PPP 2016

PATHOGENESIS OF PEMPHIGUS AND PEMPHIGOID

The open blister/mind meeting

Satellite Symposium to the 46th Annual ESDR meeting
5 to 7 September 2016

Local street map

The meeting takes place in the historical building of the Institute of Anatomy and Cell Biology.

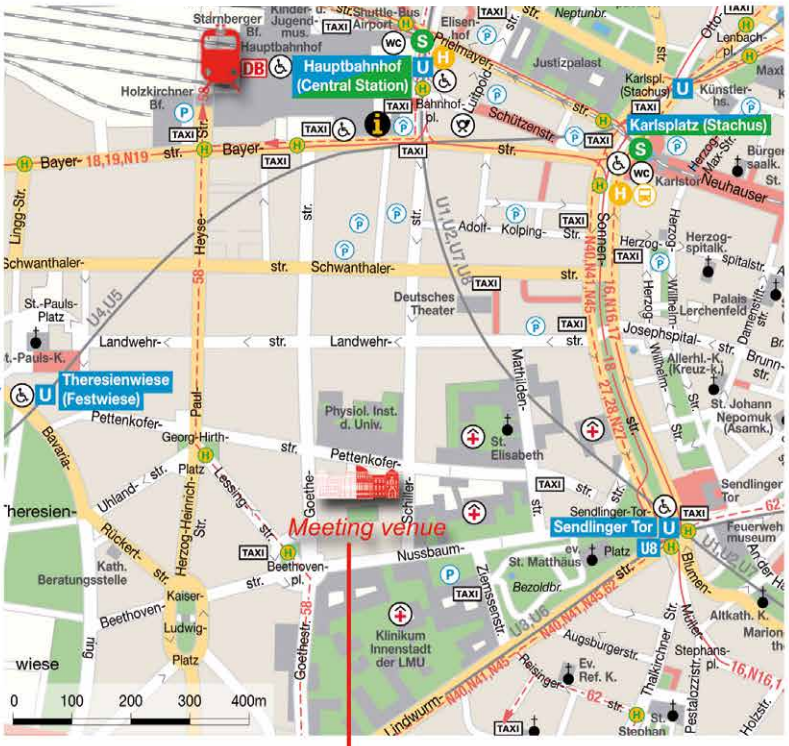
Address: Pettenkoferstrasse 11, 80336 Munich

The meeting venue is located just a few minutes walk south of the central station and is surrounded by several additional public transport stops in walking distance.
The nearest stop is the subway „Sendlinger Tor“, which is also the most convenient way to get to the ESDR meeting. Just take the blue line (U6) direction „Garching Forschungszentrum“ and exit the the train at this stop (final stop) to reach the ESDR meeting.

subway local train

Central Station (Subway and all trains)

12 min



Subway „Karlsplatz“

15 min

Subway „Theresienwiese“

8 min

Subway „Sendlinger Tor“

5 min

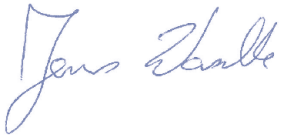
Welcome

Dear colleagues,

welcome to the "Pathogenesis of Pemphigus and Pemphigoid Meeting (PPP 2016)" which is held as an official satellite symposium of the 46th Annual ESDR meeting in Munich. We hope to create the atmosphere and spirit of previous events on bullous diseases such as the PreIID satellite symposia in Otsu 2008 and Lübeck 2013 and the meetings on pemphigus in Bern 2009 and Marburg 2011. Open and free exchange of ideas and thoughts and discussion on existing and emerging concepts are the basis for research. Thus, we added - the open blister/mind meeting - as a motto for the PPP2016. Since we are a relatively small community, gathering with you is a great pleasure for us. The Munich group is looking forward to present their historic anatomy institute located right in the heart of Munich in walking distance to the main station and numerous hotels. We hope you agree that this fantastic venue is a nice place to harbor a meeting such as the PPP2016.

In the next couple of days we look forward to 25 talks from invited speakers and 21 short talks selected from the 64 abstracts which will be presented as posters. We hope to provide a welcoming and creative atmosphere to advance our understanding on the pathogenesis of autoimmune bullous diseases.

We are happy to welcome you in Munich!



Jens Waschke
Anatomy and Cell Biology,
LMU Munich

Detlef Zillikens
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Michael Hertl
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University of Lübeck

Rüdiger Eming
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Table of contents

Scientific Program

Tuesday Sep 6 – Pemphigoid

9:00-10:30 **Epidemiology and genetics of pemphigoid diseases**
Chairs: Philippe Bernard, Detlef Zillikens

9:00 Epidemiology of pemphigoid diseases
Philippe Bernard, University of Reims, France

9:20 Expression of BP180 and BP230 in the central nervous system
Allan Seppänen, Helsinki University Hospital, Finland

9:40 Gene-environment interaction in pemphigoid diseases
John Baines, Max-Planck-Institute for Evolutionary Biology, Plön, Germany

10:00 Increased Bullous Pemphigoid Autoantibody Levels are Associated with More Severe Alzheimer's Disease
Laura Huilaja, University of Oulu, Finland

10:10 Prevalence and age distribution of pemphigus and pemphigoid diseases in Germany
Franziska Hübner, University of Lübeck, Germany

10:20 T cell receptor repertoire analysis indicates a bifunction role of T cells in Epidermolysis bullosa acquisita
Markus Niebuhr, University of Lübeck, Germany

10:30-11:00 **Coffee break**

11:00-12:30 **Immunopathology of pemphigoid diseases I**
Chairs: Wataru Nishie, Enno Schmidt

11:00 Imunopathological variants of bullous pemphigoid
Wataru Nishie, Hokkaido University, Sapporo, Japan

11:20 IL-17 in bullous pemphigoid
Frank Antonicelli, University of Reims, France

11:40 The importance of complement activation in in bullous pemphigoid
Hideyuki Ujiie, Hokkaido University, Sapporo, Japan

12:00 Loss of C-terminal domain induces neoepitopes on processed collagen XVII
Ellen Toyonaga, Hokkaido University, Sapporo, Japan

12:10 A novel mouse model for anti-laminin 332 mucous membrane pemphigoid
Eva Heppe, University of Lübeck, Germany

12:20 Role of alpha 6 and beta 4 in the pathogenesis of MMP
Razzaque Ahmed, Tufts University, Boston, USA

12:30-14:00 **Lunch break**

14:00-16:10 **Diagnosis and treatment of pemphigoid diseases and dermatitis herpetiformis**
Chairs: Luca Borradori, Jane Setterfield

14:00 BLISTER trial: what did we learn.
Karen Harman, University Hospitals of Leicester, UK

14:20 Dapsone as a corticosteroid-sparing therapy in bullous pemphigoid: a controlled prospective multicenter trial
Michael Sticherling, University of Erlangen, Germany

14:40 Diagnosis of mucous membrane pemphigoid
Jane Setterfield, King's College London, UK

15:00 The value of pattern analysis of direct IF microscopy in pemphigoid disorders
Hendri Pas, University of Groningen, The Netherlands

15:20 Dermatitis herpetiformis and the role of gluten-free diet in non-celiac gluten sensitivity
Marzia Caproni, University of Florence, Italy

15:40 Minimal requirements for diagnosis of cutaneous pemphigoid. Comparative study of direct immunofluorescence, serology and clinical features in 316 patients.
Joost Meijer, University of Groningen, Netherlands

15:50 Diagnostic value of laser scanning confocal microscopy in mucous membrane pemphigoid
Katarzyna Wozniak, University of Warsaw, Poland

16:00 A multivariant profile ELISA for one-step diagnostics of autoimmune bullous dermatoses
Gabi Ommen, University of Lübeck, Germany

16:10-16:45 Coffee break

16:45-18:15 Immunopathology of pemphigoid diseases II
Chairs: Giovanni Di Zenzo, Hiroshi Shimizu

16:45 Epidermolysis bullosa acquisita: a prototypic autoimmune bullous disease
Ralf Ludwig, University of Lübeck, Germany

17:05 Preclinical use of anti-inflammatory inhibitors in epidermolysis bullosa acquisita
Hiroshi Koga, Kurume University, Japan

17:25 Lipid mediators as drivers of effector cell recruitment in pemphigoid diseases
Christian Sadik, University of Lübeck, Germany

