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The role of the humoral immune response in psoriasis

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Summary

With about 1.6 million people affected in Germany, psoriasis is a common disease that can affect not only the skin, but also nails and joints, and which can massively restrict patients' psychosocial fabric. According to the latest research psoriasis can be assigned to systemic inflammatory diseases, which provides an explanation for the frequent occurrence of inflammatory comorbidities such as metabolic syndrome. Essential in the pathogenesis are interleukin 17 (IL-17)- producing cells, especially T helper 17 (Th17) cells, which initiate and maintain an interplay between immune cells and keratinocytes and lead to a finally self-sustaining inflammatory response. Last but not least due to extremely effective, selective IL-17-directed therapies with so-called biologicals, the crucial relevance of IL-17 for psoriasis could be demonstrated. However, it remained unclear until recently whether B cells likewise have an influence on the development of psoriasis. Case reports exist on both patients with pre-existing psoriasis, which improved during B-cell depleting therapy with rituximab, as well as on the new onset of psoriasis-like skin lesions in previously skin-healthy patients with the same therapy.

In order to learn more about the role of B cells and humoral components in the immune pathogenesis of psoriasis, in this thesis B cells and humoral immune responses in the blood of psoriasis patients and healthy volunteers were investigated. Results showed relatively few differences. The serum analysis of immunoglobulins and complement factors showed increased absolute values of immunoglobulin A (IgA), C3 and C4 in psoriasis patients as well as a positive correlation of IgA with disease activity defined as Psoriasis Area and Severity Index (PASI) score. Flow cytometric investigations of the frequency of five morphologically and ontogenetically different B cell subpopulations also demonstrated a positive correlation with disease activity for IgA positive plasma cells as well as Cluster of differentiation (CD) 138 expression. This is the cell surface expression of Syndecan-1 (CD138), a transmembrane protein that serves as a marker for plasma cells and may be relevant to the inflammatory basic conditions of psoriasis. In a subsequent investigation of 28 adequately systemically treated psoriasis patients neither IgA serum levels dropped nor substantial changes in the B cell expression pattern occured. Based on a patient with a common variable immunodeficiency (CVID) who developed psoriasis it could be mainly excluded that B cells are responsible for triggering the immune pathogenesis of psoriasis. Nevertheless, in some patients imbalances of regulatory B cell subtypes could contribute to emergence and aggravation of psoriasis.

In summary, this work revealed few but still psoriasis-specific differences in the B cell expression pattern as well as the B-cell mediated humoral components between psoriasis and healthy individuals. Whether these are epiphenomena or whether these differences have a modifying effect on the pathogenesis of psoriasis goes beyond the scope of this thesis and needs to be investigated in further studies. Possibly, it will have relevance only for a subgroup of patients in terms of a distinct endotype, in which new treatment options by modifying expression of regulatory B cells or Syndecan-1 could be considered.

Zusammenfassung

Mit rund 1,6 Millionen Betroffenen in Deutschland ist die Psoriasis eine häufige Erkrankung, die nicht nur die Haut, sondern auch Nägel und Gelenke befallen und das psychosoziale Gefüge der Erkrankten massiv beeinträchtigen kann. Neue Forschungsergebnisse ordnen die Psoriasis den systemischen entzündlichen Erkrankungen zu, was einen Erklärungsansatz für das häufige Auftreten von entzündlichen Komorbiditäten wie dem metabolischen Syndrom liefert. Entscheidend in der Pathogenese sind Interleukin 17 (IL-17)- produzierende Zellen, besonders T Helfer 17 (Th17) Zellen, die ein Wechselspiel zwischen Immunzellen und Keratinozyten initiieren sowie unterhalten und in eine sich am Ende selbst erhaltende entzündliche Reaktion münden. Nicht zuletzt durch extrem wirksame, selektiv gegen IL-17 gerichtete Therapien mit sogenannten Biologika konnte die entscheidende Bedeutung von IL-17 für die Psoriasis gezeigt werden. Unklar blieb jedoch bis zuletzt, ob B-Zellen ebenfalls einen Einfluss auf die Entwicklung der Psoriasis haben. Fallberichte existieren sowohl über Patienten mit einer vorbestehenden Psoriasis, die sich unter B-Zell depletierender Therapie mit Rituximab verbesserte, als auch über das Neuauftreten von Psoriasis ähnlichen Hautläsionen bei zuvor hautgesunden Patienten mit selbiger Therapie.

Um mehr über die Rolle von B-Zellen und humoralen Komponenten in der Immunpathogenese der Psoriasis zu erfahren, wurden in dieser Doktorarbeit B-Zellen sowie humorale Immunantworten im Blut von Psoriatikern und gesunden Probanden untersucht. Dabei stellten sich relativ wenige Unterschiede dar. Die durchgeführte Serumanalyse von Immunglobulinen und Komplementfaktoren zeigte erhöhte Absolutwerte für Immunglobulin A (IgA), C3 und C4 bei den Psoriatikern sowie eine positive Korrelation des IgA mit der Krankheitsaktivität definiert als Punktwert des Psoriasis Area and Severity Index (PASI). In der durchflusszytometrischen Untersuchung der Frequenz von fünf morphologisch und ontogenetisch unterschiedlichen B-Zellsubpopulationen konnte eine positive Korrelation mit der Krankheitsaktivität ebenso gezeigt werden für die IgA positiven Plasmazellen sowie die Cluster of differentiation (CD) 138-Expression. Hierbei handelt es sich um die Zelloberflächenexpression von Syndecan-1 (CD138), einem Transmembranprotein, das als Marker für Plasmazellen dient und möglicherweise Bedeutung für die entzündlichen Rahmenbedingungen der Psoriasis haben könnte. Bei der anschließenden Untersuchung von 28 adäquat systemisch behandelten Psoriatikern sanken allerdings weder die IgA-Serumwerte noch ergaben sich wesentliche Änderungen im B-Zellexpressionsmuster. Anhand einer Patientin mit einem variablen Immundefektsyndrom, die zusätzlich eine Psoriasis entwickelte, konnte weitgehend ausgeschlossen werden, dass B-Zellen die Immunpathogenese der Psoriasis auslösen. Möglicherweise trägt jedoch bei manchen Patienten ein Ungleichgewicht an regulatorischen B-Zellsubtypen zur Entstehung und Aggravation einer Psoriasis bei.

Zusammenfassend ließen sich in dieser Arbeit wenige, aber dennoch Psoriasis-spezifische Unterschiede im B-Zellexpressionsmuster als auch in B-Zell vermittelten humoralen Komponenten zwischen Psoriatikern und Gesunden beobachten. Ob es sich dabei um Epiphänomene handelt oder ob sich diese Unterschiede modifizierend auf die Pathogenese der Psoriasis auswirken, geht über den Rahmen dieser Arbeit hinaus und muss in weiterführenden Studien untersucht werden. Möglicherweise stellt sich eine Relevanz auch nur für eine Subgruppe von Patienten im Sinne

eines spezifischen Endotypen heraus, bei der neue Therapiemöglichkeiten durch Modifikation der Expression von regulatorischen B-Zellen oder Syndecan-1 denkbar wären.

Contents

List of abbreviations

ad Adde (fill up to)
ADAMTSL ADAMTS-like protein
AE Atopic eczema
AF Alexa Fluor
BCR B cell receptor
bp base pair
BP180, BP230 Bullous Pemphigoid 180, Bullous Pemphigoid 230
Bregs Regulatory B cells
BSA Bovine serum albumine
BSA Body Surface Area
Bv Brilliant Violet
C3, C4 Complement factor 3, complement factor 4acroCC
CCL Chemokine ligand
CD Cluster of differentiation
cDNA Complementary deoxyribonucleic acid
CI Confidence intervall
CLP Common lymphoid progenitor cells
CNS Central nervous system
CRP C-reactive protein
CVID Common variable immune deficiency
dl Deciliter
DLQI Dermatology Quality of Life Index
DMSO Dimethyl sulfoxide
DNA Deoxyribonucleic acid
DPBS Dulbecco's phosphate buffered saline
Dsg1, Dsg3 Desmoglein 1, Desmoglein 3
DTH Delayed-type hypersensitivity
EAE Experimental autoimmune encephalomyelitis
e.g. exempli gratia (for example)
EDTA Ethylenediaminetetraaceticaacid
ELISA Enzyme linked immunosorbent assay
g Unit of acceleration, g = 9,80665 m/s^2
FACS Fluorescence activated cell sorting
FCS Fetal calf serum
FSC Forward scatter
FITC Fluorescein isothiocyanate
GAG Glycosaminoglycan
HGF Hepatocyte growth factor
hi high

HLA Human Leucocyte Antigen
HSC Hematopoietic stem cells
HSPG Heparan sulfate proteoglycan
ICAM-1 Intercellular adhesion molecule 1
Ig Immunoglobulin
IL Interleukin
IFN Interferon
i.v. Intravenous
KO Knockout
mAb Monoclonal antibody
mCi Millicurie
mDC Myeloid dendritic cells
mg Milligramm
MHC Major Histocompatibility
miRNA Mitochondrial ribonucleic acid
ml Milliliter
mM Millimolar
MPP Multipotent progenitor cells
MS Multiple sclerosis
MTX Methotrexate
ng Nanogramm
NK Natural Killer
nm Nanometer
PASI Psoriasis Area and Severity Index
PBMCs Peripheral blood mononuclear cells
pDC Plasmacytoid dendritic cells
PDE4 Phosphodiesterase 4
PE Phycoerythrin
PE-Cy Phycoerythrin-Cyanine
Per-CP Peridinin-chlorophyll Protein Complex
pg Picogramme
PHA Phytohemagglutinin
PLA_2 Phospholipase A_2
PMA Phorbol 12-myristate 13-acetate
pre Precursor
pro Progenitor
PSORS Psoriasis Susceptibility
PUVA Combination therapy of psoralen and UVA (ultraviolet radiation of 315 nm to 380 nm wavelength)
RBC Red blood cell
RNA Ribonucleic acid
RPMI Roswell Park Memorial Institute

RT-PCR Real time polymerase chain reaction
SCORAD SCORing Atopic Dermatitis
SEM Standard error of the mean
SFRP Secreted frizzled-related protein
SSC Sideward scatter
Th T helper
TI Thymus-independent
TLR7 Toll-like receptor 7
TMB Tetramethylbenzidine
TNF Tumour necrosis factor
U Unit
UK United Kingdom
USA United States of America
UVB Ultraviolet radiation with a wavelength of 230 nm to 315 nm
vs Versus
xg times gravity

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1. Introduction

1.1. Overview of psoriasis

1.1.1. Definition, epidemiology and diagnosis

Psoriasis is a common chronic autoimmune disease that is nowadays seen as a systemic inflammatory process affecting more than just skin and joints [Nestle et al., 2009]. Roughly 2% of the population are affected. However, geographic variations seem to be large and data in literature are mainly estimations whereas population-based studies are rare [Christophers, 2001].
The most common form is called psoriasis vulgaris with typical regions of the skin covered with white scaly plaques on an erythematous background. Sites most likely to be affected are elbows, knees, umbilicus, rima ani and scalp [Schon and Boehncke, 2005]. Nails also show distinct changes in about 50% of the patients at diagnosis with a lifetime incidence of 80-90% [Reich, 2009]. Mild forms are pitting and yellow spots which can worsen to onycholysis and nail dystrophy. Psoriasis can affect joints as well and is then called psoriatic arthritis. As psoriasis patients have a higher risk to develop cardiovascular and metabolic diseases compared to others general risk researchers now think of psoriasis as a general inflammatory state of the whole body including vessels and possibly also nerve fibers [Nestle et al., 2009, Raychaudhuri et al., 2008, Kaye et al., 2008, Gelfand et al., 2006, Takeshita et al., 2017]. This permanent inflammation could be the reason for a lot of comorbidities and corroborates the need for lasting successful therapeutic regimes [Zeichner and Armstrong, 2016].
For patients this disease not only means a physical but also a psychological burden. They complain about itchiness, pain and burning sensations of the skin and in combination with social stigmatization the psychological burden gets even more intensified [Boehncke and Schön, 2015, Dubertret et al., 2006, Rapp et al., 1999].
Diagnosis is usually done by clinical view [Boehncke and Schön, 2015]. Only with an unclear appearance or more rare forms like psoriasis inversa a skin biopsy and its histological examination may be needed. To assess the disease severity several scores can be used. The simplest score is the Body Surface Area (BSA) score. One palm of the patient is equivalent to 1% of his or her body surface. By this the BSA score enables the investigator to indicate roughly how much of the patient's skin is affected by psoriasis or any other skin disease [Bozek and Reich, 2017]. Using the Psoriasis Area and Severity Index (PASI) score the investigator needs to indicate how many percent of the scalp, upper and lower limbs and the trunk are affected as well as the degree of erythema, induration and desquamation with a maximum of 72 points. Based on this disease severity is divided into three different severity degrees (see table 1) [Schmitt and Wozel, 2005].

Table 1 Severity degrees of PASI

PASI score	Psoriasis severity degree
< 5:	mild
5 - 20:	moderate
> 20:	severe

Another score also takes the psychological burden into account by assessing disease impact on patients' daily life. The Dermatology Life Quality Index (DLQI) consists of ten questions concerning e.g. the ability to work or study. The maximum score that can be reached is 30 with the grades of (see table 2) [Schoffski et al., 2007]:

Table 2 Meaning of the DLQI

DLQI score	Impairment by psoriasis or psoriasis therapy
0 - 1:	no effect
2 - 5:	small effect
6 - 10:	moderate effect
11 - 20:	very large effect
20 - 30:	extremely large effect

1.1.2. Etiology and pathogenesis

Both etiology and pathogenesis of psoriasis are still not fully understood and subject of numerous clinical and immunological studies.

As far as known today psoriasis has a multifactorial etiology [Chandra et al., 2015]. This means psoriasis does not have a single trigger causing the disease but there are multiple, more or less crucial factors which can together lead to the outbreak of psoriasis. Probably most important is the genetic background of a person [Bowcock, 2005]. Genom-wide association studies have revealed different susceptibility loci which were called Psoriasis Susceptibility (PSORS) genes [Lowes et al., 2014]. The key locus is PSORS1 which codes for the Human Leucocyte Antigen (HLA) allele variant HLA-C and is responsible for 35-40% of the psoriasis heritability [Chandra et al., 2015]. The strongest risk allele of PSORS1 is HLA-cw6 [Henseler and Christophers, 1985]. It could also be shown that monozygotic twins have a higher concordance rate for psoriasis than dizygotic twins with an estimated heritability between 66% and 90% arguing for a strong genetic susceptibility [Farber et al., 1974, Brandrup et al., 1978, 1982, Duffy et al., 1993, Grjibovski et al., 2007]. Closely related to genetic changes are epigenetics. There are reports about DNA methylation, chromatin modifications and mitochondrial ribonucleic acid (miRNA) deregulation in psoriasis [Fogel et al., 2017]. E.g., epigenetic downregulation of secreted frizzled-related protein (SFRP) 4, a negative regulator of the Wnt pathway, leads to hyperproliferation of keratinocytes in psoriasis [Bai et al., 2015]. However, more detailed research is needed to get deeper insight into this field of psoriasis etiology. Another important factor is a person's environment which can contain distinct trigger factors. These are physical and psychological stress, the consumption of alcohol and nicotine, streptococcal infections especially in case of the guttate form, obesity, physical stimuli summarized under the term Koebner phenomenon, drugs like β-blockers and hormonal changes e.g. during puberty or pregnancy [Griffiths and Barker, 2007, Zeng et al., 2017]. There is also increasing evidence that changes in patients' microbiota not only play a role in skin diseases like atopic eczema or acne but might also be relevant for psoriasis [Zeng et al., 2017, Langan et al., 2017].

Whereas earlier psoriasis was considered to be a pure keratinocyte-driven disease, it has been shown that it is the interplay of immune cells and epidermal keratinocytes that leads to the macro- and microscopic changes we can see in the skin of psoriasis patients [Bos et al., 2005]. The key player in the pathogenesis of psoriasis are Interleukin (IL)-17 producing cells, especially Cluster of differentiation (CD) 4+ T helper (Th) 17 cells [Hawkes et al., 2017, Kim and Krueger, 2017]. Th17 cells got their name because they produce - besides cytokines like IL-22 and Tumour necrosis factor (TNF) α - IL-17 [Martin et al., 2013]. IL-17 has an effect on differentiation, recruitment and activation of immune cells like neutrophils, but also on non-immune cells like keratinocytes and the release of antimicrobial peptides [Martin et al., 2013, Gaffen, 2009]. In the epidermis, it leads to hyperproliferation and the release of more proinflammatory cytokines by keratinocytes which recruit neutrophils and macrophages into the psoriatic plaques. Neutrophils are stimulated to produce IL-17A by themselves and thereby reinforce their own activation as well as the epidermal inflammatory processes initiated by the keratinocytes [Zeichner and Armstrong, 2016]. Th17 cells depend on IL-23 for activation and IL-17 release as well as maintenance of their phenotype [McGeachy et al., 2009, Tonel et al., 2010]. With the production of IL-23, the innate immune system becomes involved. Myeloid dendritic cells (mDC) produce IL-23 after stimulation with Interferon (IFN) α produced by plasmacytoid dendritic cells (pDC) [Hawkes et al., 2017]. IFNα release by the pDC is induced by exogenous triggers like trauma or injury [Zeichner and Armstrong, 2016]. The exact mechanism of the starting point of the psoriatic inflammation cascade is still not yet known, but in the last years three different possible autoantigens have been described that could be crucial for the initial development of psoriasis. The first publication from Lande et al. goes back to 2007 where they describe that LL37, a cathelicidin and endogenous antimicrobial peptide, is sensed by pDC when being bound to self-DNA in a complex [Lande et al., 2007]. Later on they could show that psoriasis patients have LL37-specific T cells producing IFNγ as well as IL-17 underlining the autoimmune aspect of the disease [Lande et al., 2014]. A second possible autoantigen was brought up for discussion in 2015 by Arakawa et al. They report about ADAMTS-like protein (ADAMTSL) 5, an HLA-cw6-presented melanocytic autoantigen in HLA-cw6-positive psoriasis patients that is recognised by epidermal CD8+ T cells and stimulates the production of IL-17A [Arakawa et al., 2015]. In 2016 a third possible autoantigen was described: PLA2G4D, a normally cytosolic located phospholipase A_2 (PLA_2), is recognised by CD1a-reactive T cells from psoriasis patients with the subsequent production of IL-22 and IL-17A [Cheung et al., 2016]. Figure 1 gives an overview of psoriasis immune pathogenesis:

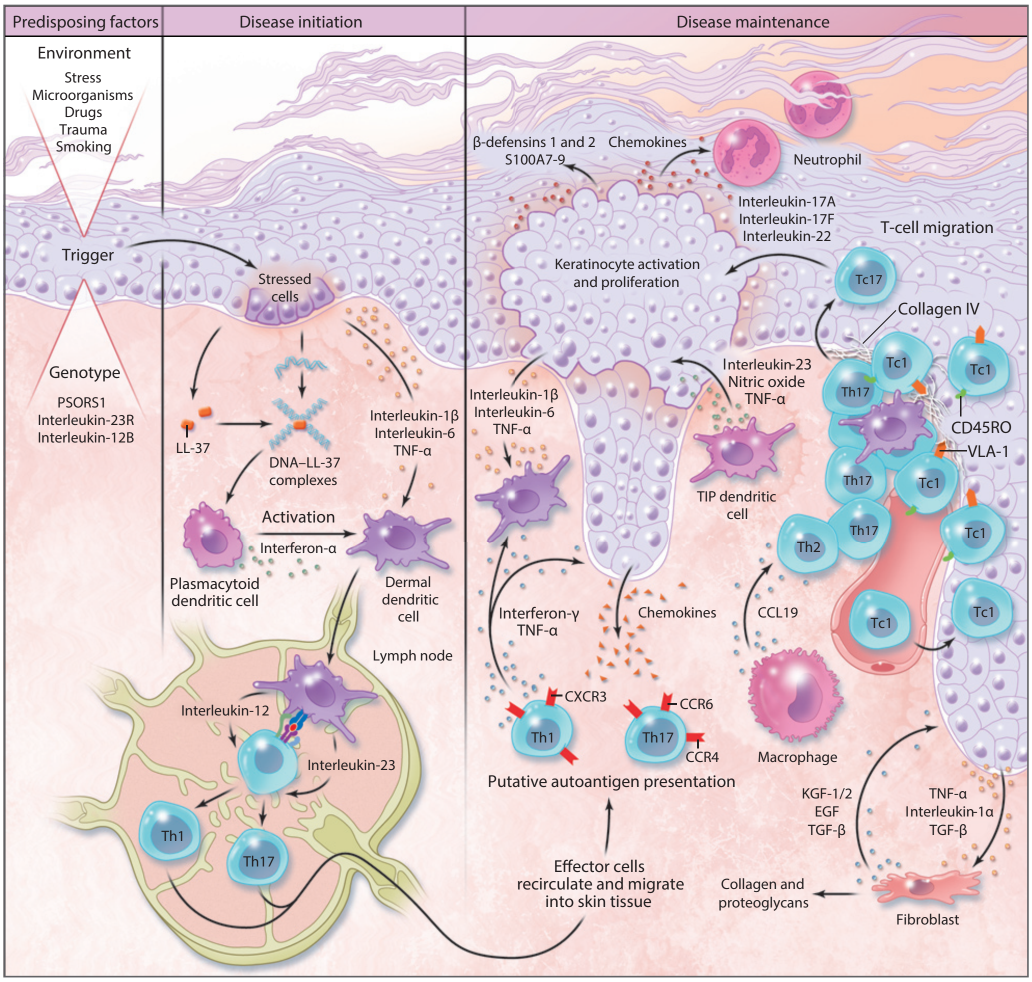

Figure 1 Proposed model of psoriasis pathogenesis: In Patients with a genetic susceptibility distinct environmental trigger factors like trauma or smoking lead to the formation of DNA-LL-37 complexes in the skin. Subsequently, plasmacytoid dendritic cells activate dermal dendritic cells which migrate to draining lymph nodes and induce differentiation of T cells into Th17 and Th1 cells via IL-23 and IL-12 release. These cells migrate to the skin where they influence other immune cells as well as keratinocytes. An inflammatory cascade mediated by TNFα, INFγ, IL-23, IL-17 and IL-22 attracts neutrophils and macrophages which enhance the inflammation and gives rise to tissue remodelling by fibroblasts and keratinocytes. Keratinocytes respond with release of antimicrobial peptides like β-defensins and histologically acanthosis and parakeratosis. Figure adapted from Nestle et al. [2009].

