polarizations as different disease related immune reactions can be compared intraindividually (Eyerich *et al.*, 2011; Garzorz *et al.*, 2015). The publications included in this thesis are based on three different scientific approaches:

1. Investigation of an exogeneous trigger

By applying an immune stimulus on the healthy skin of humans, the potential use of a new human disease models can be determined.

2. Investigation of an epithelial derived trigger

Specific neutralization of an epithelial derived cytokine in disease models allows conclusions about the impact of skin resident cells for inflammation.

3. Investigation of endogenous immune response patterns

By focusing on overlapping features of different ISD, general mechanisms of skin diseases can be investigated in a disease independent manner.

Summary of publications

Publication 1: Toll-like receptor 7/8 agonist stimulate plasmacytoid dendritic cells to initiate a Th17-deviated acute contact dermatitis in human subjects
(Garzorz-Stark *et al.*, 2017)

Background:
The number of human in vivo models for ISD is low. While models for allergic contact dermatitis exist, there are no models for psoriasis. In mice the application of imiquimod, a TLR7 and TLR8 agonist, leads to a psoriasis-like inflammation. This study aimed at determining the potential of imiquimod to induce psoriasis in humans.

Methods:
Imiquimod was applied twice a week on the skin of 18 volunteers. Skin biopsies were taken at the beginning and at three additional time points (day 4, 14 and 28) for examination of whole genome expression, infiltration of immune cells and histological changes.

Results:
Imiquimod induced a monomorphic, self-limited skin inflammation, which clinically and histologically fulfilled the criteria of an acute contact dermatitis (ACD). However, imiquimod induced contact dermatitis (ICD) only partly shared regulated genes with ACD, but also showed an overlap with human psoriasis. In contrast to ACD, plasmacytoid dendritic cells (pDC) predominantly infiltrated the skin in ICD. Imiquimod stimulated pDCs to release IL-1β, IL-6 and IL-23 via TLR7/8 and receptor independent inflammasome activation. This led to an activation of type 17 immunity as determined by high amounts of IL-17A produced by lesional T cells, infiltration of neutrophils and regulation of psoriasis-related genes (e.g. NOS2).

Conclusions:
ICD showed clinical and histological features of ACD, but shared the activation of type 17 immunity with psoriasis. Hence, application of imiquimod on human skin is a potential model to study type 17 immunity related skin reaction like psoriasis.

Doctoral candidate's individual contribution:
The doctoral candidate contributed to design the study, performed patient visits and skin biopsies, carried out experiments and was involved in writing the manuscript.

Publication 2: Neutralization of IL-17C reduces skin inflammation in mouse models of psoriasis and atopic dermatitis.
(Vandeghinste *et al.*, 2018)

Background:
IL-17C is an epithelial derived cytokine which mediates pro-inflammatory effects in the skin. MOR106, a new fully human antibody binding and neutralizing IL-17C was developed as a potential new compound to treat ISD. This study investigates effects of IL-17C blockade in murine models of ISD.

Methods:
Expression of IL-17C in human psoriasis and AE was investigated by immunohistochemistry and qRT-PCR. Neutralization of IL-17C was studied in the IL-23 injection model, which resembles psoriasis, and two models of AE (MC903 model and flacky-tail model). In all models, MOR106 was administered intraperitoneal before and during skin lesions were induced (prophylactic treatment). Effects of IL-17C blockade were measured by clinical scores, determination of ear thickness, histology, qRT-expression and whole genome expression arrays.

Results:
IL-17C gene and protein was more expressed in human AE and psoriasis than in autologous uninvolved skin. Neutralization of IL-17C led to decreased ear thickness and downregulation of type 17 immunity related genes in the IL-23 injection model. Likewise, inhibition of IL-17C signaling reduced ear thickness and Th2 related gene expression in the MC903 as well as clinical scores and cytokine production in the flacky-tail mouse model.

Conclusions:
Inhibition of IL-17C signaling markedly reduces inflammatory conditions of the skin in different mouse models. IL-17C does not specifically support type 2 or type 17 immunity, but is an epithelial derived local accelerator of inflammation. Thus, IL-17C is a potential target for the treatment of ISD.