17:45 Blocking the activating Fc gamma RIV enhances neutrophil extravasation into the skin in autoantibody-induced cutaneous inflammation
Jennifer Kloepper, University of Lübeck, Germany

17:55 Bullous pemphigoid - Increased activity of eosinophils in blister fluids and peripheral blood
Manuela Gehring, University of Hannover, Germany

18:00 Expression of IL-31 in blisters and eosinophils of patients with bullous pemphigoid
Ulrike Raap, University of Hannover, Germany

18:05 Contribution of IgE Autoantibodies to the Pathogenesis of Bullous Pemphigoid
Patricia Freire, Medical University of Vienna, Austria

18:10 High affinity IgE receptors (FcεRI+) bearing cells in the epidermis of bullous pemphigoid.
Bartłomiej Kwiek, University of Warsaw, Poland

19:00 Poster Session: Science and Wine

Wednesday Sep 7 – Pemphigus

9:00-10:30 **New insights into pemphigus autoimmunity**
Chairs: Michael Hertl, Eliane Müller

9:00 Peripheral tolerance to Dsg3-specific CD4 T cells
Masayuki Amagai, Keio University, Tokio, Japan

9:20 T cell involvement in pemphigus - from the human angle
Rüdiger Eming, University of Marburg, Germany

9:40 Deep sequencing of the isotype-specific B cell repertoire in pemphigus
Aimee Payne, University of Pennsylvania, Philadelphia, USA

10:00 Characterization of IL-21-producing cells in pemphigus vulgaris
Robert Pollmann, University of Marburg, Germany

10:15 Novel chimeric immunoreceptors for pemphigus vulgaris (PV) therapy
Christoph Ellebrecht, University of Pennsylvania, Philadelphia, USA

10:30-11:00 **Coffee break**

11:00-12:30 **Desmoglein vs. non-desmoglein autoantibody mechanisms**
Chairs: Andrew Kowalczyk, Marcel Jonkman

11:00 Role of anti-Dsg-antibodies in pemphigus - conclusions from our studies
Jens Waschke, Ludwig-Maximilians-Universität, Munich, Germany

11:20 Pemphigus autoimmunity beyond desmogleins
Sergei Grando, University of California Irvine, USA

11:40 The impact on HLA on the autoimmune response in PV
Animesh Sinha, University at Buffalo, New York, USA

12:00 Desmoglein-specific immunoadsorption abolishes the pathogenic potential of IgG from patients with pemphigus vulgaris
Stephanie Goletz, University of Lübeck, Germany

12:15 The soluble form of Fas Ligand plays a pivotal role in blister formation in pemphigus
Carlo Pincelli, University of Modena and Reggio Emilia, Italy

12:30 Polyclonal nature of pemphigus foliaceus IgG antibodies enhances pathogenic effect for blister formation in association with p38 MAPK-dependent desmoglein 1 clustering.
Kenji Yoshida, Toho University, Tokyo, Japan

12:45-14:00 **Lunch break**

14:00-16:10 **Mechanisms in pemphigus pathogenesis**
Chairs: Masayuki Amagai, Aimee Payne

14:00 Mechanistic insights in pemphigus pathogenesis from patients' skin
Marcel Jonkman, University of Groningen, The Netherlands

14:20 Effects of pemphigus IgG on desmosomal protein organization and function
Andrew Kowalczyk, Emory University, Atlanta, USA

14:40 Contributions of steric hindrance and signaling in pemphigus
Volker Spindler, Ludwig-Maximilians-Universität, Munich, Germany

15:00 Signaling mechanisms in pemphigus
Eliane Müller, University of Bern, Switzerland

15:20 Understanding pemphigus through genetics
Eli Sprecher, Tel Aviv Medical Center, Israel

15:40 Loss of flotillin expression results in weakened desmosomal adhesion and Pemphigus vulgaris-like localisation of desmoglein-3 in human keratinocytes
Antje Banning, University of Giessen, Germany

15:55 Pemphigus vulgaris autoantibodies cause invaginations of one cell into another
Ena Sokol, University of Groningen, Groningen, The Netherlands

16:10-16:45 Coffee break

16:45-18:15 Consensus session
Bringing together the different views on blister formation in pemphigus
hosted by Carien Niessen, University of Cologne, Germany

Social program

Monday Sep 5

19:00 Get together and Welcome reception

Entrance hall of the Institute of
Anatomy and Cell Biology

Wednesday Sep 7

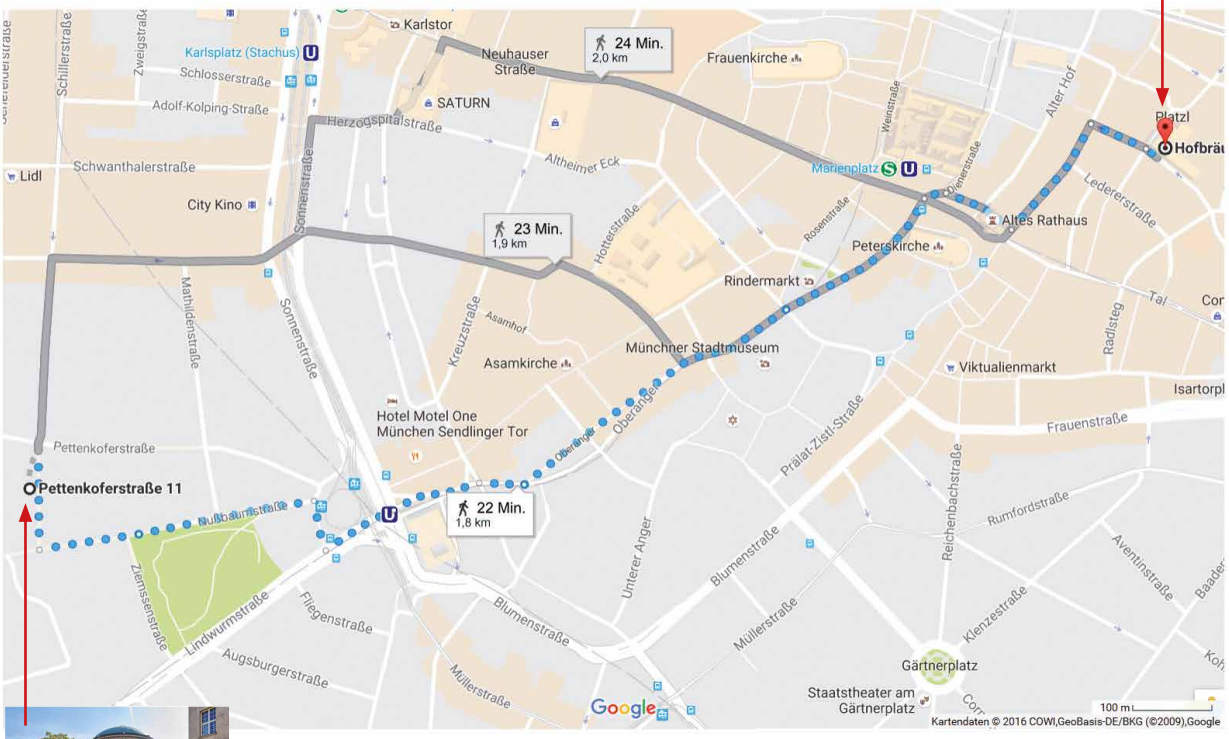
19:15 Dinner at the Hofbräuhaus

The Hofbräuhaus is located at „Platzl“ (Platzl 9, 80331 Munich). Nearest public transport stop is „Marienplatz“, just one subway stop away from „Sendlinger Tor“.