In summary, the current knowledge about psoriasis pathogenesis is already quite large but there are still missing points like the exact mechanism of the autoimmune processes. Hints may be given by other Th17 mediated diseases namely rheumatoid arthritis and multiple sclerosis. In the last years it became more evident that B cells in all likelihood play a role as important as T cells in these two diseases [Lehmann-Horn et al., 2013, Fox et al., 2010]. Clinical trials showed B cell depleting therapies as a promising therapeutic option by reducing disease activity and frequency of relapses [Lehmann-Horn et al., 2013, Calabresi, 2017, Mok, 2013, Edwards et al., 2004]. It was speculated that this was due to three different ways in which B cells can behave: As terminally differentiated plasma cells they are able to produce antibodies including autoantibodies. Additionally, B cells act as antigen-presenting cells mainly for T cells and they have the capacity to produce both pro- and anti-inflammatory cytokines and thereby influence immune processes irrespective of antibody production [Lund and Randall, 2010, Weber et al., 2011, Lehmann-Horn et al., 2013]. E.g., one subset of CD19+CD24high (hi) CD38hi B cells, seems to be capable of suppressing Th1 and Th17 differentiation while inducing regulatory T cells in healthy individuals whereas this ability might be impaired in patients with rheumatoid arthritis [Flores-Borja et al., 2013].

1.1.3. Therapy

1.1.3.1 Topical therapies[1]

In general, aim of each therapy should be the absence of any cutaneous symptoms of psoriasis. However, as psoriasis cannot be cured so far this is not a realistic aim of therapy. That is the reason why the surrogat parameter PASI 75 response was introduced. This means that a therapy should reduce the initial PASI by at least 75%. Correspondingly a DLQI score of 0 to 1 under therapy should be reached ideally which means no impairment of quality of life by the skin condition.

Topical therapies include the calcineurin inhibitors Tacrolimus and Pimecrolimus, Dithranol which is a synthetic tar derivative, glukokortikosteroids, the more historical agent tar from hard coal, the retinoid Tazaroten as well as Vitamin D3 and Vitamin D3 analogues.

A monotherapy with topical treatment is suitable for mild forms of psoriasis. In moderate- to severely affected patients a combinational therapy with phototherapy or systemic therapies is appropriate. Topical therapies should always be used adapted to the current skin condition. This demands a profound knowledge about the disease from the patient and a high motivation to continously care for the skin.

1.1.3.2 Systemic therapies[2]

The systemic therapies can be divided into two groups, the conventional systemic therapies and the relatively new biologicals. The conventional systemic treatment includes the retinoid Acicetrin with a rather weak recommondation for the use as a monotherapy, the immunosupressant Ciclosporin which should not be given as a long-term treatment because of its side effects, fumaric acid esters which are a good long-term option for patients without joint involvement, and

[1] This chapter is adapted from the German S3 guideline "Leitlinie zur Therapie der Psoriasis vulgaris Update 2011" (Guideline on the Therapy of Psoriasis vulgaris Update 2011.)

[2] This chapter is adapted from the "European S3-Guideline on the Systemic Treatment of Psoriasis vulgaris Update 2015" and the "European S3-Guideline on the Systemic Treatment of Psoriasis vulgaris Update Apremilast and Secukinumab" from 2017.

the antifolate Methotrexate (MTX) where the supplementation with 5 mg folic acid 24 hours after giving the MTX is mandatory.
The biological therapy for psoriasis contains at this stage five groups of active agents: TNFα inhibitors, anti-IL-12/IL-23 antibodies, Phosphodiesterase 4 (PDE4) inhibitors, anti-IL-17A and anti-IL-23 antibodies. Biologics are prescribed as a second-line medication for moderate to severe plaque psoriasis if phototherapy and conventional systemic therapy did not achieve a PASI 75 response, if the latter two are contraindicated or have shown untolerable side effects. The first and oldest group of biologicals contains the three anti-TNFα antibodies Adalimumab, Etanercept and Infliximab. Ustekinumab is an anti-IL-12/IL-23 antibody, Apremilast represents the group of PDE4 inhibitors and Secukinumab is the first approved anti-IL-17A antibody. Since 2017 Guselkumab is the first anti-IL-23 antibody with approval in Germany. Biologicals need to be given subcutaneous or intravenous. Because of their precise mode of action, they are mostly highly effective while also offering a good safety profile [Hawkes et al., 2017].
Only few and partly contradictory data are existing about the role of B cells in psoriasis. There are a few case reports about patients having developed psoriatic skin lesions after a B cell depleting therapy with Rituximab, a CD20 monoclonal antibody, arguing for an immune regulating function of unknown nature [Dass et al., 2007, Markatseli et al., 2009, Mielke et al., 2008, Guidelli et al., 2013]. Contrarily, reports about improvement of preexisting psoriasis under Rituximab therapy can be found as well [Moberg et al., 2010]. Besides, B cells could be detected in lesional skin from psoriasis patients [Lu et al., 2016, Mahmoud et al., 1999] and there are descriptions of higher frequencies of B cells in the sera of psoriasis patients relative to healthy controls [Gambichler et al., 2013]. Though detailed investigations about the significance of different developmental B cell subpopulations in the course of psoriasis are still lacking.

1.2. B cell development, function and pathophysiological meaning

B cells belong to the white blood cells representing one subtype of the lymphocytes. They function as the humoral part of the adaptive immune system by secreting antibodies. Besides they can present antigens, secrete cytokines and thus modulate and shape different immune responses.
B cells develop mainly in three types of tissue: bone marrow, spleen and lymph nodes (Figure 2) [Pieper et al., 2013]. All B cells are progeny of common progenitor cells, the pluripotent so called hematopoietic stem cells (HSC). Via multipotent progenitor cells (MPP) as intermediate stage the HSC differentiate into oligopotent common lymphoid progenitor cells (CLP) [Busslinger, 2004, Chao et al., 2008]. The CLP have the capacity to further differentiate into natural killer (NK) cells, B and T lymphocytes [LeBien, 2000]. The development into mature B cells occurs via diverse developmental stages which are in the order of their appearance: progenitor (pro) B cells, precursor (pre) B cells, immature naive and then mature naive B cells. Terminally differentiated B cells are represented by plasma cells as well as long-lived memory B cells [Murphy and Weaver, 2017]. During development B cells leave the bone marrow as immature naive B cells, while their subsequent development takes place in the secondary lymphoid organs. The different developmental stages vary in the composition of the gene segments encoding the heavy and light chains of the immunoglobulins as well as in the presence of surface immunoglobulin types [Ghia et al.,

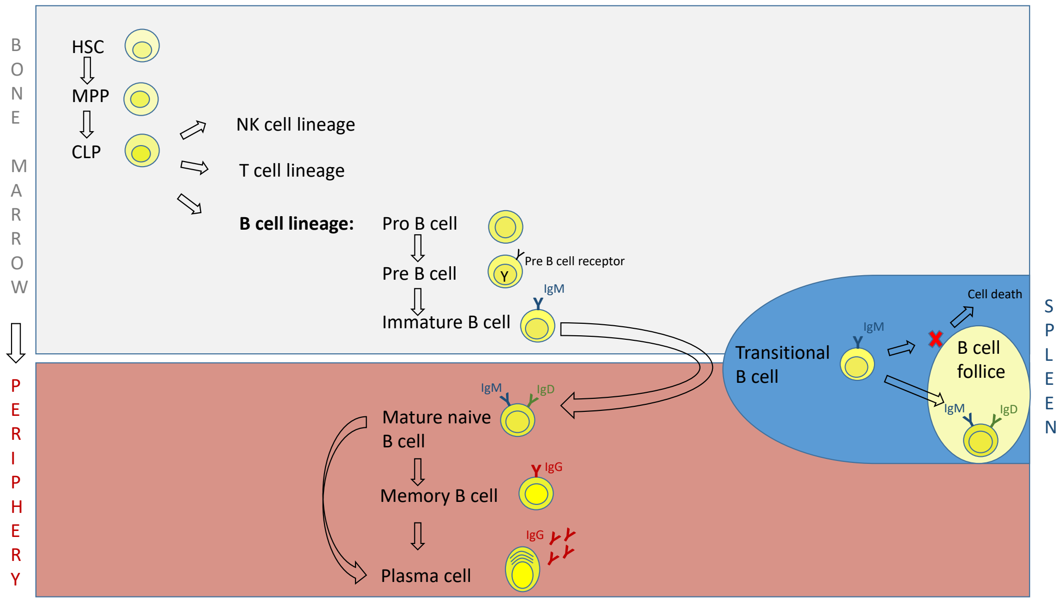

Figure 2 Developmental stages of B cell maturation: Lymphocytes derive from haematopoietic stem cells (HSC) which differentiate via multipotent progenitor cells (MPP) into common lymphoid progenitor cells (CLP). The first exclusive stage of development in B cell maturation is the pro B cell presenting no surface immunoglobulins. The subsequent pre B cell state is labelled by appearance of a pre B cell receptor. Differentiation continues with immature B cells presenting IgM and after bonemarrow release mature naive B cells which present surface IgM and IgD concomitantly. Memory B cells and plasma cells represent the terminally differentiated B cells and are able to produce other immunoglobulin classes besides IgM and IgD. In the spleen, additional maturation processes take place on the way from immature to mature naive B cells. The now called transitional B cells either get access into the spleen's B cell follicles where they undergo further maturation steps or die in case of non-access. Detailed information about immunoglobulin chain rearrangement at the individual stages of development can be found in the continuous text. Figure adapted from Murphy and Weaver [2017].

1996]. Rearrangement of the immunoglobulin chain gene segments during B cell development leads to a broad B cell repertoire with antibodies capable of recognizing more than 5 x 10^{13} different antigens [Pieper et al., 2013]. The heavy chains consist of the three gene segments named V_H, D_H and J_H. Initially, the D_H to J_H segment rearranges leading to the progression of the early pro B cell into the late pro B cell. In the late pro B cells state the rearrangement of the V_H segment to the DJ_H segment is conducted. After rearrangement of all three heavy chain gene segments B cells are capable of producing a complete heavy chain which is then part of the cytosolic pre B cell receptor (BCR). The pre BCR state is marked by the rearrangement of the light chains leading to IgM cell surface expression of then called immature B cells [Osmond et al., 1998, Pieper et al., 2013]. Before leaving the bone marrow, they are tested for autoreactivity [Chen et al., 1995, Cornall et al., 1995]. Central tolerance is guaranteed by three mechanisms: clonal deletion, anergy and receptor editing [Nemazee, 2006]. Only B cells with receptors that are not strongly binding to presented self-antigens are allowed to migrate into the spleen. In the spleen immature B cells pass through an interim stage where they are called transitional B cells. After that step they circulate in the periphery to get access to lymph node follicles [Melchers et al., 2000]. After having entered the lymph node they develop from transitional to mature B cells by upregulating immunoglobulin (Ig) D and hence presenting surface IgM and IgD concomitantly [Radbruch et al., 2006, Carsetti et al., 2004]. As mature naive B cells they recirculate through the peripheral blood and are stimulated when encountering an antigen that matches their BCR. The BCR bound antigen is transported into the intracellular compartment where it is processed and peptides are presented by Major Histocompatibility (MHC) class II molecules to antigen specific T helper (Th) cells. This T cell dependent activation leads to clonal B cell proliferation. Together with the corresponding T cell they reenter into lymph nodes where they build a so called germinal center and affinity maturation is taking place. This means that all activated B cells pass through several somatic hypermutations of their variable immunoglobulin regions and only B cells with BCR that have gained a higher antigen affinity during this process do survive. The other cells undergo apoptosis [Allen et al., 2007, Anderson et al., 2009, Crotty, 2014]. B cells with a high-affinity BCR continue to proliferate and differentiate into plasma cells or memory B cells orchestrated by cytokines [Nescakova and Bystricky, 2011]. These processes require a T cell dependent isotype switch by deoxyribonucleic acid (DNA) rearrangements in the genes coding for the constant region of the heavy chain occuring as soon as the B cells have been activated. As a consequence, B cells do not express IgM and IgD on their surface anymore like naive B cells do but IgG, IgA or IgE and become either plasma or memory B cells [Li et al., 2004, Takemori et al., 2014, Good-Jacobson and Tarlinton, 2012, McHeyzer-Williams et al., 2011]. Plasma cells are now able to leave the lymph node and recirculate into the periphery, where they act as short-lived plasma cells by producing antibodies [Shapiro-Shelef and Calame, 2005]. Others develop into long-lived antibody secreting plasma cells residing in special niches in the bone marrow [Radbruch et al., 2006, Shapiro-Shelef and Calame, 2005] or migrate into the spleen and into the medullary part of the lymph nodes [Murphy and Weaver, 2017].

Besides this T-cell dependent B cell activation there is a second type of activation triggered by certain bacterial and viral antigens called thymus-independent (TI) antigens which may have importance in the early phase of immune responses against extracellular bacteria. This type of B cell activation leads to cell proliferation and immunoglobulin release but not to affinity maturation and differentiation into memory B cells. TI antigens can be divided into TI-1 and TI-2 antigens: Depending on their concentration, TI-1 antigens, e.g. lipopolysaccharides or bacterial DNA, induce a polyclonal B cell response or antigen-specific antibody responses. Contrarily, TI-2 antigens consist of highly repetitive molecules like moieties of bacterial capsules and induce via cross-linking of the BCR IgM antibody responses or IgG production when supported by cytokine release by DCs. B cell activation through TI-2 antigens is especially important in cases of infections with capsulated bacteria which can easily escape both ingestion by phagocytes and T cell-dependent B cell activation [Murphy and Weaver, 2017, Liu et al., 2018, Fagarasan and Honjo, 2000].

Recently B cell research focused on a new subset called regulatory B cells (Bregs). Their presence was first shown in animal models for autoimmunity, infections and cancer. Most probably they exhibit their regulatory function by secreting IL-10 [Mauri and Ehrenstein, 2008, Blair et al., 2010]. The regulatory capability of these cells could be further shown by their capacity to maintain regulatory T cells and inhibit Th1 and Th17 differentiation [Flores-Borja et al., 2013].

Functionally B cells not only play a crucial role in humoral immunity by producing antibodies, they also exhibit an array of other features: B cells act as antigen-presenting cells for T cells mediating T cell activation and differentiation, also by secreting cytokines. These cytokines help maintain and regulate immune functions as well, e.g. of dendritic or T cells [LeBien and Tedder, 2008]. Moreover, B cells are key players building immunological memory and thus providing a fast and strong immune response when encountering a pathogen for the second time [Murphy and Weaver, 2017].

Eventually, B cell defects are responsible for autoimmunity, B cell malignancies and immunodeficiency emphasizing their impact within different pathophysiological conditions [Pieper et al., 2013].

1.3. Aim of the thesis

In this thesis the role of the humoral immune response for the development and maintenance of psoriasis vulgaris should be investigated. More precisely the following questions should be replied to:

1. Are B cells and the components of the humoral immune response necessary for the development of psoriasis vulgaris?
2. If so is it possible to identify distinct B cell subsets and clarify their role in the pathogenesis of psoriasis?
3. Or is it the other way round and are there B cell subsets which play a protective role and prevent people from developing psoriasis vulgaris?

2. Material and methods

2.1. Material

2.1.1. Fluorescence activated cell sorting (FACS), Enzyme linked immunosorbent assay (ELISA) and T cell experiments

Table 3 Material used for FACS, ELISA and T cell experiments

Reagents	
Ammonium chloride	Merck (Darmstadt, Germany)
Ampuwa	Fresenius Kabi (Bad Homburg v.d.H, Germany)
Aqua ad injectabilia	Berlin-Chemie (Berlin, Germany)
Bovine serum albumine (BSA)	Sigma-Aldrich (Saint-Louis, USA)
Dimethyl sulfoxide (DMSO)	AppliChem (Darmstadt, Germany)
Dulbecco's phosphate buffered saline (DPBS)	Gibco by Life Technologies (Carlsbad, USA)
Ethylendiaminetetraacetic acid (EDTA) 0.5 M	Invitrogen by Life Technologies (Carlsbad, USA)
Fetal calf serum (FCS)	GE Healthcare (Chalfont Saint Giles, United Kingdom (UK))
Fixation/Permeabilization solution	Beckton & Dickinson BD (Franklin Lakes, USA)
Glutamine	Gibco by Life Technologies (Carlsbad, USA)
GolgiPlug (containing Brefeldin A)	BD (Franklin Lakes, USA)
Human BD Fc Block	BD (Franklin Lakes, USA)
Human Serum	Sigma-Aldrich (Saint-Louis, USA)

Hydrogen peroxide solution	Sigma-Aldrich (Saint-Louis, USA)
Ionomycin	Sigma-Aldrich (St. Louis, USA)
LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit	Invitrogen by Life Technologies (Carlsbad, United States of America (USA))
Lymphoprep™	Stemcell Technologies (Vancouver, Canada)
Non-essential amino acids solution (100X)	Gibco by Life Technologies (Carlsbad, USA)
Penicillin-Streptomycin (10000 U/ml)	Gibco by Life Technologies (Carlsbad, USA)
Perm/Wash buffer (10X)	BD (Franklin Lakes, USA)
Phorphol 12-myristate 13-acetate (PMA)	Gibco by Life Technologies (Carlsbad, USA)
Potassium hydrogen carbonate	Merck (Darmstadt, Germany)
Roswell Park Memorial Institute (RPMI) medium 1640 (1X)	Gibco by Life Technologies (Carlsbad, USA)
Sodium azide	Carl Roth (Karlsruhe, Germany)
Sodium heparine 5000 U/ml	Merck (Darmstadt, Germany)
Sodium pyruvate (100 mM)	Gibco by Life Technologies (Carlsbad, USA)
Sulphuric acid (2 N) as ELISA stop solution	Merck (Darmstadt, Germany)
Tetramethylbenzidine (TMB)	Sigma-Aldrich (Saint-Louis, USA)
Thymidine (methyl-3H)	Hartmann Analytic (Hawkinge, Folkestone, United Kingdom)
Tween 20® detergent	Calbiochem (San Diego, USA)
Composition of buffer and solutions	