Doctoral candidate's individual contribution:
The doctoral candidate performed experiments and critically reviewed the manuscript for publication.

Publication 3: Type 1 immunity induces keratinocyte necroptosis and is associated to interface dermatitis

(Lauffer *et al.*, 2018)

Background:

Interface dermatitis (ID) is a histological feature of many ISD. It is defined as a dense lymphocytic infiltrate close under the epidermis and cell death of keratinoctes. This study sought to determine which regulatory changes are shared between lichen planus (LP) and cutaneous lupus erythematosus (LE), two clinically different ISD with ID.

Methods:

Whole genome expression analysis of LP and LE skin biopsies was performed. Shared regulated genes were analyzed by induced network modules. Lesional T cells were characterized for their cytokine production. Immunohistochemistry and stimulation of keratinocytes and three-dimensional skin equivalents were used to study epidermal cell death cascades. Furthermore, keratinocytes were stimulated after lentiviral induced small hairpin (sh) RNA knockdown of receptor-interacting-protein-kinase (RIP)-3. Proteins of interest were measured by western blot.

Results:

Gene regulations shared by LP and LE were dominated by type 1 immunity related cytokines (IFNγ, TNF-α). Lesional T cells produced significantly more IFN-γ than lesional T cells of psoriasis, an ISD without ID. Likewise, more t-bet expressing Th1 cells infiltrated the skin in LE and LP than in psoriasis. Cell death of keratinocytes after stimulation with mixed supernatant of LP and LE lesional T cells, was mediated by apoptosis and necroptosis. Knockdown of RIP3, a central protein of necroptosis, significantly prevented keratinocyte cell death after stimulation with type 1 cytokines.

Conclusions:

In different ISD ID is regulated by two key mechanism: Type 1 immunity and induction of necroptosis. These mechanisms are potential targets to treat ISD with ID.

Doctoral candidate's individual contribution:

The doctoral candidate contributed to design the study, performed experiments and wrote the first draft of the manuscript for publication.

Conclusions and discussion

The skin shows morphological reaction patterns in response to distinct immune triggers

The publications included in this thesis investigated general immune regulations of skin resident and infiltrating immune cells in response to exogeneous and endogenous triggers. We observed that specific stimuli induce distinct reactions of the skin. This observation is the basis for a precise classification and pathogenesis-based therapy of ISD. Exogeneous application of imiquimod led to a monomorphic reaction pattern in all patients included in the study; no matter if they concurrently suffered from psoriasis, AE, both diseases or no ISD. Similar morphology was paralleled by similar immune regulations. In response to imiquimod the release of IL-1β, IL-6 and IL-23 by pDCs favored the generation and accumulation of Th17 cells, which consecutively mediated the influx of neutrophil granulocytes to the skin. Hence, in a heterogeneous study population the skin develops an invariable immune response pattern in response to a certain stimulus. Of note, the local inflammation was dominant over the intrinsic susceptibility to generate type 2 or type 17 immune reactions. This observation confirms previous findings about the importance of the local inflammatory milieu. By investigating patients suffering from psoriasis and AE it became clear that that the skin immune system can form contrary reaction patterns at different sites of the body within one individual person (Eyerich *et al.*, 2011). While a psoriasis plaque is dominated by type 17 immunity, only a few centimeters away a patient can develop an acute contact dermatitis, which is strongly influenced by type 2 immunity (Quaranta *et al.*, 2014). Thus, immune triggers lead to reaction patterns of the skin in terms of clinical picture and molecular regulation.

Based on these findings the hypothesis raised, if - vice-versa - common regulations can be detected in ISD with shared morphological features. As a proof of concept we investigated ISD with ID, a feature, which can be clearly detected by histological examination (Sontheimer, 2009). Classically, LP is regarded as an ISD, while cutaneous LE is grouped as an autoimmune disease. However, when only analyzing the overlap of genetic regulations a common immune reaction pattern is apparent. Here, we discovered that the mechanisms leading to ID are a strong type 1 immune reaction in the skin and the induction of keratinocyte death by apoptosis and necroptosis. Thus, besides a type 2 reaction pattern in AE and a type 17 dominance in psoriasis, ID is an additional reaction pattern of ISD in response to type 1 immunity.