The Hofbräuhaus can also be reached by a splendid 20 min walk from the meeting venue through the inner city.
If you want to attend a guided walk, meet us at 18:45 in the entrance hall of the Institute of Anatomy and Cell Biology.

subway local train

Hofbräuhaus



Meeting venue

Posters

Epidemiology and genetics of autoimmune bullous diseases

POSTER 1

Psychiatric and neurological disorders are associated with bullous pemphigoid – a nationwide Finnish Care Register study

› Anna-Kaisa Försti[1], Jari Jokelainen[2] [3], Hanna Ansakorpi[4], Allan Seppänen[5], Kari Majamaa[4], Markku Timonen[3], Kaisa Tasanen[1]

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[3]Center for Life Course Epidemiology and Systems Medicine, P.O. Box 5000, 90014, University of Oulu, Oulu, Finland
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[5]Psychoses and Forensic Psychiatry, Helsinki University Hospital, Vanha Valtatie 198, 04500, Kellokoski, Finland and Vanha Vaasa Hospital, Vierinkiventie 1, 65380 Vaasa, Finland

Pemphigus autoantibodies directed against the desmosomal cadherins Desmoglein (Dsg) 1 and 3 lead to flaccid blistering of the skin and mucous membranes of the oral cavity. However, some patients develop an ocular involvement which is most frequently diagnosed as conjunctivitis, but the underlying mechanisms are not elucidated yet. We therefore characterized human and murine conjunctiva with respect to the PV autoantigens and evaluated the effects and mechanisms of PV autoantibodies applied to human conjunctiva ex vivo. We first studied a mouse model depleted of the desmosomal cadherin desmoglein 3 (Dsg3), the primary autoantigen in PV. Further, human conjunctiva specimens from surgical explants were obtained and a short-term culture model to study the alterations induced by antibody fractions of PV patients (PV-IgG) was established. Both, murine and human conjunctiva sample expressed the majority of desmosomal molecules with an expression pattern similar to the epidermis. Interestingly, Dsg3 knock out animals frequently develop eye lesions, histologically evident as microblisters in the eyelid epidermis and conjunctiva. Incubation of human specimens with IgG-fractions of pemphigus vulgaris patients (PV-IgG) for 12 h caused blistering in the suprabasal layers of the conjunctiva, resembling the effects of PV-IgG in the epidermis. Concomitantly, a reduction of Dsg1 and 3 levels was detectable. Furthermore, PV-IgG prompted activation of p38MAPK in the conjunctiva, which is a central pathomechanism leading to blistering in the epidermis. Taken together, these models indicate that the ocular involvement observed in PV patients is mainly based on conjunctival blistering. patients (PV-IgG) for 12 h caused blistering in the suprabasal layers of the conjunctiva, resembling the effects of PV-IgG in the epidermis. Concomitantly, a reduction of Dsg1 and 3 levels was detectable. Furthermore, PV-IgG prompted activation of p38MAPK in the conjunctiva, which is a central pathomechanism leading to blistering in the epidermis. Taken together, these models indicate that the ocular involvement observed in PV patients is mainly based on conjunctival blistering.
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POSTER 2

Increased Bullous Pemphigoid Autoantibody Levels are Associated with More Severe Alzheimer's Disease

› Laura Huilaja[1], Nina Kokkonen[1], Sanna-Kaisa Herukka[2], Merja Kokki[3], Anne M. Koivisto[2], Päivi Hartikainen[2], Anne M. Remes[2], and Kaisa Tasanen[1]

[1]Department of Dermatology, PEDEGO Research Unit, Medical Research Center Oulu, Oulu University Hospital and University of Oulu, Oulu, Finland
[2]Institute of Clinical Medicine – Neurology, University of Eastern Finland and Department of Neurology, Kuopio University Hospital
[3]Department of Anesthesia and Operative Service, Kuopio University Hospital, Kuopio, Finland

Bullous pemphigoid (BP) is a subepidermal blistering skin disease, which has shown a strong association with neurological diseases in epidemiological studies. The BP autoantigens BP180 and BP230 are expressed in the cutaneous basement membrane and the central nervous system. Using ELISA assays we analyzed the IgG reactivity to BP autoantigens in the sera of 115 patients with Alzheimer's disease (AD) and 40 neurologically healthy controls. Increased BP180-NC16A-antibody values were found in 19% of AD patients, whereas only 2% of controls had positive results (P=0.011). Anti-BP230 values were more often elevated in AD patients than controls, but not significantly. Despite the increased autoantibody values, a retrospective evaluation of the hospital records of the AD patients revealed neither BP diagnosis nor BP-like symptoms. BP180 and BP230 antibody values correlated well with each other, but not with age. A higher proportion of females than males had elevated anti-BP180 values (P=0.002). Furthermore, increased BP180-NC16A-antibody values correlated with cognitive decline measured by Mini-Mental State Examination (MMSE) scores and weakly with the concentration of the AD biomarker p-tau181 in cerebrospinal fluid.
Our findings further the understanding of the role of BP180 as a shared autoantigen in neuro-dermatological interactions and the association between BP and neurodegenerative diseases.

POSTER 3

Prevalence and age distribution of pemphigus and pemphigoid diseases in Germany

› Franziska Hübner[1], Andreas Recke[2], Detlef Zillikens[1], Roland Linder[3], Enno Schmidt[1,2]

[1]Department of Dermatology, University of Lübeck, Lübeck, Germany
[2]Lübeck Institute of Experimental Dermatology (LIED), University of Lübeck, Lübeck, Germany
[3]Scientific Institute of Techniker Krankenkasse for Benefit and Efficiency in Health Care (WINEG), Hamburg, Germany

While several reports have estimated the incidence of autoimmune blistering diseases (AIBDs), data about the prevalence of AIBDs are scarce. Here, we analysed the data base of a major German health insurance company, the Techniker Krankenkasse, which insures about 9.7 million individuals representing about 12% of the German population. Diagnoses were based on the ICD-10-GM 2011 classification and included all patients in 2014. To control for a slightly different demographical composition of the insured individuals regarding age and sex, data were adjusted to the general German population based on the data of the Federal Statistical Office of Germany for the year 2014. The prevalence of pemphigus and pemphigoid diseases in 2014 was calculated to 0.5‰, resulting in a total number of about 40,400 patients in the total population of 80.925 million inhabitants in Germany. Bullous pemphigoid had the highest prevalence of all AIBDs (259 patients/ million) followed by pemphigus vulgaris (95 patients/ million), mucous membrane pemphigoid (25 patients/ million), and chronic bullous dermatosis of childhood (24 patients/ million inhabitants <18 years of age). Lower prevalences were seen for pemphigoid gestationis (14/ million females), pemphigus foliaceus (10/ million) and epidermolysis bullosa acquisita (3/ million). In line with previous reports, we found bullous pemphigoid and mucous membrane pemphigoid more frequent in the elderly population, while pemphigus diseases predominantely occurred in the middle age. These data will be helpful to design clinical studies and estimate the health burden for this group of organ-specific autoimmune patients.

POSTER 4

Clinical and demographical features of autoimmune bullous diseases: A retrospective analysis of 85 patients

› Ülker Gül[1], Arzu Kılıç[2], Seray Külcü Çakmak[3], Seçil Soylu[4], Müzeyyen Gönül[5]

[1]Akdeniz University, Faculty of Medicine, Department of Dermatology, Antalya / Turkey
[2] Balikesir University, Faculty of Medicine, Department Of Dermatology, Balikesir/ Turkey
[3] Ministery Of Health Ankara Numune Research And Education Hospital, Dermatology Clinic, Ankara/ Turkey
[4] Private Lokman Hekim Hospital, Dermatology Clinic, Ankara/ Turkey
[5] Ankara Diskapi Yildirim Beyazit Education and Research Hospital, Department of Dermatology, Ankara, Turkey

Objective: Autoimmune bullous dermatoses is a group of diseases that are characterized by the presence of autoantibodies in tissue or blood against specific cutaneous antigens. The aim of our study is to evaluate the demographical and clinical features of patients with autoimmune bullous diseases that applied to our clinic.
Materials and methods: Between the years 2003 and 2011, the patients who applied to our clinic and whom were diagnosed as autoimmune bullous diseases according to clinical, histopathological and immunopathological criteria were evaluated retrospectively. Cases were evaluated according to their gender, ages, the type of autoimmune bullous disease, the onset and localization of lesions, the extensity of involvement, associated diseases, the treatment they were given and the response to the treatment.
Results: A total of 85 cases were included in our study. 49 (57.64%) of the patients were female, 36 (42.36%) were male. 57 of cases (67.05%) belonged to pemphigus (P) group and 24 of them (28.23%) to pemphigoid. The ratio of patients with P.vulgaris to the patients with pemphigoid were detected as 3.16. The age range of the onset of the symptoms was between 22 and 82 years old. The chronicity of the lesions was between 2 weeks and 120 months. On follow up, a patient with P.vulgaris was diagnosed as gastric carcinoma. Bone metastasis was detected in one patient with bullous pemphigoid who was operated for breast carcinoma previously. Renal cell carcinoma and lymphoma were detected in other two patients with bullous pemphigoid. All patients except one case responded to therapy.