Cell culture medium	RPMI medium 1640 containing L-glutamine and $NaHCO_3$ supplemented with 100 U/µg/ml penicillin/streptomycin and 10% FCS
Citrate buffer	8.41 g citric acid monohydrate 180 ml distilled water Adjust pH to 3.95 and add to 200 ml with distilled water
ELISA assay diluent	DPBS with 10 % FCS, pH = 7.0
ELISA coating buffer	containing 0.1 M Na_2CO_3, pH = 9.5
ELISA reagent diluent	1% BSA in DPBS, pH = 7.2 - 7.4
ELISA substrate solution	5500 µl citrate buffer 2.55 µl H_2O_2 55 µl TMB
ELISA wash buffer	DPBS with 0.05% detergent Tween 20
ELISA wash buffer stock (20X DPBS)	46.4 g NaH_2PO_4 8 g KH_2PO_4 320 g NaCl 8 g KCl 1900 ml distilled water Adjust pH to 7.0 and add distilled water to a total volume of 2000 ml
Fluorescence activated cell sorting (FACS) buffer	DPBS with 5% FCS, 0.02% sodium azide
Freezing medium	FCS with 5% DMSO
10X red blood cell (RBC) lysis buffer	1500 mM NH_4Cl 100 mM $KHCO_3$ 1 mM EDTA Dissolved in H_2O

T cell proliferation medium	RPMI medium with 5% human serum 1% glutamine 1% Penicillin/Streptomycin 1% non-essential amino acids 1% sodium pyruvate
Antibodies for FACS staining	
Anti-CD3 (Peridinin-chlorophyll Protein Complex (PerCp-Cy)5.5, clone UCHT1)	BD (Franklin Lakes, USA)
Anti-CD19 (Brilliant Violet (Bv)605, clone SJ25C1)	BD (Franklin Lakes, USA)
Anti-CD20 (APC-Vio770, clone REA780)	Miltenyi Biotec (Bergisch-Gladbach, Germany)
Anti-CD24 (Bv421, clone ML5)	BD (Franklin Lakes, USA)
Anti-CD27 (Alexa Fluor (AF)700, clone M-T271)	BD (Franklin Lakes, USA)
Anti-CD38 (Fluoresceinisothiocyanate (FITC), clone HIT2)	BD (Franklin Lakes, USA)
Anti-CD56 (Phycoerythrin-Cyanine (PE-Cy)7, clone B159)	BD (Franklin Lakes, USA)
Anti-CD138 (Phycoerythrin (PE), clone 359103)	R&D Systems (Minneapolis, USA)
Anti-IgA (APC, clone REA1014)	Miltenyi Biotec (Bergisch-Gladbach, Germany)
Anti-IL-10 (eFluor 660®, clone JES3-9D7)	eBioScience (Frankfurt a.M., Germany)
ELISA kits	
Human IL-4 ELISA Set (555194)	BD (Franklin Lakes, USA)
Human IL-17 DuoSet ELISA (DY317)	R&D Systems (Minneapolis, USA)
Human IL-22 DuoSet ELISA (DY782)	R&D Systems (Minneapolis, USA)
INF-γ DuoSet ELISA (DY285B)	R&D Systems (Minneapolis, USA)
TNF-α DuoSet ELISA (DY210)	R&D Systems (Minneapolis, USA)

T cell experiment kits and stimulants	
IL-2 Proleukin S	Novartis (Basel, Switzerland)
Mouse anti-human CD3 (clone UCHT1)	BD (Franklin Lakes, USA)
Mouse anti-human CD28 (clone CD28.2)	BD (Franklin Lakes, USA)
Phytohemagglutinin (PHA)	Sigma-Aldrich (Saint-Louis, USA)
Consumables	
Cell culture plate 24-well, flat, untreated	Corning (Corning, New York, USA)
Cell culture plate 96-well, round botton	Sarstedt (Nümbrecht, Germany)
Cellstar centrifuge tubes, 15 ml and 50 ml	Greiner Bio-One (Frickenhausen, Germany)
Corning® 96 well microtitration plate, high bind, flat bottom	Sigma-Aldrich (Saint Louis, USA)
CryoPure tube 1.8 ml	Sarstedt (Nümbrecht, Germany)
FACS cluster tubes	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
Falcon tubes, 5 ml	Corning (Corning, New York, USA)
Filtropur vacuum filter, pore size 0.22 µm	Sarstedt (Nümbrecht, Germany)
MeltiLex solid scintillator for MicroBeta filters	PerkinElmer (Waltham, Massachusetts, USA)
Leucosep tube 15 ml and 50 ml	Greiner Bio-One (Frickenhausen, Germany)
Pipette tips epT.I.P.S. 0.1-10 µl	Eppendorf (Hamburg, Germany)
Pipette tips epT.I.P.S. 0.5-20 µl	Eppendorf (Hamburg, Germany)
Pipette tips epT.I.P.S. 2-200 µl	Eppendorf (Hamburg, Germany)

Pipette tips epT.I.P.S. 50-1000 µl	Eppendorf (Hamburg, Germany)
Pipette tips Diamond 2-200 µl	Gilson (Middleton, USA)
SafeSeal tube 1.5 ml and 2.0 ml	Sarstedt (Nümbrecht, Germany)
Serological pipette 1 ml, 2 ml, 5 ml, 10 ml and 25 ml	Greiner Bio-One (Frickenhausen, Germany)
Syringe 50 ml	Sarstedt (Nümbrecht, Germany)
Technical devices	
BD FACSDiva Software, Version	BD (Franklin Lakes, USA)
Biological Safety Cabinet Heraeus HeraSafe	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
Centrifuge Heraeus Megafuge 40R	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
CoolCell cell freezing container	Biocision (San Rafael, USA)
Cryostorage system LS6000	Tec-Lab (Taunusstein, Germany)
Epoch Microplate Spectrophotometer	Biotek (Winooski, USA)
Falcon Pipet Controller	Corning (Corning, New York, USA)
Flow cytometer, LSR Fortessa	BD (Franklin Lakes, USA)
FlowJo Software, Version 10	FlowJo LLC (Ashland, USA)
Freezer -20 ° C	Liebherr (Bulle, Germany)
Freezer HeraFreeze -80 ° C	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
HydroSpeed™ microplate washer	Tecan (Männedorf, Switzerland)
Multi channel pipette Transferpette 20-200 µl	Brand (Wertheim, Grmany)

Single channel pipette Research plus 0.1-2.5 µl	Eppendorf (Hamburg, Germany)
Single channel pipette Research plus 0.5-10 µl	Eppendorf (Hamburg, Germany)
Single channel pipette Research plus 10-100 µl	Eppendorf (Hamburg, Germany)
Single channel pipette Research plus 100-1000 µl	Eppendorf (Hamburg, Germany)
Vacuum pump Vacuu Hand Control	Vacuubrand (Wertheim, Germany)

2.1.2. Immunofluorescence

Table 4 Material used for immunohistology

Reagents	
2-Propanol	Otto Fischer (Saarbrücken, Germany)
BOND Primary Antibody Diluent AR 9352	Leica Biosystems (Wetzlar, Germany)
Citric acid monohydrate	Carl Roth (Saarbrücken, Germany)
DAPI Nucleic Acid Stain	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
Disodium phosphate	Merck (Darmstadt, Germany)
Ethanol (96% and 70%)	Otto Fischar (Saarbrücken, Germany)
Hydrochloric acid (5 N)	Merck (Darmstadt, Germany)
Hydrogenperoxide solution	Sigma-Aldrich (Saint-Louis, USA)
Potassium chloride	Merck (Darmstadt, Germany)
Potassium dihydrogenphosphate	Merck (Darmstadt, Germany)

Roticlear	Carl Roth (Karlsruhe, Germany)
Sodium chloride	Carl Roth (Saarbrücken, Germany)
Sodium hydroxide (10 N)	Pharmacy of the University Hospital Rechts der Isar (München, Germany)
Sudan Black B	Sigma-Aldrich (Saint-Louis, USA)
Trizma Base Primary standard and buffer	Sigma-Aldrich (Saint-Louis, USA)
Tween 20® detergent	Calbiochem (San Diego, USA)
Composition of buffer and solutions	
10X Tris buffer	60.5 g Trizma Base Add to 700 ml distilled water Add 5 N HCl to adjust pH to 7.6 Add 90 g NaCl Add distilled water to a total volume of 1000 ml
10X PBS	80 g NaCl 2 g KCl 14.4 g Na_2HPO_4 2.4 g KH_2PO_4 Add to 800 ml with distilled water Adjust pH to 7.4, add distilled water to a final volume of 1000 ml
Citrate buffer	Add 4.2 g Citric acid monohydrate to 2 l distilled water Adjust pH to 6.0 with 10 N NaOH
Sudan Black solution 0.1 %	0.1 g Sudan Black B 100 ml Ethanol (70%)
Antibodies and sera for immunofluorescence stainings	

Alexa Fluor® 488 goat anti-rabbit IgG (polyclonal)	Life Technologies (Carlsbad, USA)
Mouse anti-CD31 (clone JC70)	Cell Marque (Rocklin, USA)
Normal donkey serum	Sigma-Aldrich (Saint-Louis, USA)
Normal goat serum	Novex by Life Technologies (Carlsbad, USA)
NorthernLights™ 557 donkey anti-mouse IgG	R&D Systems (Minneapolis, USA)
Rabbit anti-IgA (polyclonal)	Zytomed (Berlin, Germany)
Consumables	
Microscope cover slips 24x60 mm and 24x40 mm	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
Microscope slides Superfrost™ Plus	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
Microtome blades	Feather Safety Razor (Osaka, Japan)
Nail polish Last Forever	p2 Kosmetik (Wien, Austria)
Rotilabo®-disposable weighing trays	Carl Roth (Karlsruhe, Germany)
Mounting medium VectaShield®	Vector Laboratories (Burlingame, USA)
PAP PEN MaxTag™ hydrophobic barrier pen	Rockland Immunochemicals (Limerick, USA)
Staining dishes	Diverse common manufacturers
StainTray slide staining system	Sigma-Aldrich (Saint-Louis, USA)
Stirring bars and metal spoons	Divers common manufacturers
Syringe filter Millex-HV 0.45 µm	Merck Millipore (Cork, Ireland)

Syringe Omnifix® Solo 50 ml	B. Braun Melsungen (Melsungen, Germany)
Technical devices	
Heating oven	Memmert (Schwabach, Germany)
Heidolph MR3001 magnetic stirring hotplate	Heidolph Instruments GmbH & Co. KG (Schwabach, Germany)
IX73 Inverted microscope	Olympus (Tokio, Japan)
Microtome RM 2255	Leica Biosystems (Wetzlar, Germany)
pH meter inoLab pH7110	WTW (Weilheim i. Obb., Germany)
Precision scale Kern 770	Kern & Sohn GmbH (Balingen, Germany)
Pressure cooker 4.5 l Perfect	WMF Group (Geislingen a.d. Steige, Germany)
Single cooking plate Type THL 1597	Rommelsbacher (Dinkelsbühl, Germany)
Software cellSens Version	Olympus (Tokio, Japan)
Software ImageJ Version 1.50i	OpenSource

2.1.3. Ribonucleic acid (RNA) experiments

Table 5 Material used for RNA experiments

Reagents	
FastStart Universal SYBR Green Master (Rox)	Roche Diagnostics GmbH (Mannheim, Germany)
High Capacity cDNA Reverse Transcription Kit	Applied Biosystems (Foster City, California, USA)
miRNeasy Mini Kit	Qiagen (Venlo, Netherlands)
Primer	

QIAzol® Lysis Reagent	Qiagen (Venlo, Netherlands)
RNA 6000 Nano Kit	Agilent Technologies (Santa Clara, California, USA)
RNAlater RNA Stabilization Reagent	Qiagen (Venlo, Netherlands)
RNase-Free DNase Set	Qiagen (Venlo, Netherlands)
RNase Zap® RNase decontemination solution	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
TrueSeq Stranded Total RNA Kit	Illumina (San Diego, California, USA)
UltraPure DEPC-treated water	Invitrogen by Life Technologies (Carlsbad, USA)
Consumables	
Cellstar centrifuge tubes, 15 ml	Greiner Bio-One (Fricken-hausen, Germany)
Neopretex® gloves	Meditrade (Kiefersfelden, Ger-many)
PCR plate, 384-well, standard	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
Pipette tips epT.I.P.S. 0.1-10 µl	Eppendorf (Hamburg, Ger-many)
Pipette tips epT.I.P.S. 0.5-20 µl	Eppendorf (Hamburg, Ger-many)
Pipette tips epT.I.P.S. 2-200 µl	Eppendorf (Hamburg, Ger-many)
Pipette tips epT.I.P.S. 50-1000 µl	Eppendorf (Hamburg, Ger-many)
Safe-Lock microcentrifuge tubes 0.5, 1.5 and 2.0 ml	Eppendorf (Hamburg, Ger-many)
SafeSeal tubes 1.5 ml and 2 ml	Sarstedt (Nürnbrecht, Germany)
Stainless steel beat 5mm	Qiagen (Venlo, Netherlands)

Sterile scissors and forceps	Diverse common manufacturers
Tubes, 0.2 ml, flat cap	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
Technical devices	
2100 Bioanalyzer	Agilent Technologies (Santa Clara, California, USA)
Centrifuge 5427 R	Eppendorf (Hamburg, Germany)
Digital precision thermometer GTH 175/MO	Greisinger electronic GmbH (Regenstauf, Germany)
Heraeus Biofuge Pico	Heraeus (Hanau, Germany)
HiSeq 4000 system	Illumina (San Diego, California, USA)
Magnetic stirring plate IKAMAG REO	IKA®-Werke GmbH & Co. KG (Staufen, Germany)
NanoDrop ND1000 UV-vis spectrophotometer	Thermo Fisher Scientific (Waltham, Massachusetts, USA)
Single channel pipette Research plus 0.5-10 µl	Eppendorf (Hamburg, Germany)
Single channel pipette Research plus 10-100 µl	Eppendorf (Hamburg, Germany)
Single channel pipette Research plus 200 µl	Eppendorf (Hamburg, Germany)
Single channel pipette Research plus 100-1000 µl	Eppendorf (Hamburg, Germany)
Techne TC-412 Thermal Cycler	Bibby Scientific Limited (Staffordshire, UK)
TissueLyser II plus TissueLyser Adapter Set 2x24	Qiagen (Venlo, Netherlands)
ViiA ™ 7 Real-Time PCR System plus 384-well cooling plate and software	Applied Biosystems (Foster City, California, USA)

Vortex Genie	Bender & Hobein AG (Zurich, Switzerland)
VTX-3000L Mixer Uzusio	LMS Co., Ltd. (Tokyo, Japan)

2.1.4. Sample acquisition

Table 6 Material used for sample aquisition

Reagents	
Skin antiseptic octeniderm®	Schülke & Mayr (Norderstedt, Germany)
Paraformaldehyde	Fischar Otto (Saarbrücken, Germany)
Xylonest 1 % with adrenaline 1:200000, 50 ml	AstraZeneca (London, UK)
Consumables	
Biopsy Punch 6 mm	Stiefel Laboratorium GmbH (Offenbach a.M., Germany)
Blood collection tube S-Monovette® clotting activator/serum, 9 ml	Sarstedt (Nümbrecht, Germany)
Blood collection tube S-Monovette® EDTA, 2.5 ml	Sarstedt (Nümbrecht, Germany)
Blood collection tube S-Monovette® Sodium heparin, 7.5 ml	Sarstedt (Nümbrecht, Germany)
Dafilon 4/0 (1.5) suture, 45 cm	B. Braun Melsungen (Melsungen, Germany)
Disposable scalpel No. 11	Feather Safety Razor (Osaka, Japan)
Raucodrape® fenestrated drape 90x75 cm	Lohmann & Rauscher (Neuwied, Germany)
Safety-Multifly® needle 21G und 23G	Sarstedt (Nümbrecht, Germany)
Sterican single-use needle 20G und 30G	B. Braun Melsungen (Melsungen, Germany)
Sterile surgical set including scissors and forceps	Divers common manufacturers
Syringe 2 ml	B. Braun Melsungen (Melsungen, Germany)

2.2. Methods

2.2.1. Study cohorts

In the study included were 35 treatment-naive patients suffering from psoriasis vulgaris with PASI > 5. Included were patients without any therapy at all at the time of admission, patients receiving exclusively topical treatment with e.g. corticosteroids or Dithranol but also patients with a systemic treatment for itchiness like intravenous (i.v.) antihistamines. The data collected in every psoriasis patient were: age, sex, current therapy, psoriasis severity degree measured as PASI and impairment of quality of life indicated as DLQI. Serum was analysed for IgA, IgE, IgG and IgM and complement factors 3 and 4 (C3, C4).
Two age- and sex-matched control cohorts were built up, one consisting of 20 volunteers with no history of chronic skin disease, and one with 13 patients suffering from atopic eczema (AE).
To study whether effective systemic immunosuppressive treatment would influence patients' B cell and humoral profile, a second psoriasis cohort (52.1 ± 2.1 years; PASI: range 0 to 4.7; mean: 1.7) with 28 well-treated patients (PASI < 5) was built up also. Part of the treated cohort were patients receiving methotrexate (n=8), TNF inhibitors (n=8), anti-IL-12/IL-23 antibodies (n=5), dimethyl fumarate (n=3), the PDE-4 inhibitor Apremilast (n=3) and Acitretin (n=1). A third mixed treated and treatment-naive psoriasis cohort (n=7; 48.3 ± 6.3 years; PASI: range 0.0 to 29.0, mean: 7.0) was studied for additional FACS experiments which will be explained in detail later (see chapter 3.2.2).
Every study participant was in advance informed about the study approach and the voluntary participation and gave his or her written informed consent. The ethics commission of the faculty of medicine of the Technical University of Munich had approved the study (project number 5060/11).

Table 7 Epidemiological and clinical data of the three main study cohorts

Parameter	Patients with psoriasis (n = 35)	Healthy controls (n = 20)	Patients with AE (n = 13)	P value
Age (years)	41.7 ± 2.5 (15 to 79)	48.2 ± 3.2 (22 to 70)	37.5 ± 5.8 (11 to 72)	0.11
Gender n (%) Female Male	 10 (28.6 %) 25 (71.4 %)	 7 (35.0 %) 13 (65.0 %)	 7 (53.8 %) 6 (46.2 %)	0.27
Disease score	15.0 ± 1.6 (6.2 to 43.2)	Indication not appropriate	38.2 ± 3.9 (13.3 to 55.5)	

As table 7 explains the three main study cohorts showed no significant deviation in age (Kruskal Wallis test: p = 0.11) or gender (Chi-square test: p = 0.27) composition. All of them had a medium mean age. The gender distribution for psoriasis patients and the healthy control cohort was about one third of women and two thirds of men. Among the study cohort consisting of patients with AE, gender distribution was evened but not statistically significant from the two other cohorts. The mean PASI of the psoriasis cohort was 15.0 which represents a moderate disease severity. In the AE cohort the mean SCORing Atopic Dermatitis (SCORAD) value was 38.2 which also indicates a moderate severity.
The third study cohort of AE patients was included to prove that differences found between healthy controls and the psoriasis cohort were specific for psoriasis and not a general phenomenon of inflammatory skin

conditions. In the description of the results the main focus was put on the psoriasis and healthy volunteer cohorts whereas the AE cohort was considered in case of significant differences between the first two cohorts.

2.2.2. Collection of blood samples

From every study participant 40 ml of blood were taken from the median cubital vein. 37.5 ml were used for the serum parameter analysis, another 2.5 ml mixed with EDTA for anticoagulation were prepared for the FACS analysis.

2.2.3. Surface staining of blood cells

2.2.3.1 Blood preparation

10 ml of 1X RBC lysis buffer (that is 9 ml H_2O and 1 ml 10X RBC lysis buffer) and 1 ml blood of each donor were mixed well in a 50 ml Falcon tube and incubated for 15 minutes at room temperature. After centrifugation with 1200 rpm for ten minutes the supernatant with the lysed red blood cells was drained and the cell pellet with the remaining blood cells was resuspended in 50 ml DPBS. After another centrifugation with 1200 rpm for ten minutes the supernatant was completely taken off by aspiration and the cell pellet was resuspended in 200 µl DPBS and then placed on ice.