Immune response patterns of the skin allow transfer of therapeutic concepts

These findings led to the hypothesis that a limited number of local immune deviations might be detectable in a multitude of ISD and a new grouping of ISD in categories of immune polarizations was suggested (Eyerich and Eyerich, 2017). As a result, therapies, which are effective and approved for one ISD might be transferable to others with a similar immune regulation. Practical observations support this hypothesis. In particular, blocking type 17 immunity in ISD with a high number of neutrophils granulocytes, like hidradenitis suppurativa, pustular psoriasis or pyoderma gangrenosum showed convincing efficacy (Bohner *et al.*, 2016; Guenova *et al.*, 2011; Schuch *et al.*, 2018). Immunological counteraction is another potential therapeutic strategy. In particular, it was demonstrated that psoriasis can efficiently be treated by the injection of IL-4, an observation, which is based on the fact that IL-4 inhibits the development of Th1 and Th17 cells (Ghoreschi *et al.*, 2003; Guenova *et al.*, 2015). Hence, classifying ISD by their immune polarization allows new therapeutic approaches. However, this concept only deals with established ISD and not with the initial disease trigger causing the respective pattern.

Unknown disease triggers hamper causative therapeutic concepts

Identification of initial disease triggers might lead to causative therapeutic concepts. A positive example are bullous skin diseases. Here, autoantibodies directed against adhesion molecules of keratinocytes lead to a destruction of the epidermal architecture and a consecutive inflammation. Therapeutic elimination of antibodies is a highly effective approach (Eming and Hertl, 2006). For the majority of ISD, however, there is no causative therapy as the initial disease trigger is unknown. Several autoantigens were proposed to be involved in the pathogenesis of psoriasis, such as self-DNA, antimicrobial peptides or melanocyte structures (Arakawa *et al.*, 2015; Chamilos *et al.*, 2012; Lande *et al.*, 2014). However, apart from technical limitations, there is no evidence that depleting autoreactive T cells in ISD is effective. Further factors like commensal bacteria or metabolic comorbidities might also contribute to the multifactorial disease pathogeneses and maintain the chronicity of ISD (Brunner *et al.*, 2017b; Kaesler *et al.*, 2014). Hence, though effective compounds for the treatment are approved, a healing of ISD appears infeasible and patients need life-long symptomatic

treatment. Though the safety profile of novel biologic treatments is in general favorable, long-term adverse reactions cannot be excluded.

Skin resident accelerators of inflammation – IL-17C as an additional target?

Inflammation of the skin is mediated by infiltrating immune cells and skin resident cells. In terms of new therapeutic strategies, targeting skin specific cytokines might enhance specificity and reduce systemic side effects. In our study, we identified IL-17C as an important skin specific mediator of inflammation. Selective neutralization markedly decreased inflammation of the skin in murine models. Of note, this inhibitory effects were not specific for a type 2 or type 17 mediated inflammation. In accordance, we detected an enhanced IL-17C expression compared to healthy skin in both, psoriasis and AE. Hence, IL-17C seems to accelerate inflammatory conditions of the skin independent from the adaptive immune polarization. Recently, it was described that IL-17C is also involved in mediating growth of peripheral sensory neurons (Peng *et al.*, 2017). Though this study focused on the role of IL-17C during herpes simplex virus infection, this effect might also contribute to neuronal changes in ISD, which can influence the sensing of itch. Thus, depleting IL-17C might a promising approach to treat ISD. However, IL-17C is not solely produced by keratinocytes but also by other epithelial cells in the gut and airways (Ramirez-Carrozzi *et al.*, 2011). Mouse models pointed towards an important role for IL-17C in the control of intestinal infections (Song *et al.*, 2011). Therefore, bacterial infections are potential adverse events when blocking IL-17C signaling in humans. However, in a phase I clinical trial testing MOR106, a neutralizing antibody against IL-17C, in healthy volunteers, no major safety concerns have been reported and a phase II clinical trial in patients suffering from AE is currently recruiting patients (Thaçi *et al.* 2018).