POSTER 5

HLA impacts gene expression related to autoimmune activation and protection in Pemphigus

› Dey-Rao, R.; Seiffert-Sinha, K.; Sinha, A.A.

Department of Dermatology, Jacobs School of Medicine and Biomedical Sciences, University at Buffalo, NY, USA

Pemphigus vulgaris (PV) is strongly associated with the HLA types DRB1*0402 and DQB1*0503. The full functional importance of HLA type to the autoimmune response in PV remains to be determined. Prior work by our group investigating genome-wide expression profiles in patients and healthy controls by microarray demonstrated a distinct separation by unsupervised hierarchical clustering between PV patients (both matched and unmatched for HLA risk alleles) and HLA-matched controls, defined as the PV-associated "disease signature". In new work, we compared transcriptional profiles in HLA-unmatched controls (non-carriers of the PV associated HLA risk alleles) to PV patients and HLA-matched controls. We demonstrate that PV patients typing as DRB1*0402 or DQB1*0503 group with HLA-matched healthy individuals. In contrast, PV patients negative for these risk alleles group with HLA-unmatched controls. These data reveal HLA as a key driver of gene expression, leading us to define a PV-associated "HLA signature" by ontology enrichment analysis, particularly in pathways and processes related to immune/inflammatory response, cytoskeletal reorganization, MAPK signaling, cell adhesion and apoptosis. Thus, even among healthy individuals, the presence of PV HLA risk alleles results in (at least partial) autoimmune acti-

vation. However, additional upregulated pathways and processes in the "disease signature" are absent from the "HLA signature", i.e. lacking in "at risk", but healthy, individuals. Previous work from our laboratory suggests that healthy individuals carrying PV-associated HLA alleles may be "protected" from progression to disease by the down-regulation of several "disease signature" associated genes/pathways. We propose that HLA impacts both autoimmune activation and protection in PV.

Diagnosis, antibody profiles and novel disease entities

POSTER 6

Reappraisal of the histopathological features of pemphigus and pemphigoid

› Chika OHATA, Norito ISHII, Hiroshi KOGA, Takekuni NAKAMA

Department of Dermatology, Kurume University School of Medicine

Bullous pemphigoid (BP) and pemphigus are the most common autoimmune bullous diseases and are diagnosed on the basis of clinical manifestation, histopathological findings, results of direct immunofluorescence, and serological test results. Because subepidermal bulla and acantholysis in a biopsy specimen are the hallmarks of BP and pemphigus, respectively, other histopathological features are often ignored. We retrospectively studied histopathological features of 110 BP cases, 35 pemphigus vulgaris (PV), and 27 pemphigus foliaceus (PF) cases. Of the 110 BP cases, subepidermal bulla was seen in 98 cases, of which 65 also showed linear necrosis of basal cells, 25 showed intraepidermal bulla, and 26 showed adnexal involvement. Adnexal involvement was confirmed by the presence of floating adnexal epithelium within the bulla in 24 cases. Acantholysis was seen in 20 cases harboring subepidermal bulla, whereas vacuolar alteration was seen in 34 cases with and 8 cases without subepidermal bulla. Although eosinophils were the predominant inflammatory cells within the bulla and in the dermis and epidermis in most cases, neutrophils predominated in the epidermis in 15 cases and within the bulla in 16 cases. In PV, acantholysis was noted in the suprabasal area in 3 cases, in the lower half of the epidermis in 19 cases, and throughout the epidermis in 13 cases. In PF, acantholysis was observed in the granular layer in 6 cases, in the upper half of the epidermis in 14 cases, and throughout the epidermis in 7 cases. It is important to know unusual histopathological features of BP, PV, and PF.

POSTER 7

Minimal requirements for diagnosis of cutaneous pemphigoid. Comparative study of direct immunofluorescence, serology and clinical features in 316 patients.

› Meijer JM, De Lang EWG, Diercks GHF, Pas HH, Jonkman MF

Center for Blistering Diseases, Department of Dermatology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands

In order to determine the minimal laboratory requirements for diagnosis of cutaneous pemphigoid, this single-center retrospective study assessed the diagnostic value of direct immunofluorescence (DIF) microscopy and of several immune serological tests, including indirect immunofluorescence microscopy on 1M NaCl-split skin (IIF SSS), IIF on monkey oesophagus (IIF MO), immunoblot and anti-BP180 NC16A and anti-BP230 ELISA's. Paired data of 316 newly diagnosed patients with cutaneous pemphigoid with a mean age of 71.9 years were compared to 811 controls. In addition, the study assessed DIF serration pattern analysis and differences in biopsy sites for DIF microscopy.

DIF showed a sensitivity of 88.3% and specificity of 96.1%. Sensitivities of serological assays were 80.1% (IIF SSS), 60.2% (IIF MO), 74.6% (immunoblot), 68.6% (MBL ELISA anti-BP180 NC16A) and 52.4% (MBL ELISA anti-BP230), with high specificities in all tests. IIF SSS showed a very high positive predictive value (97.3%). Sensitivity of perilesional skin biopsies for DIF was significantly higher compared to lesional skin and unaffected skin. 20% of patients presented with the nonbullous subtype of cutaneous pemphigoid, but no preference was found in biopsy site for DIF. BP230 was significantly more often targeted as autoantigen in patients with nonbullous cutaneous pemphigoid compared to pemphigoid patients with blisters.
The current gold standard DIF misses approximately 10% of diagnoses of cutaneous pemphigoid, which additionally can be found with IIF SSS. Both ELISA's showed low sensitivities and should not be recommended as standalone test. The minimal required diagnostic tests for the diagnosis of cutaneous pemphigoid are DIF plus IIF SSS.

POSTER 8

BP180 autoimmunity-related localized blistering dermatoses are intellectually challenging for dermatology clinicians: focal cutaneous phenomena have pathogenic relevance

› Marian Dmochowski, Justyna Gornowicz-Porowska, Pawel Bartkiewicz, Monika Bowszyc-Dmochowska

Autoimmune Blistering Dermatoses Section, Dermatology, University School of Medical Sciences, Poznan, Poland

BP180-mediated bullous pemphigoid (BP), plausibly the most common autoimmune blistering dermatosis affecting the elderly, has rich clinical presentations as from 13 to 15 clinical varieties were described giving from 213-1 to 215-1 mathematical combinations. Here, we present three cases of BP180 autoimmunity-related localized blistering dermatoses causing diagnostic difficulties at the clinical level in whom diagnoses were reached following meticulous analysis of clinical/direct immunofluorescence (DIF)/ELISA data. In a nonagenarian female, in whom zoster was suspected, the diagnosis of initially localized, paraneoplastic, conceivably, as suggested by PubMed search, ramipril-modulated BP developing after surgery for malignancy of left breast was established. In a female in her seventies, initially treated with just topical disinfectant, ethacridine lactate, for suspected infection, the diagnosis of localized, trauma-induced BP developing after removal of discomfort-causing plaster cast used for stabilisation of right forearm fracture was established. In a male in his sixties, suspected of having discoid lupus erythematosus as chronic active inflammation was merely seen by a pathologist in H+E specimen, the diagnosis of Brunsting-Perry pemphigoid plausibly modified by levetiracetam, as suggested by PubMed search, used for pre-existing epilepsy, presenting as a single scarring/blistering lesion almost entirely affecting his right cheek and conjunctiva was established. Thus, focal cutaneous phenomena, still requiring experimental exploration, have pathogenic relevance in BP180 autoimmunity-related localized blistering dermatoses posing intellectual challenge for dermatology clinicians, seemingly bewildered by imprecise nosology now, who should remember that imaging, namely DIF, and molecular-biochemical, namely ELISA, ideally multiparametric one, techniques are to be performed in diagnosing localized blistering eruptions.