2.2.3.2 B cell surface staining for FACS analysis

In order to stain the cells with fluorochrome conjugated antibodies the samples were pipetted into a 96-well U-bottom plate. 20 µl of the cell suspension were prepared with FACS buffer as unstained control. The rest of the cell suspension was used for staining. After centrifugation with 800 xg for 1 minute the cell pellet for the unstained cells was resuspended in FACS buffer and then stored in FACS cluster tubes at 4 °C. The cells that should be stained were washed with DPBS at 800 xg for 1 minute and the remaining cell pellet was resuspended in 100 µl Aqua solution (1:1000 diluted in cold DPBS) and incubated for 30 minutes at 4 °C. The staining with Aqua is used for differentiation of living and dead cells. In living cells the dye interacts only with cell surface proteins whereas in dead cells it can penetrate the cell membrane. As a consequence, it can form more bonds in the cytoplasm and therefore shows a stronger fluorescence for Aqua compared to living cells. After the incubation the cells were washed first with DPBS followed by a second washing step with FACS buffer. Then the cells were incubated for 10 minutes at room temperature with 10 µl Human BD Fc Block (1:10 diluted with FACS buffer). This prevents unspecific binding of the fluorochrome conjugated antibodies that were used for staining to the Fc receptor. After adding 10 µl antibody mix the cells were incubated for 30 minutes at 4 °C. The antibody mix contained the following antibodies:

- PerCp-Cy5.5 anti-CD3 (BD) 1:100 dilution
- Bv605 anti-CD19 (BD) 1:20 dilution
- Bv421 anti-CD24 (BD) 1:100 dilution
- FITC anti-CD38 (BD) 1:10 dilution
- PE-Cy7 anti-CD56 (BD) 1:100 dilution
- PE anti-CD138 (R&D Systems) 1:10 dilution
- APC-Vio770 anti-CD20 (Miltenyi Biotec) 1:50 dilution

- APC anti-IgA (Miltenyi Biotec) 1:50 dilution

Before storing the stained cells in FACS buffer in FACS cluster tubes at 4 °C until FACS measurement, they were washed again with FACS buffer.

2.2.4. Intracellular staining of blood cells

The whole protocol for the intracellular B cell staining was adapted from Blair et al. [2010].
Briefly, lymphocytes and PBMCs were isolated from heparinized blood. After a stimulation period, the aforementioned surface staining followed by an additional intracellular staining procedure was performed.

2.2.4.1 Blood preparation

For the intracellular staining it was necessary to separate lymphocytes and PBMCs from the remaining blood cells via density gradient centrifugation. Therefore 50 ml Leucosep tubes were filled with 15 ml Lymphoprep medium and centrifugated for 30 seconds with 1000 xg at room temperature so that the Lymphoprep medium was located below the porous barrier membrane. The heparinized blood was diluted 1:2 with sodium chloride solution and up to 30 ml of the diluted blood were poured into the Leucosep tubes. After centrifugation with 1000 g for ten minutes at room temperature the upmost located plasma layer fraction was discarded and the lymphocytes/PBMCs cell fraction was harvested and washed three times with DPBS.

2.2.4.2 Intracellular B cell staining for FACS analysis

First 10^6 PBMCs were resuspended in 1 ml of culture medium and put into a 24 well plate. Then the cells were stimulated with 1 mg/ml purified stimulatory mouse anti-human CD40 monoclonal antibody (BD Biosciences; 0.5 µg CD40 mAb/10^6 cells) for 72 hours at 37 °C and 5% CO_2. For the last six hours, GolgiPlug along with PMA (10 ng/ml) and Ionomycin (1 µg/ml) was added. After stimulation the cells were centrifugated and resuspended in DPBS. Surface staining was performed in a 96 U-bottom plate as described above. For the subsequent intracellular staining, the cells were fixed with Fixation/Permeabilization solution and incubated for 20 minutes at 4 °C. After two washing steps with FACS buffer, the cells were first treated with 1X Perm solution (10X Perm/Wash buffer 1:10 diluted with sterile water) and then stained with 10 µl antibody mix (Anti-IL-10 (eBioScience), 1:50 dilution in Perm solution) by incubation for 30 minutes at room temperature in the dark while shaking. The cells were washed with FACS buffer and resuspended in FACS buffer and stored in FACS cluster tubes at 4 °C until measurement.

2.2.5. Cell detection

After having stained the cells with fluorochrome conjugated antibodies they were analysed via flow cytometry. In this process the cells are aspirated through a capillary, illuminated by multiple lasers with light of different wavelengths and then the scattered light as well as the fluorescence emitted by the added fluorescent dye conjugated antibodies are detected. Thereby the forward scatter (FCS) as measure of cell volume and the sideward scatter (SSC) as measure of the granularity of the cells can be distinguished. The cell detection with the flow cytometer LSR Fortessa (BD) was continued until 30000 B cells of one sample had been recorded.

2.2.6. Gating strategy of the B cells

The analysis of the FACS results was performed with the FlowJo software Version 10. For a better understanding of the gating strategy the receptors stained on the cell surface will be explained briefly:
The expression of the six cell surface markers CD19, CD24, CD38, CD138, CD3 and CD56 was measured while CD3 and CD56 were used for detection of T cells, NKT and NK cells. The CD19 positive cells were divided into five subpopulations according to their CD24 and CD38 expression. CD19 as a B cell marker is expressed from early stages of B cell development on [Tedder and Isaacs, 1989]. CD38 and CD24 are two developmentally regulated markers that are co-expressed by all B cells [Carsetti et al., 2004]. CD38 is considered a marker of differentiation and activation of B and T cells whose level of expression during B cell maturation is strictly regulated [Rodriguez-Alba et al., 2008] . CD24 is produced by many cell types like most of the haematopoetic cells but also by keratinocytes [Magnaldo and Barrandon, 1996] and epithelial cells of different types of tissue [Ye et al., 2005, Sleeman et al., 2006, Shirasawa et al., 1993]. All these cells have in common that they express CD24 mainly as immature cells. After having completed maturation they usually don't express CD24 on the surface anymore [Tan et al., 2016, Allman et al., 1992]. CD138, also called Syndecan-1, serves the identification of plasma cells [Costes et al., 1999, O'Connell et al., 2004, Chilosi et al., 1999].

Figure 3 shows the five by definition CD19 positive B cell subpopulations that were paid most attention to in this study.

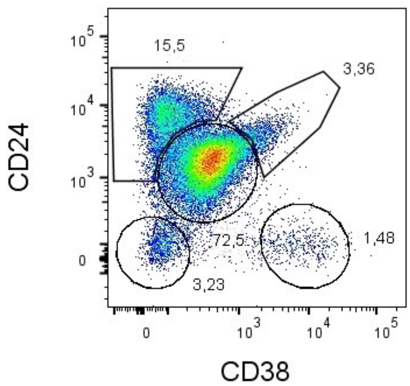

Figure 3 Example of gating strategy: Part of FACS data of one representative psoriasis patient with the five B cell subpopulations of CD24+CD38- memory B cells, CD24 intermediate CD38 intermediate mature naive B cells, CD24+CD38+ transitional/regulatory B cells, CD24- CD38+ plasmablasts/plasma cells and CD24-CD38- new memory B cells. Figure adapted from Thomas et al. [2018]

According to the B cells' CD24 and CD38 surface expression the following populations can be distinguished (s. figure 3):

- CD24+ CD38- memory B cells [Carsetti et al., 2004, Lin et al., 2017]
- CD24 intermediate CD38 intermediate mature naive B cells [Lin et al., 2017]
- A CD24+ CD38+ population, described as transitional B cells [Carsetti et al., 2004] or rather as regulatory B cells [Blair et al., 2010, Flores-Borja et al., 2013, Mauri and Ehrenstein, 2008, Lin et al., 2017]
- CD24- CD38+ B cells, by Lin et al. described as population consisting of plasmablasts and plasma cells [Lin et al., 2017]

- CD24- CD38- B cells, described by Buffa et al. as new memory B cells which are found at higher frequencies in the elderly [Buffa et al., 2013]

To detect plasma cells independently of their precursors, the plasmablasts, a separate gating step was performed and plasma cells depicted as CD19+ CD24- CD38+ CD138+. Backgating of CD20 was performed in exemplary chosen patients to show that CD19+ CD24- CD38+ CD138+ plasma cells are CD20- as indicated in literature [Martin and Chan, 2006, Bour-Jordan and Bluestone, 2007].

2.2.7. Stimulation of PBMCs

To measure their proliferation, stimulated PBMCs were labeled with radioactive thymidine.

2.2.7.1 Isolation of PBMCs

50 ml of peripheral blood of patient A.B. was collected in a syringe pre-filled with 100 µl heparine. After diluting the blood with DPBS (1:2 dilution) it was carefully layered on Lymphoprep solution. After centrifugation for 15 minutes at room temperature and 950 xg, PBMCs were collected and washed several times with DPBS supplemented with 5 Millimolar (mM) EDTA.

2.2.7.2 Stimulation of PBMCs and ^{3}H-Thymidine Proliferation Assay

For measuring PBMC proliferation, proliferating cells were labeled with radioactive thymidine. First, PBMCs were stimulated with anti-CD3, anti-CD28, Phytohemagglutinin (PHA) and pertussis toxin. Then, proliferation was measured. For a 96-well proliferation assay, supernatants from stimulated T cells were replaced with 90 µl fresh culture medium. Then, ^{3}H-Thymidine was diluted 1:50 in DPBS and 10 µl of this dilution were pipetted in each 96-plate well leading to a working concentration of 2µCi. The plate was wrapped in Alu foil and cells were incubated for 6 to 8 hours or overnight at 37°C and 5% CO_2. Subsequently, the plate was stored at -20 °C until readout. Therefore, proliferating cells with built-in ^{3}H-Thymidine were separated from the radioactive supernatant by aspirating the cell suspension through a filter which was unpermeable for the cells but permeable for unbound ^{3}H-Thymidine. After drying the filter was then melted together with a scintillation foil which made radioactivity readable for the β-counter. Radioactivity on the filter was indicated as counts per minute (cpm) and correlated with the amount of newly formed DNA and thus the cells' proliferation.

2.2.8. Stimulation of lesional T cells

2.2.8.1 T cell isolation from biopsy

Freshly taken skin biopsies were incubated at 37,5 °C and 5% CO_2 for one hour in 24-well plates precoated with 0.75 µg/ml anti-CD3 and containing T cell culture medium supplemented with 0.75 µg/ml anti-CD28 and 60 U/ml IL-2. Three times a week, medium was replaced with fresh medium containing 60 U/ml IL-2 and emigrated T cells were expanded.

2.2.8.2 T cell stimulation

T cells were seeded into a 96-well plate (150000 T cells/per well) and stimulated with 0.75 µg/ml anti-CD3 and 0.75 µg/ml anti-CD28 in T cell medium or left unstimulated as control. After 48 hours of incubation, supernatants were collected and ELISA was performed as described below.

2.2.9. ELISA

The concentration of the cytokines IL-4, IL-17, IL-22, TNF-α and INF-γ in T cell supernatants was measured by ELISA. A common ELISA set was used in accordance with the manufacturer's instructions: By using a first cytokine-specific antibody (called capture antibody) the corresponding cytokine was bound in the wells of a 96-well microtiter plate and a second cytokine-binding antibody (detection antibody) which was linked to an enzyme was added. By adding a solution with a colorimetric substrate that could be converted by the enzyme leading to colour change the absorption could be measured at a certain wavelength and the cytokine concentration in the serum was determined by comparison with a standard curve.

2.2.10. RNA experiments

RNA experiments are based on the work presented by Garzorz-Stark et al. [2016].

2.2.10.1 RNA isolation from biopsy

To isolate RNA from the skin samples, the miRNeasy Mini Kit (Qiagen) was used. The biopsies were transferred from RNA later RNA stabilization reagent to 700 µl of Qiazol Lysis reagent and cut into small pieces with sterile scissors. After homogenization using the TissueLyser the subsequent RNA isolation was performed following the manufacturer's instructions including a DNase digestion using RNase free DNase set. The resulting RNA concentration was determined by NanoDrop.

2.2.10.2 Complementary deoxyribonucleic acid (cDNA) synthesis

The transcription of the eluated RNA into cDNA was performed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) following the manufacturer's instructions for the protocol "Kit without RNase inhibitor". RNA input was 500 ng per sample. Target genes were NOS2 and CCL27. Primers amplifying these two genes were designed by help of the Open Source software Primer3. Sequences were:

- NOS2:
  5'- GTTCTCAAGGCACAGGTCTC-3‘
  5'- GCA GGT CAC TTA TGT CAC TTA TC-3‘
- CCL27:
  5'- AGGTCATCCAGGTGGAACTGC-3‘
  5'- TCAAACCACTGTGACAGGCTG-3‘
- 18S:
  5'-GTAACCCGTTGAACCCCATT-3‘
  5'-CCATCCAATCGGTAGTAGCG-3‘

2.2.10.3 Real time polymerase chain reaction (RT-PCR)

RT-PCR was done using the Fast Start Universal SYBRGreen Master (Rox) (Roche Diagnostics) according to the manufacturer's instructions and the ViiA 7 Real-Time PCR system (Applied Biosystems) for detection of fluorescence. 18S ribosomal RNA served as housekeeping gene (see above). RT-PCR data were analyzed by $2^{-\Delta\Delta Ct}$ method with CT values from non-lesional skin as calibrator.

2.2.10.4 RNA sequencing

RNA isolation from biopsy was performed as described above. Subsequently, using the RNA 6000 Nano Kit (Agilent Technologies) and the 2100 Bioanalyzer (Agilent Technologies) according to the manufacturer's protocol RNA amount and quality were determined. By performing a Smear Analysis from 200 to 10000 base pairs (bp) the percentage of RNA fragments > 200 nucleotides (called DV_{200} metric) was calculated from the Bioanalyzer traces. DV_{200} metric served as a quality marker for RNA. Finally, RNA libraries were prepared from 200 ng total RNA using a TrueSeq Stranded Total RNA Kit (Illumina) according to the manufacturer's protocol and sequenced in an Illumina HiSeq 4000 system (paired-end, 2x 150 bp, output each 40 million cluster).

2.2.11. Skin sample acquisition

After desinfection and having injected the local anaesthetic Xylonest 1 % with 1:200 000 adrenaline addition the skin sample was taken with a 6 mm biopsy punch. One piece of each biopsy was sent to routine histology to confirm the diagnosis, another one used for immunohistology later was kept in 10 % formalin until embedding in paraffin and one was stored in RNA later RNA Stabilization Reagent until isolation of RNA. The site of sample acquisition was sutured with one stitch. All patients had been informed about the procedure and possible risks and had given their written consent. In total we obtained lesional skin samples from 18 psoriasis patients, lesional skin from one patient with atopic eczema and non-lesional skin samples from four healthy volunteers.

2.2.12. Immunofluorescent staining

At first the biopsies were cut into 2.5 µm thick sections with a microtome and mounted on superfrost slides. The slides were incubated for 25 minutes at 65 °C for initial deparaffinization. The final deparaffinization was reached by immersing the slides in Roticlear twice for ten minutes respectively. To rehydrate the slides several immersion steps in a descending ethanol series were performed: Twice for five minutes in 100% 2-Propanol, once for five minutes in 96% ethanol, once for five minutes in 70% ethanol and last for five minutes in distilled water. Subsequently the sections were kept in boiling citrate buffer (ca. 96 °C) in a pressure cooker for seven minutes for demasking the antigen structures of the tissue. After a washing step with 1X Tris buffer, a 15-minute blocking step with 3 % hydrogen peroxide solution and another washing step with Tris buffer the sections were incubated for one hour with 10 % goat serum and 10 % donkey serum in antibody diluent. The choice of the host of the sera was dependent on the host of the primary antibodies in order to avoid unspecific bindings between primary antibody and tissue. The following incubation with the two primary antibodies (Anti-IgA (Zytomed) undiluted and anti-CD31 (Cell Marque) in a 1:50 dilution) was performed at room temperature for one hour and then overnight at 4 °C. Before and after the one-hour dark incubation at room temperature with the secondary antibodies (Alexa Fluor 488 goat anti-rabbit antibody (Life Technologies) in a 1:500 dilution and NorthernLights 557 donkey anti-mouse antibody (R&D Systems), also 1:500 diluted) the sections were rinsed with Tris buffer. After that the sections were immersed in 0.1 % Sudan Black solution for 20 minutes, washed with 0.02 % Tween 20 detergent in PBS and then immersed in distilled water twice for five minutes. For the last staining step the slides were incubated for two minutes in DAPI 1:1000 diluted in distilled water and then again immersed in distilled water for five minutes. Finally, the microscope slides were mounted with VectaShield mounting medium and cured by sealing with nail polish. Images were taken with an Olympus IX73 inverted microscope with DAPI in the blue channel, CD31 in the red and IgA in the green channel and analysed as described in chapter 2.2.14.2 Immunofluorescence.

2.2.13. Serum immunoglobulins and complement factors

Measurement of immunoglobulins and complement factors in the sera of patients was conducted with a Cobas 8000 Modular Analyzer (Roche, Basel, Switzerland) using the module c 502 as well as the appropriate commercially available Roche reagents.

2.2.14. Statistical analysis

2.2.14.1 Data from whole blood and serum samples

The data resulting from the FACS experiments as well as the results for immunoglobulins and complement factors were analysed with the software GraphPad Prism 7.00. To compare the three main study cohorts Kruskal Wallis test followed by Dunn's multiple comparison test was performed after having tested for Gaussian distribution, which was not the case. For comparison of the treatment-naive psoriasis patients with the treated psoriasis cohort, Mann Whitney test was used. By means of Spearman's rank correlation coefficient the collected parameters were checked for a correlation with disease activity measured as PASI. All values and graphs were expressed as means with standard error of the mean (SEM).

2.2.14.2 Immunofluorescence

To distinguish whether IgA in the skin was deposited in the tissue or in dermal vessels CD31 as endothelial marker was stained so that the image sections could be chosen by scanning for fluorescence in the red channel. In the respective image section the amount of IgA was evaluated by measuring the mean intensity in the green channel after normalization to unspecific background fluorescence. The IgA expression in psoriatic skin samples was compared to the one in eczematous skin lesions and healthy skin.

2.2.14.3 RNA experiments

cDNA synthesis and RT-PCR

A logistic regression model was used to specify the diagnosis in six clinically and/or histologically unclear patients. Based on their NOS2 and CCL27 expression on RNA level a probability of having psoriasis or eczema and therefore a clear diagnosis could be indicated and patients not having psoriasis were excluded from the psoriasis cohort. Further details about this classifier can be found in Garzorz-Stark et al. [2016].

RNA sequencing

Data analysis of RNA sequencing and network analysis were performed by Ritcha Batra using R software following the GATK protocol for variant calling [McKenna et al., 2010].
Using the STAR 2-pass method RNA sequencing reads were mapped to human reference genome HG38 [Dobin et al., 2013]. This method involves identifying splice junctions in first alignment and using junctions information for second alignment. Next, bases hanging over intronic regions were clipped using SplitNCigarReads and HaplotypeCaller was used to identify variants. Variants were annotated using the wANNOVAR tool (http://wannovar.wglab.org\). To build the common variable immunodeficiency network, the interactions of the 13 CVID associated genes from Genetics home reference, NIH (https://ghr.nlm.nih.gov/condition/common-variable-immune-deficiency#sourcesforpage) were extracted using STRING database.