Future health care for inflammatory skin diseases

The progress achieved in the treatment of psoriasis and AE is remarkable. After identifying disease specific key mediators, therapeutic antibodies were developed and approved within a short period of time (Noda *et al.*, 2015). At the moment, the biggest number of approved systemic therapies for ISD is available for the treatment of psoriasis. However, though the number of psoriasis patients receiving a systemic treatment increases (Langenbruch *et al.*, 2016), concerns about correct prescription and high therapy related costs still hamper the current health care situation (Nast *et*

al., 2008). Many patients experience secondary loss of efficacy after some years of drug intake. Therefore, new therapeutic concepts leading to long-term disease modification are desirable. One hypothesis is that starting a systemic treatment as early as possible might influence the future course of disease (Iversen *et al.*, 2018). This “hit-hard-and-early” concept is established in the treatment of infectious diseases like human immunodeficiency virus. Our studies provide evidence that different factors might regulate the initial immune response and the chronic inflammation. In particular, application of imiquimod on human skin induced a type 17 immune response, but it was a self-limited inflammation and did not result in a typical psoriasis lesion. Thus, treating an ISD at an early stage might interrupt a cascade of additional factors perpetuating inflammation.

Another hypothesis is the concept of redirecting the immune system. ISD are a result of a misguided inflammatory response. An immune response which is naturally required for host defense against viral, fungi or parasitic infections occurs in the skin in a self-perpetuating manner. Very similar, allergic rhino conjunctivitis is based on a misleaded immune response towards harmless environmental particles. Here, a specific immune therapy, which is based on the injection of increasing doses of allergen, can re-educate the immune response towards tolerance and reduce the amount of drugs required for disease control (Mortuaire *et al.*, 2017). However, according to our current understanding ISD are mostly not based on a reaction against one or a limited number of (auto-)antigens. Furthermore, we and others confirmed the role of keratinocyte derived pro-inflammatory signals like IL-17C. Therefore, the potential of an antigen based immune modulation strategy appears limited.

Another approach is to target exogeneous triggers of ISD. In AE, colonization of skin lesions with Staphylococcus aureus is a frequent phenomenon. It was shown that Staphylococcus aureus antigen is a pro-inflammatory stimulus and might be accumulating just before a disease flare (Kong *et al.*, 2012). On the other hand, it was shown that nonpathogenic bacteria mediate anti-inflammatory effects (Volz *et al.*, 2014). Therefore, a therapeutic modulation of the skin microbiota could be beneficial in ISD. Indeed, topical application of nonpathogenic bacteria leads to an improvement of AE (Gueniche *et al.*, 2008; Myles *et al.*, 2018). Thus, understanding the interaction between skin immune system and commensal skin microbiota is an increasing field of research allowing new therapeutic concepts.

Summary and outlook

While for key ISD such as psoriasis and AE increasing knowledge about the pathogenesis resulted in development of specific symptomatic therapies, most ISD are poorly understood and effective therapies are lacking. This PhD thesis contributes to molecular-directed decision-making of ISD rather than decision according to historic description. The publications included in this thesis demonstrate how the skin immune system is guided by exogeneous and endogenous triggers towards different reaction patterns and how epithelial derived cytokines contribute to local inflammation. Identification of common triggers eliciting distinct patterns such as interface dermatitis will lead to a better access of specific therapies in a broader context of ISD. Similar, the general inflammatory circuit elicited by IL-17C or skin inflammation elicited by TLR7/8 stimulation is shared by several ISD. Based on these findings, there is a rationale to transfer established therapies to rare disease entities with similar immune directions or to target keratinocyte specific pro-inflammatory cytokines. The future of ISD treatment will be based on a classification of key triggers and molecular events elucidating distinct immune responses. Thus, after decades of morphological description and unspecific treatments, this is the first step towards precision medicine in ISD.

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Appendix