POSTER 9

The effect of endoscopic ear, nose and throat examination on pemphigus severity scoring systems

› Bilgiç Temel A[1], Temel I.C[2], Bostancı Toptaş A[2], Turhan M2, Bozkurt S[3], Uzun S[1]

[1]Akdeniz University, Faculty of Medicine, Dermatology and Venereology Department, Antalya
[2]Akdeniz University, Faculty of Medicine, Otolaryngology Department, Antalya
[3]Akdeniz University, Faculty of Medicine, Biostatistics and Medical Informatics Department, Antalya

Background: Pemphigus vulgaris (PV) is a potentially life-threatening, autoimmune blistering disease of the skin and mucous membranes. Since endoscopic ear nose throat (ENT) examination is not a routinely performed procedure, ENT involvement of PV remains unrecognized.
Aim of the study: Available severity scoring systems for PV do not include ENT examination. This study designed to evaluate the real extent of PV involvement and the effect of routine ENT examination on PV scoring systems (PDAI and ABSIS).
Material and Methods: Patients with newly diagnosed as PV or patients referred with exacerbation or remission were allowed to be included in our study. All patients were evaluated for ENT manifestations by direct and endoscopic examination at the medical visit to our hospital, whether or not they had symptoms. The severity scores (PDAI and ABSIS) of the patients were calculated before and after ENT examination and the results were compared for correlation and significance.
Results: ENT score was found to be highly correlated with PDAI mucosal score ($r=0.918$, $p<0.05$), ABSIS mucosal score ($r=0.869$, $p<0.05$). The severity of the mucosal involvement in PDAI and/or ABSIS was not a reflection of the exact disease severity. The mucosal involvement was more severe when scored by ENT score than mucosal affection assessed by PDAI or ABSIS. ENT score was significantly associated with symptoms and endoscopic findings, especially PDAI ≥15 and/or ABSIS≥17 ($p<0.05$).
Conclusion: ENT endoscopic examination could result in more accurate grading in pemphigus. Especially it should be considered to perform in patients especially when PDAI≥15 and/or ABSIS≥17, regardless of ENT symptoms.

POSTER 10

High prevalence of autoantibodies targeting collagen XVII in mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is a heterogeneous autoimmune blistering disorder which mainly affects mucosal regions. Various molecules of the basement membrane zone have been reported as autoantigens in MMP. Based on the reactivity of indirect immunofluorescence using 1M NaCl salt-split skin (ss-IIF), MMP autoantibodies (autoAbs) can be classified into two groups. AutoAbs directing COL17 and BP230 react with the epidermal side, whereas those directing laminin-332 react with the dermal side. Previous studies using immunoblotting or ELISA with bacterial recombinant proteins have shown that around 50-70% of MMP autoAbs directing the epidermal side in ss-IIF react with the C-terminus of COL17, which is thought to be the main epitope for this type of MMP autoAb. However, other regions of COL17 can be targeted by MMP autoAbs. Based on these findings, we hypothesize that an ELISA using full-length recombinant COL17 (full-length COL17 ELISA) will be useful for detecting MMP autoAbs, because the system can detect autoAbs targeting entire regions of COL17. We performed full-length COL17 ELISA using 17 MMP sera. The full-length COL17 ELISA detected IgG autoAbs in 12 of 17 MMP cases (70.6%), indicating that COL17 is the major autoantigen in MMP. Notably, all 11 MMP cases in which autoAbs reacted with epidermal side in ss-IIF showed positive reactivity by full-length COL17 ELISA, suggesting that MMP autoAbs reacting with the epidermal side in ss-IIF target COL17. In conclusion, the present study shows that COL17 is the main autoantigen in MMP, especially in cases with autoAbs targeting the epidermal side in ss-IIF.

POSTER 11

Diagnostic value of linear fluorescence of the basement membrane of sweat ducts in bullous pemphigoid

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Linear IgG deposits along the basement membrane of adnexa have been found useful for the diagnosis of bullous pemphigoid (BP), but no controlled studies have been performed. We aimed to evaluate the fluorescence pattern and intensity of sweat gland ducts (SGD) and other adnexal structures in BP and to compare them with a control group in order to assess their diagnostic performance.
DIF sections of 64 BP and 82 control patients were examined for the fluorescence pattern and intensity of IgG. SGDs in papillary, mid and deep dermis, hair follicles and sebaceous glands were assessed separately. The fluorescence intensity was graded semi-quantitatively.
All BP patients showed linear immunofluorescence along the epidermal basement membrane with IgG, whereas control skin samples did not. Positive SGDs were found in 58 (90.6%) BP and 44 (53.7%) control patients, the difference was statistically significant. The sensitivity of positive SGDs was high (90.6%), but the specificity low (46.3%). Only strong fluorescence intensity was associated with higher specificity (up to 100%).
In conclusion, positive SGDs in DIF are highly sensitive for BP; however, only strong fluorescence has acceptable specificity. Evaluation of the location, fluorescence pattern and intensity of SGDs can be useful in the diagnosis of BP, but SGDs fluorescence without linear fluorescence of the epidermal basement membrane cannot be recommended as the basis for diagnosis of BP if the signal is low.

POSTER 12

Paraneoplastic pemphigus-specific autoantibodies cause various staining patterns on IIF substrates

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Paraneoplastic pemphigus (PNP) is an autoimmune blistering disease which is coinciding with the presence of certain neoplasms. Autoantibodies, indicative for PNP are directed against diverse antigens within the epidermal spinous layer, mostly plakin proteins but also desmogleins, plectins, BP230 or α-2-macroglobin-like-1. In indirect immunofluorescence (IIF), rat bladder, which is rich in plakins, is recommended as substrate for determination of circulating autoantibodies in PNP. However, varying combinations of co-existing autoantibodies challenge the recommendation of a single substrate and the definition of a specific fluorescence pattern. We analysed the reactivity of PNP patient sera on different tissues and assembled a compilation of emerging fluorescence patterns.
Sera of 20 clinically confirmed PNP patients were tested in IIF applying following substrates: primate oesophagus, bladder, gall bladder, liver, pancreas and diaphragma, rat bladder, human fetal epidermis and the purified antigens desmoglein 1 and 3 as well as BP180 and BP230.
Rat bladder (100%) as well as primate oesophagus (85%) and diaphragma (80%) revealed the highest reactivity with PNP patient samples. In particular, the mucosal membrane and the transitional epithelium of bladder, the oesophageal desmosomes and the Tunica serosa were stained by IgG. Monospecific reactions were most frequently observed with desmoglein 3 (60%).
Our results confirm bladder as most sensitive substrate for the detection of PNP-specific autoantibodies. However, different fluorescence staining of the mucosa and the transitional epithelium may be relevant. Still, diverse reactions of the individual samples were seen with the tissues in general suggesting a benefit of parallel testing on different substrates.

POSTER 13

Detection of IgG autoantibodies against desmocolin – 3 in Greek patients with pemphigus vulgaris and pemphigus foliaceus

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Pemphigus represents a spectrum of autoimmune bullous disorders caused by autoantibodies against desmosomal cadherins. The most common clinical forms are pemphigus vulgaris (PV) and pemphigus foliaceus (PF). Basic target antigens in PV are desmoglein-1 (DSG-1) and desmoglein-3 (DSG-3), while in PF is DSG-1. Among the numerous proteins that are considered responsible for the cohesion of keratinocytes in epidermis, desmocolin-3 (Dsc-3) has been initially reported to participate in epidermal blistering in mice. In recent years, there have been reports, such as case series of patients with pemphigus herpetiformis or with paraneoplastic pemphigus and pemphigus cases with atypical immunological profile, in which autoantibodies against Dsc-3 have been detected. In PV, a limited number of studies showed no presence of IgG or IgA autoantibodies against Dsc-3.
Aim of this study was to examine sera from Greek patients with PV and PF for the presence of IgG autoantibodies against Dsc-3. Fifty three patients were recruited, 45 patients with PV and 8 with PF. Diagnosis of PV or PF was established with histology, immunofluorescence techniques (direct and indirect) and commercial ELISAs (anti DSG-1, anti DSG-3). Immunoblotting for the detection of autoantibodies against Dsc-3 was performed in sera from all cases, according to the published protocol. Dsc-3 autoantibodies were not detected in both groups (PV and PF). Our results confirm the option that the pathogenetic role of Dsc-3 in epidermal blistering in PV and PF remains controversial.

POSTER 14

Multiple target antigens in autoimmune bullous disorders: does the phenotype represent epitope spreading?