3. Results

3.1. Analysis of humoral immune response

3.1.1. Analysis of serum parameters

The results of the immunoglobulin and complement factor group comparison are shown in Figure 4. In pso-

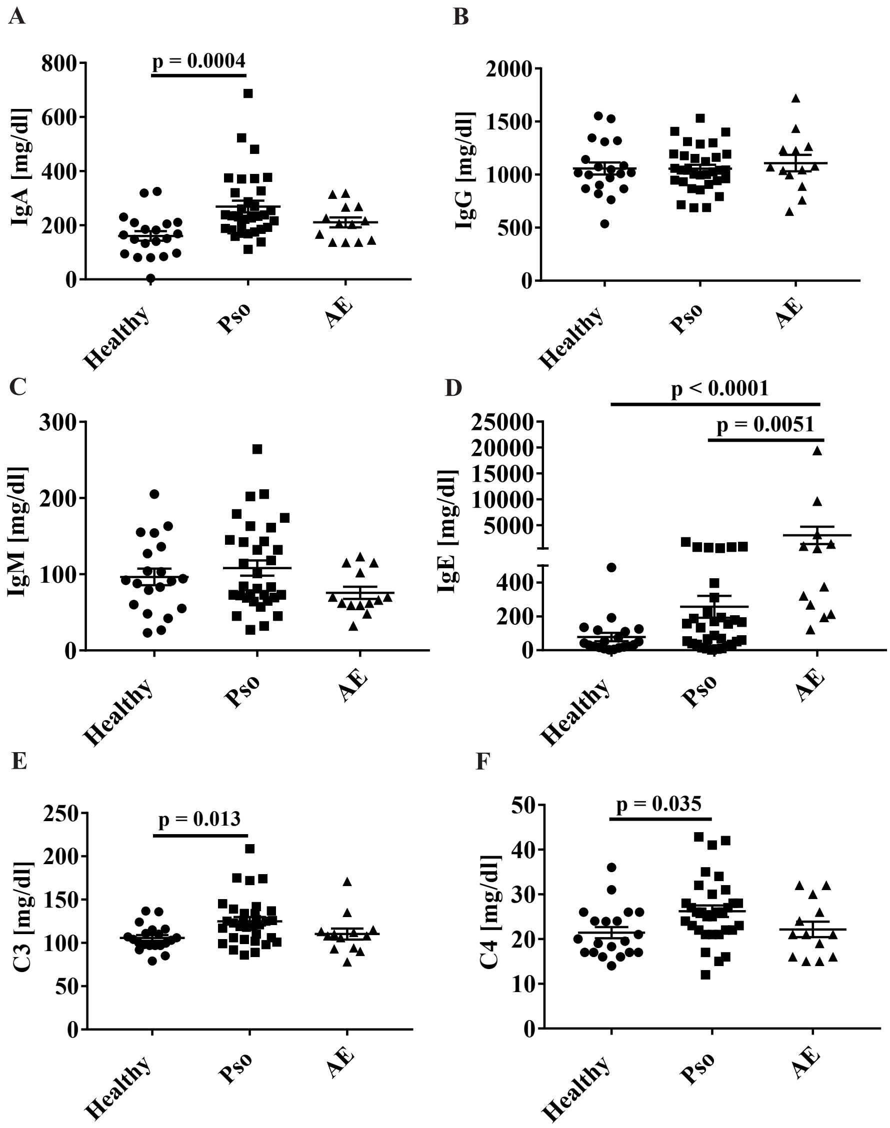

Figure 4 Group comparison of serum parameters: Significant differences in immunoglobulin and complement factor levels between the healthy controls and the psoriasis cohort were identified for IgA, C3 and C4. For IgG, IgM and IgE levels there were no statistical differences between these two cohorts. Bars show means with SEM. Statistical analysis by Kruskal Wallis test. Figure modified from Thomas and Kuepper et al. [2018].

riasis patients, IgA levels were significantly higher with a mean concentration of 269.2 milligramme/milliliter (mg/dl) compared to 160.5 mg/dl in healthy controls (p = 0.0004) (Figure 4A). Regarding IgG and IgM there were no significant differences between the three study cohorts. Psoriasis patients showed a mean

IgG level of 1056 mg/dl, healthy controls 1058 mg/dl and the AE cohort 1108 mg/dl. IgM analysis revealed a mean concentration of 108.2 mg/dl in psoriasis patients, 96.43 mg/dl in healthy volunteers and 75.62 mg/dl in AE patients. IgE levels were not significantly altered between healthy controls and the psoriasis cohort. As anticipated by the pathophysiology of atopic diseases, the mean IgE level in the AE patients was significantly higher than in the two other cohorts: Pso 256.8 mg/dl, Healthy: 78.39 mg/dl, AE: 3050 mg/dl (Healthy versus (vs) AE: $p < 0.0001$; Pso vs AE: $p = 0.0051$) (Figure 4D). In accordance with the IgA data, statistically significant differences in the complement factor levels between the healthy control group and the psoriasis cohort were also detected. The C3 levels ($p = 0.013$) (Figure 4) as well as the C4 levels ($p = 0.035$) (Figure 4F) were higher in the psoriasis patients (Pso: 125 mg/dl for C3, 26.21 for C4; Healthy: 105.7 mg/dl for C3, 21.44 mg/dl for C4).
Subsequently, all serum parameters were checked for a possible correlation with disease severity. Here, a positive correlation between IgA and PASI in the psoriasis patients was identified ($r = 0.40$ [95% CI: 0.05 to 0.66], $p = 0.02$) (Figure 5A), while disease severity score in AE patients (SCORAD) did not correlate with IgA levels ($r = 0.15$ [95% CI: -0.45 to 0.66], $p = 0.62$) (Figure 5G). IgA was exclusively positively correlated with disease severity, while the remaining humoral factors (Figure 5B-F) did not show a significant correlation with PASI (IgG: $r = 0.03$ [95% CI: -0.34 to 0.38], $p = 0.89$; IgM: $r = 0.17$ [95% CI: -0.2 to 0.5], $p = 0.36$; IgE: $r = 0.23$ [95% CI: -0.14 to 0.54], $p = 0.21$; C3: $r = -0.098$ [95% CI: -0.44 to 0.27], $p = 0.59$; C4: $r = -0.14$ [95% CI: -0.48 to 0.23], $p = 0.44$).

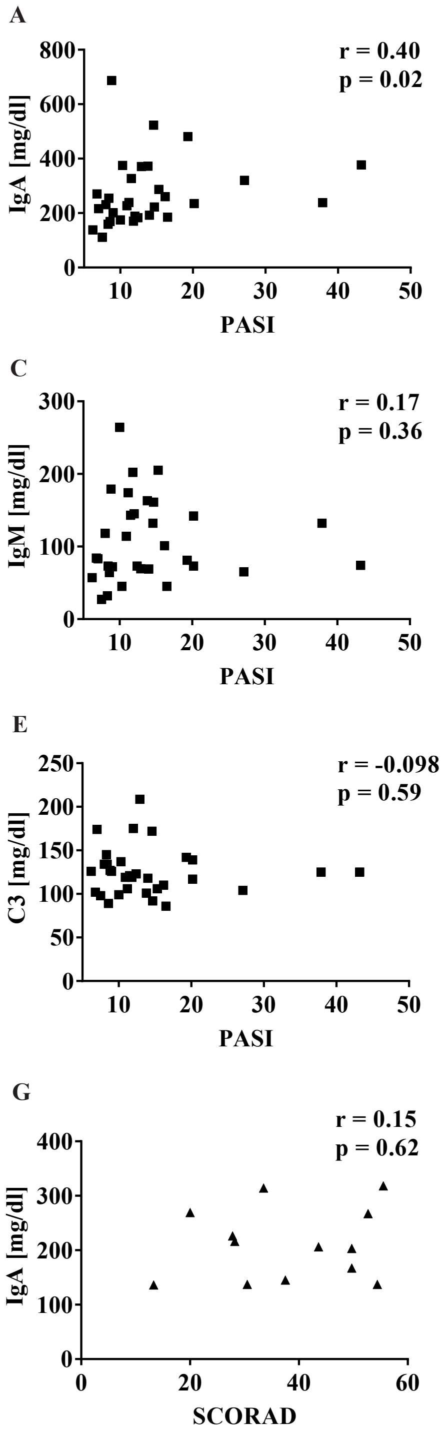

Figure 5 Correlation analysis of serum parameters and disease severity score: A positive correlation between IgA and PASI but not with SCORAD was detected. For the other humoral factors - IgG, IgM, IgE, complement factors C3 and C4 - there was no significant correlation with PASI. Statistical analysis by Spearman's rank correlation coefficient. Figure modified from Thomas and Kuepper et al. [2018].

3.1.2. Deposits of IgA in immunohistology stainings

Following the detection of increased IgA levels in the serum of psoriasis patients aim was to validate these results on skin level. Therefore immunofluorescent stainings of IgA (green), CD31 as endothelial marker (red) and DAPI as nuclear counterstaining (blue) were performed in skin samples of psoriasis patients (n=18), AE patients (n=1) and in healthy skin (n=4) (Figure 6). There was no IgA detected in the tissue but intravascular deposits of IgA in dermal and subcutaneous blood vessels. In accordance to the serum, IgA levels were higher in psoriasis patients compared to the two other cohorts.

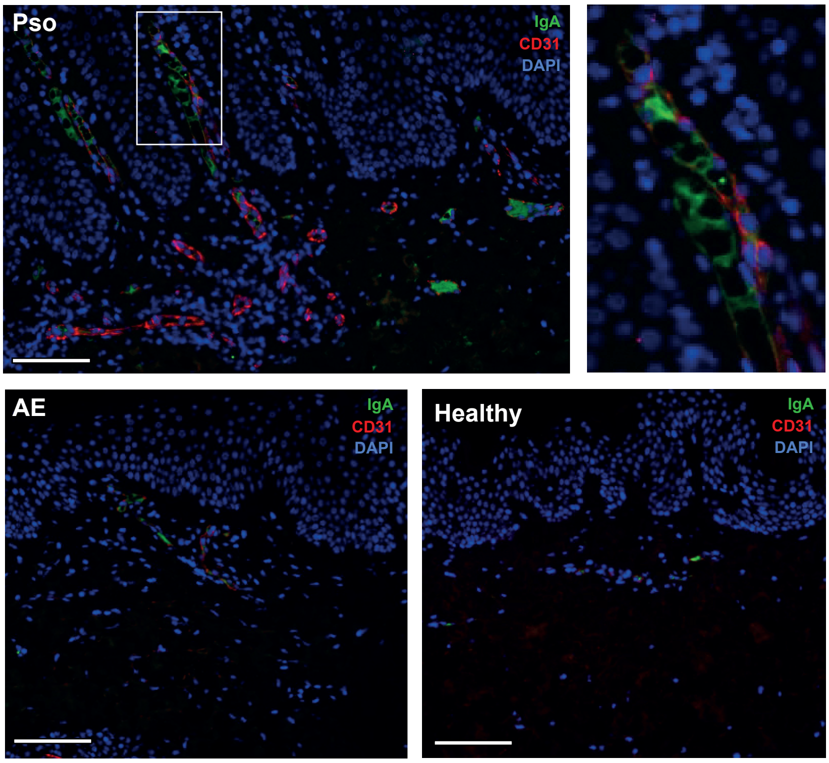

Figure 6 Cutaneous IgA deposits: IgA is shown in green, CD31 in red and DAPI nuclear staining in blue. IgA deposits were higher in psoriasis (n=18) compared to healthy skin (n=4) and skin from AE patients (n=1). IgA was exclusively detected intravascular. Figure modified from Thomas and Kuepper et al. [2018].

3.2. FACS analysis of B cell subpopulations

3.2.1. Traditional CD24/CD38 gating

The observation of increased IgA levels as product of B cells in psoriasis patients led to the analysis of different B cell subpopulations in the three study cohorts (Figure 7). In none of the five subpopulations - CD24+CD38- primarily memory B cells, CD24 intermediate CD38 intermediate mature naive B cells, CD24+CD38+ transitional/regulatory B cells, CD24- CD38+ plasmablasts/plasma cells and CD24-CD38- new memory B cells - a significant difference between healthy controls and psoriasis patients were detected. Since plasma cells are by definition producers of immunoglobulins including IgA [Gommerman et al., 2014], this subpopulation was analysed in more detail in the different cohorts. CD138 served as plasma cell marker defining the CD19+CD24-CD38+CD138+ plasma cell population. Although frequencies of plasma cells were not altered between the different groups (Figure 7B), a significantly positive correlation between plasma cells and IgA (r = 0.41 [95% CI: 0.06 to 0.67], p = 0.02) as well as between plasma cells and PASI (r = 0.39 [95% CI: 0.05 to 0.64], p = 0.02) was found (Figure 7D).

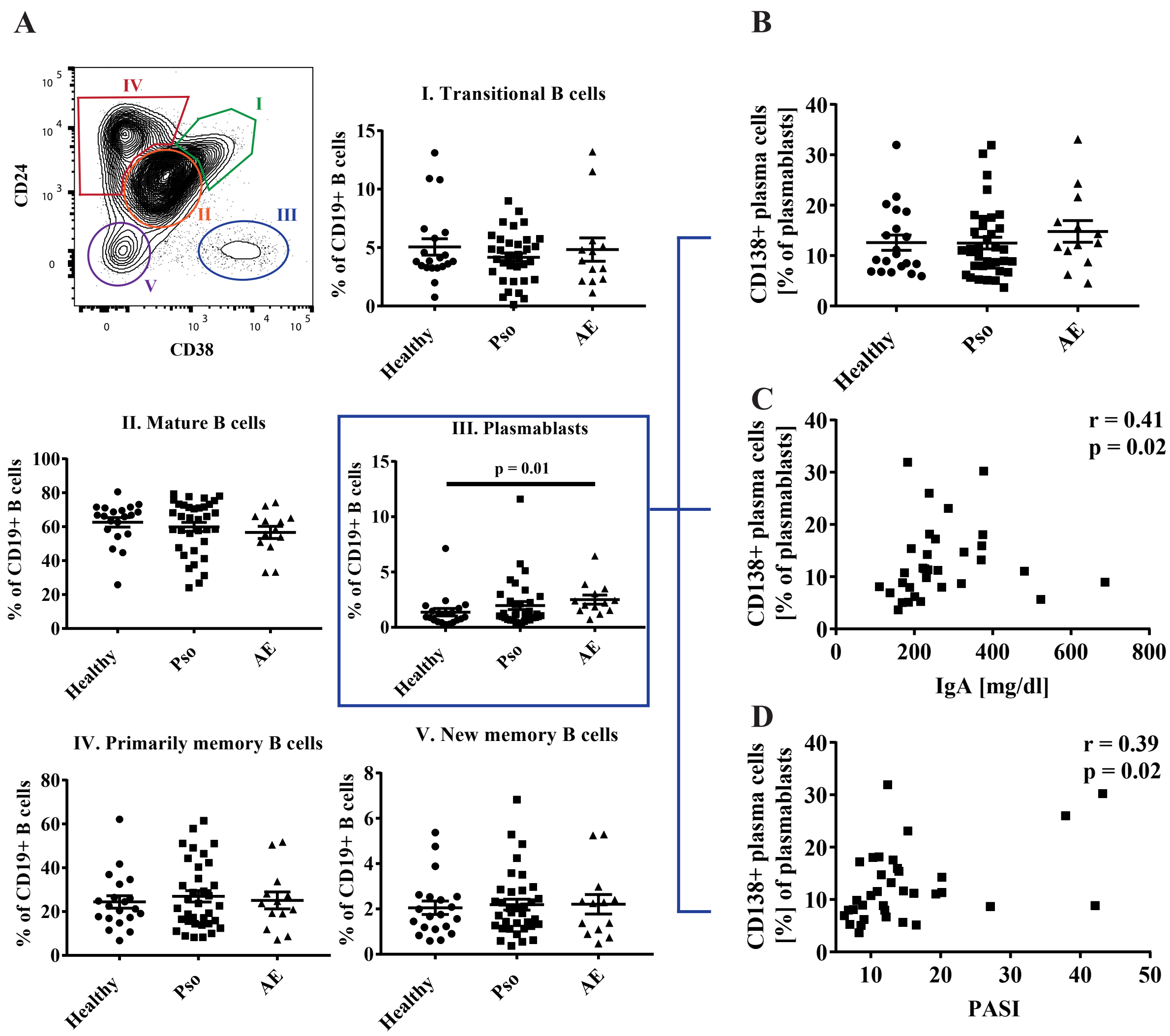

Figure 7 Group comparison of B cell subpopulations: No differences between healthy controls and psoriasis patients were detected in the group analysis for the five B cell subpopulations as well as for an additional plasma cell subpopulation. Of note there was a positive correlation between plasma cells and IgA or rather PASI. Statistical analysis by Kruskal Wallis test **(A, B)** and Spearman's rank correlation coefficient **(C, D)**. Figure modified from Thomas and Kuepper et al. [2018].

3.2.2. IgA+ plasma cells

Since positive correlation of plasma cells with IgA as well as positive correlation of plasma cells with PASI was observed, the frequency of plasma cells with membrane bound IgA was analysed concerning their correlation with PASI in an additional cohort of seven psoriasis patients. The gating strategy is shown in Figure 8A. Plasma cells defined as CD19+CD24-CD38+CD138+ cells (see chapter 3.2), were analysed for their IgA surface expression. Backgating of CD20 and IgA expression showed that CD20-IgA+ B cells corresponded to the population of plasmablasts and plasma cells (each shown in green) whereas CD20+IgA+ B cells represented the primarily memory B cells (each shown in orange). Investigating the relationship between the levels of IgA+ plasma cells and disease severity, correlation analysis revealed a significant positive correlation between IgA+ plasma cells and PASI ($r = 0.883$, $p = 0.015$) (Figure 8B).

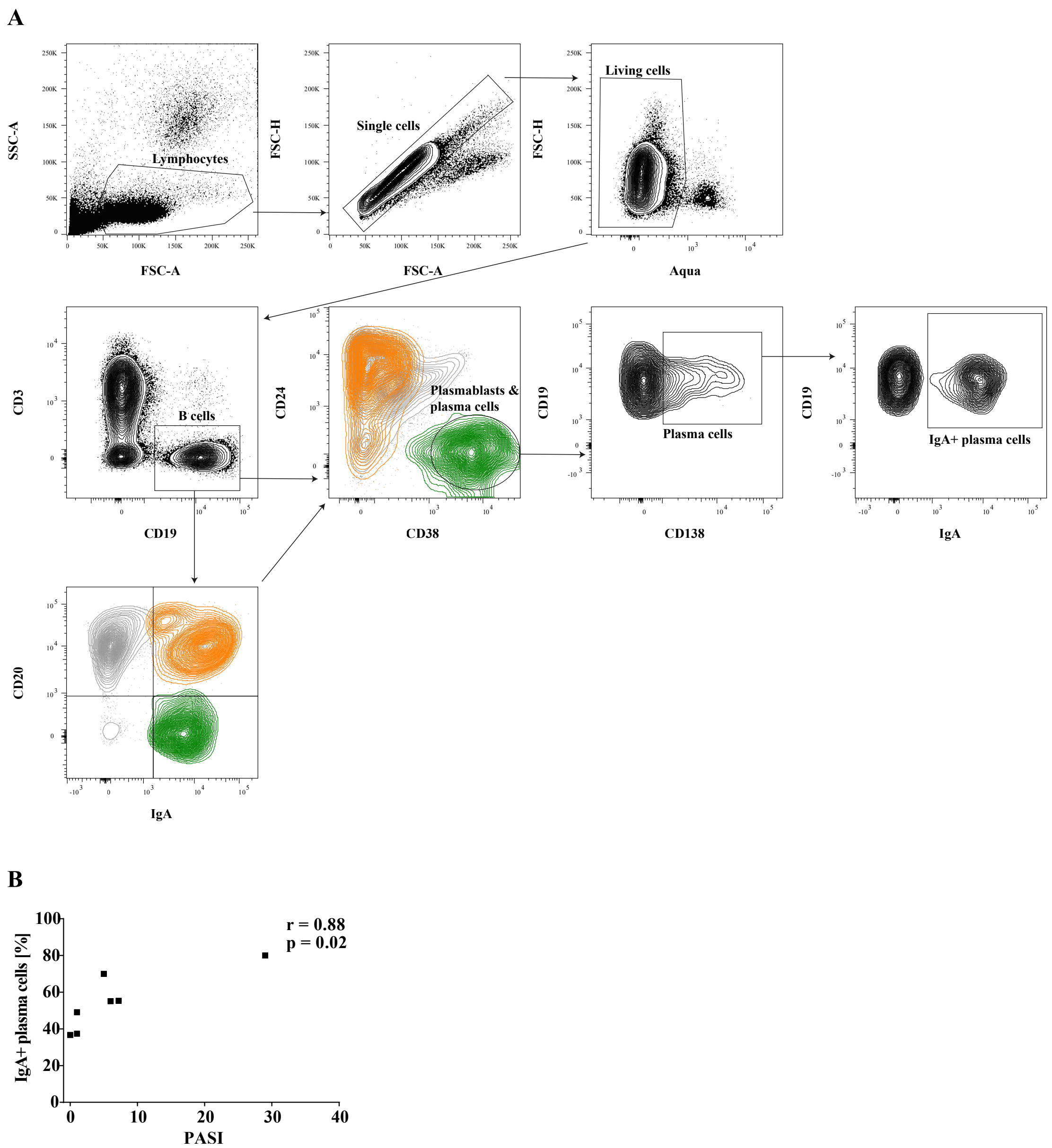

Figure 8 IgA surface staining: Gating strategy of the flow cytometric detection of lymphocytic IgA surface expression consisted of the following steps: After gating on lymphocytes and exclusion of doublets and dead cells, the well-known depiction of CD19 positive B cells according to their CD24 and CD38 expression was used. Backgating on CD20 and IgA expression revealed that CD20-IgA+ B cells (orange) were mainly to be found in the plasmablast and plasma cell population whereas the CD20+IgA+ B cells (green) were above all present in the population of primarily memory B cells. Inside the CD24-CD38+ population of plasmablasts and plasma cells, plasma cells were determined by gating on the CD19+CD138+ subpopulation as already known from Figure 7B. As a last step, IgA+ plasma cells were selected by gating on the CD19+IgA+ subpopulation. **(A)**. There was a strong correlation between IgA+ plasma cells and PASI (n=7). Statistical analysis by Spearman's rank correlation coefficient **(B)**. Figure modified from Thomas and Kuepper et al. [2018].

3.2.3. Intracellular IL-10 staining

Since IL-10 producing regulatory B cells are described to inhibit Th17 and Th1 differentiation [Blair et al., 2010, Flores-Borja et al., 2013] - key players in psoriasis - a decreased number of regulatory B cells (Bregs) could be associated with the immune pathogenesis of psoriasis. Due to the inconsistent literature concerning the characterization of human regulatory B cells, flow cytometric experiments to detect Bregs were performed analysing blood from two healthy volunteers. This approach was considered to investigate whether regulatory B cells defined as IL-10+ corresponded to the subpopulation of CD24highCD38high B cells as described by Blair et al. [2010] or are rather scattered throughout all B cell subpopulations [Kristensen et al., 2015]. The intracellular cytokine staining after B cell surface panel staining revealed low frequencies of IL-10+ B as well as T lymphocytes (Figure 9A and B). Regarding the distribution pattern of IL-10+ B cells, IL-10 production could not be attributed to one specific B cell subset but was rather produced by all subsets predominantly appearing in the subpopulation of memory B cells, mature naive B cells and transitional B cells (Figure 9C: B cells (CD19+) depicted in blue, regulatory B cells (CD19+IL10+) in grey). In particular, they were not limited to the CD24hiCD38hi subpopulation.

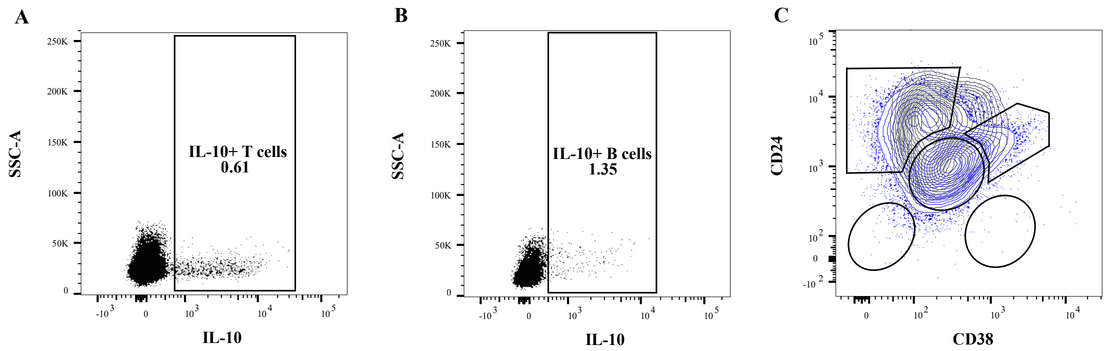

Figure 9 Intracellular IL-10 staining: Intracellular staining of IL-10 showed only small amounts of IL-10+ T (A) and B cells (B). Based on the CD24/CD38 gating strategy, IL-10+ B cells revealed a scattered distribution pattern over all B cell subpopulations (blue = CD19+ cells, grey = CD19+IL10+ cells) (C).

3.3. Investigation of a patient suffering simultaneously from common variable immunodeficiency and psoriasis

To answer the question whether B cells play a critical role in psoriasis pathogenesis, knockout (KO) models are a useful tool. In the human system genetic deficiency can mimic such KO models. Here, a patient (A.B.) suffering from common variable immunodeficiency (CVID) - a heterogenous disease with frequent occurence of infections due to lacking B cells and immunoglobulins - simultaneously showed a psoriatic phenotype, which offered a rare opportunity to study psoriasis under B cell deficient conditions. The coincidence of the two diseases in one patient might give a chance to answer the question whether proper B cells and their related cytokines are necessary or rather minor for the development of psoriasis.

Patient A.B., suffering from CVID since birth, developed psoriasis for the first time at the age of 30 years. Psoriasis could be clearly diagnosed both clinically (Figure 10A) and histologically (Figure 10B): A.B.'s skin was covered all over the body with sharply demarcated scaly plaques on erythematous background. Histology showed acanthosis with elongated rete ridges, lymphocytic infiltration and Munro's micro-abscesses. PASI on admission was 30 and family history for psoriasis positive.

First, RNA sequencing of lesional skin and analysis of genetic variants was performed for further description of A.B.'s genetic defect. The network analysis revealed 6 synonymous and 1 nonsynonymous variants in NFKB1, NFKB2 and PRKCD, which represent three of the 13 (according to OMIM database [Hamosh and Kniffin, 2003]) described CVID genes and 1 nonsynonymous variant in the ligand of the TNFRSF13B receptor called TNFSF13 (Figure 10C).

To exclude functional impairment of PBMCs and T cells of A.B. in the course of CVID, cell activation

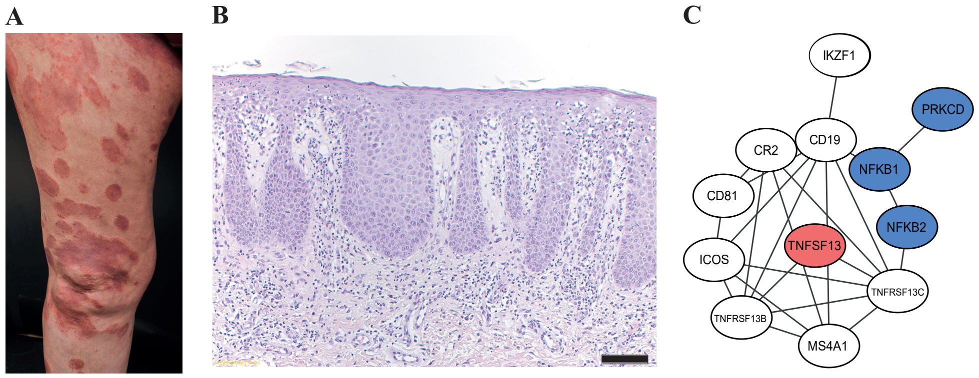

Figure 10 Characterization of index patient A.B.: Clinical (**A**) and histological (**B**) images of A.B. showed a clear psoriasis phenotype. RNA sequencing of lesional skin from A.B. and analysis of genetic variants revealed 6 synonymous and 1 nonsynonymous variants in three (NFKB1, NFKB2 and PRKCD, depicted in blue) of the 13 described CVID genes and 1 nonsynonymous variant in TNFSF13 (red), the ligand of the TNFRSF13B receptor (**C**). Figure adapted from Thomas and Kuepper et al. [2018].

assays were performed. PBMCs stimulated with anti-CD3 plus anti-CD28, Phytohemagglutinin (PHA) as well as pertussis toxin showed regular proliferation measured by radioactive thymidine incorporation (Figure 11A). T cell function was measured by release of the cytokines IL-4, IL-22, TNF-α, IFN-γ and IL-17 after stimulation of lesional skin T cells and showed comparable cytokine secretion to a cohort of eleven randomly chosen psoriasis patients from the untreated cohort. In particular, TNF-α, IFN-γ and IL-17 were most abundant. Thus not only proper T cell function of A.B. was shown but also a clear Th17 profile typical for psoriasis (Figure 11B).

Furthermore, investigating the B cell and humoral profile, A.B. showed nearly a complete lack of B cells

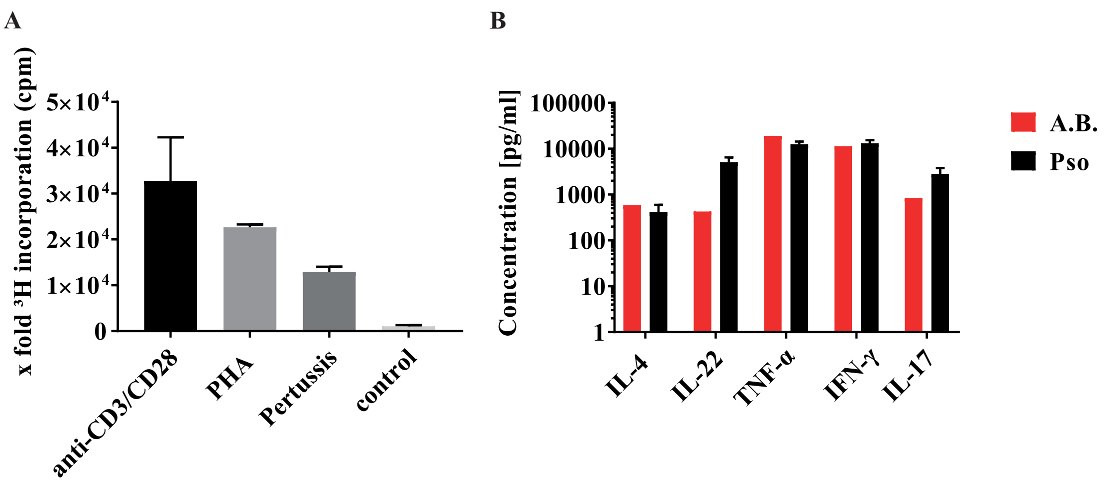

Figure 11 PBMC and T cell functionality of patient A.B.: PBMCs of patient A.B. showed substantial proliferation after stimulation with anti-CD3 and anti-CD28, PHA and pertussis toxin as assessed by ³H-Thymidine incorporation (**A**). Stimulated T cells from lesional skin of patient A.B. released comparable amounts of TNF-α, IFN-γ and IL-17 as eleven psoriasis patients from the untreated cohort indicating a psoriasis-typical Th17 profile (**B**). Figure adapted from Thomas and Kuepper et al. [2018].

and immunoglobulins as expected by the CVID pathogenesis: Frequency of CD19+ B cells in A.B. was 2.32% of lymphocytes in comparison to 9.4 $\pm$ 0.8% among healthy controls and 11.1 $\pm$ 0.7% for psoriasis patients (Figure 12B). IgA levels were plainly reduced with 57.0 mg/dl compared to healthy controls (160.5 $\pm$ 17.6 mg/dl) and psoriasis patients (269.2 $\pm$ 21.6 mg/dl) (Figure 12A). Investigation of B cell

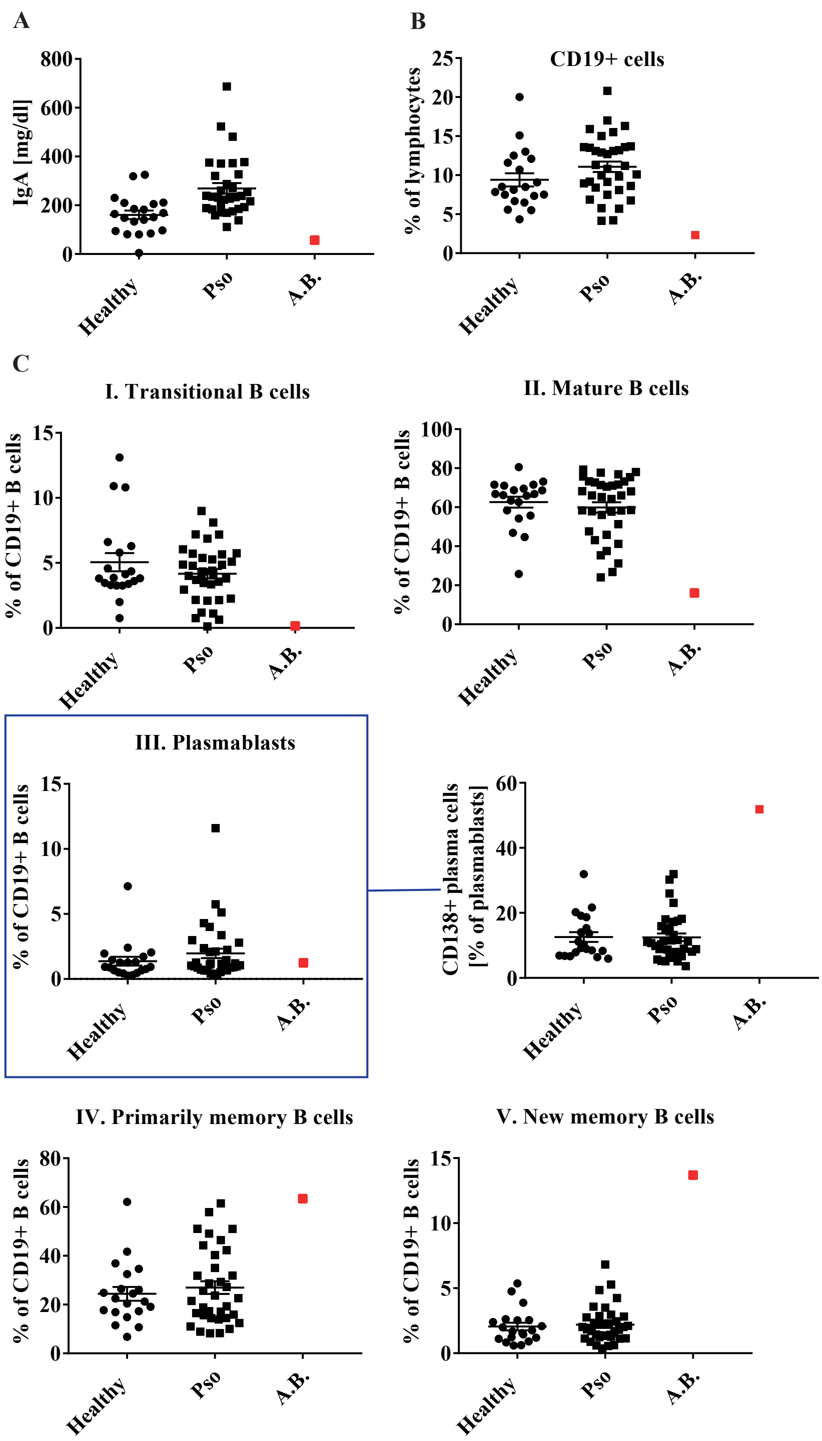

Figure 12 Comparison of serum and FACS data with patient A.B.: Resulting from the clearly reduced B cell levels (2.32% of lymphocytes, **B**), patient A.B. presented virtually no IgA (57 mg/dl, **A**). B cell subpopulation analysis showed lower percentages of transitional and mature B cells as well as plasmablasts and in return higher percentages of primarily memory and new memory B cells (**C**). CD138+ plasma cells were elevated as well in A.B. Figure adapted from Thomas and Kuepper et al. [2018].

subsets (Figure 12C) showed relatively decreased percentages of transitional B cells (0.16%), mature B cells (16.0%) and plasmablasts (1.24%). Frequencies of CD138+ plasma cells (51.9%), primarily memory B cells (63.4%) and new memory B cells (13.7%) were increased.

3.4. Comparison with systemically treated psoriasis patients under disease-control

Another way to evaluate the relevance of the findings in the cohort of untreated psoriasis patients was to compare them with data from succesfully treated patients. These included 28 patients with low disease scores of PASI < 5 under systemic immunosuppressive medication. In the serum analysis, no significant changes concerning IgA levels between treatment-naive and treated psoriasis patients were observed (Figure 13A): Mean IgA level in the untreated patients was 269.2 $\pm$ 21.6 mg/dl, in the treated cohort 260.1 $\pm$ 23.6 mg/dl ($p = 0.66$). Also the comparison of the five B cell subpopulations did not reveal differences in cellular frequencies despite a significant drop of plasmablasts in the cohort of treated psoriasis patients (Pso untreated: 2.69%; Pso treated: 1.3%; $p = 0.02$; Figure 13B). However, frequencies of CD138+ plasma cells did not differ significantly between the two cohorts ($p = 0.14$; Figure 13B). Correlation analysis revealed no significant correlation between CD138+ plasma cells and IgA or PASI in the cohort of treated psoriasis patients (Figure 14). These values had been positively correlated in the treatment-naive psoriasis patients.

3.5. Analysis of CD138 expression pattern

CD138 - known as plasma cell marker - was brought more into focus after having analysed its expression on the five B cell subpopulations. Compared with healthy controls and AE patients the percentage of CD138+ B cells in all subpopulations except for the plasmablasts was higher in psoriasis patients (Figure 15), even if not significantly. This finding led to investigations of CD138 expression on further cell populations to check whether it could serve as a potential biomarker for psoriasis. First, the CD138 expression on total B cells was analysed, independently of any subpopulation. The group comparison showed higher frequencies of CD138+ B cells within psoriasis patients. Although these differences did not reach statistical significance (Figure 16A), correlation analysis revealed a positive correlation between all CD138+ B cells and disease severity in the psoriasis cohort ($r = 0.52$ [95% CI: 0.21 to 0.72], $p = 0.002$) but not in the AE cohort ($r = -0.02$ [95% CI: -0.58 to 0.55], $p = 0.94$) (Figure 16B). In order to test how dominant this effect was, CD138 expression was analysed without further gating on the whole lymphocyte population (Figure 17). The mean frequency of CD138+ cells was highest in the psoriasis patients with statistical significance for the comparison with the AE cohort (Pso vs AE: $p = 0.01$) (Figure 17A). Moreover, the percentage of CD138+ lymphocytes and disease severity correlated significantly in the psoriasis cohort ($r = 0.50$ [95% CI: 0.19 to 0.72], $p = 0.003$) but not in the AE cohort ($r = -0.10$ [95% CI: -0.63 to 0.49], $p = 0.75$) (Figure 17B).

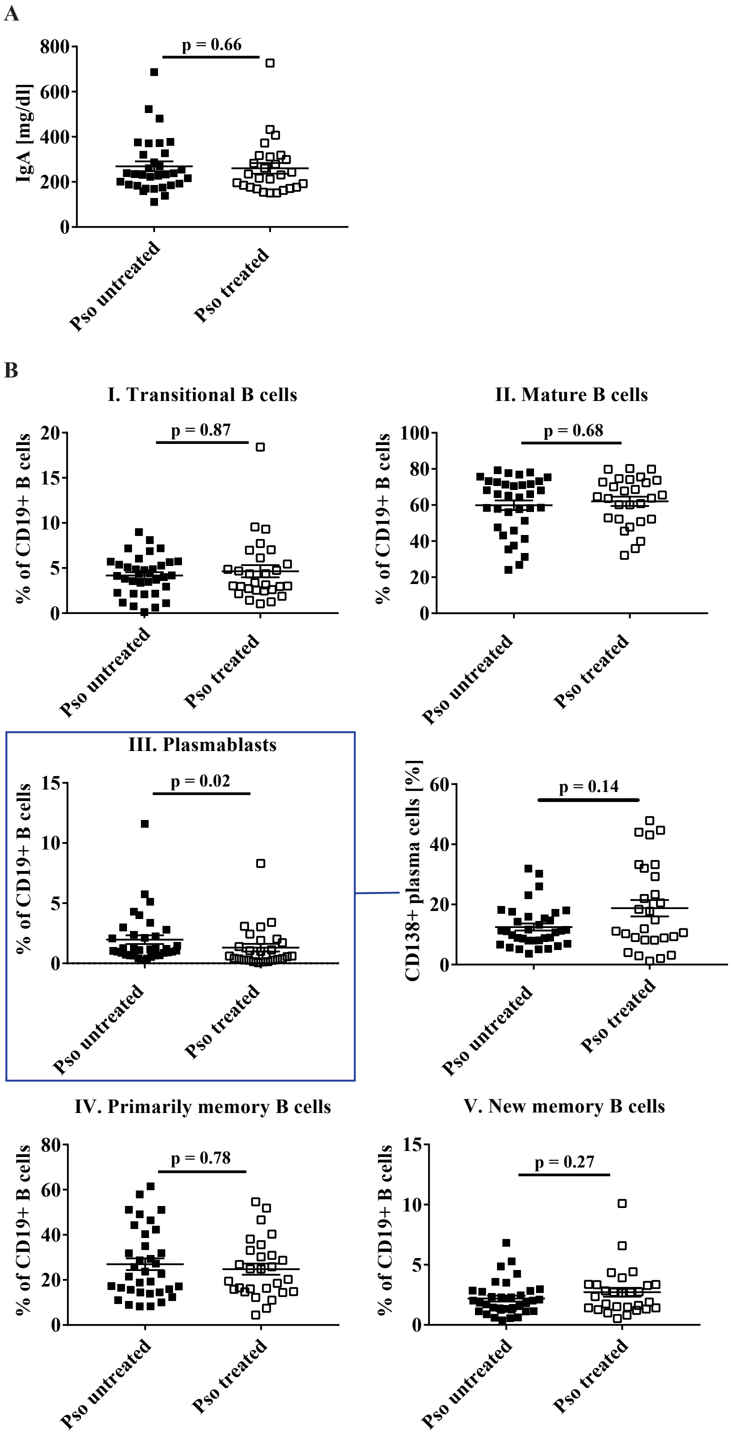

Figure 13 Group comparison of previous findings with systemically treated patients with minimal disease activity: Comparing IgA levels and frequencies of B cell subpopulations from the initial 35 treatment-naive psoriasis patients with 28 well-treated psoriasis patients (PASI < 5) under systemic immunosuppressive therapy shows comparable levels of IgA as well as comparable frequencies of transitional B cells, mature B cells, primarily memory B cells and new memory B cells. In the plasmablast subpopulation, there is a significantly lower frequency of cells in the treated cohort (p = 0.02). Statistical analysis by Mann Whitney test. Figure adapted from Thomas and Kuepper et al. [2018].