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Epitope spreading may explain the phenotypic variety of both intraepidermal and subepidermal autoimmune bullous diseases (AIBD). There are many cases of (AIBD) in the literature with atypical clinical and immunological profile, in which reactivity against multiple antigens is detected.
We present four distinct cases, three in the spectrum of subepidermal AIBD and one in the pemphigus spectrum, with a complex reactivity. Diagnosis was based on the classic diagnostic methods, such as histology, immunofluorescence techniques (direct and indirect) and commercially available ELISAs. Additionally, when the findings were not pathognomonic, the identification of target antigens was achieved with immunoblotting.
In the first case, with clinical and histological features of bullous pemphigoid, the target antigens were 135kDa, 160kDa, 180kDa and 190kDa. The second case, with atypical clinical features but subepidermal detachment in histology, revealed reactivity against 140kDa (Laminin-332, β_3 subunit) and 190kDa. The third patient had crusted lesions on the trunk, histology of a subepidermal blistering disease and antibodies against three proteins of low molecular weight, about 60kDa, 50kDa and 40kDa respectively. The last case, although pemphigus vulgaris was diagnosed with all established diagnostic procedures, an additional detection of antibodies with molecular weights 100kDa and 127kDa, by immunoblotting, expanded the patient's immunological profile.
The first and the last case were quite recalcitrant to therapy cases and a possible underlying paraneoplastic etiology remains to be elucidated during short and long term follow up.

POSTER 15

Diagnostic value of laser scanning confocal microscopy in mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is a heterogeneous group of autoimmune blistering disorders with high predilection to mucosa involvement.
The aim of the study was to differentiate autoimmune blistering disorders with exclusive or predominant involvement of mucous membranes using laser scanning confocal microscopy (LSCM) with subsequent antigen characterization by immunoblotting. We enrolled to the study 29 patients who fulfilled clinical criteria for MMP (chronic erosions located in the oral cavity, conjunctive, pharynx and larynx) and presented linear IgG/IgA deposits along the basement membrane zone (BMZ) and circulating IgG anti-BMZ antibodies by immunofluorescence.
In 5 out of 29 patients LSCM showed IgG deposits located above laminin 5, typically for bullous pemphigoid (BP). In this group NC16a antigen was the main antigen. In 19 of 29 – IgG deposits were located below laminin 5 and above type IV collagen, characteristic of MMP. Some sera in this group reacted with different epitopes of BP180 kD antigen, however majority of them were negative by immunoblotting. In one of 29 patients IgG colocalized with laminin 5 in LSCM and his serum reacted with laminin 5, typically for antiepiligrin cicatricial pemphigoid. In 4 of 29 cases IgG was observed below type IV collagen, characteristic of epidermolysis bullosa acquisita (EBA).
Our study disclosed that mucous membrane involvement is not patognomonic for MMP and may also be observed in BP and EBA cases. LSCM is useful in routine diagnostics in cases of subepidermal blistering disorders with mucosa involvement, particularly with undetectable circulating anti-BMZ antibodies. Prompt diagnostics of this disorders is crucial in a term of therapy and prognosis.

POSTER 16

A multivariant profile ELISA for one-step diagnostics of autoimmune bullous dermatoses

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Autoimmune bullous dermatoses (AIBD) are characterized by autoimmunity to structural proteins of desmosomes (pemphigus) and dermal-epidermal junction (pemphigoid disease) resulting in extensive blistering of skin and/or mucous membranes. Major antigens are BP180, BP230, collagen type VII, desmoglein 1 and 3, and envoplakin. Here we present an ELISA for parallel determination of relevant serum autoantibodies.
The ELISA is based on recombinant fragments of the immunodominant region of BP180, BP230, collagen type VII, desmoglein 1 and 3, and envoplakin as antigenic substrates, each applied in a separate reagent well. Its performance was compared to conventional stepwise diagnostic decision strategy including immunofluorescence with frozen tissue sections, antigen dots, and immunoblots.
Analyzing 158 sera from AIBD patients and 145 control individuals, sensitivities from 60% (anti-BP230, bullous pemphigoid) to 100% (anti-desmoglein 3, pemphigus vulgaris) and specificities from 97.3% (anti-BP180 NC16A) to 100% (anti-collagen type VII) were reached. In a prospective study with 289 consecutive sera from patients with suspected AIBD, the ELISA and the conventional diagnostic strategy yielded identical results in 89% of patients.

The ELISA represents a sensitive and specific multiparametric test system for one-step diagnostics of pemphigus and pemphigoid diseases, in high accordance to conventional stepwise diagnostics. Discrepancies could be attributed to the absence of certain target structures (e.g. p200, laminin 332, BP180 ectodomain), and the lack of IgA autoantibody detection.

POSTER 17

Pemphigus herpetiformis: clinical, pathologic and immunopathologic features in a series of six patients

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Pemphigus herpetiformis (PH) is a rare distinct disease entity combining the clinical features of dermatitis herpetiformis with immunopathologic findings of pemphigus. To date approximately hundred cases of PH have been reported in the literature but autoantibody profile was assessed in few of them, showing reactivity mostly with desmoglein 1 (Dsg1).
The aim of our study was to analyze the clinico-laboratory characteristics in six PH patients whose diagnosis was confirmed based on clinical, histologic, direct (DIF) and indirect immunofluorescence (IIF) findings. Serum autoantibody specificity was determined in five patients using ELISA anti-Dsg1 and 3.
All patients were women, aged 24-82. Associated conditions were present in five of them, including psoriasis, thymoma, breast and uterine cancers, and latent syphilis. Histology and DIF findings were compatible with superficial pemphigus in most cases. IIF was positive in five out of six patients on monkey eosaphagus and negative in all using rat bladder substrate. High values of anti-Dsg1 antibodies were present in all five tested sera whereas only one was strongly positive for anti-Dsg3.
Our results correspond to the published data where Dsg1 and rarely Dsg3 appear to be the major antigenic targets in PH. It remains unclear, however, why the corresponding IgG autoantibodies induce the unique clinico-pathologic manifestations of PH but not the features of the classic pemphigus foliaceus and vulgaris. The role of underlying diseases present in most of our patients could be suspected as contributing to the occurrence of this unusual variant of pemphigus.

Cell biology of desmosomes, disease pathways in pemphigus

POSTER 18

The soluble form of Fas Ligand plays a pivotal role in blister formation in pemphigus

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Although anti-desmogleins (dsgs) and non-dsgs antibodies (Ab) are essential for the development of pemphigus, the molecular mechanisms underlying cell-to-cell detachment (acantholysis) are still to be fully clarified. We have demonstrated that PVIgG induce the up-regulation and release of Fas Ligand (FasL) in keratinocytes, while high levels of FasL are present in sera from pemphigus patients. We investigated the role of FasL in the induction of the

acantholytic process in pemphigus. Human rFasL induces a dose- and time-dependent degradation of both dsg1 and dsg3 in association with the acantholysis in vitro. Keratinocytes treated with FasL siRNA are protected from dsg cleavage and acantholysis induced by PVIgG. Using the passive transfer mouse model, we demonstrated that PVIgG induce the increase of FasL between 1 and 3 hr at the skin level, while anti-FasL Ab blocks blister formation. To definitely dissect the role of FasL in the pathogenesis of pemphigus, we injected PVIgG into two different gene-targeted mutant mice that selectively lack either the secreted soluble FasL (sFasL; $FasL^{Ds/Ds}$: C57BL/6-Del494) or the membrane-bound FasL (mFasL; $FasL^{Dm/Dm}$: C57BL/6-Del478). 20 hrs after PVIgG treatment, clinical blisters are visible only in $FasL^{Dm/Dm}$ animals, lacking the mFasL, but still secreting sFasL, in a way similar to WT animals. On the other hand, a statistically significant decrease in the relative acantholytic area is observed only in $FasL^{Ds/Ds}$ animals. Taking together, these results point to a pivotal role of the soluble form of FasL in the blister formation in pemphigus.