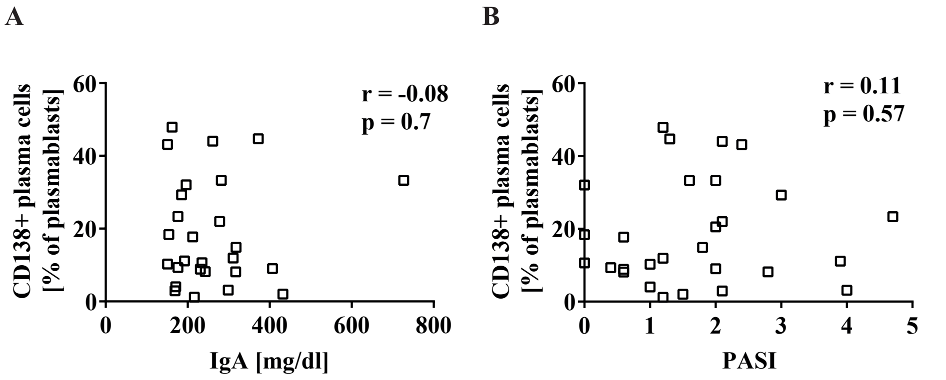

Figure 14 Correlation analysis of the cohort of treated psoriasis patients: Among the treated psoriasis patients no correlation between CD138+ plasma cells and IgA (**A**) or between CD138+ plasma cells and PASI (**B**) was observed. Statistical analysis by Spearman's rank correlation coefficient. Figure adapted from Thomas and Kuepper et al. [2018].

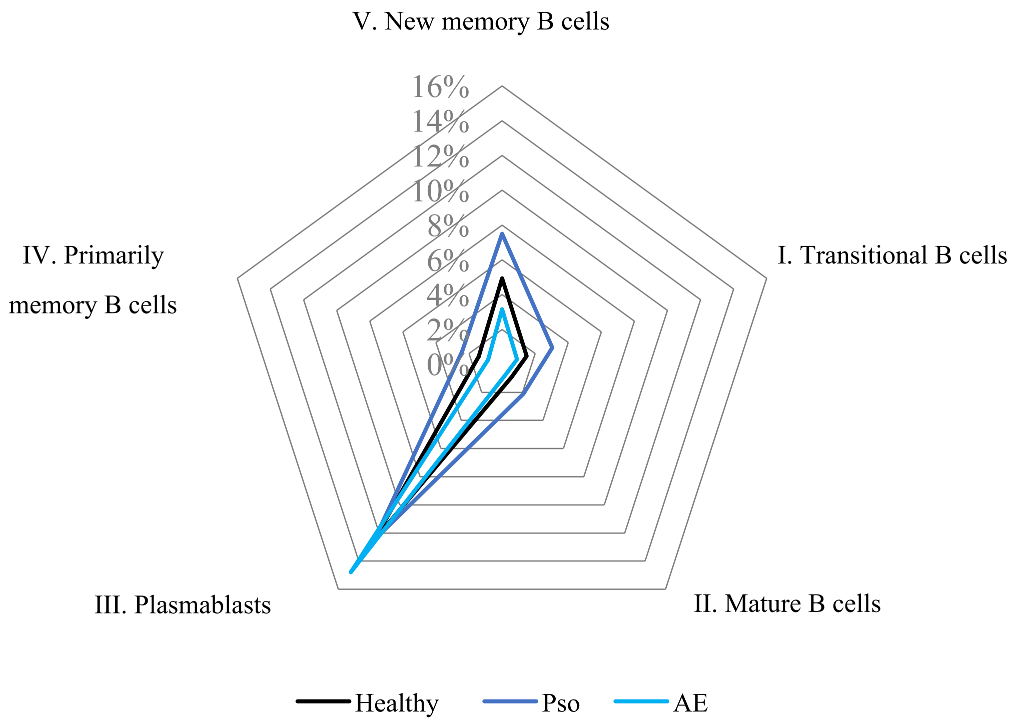

Figure 15 CD138 expression on B cell subpopulations: Percentage in radar plot signified that psoriasis patients showed the highest percentages of CD138+ B cells in all of the five subpopulations except for plasmablasts.

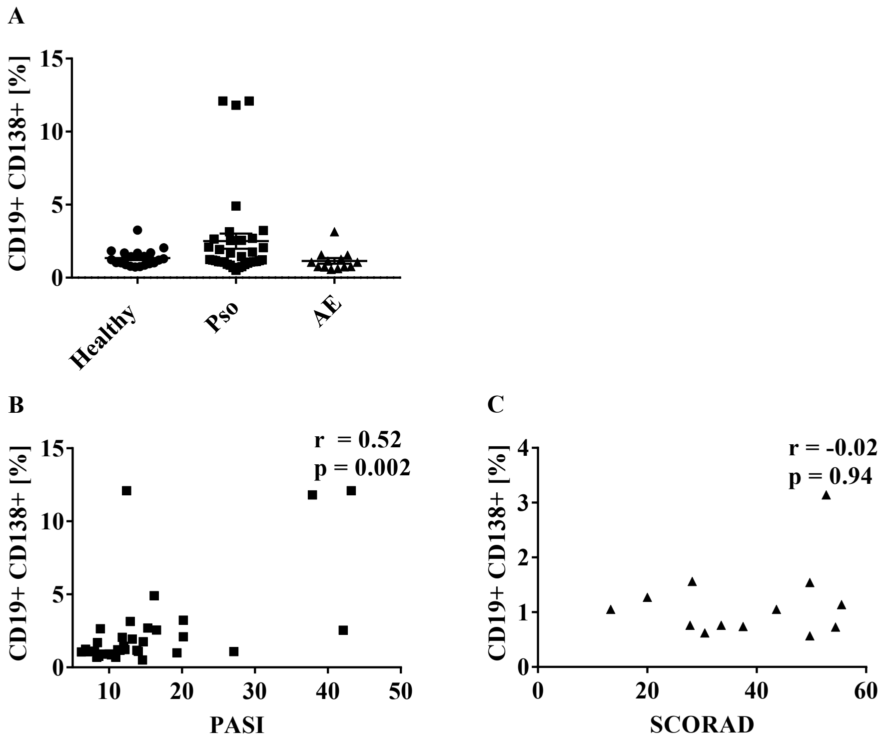

Figure 16 CD138 expression on total B cells: The psoriasis patients showed the highest proportion of CD138+ B cells as well as a positive correlation with disease severity. In the AE patients a positive correlation was not given. Statistical analysis by Kruskal Wallis test **(A)** and Spearman's rank correlation coefficient (**B, C**).

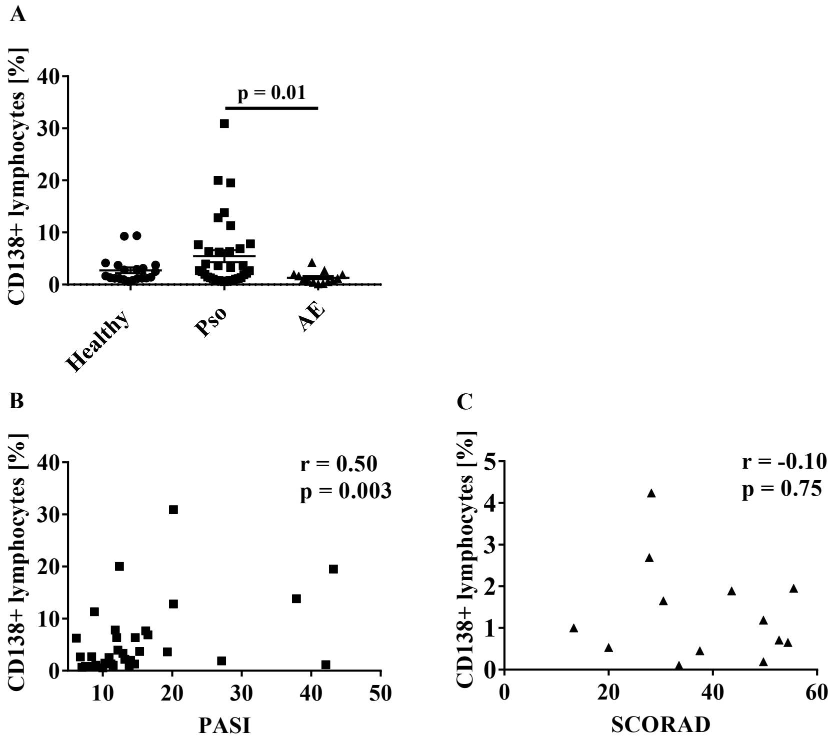

Figure 17 CD138 expression on total lymphocytes: The percentage of CD138+ cells of all lymphocytes was highest in the psoriasis cohort where it was in contrast to the AE cohort positively correlated with disease severity. Statistical analysis by Kruskal Wallis test **(A)** and Spearman's rank correlation coefficient **(B, C)**.

3.6. Prospective analysis of CD138 before and after systemic treatment

Ideally a new marker cannot only indicate disease severity but also predict a therapeutic outcome for a certain medication. Therefore, some patients from the untreated cohort were reanalysed concerning CD138 expression after systemic treatment for several weeks. The largest group of patients with the same therapy were four patients receiving anti-IL-17 therapy. In vitro comparison of CD138 expression on all lymphocytes before and after systemic treatment revealed a trend in decreasing CD138 levels in the course of therapy (Wilcoxon test: $p = 0.13$) (Figure 18).

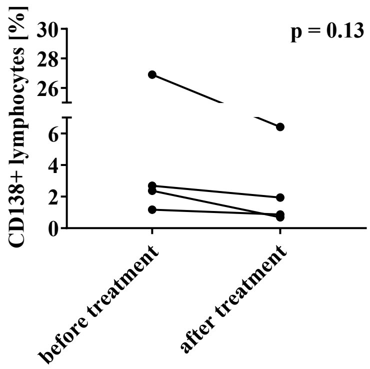

Figure 18 CD138 reduction in four patients with anti-IL-17 therapy: Levels of CD138+ lymphocytes drop in patients after anti-IL-17 treatment compared to levels before therapy (n=4). Statistical analysis by Wilcoxon test.

4. Discussion

4.1. Summary of aims and results

Psoriasis is a worldwide common T-cell mediated autoimmune disease affecting skin, nails and joints. Its physical and psychosocial impact on patients' life is huge and the impairment of quality of life is comparable to other major chronic diseases. Although there have been case reports for decades about new onset of psoriasis in patients receiving B-cell depleting therapies the meaning of the humoral immune response for the pathogenesis of psoriasis remained unclear. Therefore, aim of this thesis was to give new insights whether B cells do play a role for psoriasis and in more detail identify distinct B cell subsets that could modify the immunological pathways leading to psoriatic eruptions. Compared to healthy voluntary control subjects, psoriasis patients showed significantly higher complement factor and IgA levels whereas other immunoglobulins did not differ from those in the healthy controls. Increased IgA levels could be confirmed in terms of IgA deposits in psoriatic skin samples. Although, frequencies of the five commonly described B cell subsets of transitional B cells, mature B cells, plasmablasts, primarily memory B cells and new memory B cells as well as additionally analysed CD138+ plasma cells were not altered in peripheral blood compared to the healthy control cohort, a correlation analysis revealed a significant positive correlation between plasma cells and IgA as well as between plasma cells and disease severity in psoriasis patients. Moreover, IgA+ plasma cells were shown to strongly correlate with PASI. Comparing these data to a second psoriasis cohort with disease-controlling systemically treated patients neither a drop of IgA levels nor major alterations in frequencies of B cell subsets were observed. Studying a patient concurrently suffering from CVID and psoriasis proved that psoriasis can develop in the absence of B cells and immunoglobulins, while a protective role of e.g. Bregs could not be excluded. An in-depth investigation of CD138 - also called Syndecan-1 - showed a stable positive correlation of PASI and all CD138+ B cells as well as all CD138+ lymphocytes. Additionally, in four psoriasis patients with anti-IL-17 treatment decreased levels of CD138+ lymphocytes were measured after therapy.

In search of the relatively newly discovered regulatory B cell subsets the approach to detect IL10 producing B cells could neither show one specific subset as the main pool of IL10+ cells nor measure a relevant amount of IL-10+ B cells at all.

All the significant differences were double-checked in atopic eczema patients to show that the findings are specific for psoriasis and not an epiphenomena of inflammatory processes in general.

In summary, these results point to the fact that the humoral immune system is not generally involved in the pathogenesis of psoriasis. However, factors of the humoral immune system may be used as descriptive biomarkers for disease severity.

4.2. Discussion of cohorts

The three main study cohorts consisted of 35 psoriasis patients, 20 healthy controls and 13 patients with atopic eczema. There were no significant differences in age or sex between the cohorts which means that differences to be seen are unlikely due to different baseline characteristics. In the statistical analysis all age-correlated parameters were excluded to avoid confounding effects and mistakenly interpret data as positively correlated.

Critical point of the analysis was the heterogeneity among the psoriasis patients. Despite defining a period of minimum six weeks without any systemic treatment as criterion for inclusion some patients still seemed to vary widely in B cell levels. This might be due to other comorbidities and even subclinical contact to pathogens requiring antibody response but, probably to the most part, it's due to a kind of intrinsic large variability present in chronic inflammatory skin diseases.

4.3. Characterization of humoral and cellular phenotypes of B cell biology

4.3.1. Are immunoglobulins relevant for psoriasis development?

In this study, investigation of the humoral immune response (Figures 4 and 5) showed significantly higher IgA levels in psoriasis patients compared to healthy controls which were moreover positively correlated with disease severity indicated as PASI. C3 and C4 levels were also significantly increased in the psoriasis cohort. Levels of IgG, IgM and IgE did not differ between psoriasis patients and healthy volunteers. Additionally, correlation between IgA levels and disease severity score could not be confirmed in the AE patients. Interestingly, increased levels of IgA in psoriasis patients were observed on skin level as well: In dermal vessels, the amount of IgA deposits was higher in psoriasis patients compared to healthy controls and AE patients (Figure 6). In summary, psoriasis patients show a characteristic, yet unspecific humoral profile with elevated IgA, C3 and C4 serum levels. These results are in line with other studies about psoriasis, but literature is quite contradictory at that point.

Fraser et al. measured immunoglobulin levels in 27 psoriasis patients and detected a significant elevation of IgA and a significant decrease of IgM levels compared to 47 healthy controls. This pattern was very similar to what they observed in dermatitis herpetiformis [Fraser et al., 1969]. Lai a Fat and van Furth also measured serum immunoglobulin levels in various skin diseases and detected a significant IgG elevation in 15 psoriasis patients compared to 156 healthy donors but no IgA elevation. Because of the small amount of psoriasis patients, they themselves advise to handle their results with care [Lai and van Furth, 1974]. Guilhou et al. compared serum immunoglobulin levels between 60 psoriasis patients and 300 controls which revealed a significant IgA and IgG elevation in the psoriasis group [Guilhou et al., 1976]. Fattah et al. showed an IgA and IgG elevation in 35 psoriasis patients as well [Fattah et al., 1986]. The different results are possibly due to different measurement methods and maybe also due to differing criteria how to include patients.

What has been a relatively stable result over the decades and emerged also in this study (Figure 4A) is the IgA elevation in psoriasis patients' sera. This could represent either a general phenomena occuring in various inflammatory conditions or a specific contribution of distinct humoral factors to the pathogenesis of psoriasis. The fact that in the AE patients of this study neither increased IgA levels (Figure 4A) nor a correlation of IgA with disease severity (Figure 5G) became evident contradicts a general inflammatory epiphenomena. On the other hand, IgA deposits were found exclusively in dermal vessels and not in dermal or epidermal tissue. This could rather indicate a non-specific role of IgA. Looking at dermatitis herpetiformis where IgA deposits exhibit a clear pathophysiological role, IgA is located at the dermal-epidermal junction and not only intravascular [Hall and Lawley, 1985]. IgA deposits in vessels however can also be found in the apparently healthy skin from patients with Henoch-Schönlein purpura, IgA nephropathy, alcoholic liver disease [Hene et al., 1986] and ankylosing spondylitis [Collado et al., 1988]. Possibly, intravascular IgA indeed has a distinct pathophysiological role in psoriasis patients, namely to attract neutrophils and facilitate their diapedesis. This would mean that IgA does not have to be deposited directly at the site of lesion but can enhance inflammation from adjacent dermal capillaries and venules. Heineke et al. suggested a similar mechanism for Henoch-Schönlein purpura: By producing IgA antibodies specifically binding to endothelial cells as well as neutrophils presenting with Fc-α receptors, a cascade is initiated with endothelial bound IgA recruiting and activating neutrophils which then leave the vascular lumen and

subsequently cause tissue damage [Heineke et al., 2017].
Considering the probably most important role of IgA - the anti-microbial defense function of secretory IgA after being secreted to mucosal surfaces of the body [Brandtzaeg, 2013] - and the fact that psoriasis patients get less skin infections than atopic eczema patients one might argue that there is a causal relationship. This would require that IgA covering the skin surface as part of sweat binds to pathogens and initiates immunological response such as complement system activation. This might be reflected by higher IgA serum levels (Figure 4A) but epidermal thickness and hence a stronger barrier of psoriasis patients seem more important than secretory IgA. Moreover, this study did not distinguish between monomeric and dimeric, namely secretory IgA so it is not clear whether dimeric IgA in the serum is elevated.
Besides IgA, IgE has been measured in psoriasis patients' sera long since. Whereas this study neither detected elevated IgE levels in psoriasis patients compared with healthy controls at all (Figure 4D) nor a correlation of IgE with disease severity (Figure 5D) which is in line with other studies [Del Puerto et al., 2012, Lajevardi et al., 2014], Ovcina et al. detected a significant elevation of IgE levels in psoriasis [Ovcina-Kurtovic and Kasumagic-Halilovic, 2010]. Considering that IgE elevation is part of atopic diseases it seems rather unlikely that IgE levels are also elevated in psoriasis per se. However, there might be psoriasis patients suffering from allergic rhinoconjunctivits concomitantly which could explain higher IgE levels compared to healthy control cohorts.
Higher C3 and C4 levels in psoriasis patients (Figure 4E, F) match data from literature which shows higher complement factor levels for psoriasis without exception [Ozturk et al., 2001, Kapp et al., 1985, Acevedo and Hammar, 1989]. The significance of these changes remains unclear. It may reflect an activated state of the complement system. One of its endpoints, the formation of the membrane attack complex, would then lead to the recruitment of immune cells and the beginning of an inflammatory cascade. There could be a link between the IgA and C3 elevation in terms of an IgA-dependent activation of pathways leading to a complement activation. This process may occur in circulation or after IgA immune complexes have been deposited in the skin comparable to what is happening in the mesangium in IgA nephropathy [Maillard et al., 2015]. Whether C3 and C4 deposits occur in the skin was not investigated in this thesis but could be interesting for further studies.

4.3.2. B cells - indispensable for psoriasis immune pathogenesis?

In this thesis B cell subsets were defined by their CD24, CD38 and CD138 surface expression profile as standard method according to literature [Carsetti et al., 2004, Buffa et al., 2013, Lin et al., 2017, Blair et al., 2010, Flores-Borja et al., 2013, Mauri and Ehrenstein, 2008, Robillard et al., 2014, Tembhare et al., 2014]. Hence, initially studied B cell subsets were: CD24hiCD38hi transitional B cells, CD24intCD38int mature B cells, CD24-CD38+ plasmablasts, CD24+CD38- primarily memory B cells, CD24-CD38- new memory B cells. For none of them, a significant difference between psoriasis patients and healthy controls was observed (Figure 7A). Also for the additionally investigated CD138+ plasma cells (CD19+CD24-CD38+CD138+) mean frequencies of psoriasis patients and healthy volunteers were equal (Figure 7B), but correlation analysis revealed a positive correlation of CD138+ plasma cells and IgA (Figure 7C) as well as of CD138+ plasma cells and PASI (Figure 7D) among the psoriasis patients. The correlation with PASI was even stronger when considering IgA+ plasma cells only (Figure 8).
Finding no significant differences in B cell subset frequencies between patients and healthy volunteers does not point out to a disease-specific contribution of B cells to psoriasis immune pathogenesis. But still positive correlation with IgA levels and disease severity was evident for the plasma cell subset. The latter one got even stronger when considering IgA+ plasma cells exclusively. So this might be a hint that IgA-producing plasma cells play a minor role in psoriasis pathogenesis. On the other hand this could also be expression of inflammatory epiphenomena.
It should be noted critically that there is no clear consistent gating strategy for plasma cells in flow cytometry described. Plasma cells have been described as CD10–CD19+CD20–CD27++CD38++ [Neumann

et al., 2015], as CD19lowCD38hiCD27hiCD24- [Iodice et al., 2014] or as CD19+CD20–CD38++ [Caraux et al., 2010], whereas others indice that it is enough to measure strong CD38 and CD138 expression [Rawstron, 2006, Tembhare et al., 2014]. The approach chosen in this thesis was to depict plasmablasts/plasma cells as CD19+CD24-CD38+ and to characterize sole plasma cells as CD138+ cells within that subpopulation. Backgating of CD20 in seven psoriasis patients revealed that the subpopulation of CD19+CD24-CD38 plasmablasts and plasma cells indeed was mainly CD20- (Figure 8A) as indicated by some of the authors mentioned above.