POSTER 19

IFNγ and IL-17A producing desmoglein 3 and bullous pemphigoid antigen 180 specific T cells in lichen planus

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Lichen planus (LP) is a chronic inflammatory disorder of skin and mucous membranes whose immune pathogenesis has been linked to a T cell-mediated immune response against epidermal keratinocytes. Some clinical phenotypes of LP show striking similarities with pemphigus vulgaris (PV) and bullous pemphigoid (BP), respectively. In the present study, we analysed reactivity and cytokine profile of peripheral T lymphocytes from 28 LP patients, 7 BP patients and 18 healthy controls against the autoantigens of BP, BP180 and PV, Dsg3 and Dsg1, respectively, by ELISPOT analysis. Ex vivo stimulated T cells were monitored for the release of interferon γ (IFNγ), interleukin-5 (IL-5) and interleukin 17A (IL-17A). There was a statistically significant higher number of IFNγ and IL-17A-secreting T cells reactive with the immunodominant NC16a domain but not with the COOH-terminus of BP180) in patients with cutaneous LP. Moreover, several LP patients with preferential oral involvement showed IFNγ-dominated T cell reactivity against Dsg3, the autoantigen of PV. IL-5-secreting T cells reactive with BP180 and Dsg3 were not significantly increased in LP. In contrast to LP, the studied BP patients showed IL-5-dominated Th2 responses against the NC16a domain of BP180. These findings show for the first time that LP is associated with a Th1/Th17 dominated T cellular response against BP180 and Dsg3, the autoantigens of BP and PV, respectively, which are linked to a Th2-driven pathogenesis. We thus propose that LP is the Th1/Th17 dominated pole of a spectrum of autoimmune skin disorders which target autoantigens of the skin, such as BP180 and Dsg3.

POSTER 20

Polyclonal nature of pemphigus foliaceus IgG antibodies enhances pathogenic effect for blister formation in association with p38 MAPK-dependent desmoglein 1 clustering.

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Polyclonal anti-desmoglein 1 (Dsg1) autoantibodies in pemphigus foliaceus (PF) patients sera contain both pathogenic (P) and non-pathogenic (NP) autoantibodies as shown by isolation of monoclonal antibodies (mAbs). To study how those mAbs contribute to blister formation, effects of a single mAb or a mixture of mAbs were compared in organ cultured human skin and in primary keratinocytes. A mixture of P and NP IgG mAbs showed IgG deposition throughout the epidermis but an aberrant granular pattern co-localized with Dsg1 and induced blister formation in the superficial epidermis. In comparison, a single P mAb caused blisters without inducing this Dsg1 clustering. Studies using IgG and scFv (single chain variable fragments) forms of P and NP mAbs indicated that Dsg1 clustering required both cross-linking of Dsg1 and trans-interaction blocking by a P mAb. Furthermore, studies using small molecule inhibition of p38 MAPK showed that this Dsg1 clustering required p38 MAPK signaling. When pathogenic activity was measured by an in vitro dissociation assay, the mixture of P and NP IgG mAbs disrupted keratinocytes adhesion more than the single P mAb. This pathogenic effect was only partially suppressed by the p38MAPK inhibitor. These findings indicated polyclonal mixture of anti-Dsg1 IgG antibodies enhanced pathogenic activity for blister formation associated with p38MAPK-dependent Dsg1 clustering. In conclusion, not only pathogenic Abs but also non-pathogenic Abs coordinately contribute to blister formation in PF.

POSTER 21

Characterization of IL-21-producing cells in pemphigus vulgaris

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The production of auto-antibodies (auto-ab) in pemphigus vulgaris (PV) depends on different CD4+ T cell subsets producing a variety of cytokines that are crucial for the induction of the auto-ab response. However, the role of disease promoting cytokines in PV has not yet been fully characterized. Our work focuses on IL-21, a pleiotropic cytokine promoting B cell proliferation and antibody production that is produced by several T helper (Th) cell subsets, including T follicullar helper (Tfh) cells, Th17 as well as CD4+ natural killer T (NKT) cells. In a cross-sectional study including PV patients and healthy controls, peripheral blood was analysed for IL-21 production by CD4+ T cells and for IL-21 cytokine plasma levels. We observed increased concentrations of IL-21 in plasma of PV patients compared to healthy controls. For the first time, we could detect autoreactive Dsg3-specific T cells producing IL-21 in peripheral blood of PV patients upon ex vivo stimulation with recombinant Dsg3 by ELISpot-assay. Th17 and CD4+CXCR5+ Tfh cells are both predominant cellular sources of IL-21 and were significantly elevated in peripheral blood of PV patients suggesting a potential implication of IL-21-producing T cells in PV pathogenesis. Finally, ongoing experiments aim at further analyzing IL-21 production also in other CD4+ cell populations such as NKT cells in peripheral blood of PV patients. The more defined characterization of IL-21-producing cells in PV will lead to a better understanding of disease pathogenesis and finally it may contribute to novel, specific therapeutic options for PV in the future.

POSTER 22

Desmoglein-specific immunoadsorption abolishes the pathogenic potential of IgG from patients with pemphigus vulgaris

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Pemphigus vulgaris (PV) is a life-threatening autoimmune blistering skin disease affecting the skin and surface-close mucous membranes. Patients predominantly develop autoantibodies against desmoglein 1 and 3 (Dsg 1, Dsg 3) that reduce cell-cell-adhesion between keratinocytes. However, fundamental doubt has been raised whether anti-Dsg 1 and anti-Dsg 3 autoantibodies alone can reproduce the pathological hallmarks of PV, i.e. desmosome degradation, acantholysis, and intraepidermal splitting. In this study, we investigated the pathogenic potential of autoantibodies from three PV patients using three different pemphigus models; the desmosome degradation assay, the dispase assay, and the neonatal mouse model. PV IgG was affinity-purified by the use of the entire Dsg 3 (and Dsg 1) ectodomain recombinantly expressed in HEK293 cells. Dsg 3/1-depleted IgG of the three PV patients did not induce internalization of Dsg 3, did not cause acantholysis in the dispase-based dissociation assay, and did not lead to macroscopic or microscopic blister formation when injected into neonatal mice (n=3). In contrast, the Dsg 3-specific IgG fractions showed a concentration-dependent internalization of Dsg 3, an increased fragmentation of keratinocyte monolayers, and induced macroscopic blistering in neonatal mice. In summary, our observations suggest that anti-Dsg 3/1 autoantibodies are a prerequisite for the induction of major pathophysiological characteristics in PV.

POSTER 23

Pemphigus autoantibodies induce blistering in human conjunctiva

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Pemphigus autoantibodies directed against the desmosomal cadherins Desmoglein (Dsg) 1 and 3 lead to flaccid blistering of the skin and mucous membranes of the oral cavity. However, some patients develop an ocular involvement which is most frequently diagnosed as conjunctivitis, but the underlying mechanisms are not elucidated yet. We therefore characterized human and murine conjunctiva with respect to the PV autoantigens and evaluated the effects and mechanisms of PV autoantibodies applied to human conjunctiva ex vivo. We first studied a mouse model depleted of the desmosomal cadherin desmoglein 3 (Dsg3), the primary autoantigen in PV. Further, human conjunctiva specimens from surgical explants were obtained and a short-term culture model to study the alterations induced by antibody fractions of PV patients (PV-IgG) was established. Both, murine and human conjunctiva sample expressed the majority of desmosomal molecules with an expression pattern similar to the epidermis. Interestingly, Dsg3 knock out animals frequently develop eye lesions, histologically evident as microblisters in the eyelid epidermis and conjunctiva. Incubation of human specimens with IgG-fractions of pemphigus vulgaris patients (PV-IgG) for 12 h caused blistering in the suprabasal layers of the conjunctiva, resembling the effects of PV-IgG in the epidermis. Concomitantly, a reduction of Dsg1 and 3 levels was detectable. Furthermore, PV-IgG prompted activation of p38MAPK in the conjunctiva, which is a central pathomechanism leading to blistering in the epidermis. Taken together, these models indicate that the ocular involvement observed in PV patients is mainly based on conjunctival blistering.

POSTER 24

Keratin filaments control binding properties of desmosomal cadherins – contribution to loss of cell cohesion in pemphigus?

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Keratin retraction is considered a microscopical hallmark in pemphigus. It describes the collapse of the intermediate filament (IF) cytoskeleton and its detachment from desmosomes, which correlates well with loss of intercellular adhesion. Indeed, it is an early event in human keratinocytes when exposed to IgG-fractions of pemphigus vulgaris patients (PV-IgG) as revealed by immunostaining and atomic force microscopy (AFM). However, the significance of these keratin filament alterations for PV-IgG-induced loss of cell cohesion is unknown. To investigate the relevance of keratin filaments for the binding properties of desmosomal cadherins we here used murine keratinocytes lacking all keratin filaments (KtyII k.o.) and compared them with their wildtype (KtyII wt) controls. The k.o. cell line displayed reduced intercellular adhesion compared to KtyII wt as demonstrated by dissociation assays. Surprisingly, protein levels and membrane localization of Dsg3 was increased in KtyII k.o. cells. In line with this, by using AFM, a higher frequency of Dsg3-mediated binding events on the plasma membrane was observed in KtyII k.o. cells. However, the binding forces of individual Dsg3-mediated binding events were significantly reduced compared to KtyII wt cells. These data show for the first time that keratins, potentially by anchoring the desmosomal complex or by the regulation of intracellular signaling, control the surface levels and binding properties of Dsg3. Further, it introduces a possible mechanism how keratin filament retraction contributes to reduced intercellular adhesion in PV.