Based on case reports about new onset of psoriasis in patients receiving B cell depleting therapy [Dass et al., 2007, Markatseli et al., 2009], the question arose about the existence of a protective, immunosuppressive B cell subset namely regulatory B cells. The mechanism how these regulatory B cells control overabundant immune reactions appeared to be production of IL-10 [Iwata et al., 2011, Mauri and Ehrenstein, 2008] which results in development of regulatory T cells and control of T cell activation and autoimmunity [Barsotti et al., 2016]. In the traditional CD24/CD38 gating pattern defined as CD24hiCD38hi [Blair et al., 2010, Flores-Borja et al., 2013, Mauri and Ehrenstein, 2008, Lin et al., 2017], data presented in this thesis does not support the idea of reduced regulatory B cell frequencies in psoriasis patients: There was no difference in frequencies of CD24hiCD38hi regulatory B cells between psoriasis patients and healthy controls (Figure 7A). This is in contrast to results from Czarnowicki et al. who indeed detected significantly smaller percentages of CD24hiCD38hi B cells in their psoriasis patients compared to healthy controls [Czarnowicki et al., 2016]. To further analyse these seemingly contradictory results, PBMCs were stimulated with CD40 ligand and finally, intracellular IL-10 expression was measured (Figure 9). Results did not support the assumption that the CD24hiCD38hi B cell subset corresponds to IL-10 producing regulatory B cells as proposed by Blair et al. [Blair et al., 2010]. The IL-10+ B cells analysed here were scattered over different B cell subpopulations defined by CD24/CD38 gating predominantly appearing in the subpopulation of memory B cells, mature naive B cells and transitional B cells. Similar results have been presented by Kristensen et al. in patients suffering from Graves' disease and Hashimoto's thyroiditis [Kristensen et al., 2015]. Considering that transitional B cells are the first B cells to leave the bone marrow and use the blood flow as vehicle to reach lymph nodes where they continue their maturation it does not seem obvious why they should present the same pattern of surface differentiation markers as fully mature regulatory B cells. As the overall counts of IL-10+ B cells were extremely low in initially investigated PBMCs from healthy volunteers IL-10+ B cells were not further studied in all patients. Therefore data cannot be compared to the results by Hayashi et al. who detected decreased levels of IL-10-producing regulatory B cells in psoriasis patients [Hayashi et al., 2016]. Possibly, peripheral blood is not adequate to study B regs, whereas investigating lymphocytes deriving from primary and secondary lymphoid organs such as bone marrow or lymph nodes might be more useful.

In mouse models with Aldara cream the role of IL 10-producing B cells has been studied intensively. Aldara contains Imiquimod, a Toll-like receptor 7 (TLR7) agonist which leads to an inflammatory reaction histologically resembling to human psoriasis when topically applied to the skin of mice. Not all patterns are matching but still it is nowadays seen as a satisfying animal model for psoriasis.

Yanaba et al. e.g. compared the skin inflammation's severity after treatment with Aldara cream in wildtype mice and B cell depleted mice. They observed a stronger inflammatory status in the B cell depleted mice which was partly reversible after transferring IL-10-producing B cells from wildtype mice into the initially B cell depleted mice [Yanaba et al., 2013]. Their results bear out the hypothesis that B cells are dispensable for the development of psoriasis. Nevertheless, their results give rise to the suspicion that regulatory B cells could also play a role for psoriasis pathogenesis in humans.

Alrefai et al. go one step further and present NFATc1 as possible negative mediator between Aldara-induced skin inflammation and activation of IL-10-producing B cells. They investigated B cell depleted mice as well as mice with IL-10-deficient B cells which showed extraordinary strong skin inflammation after having applied Aldara cream on their back. By blocking NFATc1 in B cells the skin reaction got much milder. They identified NFATc1 as suppressor of IL-10 expression by binding to the transcriptional repressor HDA1C and the Il10 gene and suggested NFATc1 as a possible target of psoriasis therapy in humans [Alrefai et al., 2016]. According to data from this thesis targeting B cells for treatment of human psoriasis patients is most likely only an option in distinct patients.

Investigation of a patient (A.B.) with hereditary CVID presenting with full phenotype of psoriasis (Figure 10) offered the chance to clarify the question whether intact B cell and humoral reponse are necessary or rather dispensable for the development of human psoriasis. A.B. showed very low levels of serum IgA and nearly absent B cells in peripheral blood as expected (Figure 12A, B). Transitional and mature B cells as well as plasmablasts were also decreased whereas frequencies of primarily and new memory B cells and plasma cells were relatively increased (Figure 12C). Despite the absence of B cells and immunoglobulins, T cell function of A.B. was intact (Figure 11A) and showed a psoriasis-typical Th17 cytokine profile (Figure 11B).
The coincidence of CVID and psoriasis with a high disease severity score in this patient rather argues for a negligible role of B cells in psoriatis pathogenesis. It seems conceivable that low frequencies of CD24hiCD38hi regulatory B cells might have reinforced psoriatic inflammation but in the cohort of untreated psoriasis patients overall frequency of CD24hiCD38hi B cells was not decreased. So considering A.B.'s intact T cell function and positive family history for psoriasis but not CVID T cell contribution for psoriasis development is likely to be plainly larger than the one by protective B cells. This argumentation is in line with genome-wide association studies which have revealed aberrations mainly in T cell and less in B cell related pathways [Tsoi et al., 2012, 2015, 2017].
In addition, gene variant analysis of A.B. revealed variants in three of the at present described CVID genes NFKB1, NFKB2 and PRKCD and a hereby newly described variant in the ligand of the TNFRSF13B receptor which is crucial for the regulation of humoral immunity [Jonsson et al., 2017]. Network analysis (Figure 10C) indicated strong relations between TNFSF13, TNFRSF13B receptor and the other CVID factors.

A comparison of serum parameters and B cell subsets between untreated psoriasis patients with high disease activity and disease-controlled systemically treated psoriasis patients did not reveal conspiciuous changes through therapy: IgA levels in the treated cohort did not drop (Figure 13A) and despite significantly lower frequencies of plasmablasts but not plasma cells frequencies of B cell subpopulations remained unchanged (Figure 13B). A positive correlation of CD138+ plasma cells with IgA (Figure 14A) or PASI (Figure 14B) was not given in the treated psoriasis cohort.
This again contradicts an overall psoriasis-specific role of B cells and provides an explanation why B cell depleting therapies have been far less successful than T cell directed drugs [Ronholt and Iversen, 2017]. Possibly, they are a suitable therapeutic option in distinct psoriasis subsets.

In summary, data from this thesis does not support the idea of lacking regulatory B cells as a major driver of psoriatic inflammation but it cannot be excluded that this mechanism might be important in distinct subsets of the patients. The significance of IgA+ plasma cells for psoriasis pathogenesis cannot be conclusively assessed, but might be a starting point for further studies.

4.4. CD138 - more than a plasma cell marker?

In quest of a new biomarker for psoriasis, CD138 was investigated in more detail because its relative expression was increased in four of the five B cell subsets (Figure 15). CD138 expression on both total B cells (Figure 16) and total lymphocytes (Figure 17) strongly correlated with disease severity in psoriasis patients but not in AE patients. Moreover, CD138+ lymphocyte levels diminished in the course of anti-IL-17 therapy in four psoriasis patients compared to levels before therapy, even if not significantly (Figure 18).

Briefly, CD138 (Syndecan-1) is a heparan sulfate proteoglycan (HSPG) above all expressed on the cell surface of epithelial and plasma cells [Bartlett et al., 2007]. Diverse roles for this transmembrane molecule have been described. It is important for inflammatory conditions, malignancy and wound healing [Derksen et al., 2002, Szatmari et al., 2015, Stepp et al., 2007, 2015]. It consists of a core protein with cytoplasmic domain, transmembrane part and glycosaminoglycan (GAG) chains connected to an extracellular domain [Teng et al., 2012]. Each of the three parts seems to have distinct functions that up to date remain largely unknown.

The results of this thesis suggest that there might be a connection between Syndecan-1 expression and disease severity in psoriasis patients which is not apparent in AE. The fact that CD138 was present on all five B cell subsets and not only on plasma cells, seems quite surprising at first sight because so far CD138 has been only described on terminally differentiated B cells and malignant myeloma tumour cells but not on other lymphocytes [Dhodapkar et al., 1998, Wijdenes et al., 1996, Derksen et al., 2002]. So possibly in psoriasis CD138 expression arises pathologically premature on plasmablasts, e.g. as precursors of plasma cells responsible for increased IgA serum levels. Another possibility is that CD138 serves as transmembrane receptor on lymphocytes activating intracellular proinflammatory pathways and therefore correlates with PASI, comparable to what Wang et al. have described for CD138 on endothelial cells in several inflammation models [Wang et al., 2005]. Data presented in this thesis show a higher percentage of CD138 expression on total lymphocytes (Figure 17A) than on total B cells (Figure 16A), so the question comes up whether CD138 - although being known as a plasma cell marker - has a greater importance for non-B cells than for B cells. Continuing this idea, intrinsically increased lymphocytic CD138 expression based on genetic background of psoriasis patients could enhance proinflammatory cytokine release by distinct lymphocytes resulting in activation of other inflammatory cells. A proinflammatory role for CD138 has also been described by Li et al. who reported about a mouse model of bleomycin-induced acute lung injury where Syndecan-1 shedding serves the transepithelial migration of neutrophils from the perivascular lung tissue into the alveoli [Li et al., 2002]. Similar mechanisms are conceivable for the immune pathogenesis of psoriasis where neutrophil migration to the site of lesion plays an important role as well. As a restriction, it needs to be stated that there are also reports about an immunosuppressive effect of CD138 shedding [Gotte et al., 2002, 2005]. Data from this thesis indicating a positive correlation between CD138 expression and disease severity however make an immunosuppressive role of CD138 in the context of psoriasis rather unlikely.

For further assessment of CD138 as a possible biomarker for psoriasis, investigations of its cellular origin and functional analysis of its significance for the pathophysiology of psoriasis are needed. Experiments to perform could be: Magnet purification of CD138+ PBMCs and characterization of subsets by FACS, stimulation with different cytokine cocktails and measurement of intracellular cytokine expression by FACS or extracellular cytokine release by ELISA as well as blocking CD138 by a neutralizing antibody after differentiation of B cells into plasma cells. At best, CD138 will turn out to be a new therapeutic target for treating psoriasis, at least for specific endotypes. In a murine MS model, a proteoglycan binding agent called Surfen was shown recently to reduce inflammation [Warford et al., 2018].

5. Summary and outlook

With about 1.6 million affected people, psoriasis is one of the most common skin diseases in Germany. It's chronic character and huge negative impact on patients' quality of life underscore the need for a deeper insight into it's pathogenesis in order to develop effective personalized targeted therapies. In the last years and decades big progress was made on the field of T cell research with the discovery of the IL-23/Th17 axis but still only little is known about B cells' contribution to psoriasis pathogenesis.
Therefore, this thesis was performed to investigate the influence of the humoral immune response on the development and maintenance of psoriasis.
Blood from 35 psoriasis patients, 20 healthy controls and 13 AE patients was compared regarding the distribution of different B cell subpopulations in flow cytometry experiments and serum parameter levels for complement factors and immunoglobulins. Furthermore, skin biopsies were stained for IgA expression.
The serum parameter analysis revealed an exclusive elevation of IgA, C3 and C4 in psoriasis patients compared to the healthy volunteers. No significant changes in these two groups could be observed for IgG, IgM or IgE. Moreover, IgA distribution among the psoriasis patients was positively correlated with disease severity represented as PASI score. Immunohistochemistry stainings for IgA in the skin as main site of psoriatic inflammation showed higher intravascular IgA deposits in psoriasis compared to the two other cohorts. Contrarily, flow cytometry experiments did not show significant differences in B cell distribution, namely the transitional and mature B cells, plasmablasts as well as primarily and new memory B cells. Also a more detailed analysis of the CD138+ plasma cells did not reveal significant differences in the group comparison, but showed a positive correlation with both disease severity and IgA levels in the serum. This correlation with disease severity could also be seen when contemplating the CD138 expression of all B cells and of all lymphocytes as well as for IgA+ plasma cells. In a cohort of adequately systemically treated psoriasis patients (n=28) IgA levels as well as B cell subsets remained widely unchanged compared to the untreated patients of the initial psoriasis cohort. Finally, a patient with a hereditary B cell deficiency suffering concomitantly from psoriasis offered the opportunity to clarify whether B cells are of high relevance or rather irrelevant for the development of psoriasis.
These data show a clear yet unspecific humoral profile for psoriasis with elevated IgA, C3 and C4 serum levels. Changes on the cellular level do not appear as elevation of a distinct subpopulation but are mainly results of the correlation analysis with disease severity. Whether these changes contribute causally to the psoriasis pathogenesis or should be interpreted as unspecific epiphenomena in the course of inflammation cannot be evaluated with certainty on the basis of the existing data. Considering data from the treated psoriasis cohort as well as from the CVID patient it is rather unlikely that B cells are major drivers of psoriasis pathogenesis. But even if not of importance for all patients, it may be possible that B cells and B cell derived humoral components play a significant role in a rare subgroup of patients where conventional T-cell directed antipsoriatic drugs fail.
Finally, figure 19 shows a simplistic schematic drawing how results presented in this work might contribute to psoriasis pathogenesis at least in a distinct, yet undefined endotype:
IgA secreted in increased quantity by plasma cells located in special bone marrow niches recirculates in the periphery (**1**). When reaching small vessels nearby lesional sites IgA binds specifically to endothelial cells. That in turn recruits neutrophil granulocytes which bind via Fc-α receptor to the endothelial fixed IgA molecules. Activation of their Fc-α receptor facilitates neutrophil diapedesis. Neutrophils then migrate to the psoriatic lesion where they enhance inflammatory processes described previously in chapter 1.1.2 (**2**). In this context, CD138 expressing lymphocytes activate endothelial and other immune cells via CD138-dependent cytokine release likewise amplifying the proinflammatory environment (**3**).

For deeper insight, functional analysis of different B cell subsets is required. Especially the role of po-

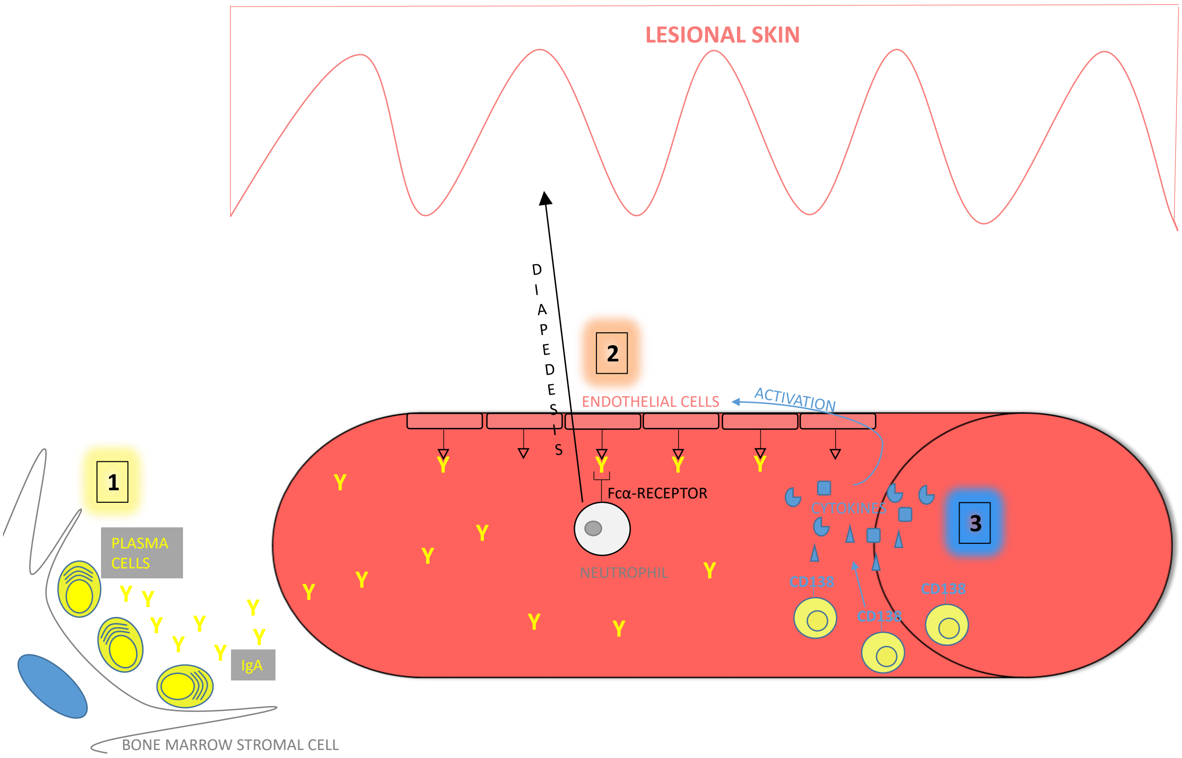

Figure 19 Hypothetical model how main study results might influence psoriasis immune pathogenesis in a subgroup of patients: Increased IgA serum levels produced by bone marrow derived plasma cells (**1**). Endothelium-IgA-neutrophil interactions facilitating neutrophil diapedesis (**2**). CD138-dependent cytokine release enhancing psoriatic inflammation (**3**).

tential immunosuppressive B cells in the field of human psoriasis should be investigated in more detail. Interesting would be an in-depth characterization of psoriasis patients non-responding to anti-IL-17 and anti-IL-23 therapies. This could possibly identify rare patients who could benefit from B cell directed therapies.

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7. Publications resulting from this thesis

Thomas, J.* , **Küpper, M.*** , Batra, R. , Jargosch, M. , Atenhan, A. , Baghin, V. , Krause, L. , Lauffer, F. , Biedermann, T. , Theis, F. , Eyerich, K. , Eyerich, S. and Garzorz-Stark, N. (2018), Is the humoral immunity dispensable for the pathogenesis of psoriasis?. J Eur Acad Dermatol Venereol. doi:10.1111/jdv.15101.
* = both authors contributed equally.

Poster presentation at 7th ADF (Arbeitsgemeinschaft Dermatologische Forschung) Winter School (Title: Is the humoral immunity dispensable for the pathogenesis of psoriasis?), Zugspitze, February 2018

Scientific talk at the ADF 44th Annual Conference with the title: Characterization of multiple B cell subsets in peripheral blood and skin biopsies of psoriasis patients identifies a correlation of plasma and regulatory B cells and disease severity, Göttingen, March 2017

Küpper, M. , Thomas, J. , Garzorz-Stark, N. , Krause, L. , Müller, N.S. , Biedermann, T. , Theis, F. , Schmidt-Weber, C. , Eyerich, K. , Eyerich, S. , 330 Characterization of multiple B cell subsets in peripheral blood of psoriasis patients identifies a correlation of regulatory B cells and disease severity. Journal of Investigative Dermatology , Volume 136 , Issue 9 , S217 (2016). doi: https://doi.org/10.1016/j.jid.2016.06.350

Poster presentation by Jenny Thomas at EAACI (European Academy of Allergy and Clinical Immunology) Congress 2018, Munich, May 2018

Thomas, J. , Garzorz-Stark, N. , **Küpper, M.** , Krause, L. , Müller, N.S. , Biedermann, T. , Theis, F.J. , Schmidt-Weber, C.B. , Eyerich, K. , Eyerich, S. , Characterization of multiple B cell subsets in peripheral blood of psoriasis patients identifies a correlation of plasma and regulatory B cells with disease severity. Exp. Dermatol. 25, 27-28 (2016). doi:10.1111/exd.13200