POSTER 25

Desmoglein 3 balances quiescence versus activation in the hair follicle stem cell niche

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Hair follicle (HF) stem cells (SC) are set aside in a specialized niche called the bulge to support lifelong HF cycling including HF regeneration. How the HF bulges are repaired in case of injury is not well understood. Here we used the skin disease pemphigus vulgaris (PV) with anti-desmoglein 3 antibodies (AK23) as a model to disrupt intercellular adhesion in the mouse resting (quiescent) bulge HF stem cell (BuSC) niche. This mouse model mimics the acantholysis seen in PV patients, allowing us to investigate both the pathogenic and repair phases of the disease.
Here, we focused on the long-term repair mechanisms and in particular on the specific signaling pathways modulating repair. Combining functional analyses with SC marker expression and associated pathways we defined these repair-associated processes. In short, loss of Dsg3 function followed by disrupted cell-cell adhesion (inducing bulge lesions without hair loss) activates quiescent BuSC resulting in a loss of stemness, as seen by colony forming efficiency assay and reduced BuSC markers (such as CD34). This is then followed by a recovery in stemness and repair through proliferative mechanisms different from anagen induction by involving the EGFR pathway.
Our findings uncovered ingenious protective measures of the injured HF niche to recover its multipotent BuSC without anagen induction; therefore having potential use for PV patients as we can further target inductive mechanisms. However we must be careful as we have shown that EGFR inhibition, while preventing blistering, hampers repair.

POSTER 26

Loss of flotillin expression results in weakened desmosomal adhesion and Pemphigus vulgaris-like localisation of desmoglein-3 in human keratinocytes

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Desmosomes are adhesion plaques that mediate cell-cell adhesion in many tissues, including the epidermis, and generate mechanical resistance to tissues. The extracellular domains of desmosomal cadherin proteins, desmogleins and desmocollins, are required for the interaction with cadherins of the neighbouring cells, whereas their cytoplasmic tails associate with cytoplasmic proteins which mediate connection to intermediate filaments. Disruption of desmosomal adhesion by mutations, autoantibodies or bacterial toxins results in severe human disorders of e.g. the skin and the heart. Despite the vital role of desmosomes in various tissues, the details of their molecular assembly are not clear. We here show that the two members of the flotillin protein family directly interact with the cytoplasmic tails of desmogleins. Flotillins are ubiquitously expressed and are associated with membrane rafts. Flotillins function in various cellular processes such as signalling and membrane trafficking. Here we show that depletion of flotillins in human keratinocytes results in weakened desmosomal adhesion and reduced expression of desmoglein-3, most likely due to a reduction in the desmosomal pool due to increased turnover. In the absence of flotillins, desmoglein-3 shows an altered localisation pattern in the cell-cell junctions of keratinocytes, which is highly similar to the localisation observed upon treatment with pemphigus vulgaris autoantibodies. Thus, our data show that flotillins are of importance for the desmosomal cell adhesion.

POSTER 27

p38MAPK signalling contributes to blistering and reduction of desmosome size induced by pemphigus autoantibodies in human epidermis.

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Introduction: Pemphigus vulgaris (PV) is a skin blistering disease caused by autoantibodies targeting the adhesion proteins desmoglein 1 and 3. These proteins together with others form the desmosomes which are adhesive contacts vital for cell cohesion. The mechanisms underlying pemphigus skin blistering are not fully elucidated, but p38MAPK activation is one of the signalling events necessary for full loss of cell cohesion. However, it is unclear whether intracellular signalling mediates the hallmark changes in desmosome morphology. In this study, we thus tested the p38MAPK dependency of blister formation and of the ultrastructural changes induced by PV autoantibodies in human skin.
Methods: Ex vivo human skin model, transmission electron microscopy, serial cryosectioning, H&E, immunostaining.
Results: Injection of IgG fractions of PV patients (PV-IgG) as well as the monoclonal Dsg3 antibody from a PV mouse model AK23 induced p38MAPK activation, interdesmosomal widening, formation of split desmosomes and a reduction of desmosome size. In contrast, full epidermal blister formation and lower desmosome numbers were evident in tissue samples exposed to PV-IgG only. Pharmacologic inhibition of p38MAPK blunted the reduction of desmosome size, ameliorated interdesmosomal widening and prevented blister formation.

Conclusion: Our data demonstrate for the first time that skin blistering can be prevented by inhibition of p38MAPK in human epidermis. Moreover, typical morphologic alterations induced by PV-IgG such as interdesmosomal widening and the reduction of desmosome size at least in part require p38MAPK signalling.

POSTER 28

Autoantibody profiles determine signaling patterns and loss of keratinocyte cohesion in pemphigus

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We characterized the mechanisms engaged by pemphigus autoantibody fractions from patients with different clinical phenotypes and autoantibody profiles. All pemphigus vulgaris (PV) autoantibody fractions as well as AK23 caused loss of cell cohesion in the dispase-based dissociation assay and compromised binding of Dsg3 but not Dsg1 as revealed by AFM. Strong alterations in Dsg3 distribution were caused by both mu-cocutaneous PV-IgG (antibodies targeting Dsg1 and Dsg3) as well as by a fraction from an atypical case of pemphigus where skin blistering was associated with antibodies against Dsg3 only. The pathogenic effects were accompanied by activation of p38MAPK and Src. In contrast, rapid Ca++ influx and Erk activation were induced by mucocutaneous PV-IgG and pemphigus foliaceus (PF)-IgG only. Interestingly, selective inhibition of p38MAPK, Src or PKC each was effective to block loss of keratinocyte cohesion in response to all autoan-tibody fractions. In contrast, the Erk inhibitor U0126 was protective against mucocutaneous IgG only. These results indicate that i) direct inhibition of Dsg3 binding and activation of p38MAPK and Src can be caused by antibodies targeting Dsg3; ii) other signaling mecha-nisms such as Ca++ influx and Erk activation are stimulated by IgG containing autoantibod-ies against Dsg1 and may be related to epidermal blistering; iii) p38MAPK, Src and Ca++-induced PKC activation appear to be part of the machinery regulating baseline desmo-some turnover whereas Erk is recruited in pemphigus. This concept helps to explain why in several models of pemphigus inhibition of one single signaling pathway often is protective.

POSTER 29

Pemphigus vulgaris autoantibodies cause invaginations of one cell into another

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Pemphigus vulgaris (PV) is an autoimmune blistering skin disease were autoantibodies against desmoglein 3 (Dsg3) cause blistering of the mucous membranes. This occurs due to the loss of cell-cell adhesion in the suprabasal layers of the mucous epithelium. Dsg3 is one of the four known isoforms of the desmogleins, which are building proteins of desmosomes, cell-cell contacts that interconnect neighboring cells to each other. How autoantibodies against Dsg3 cause loss of cell-cell adhesion is not completely understood, although several hypotheses exist. We studied the effects of Dsg3 autoantibodies from pemphigus vulgaris patients on Dsg3 in the primary human keratinocytes using light microscopy, time-lapse imaging and correlative light and electron microscopy. PV autoantibodies (PV

IgG) induce internalization and relocalization of transmembrane Dsg3 into clusters and arrays in primary human keratinocytes. Arrays of labeled PV IgG are dynamic structures that tend to change shape and fuse/defuse when followed in real time. PV IgG/Dsg3 arrays colocalize with keratin filaments and other components of desmosomes. When analyzed using correlative light and electron microscopy, arrays are utrastructuraly invaginations of one cell into another with rudimentary desmosomal plaques present. Although PV IgG induced relocalization of Dsg3 and other desmosomal components, loss of cell-cell adhesion was not induced due to the persistance of desmosomes by compensatory desmoglein 2 in culture. Our results support the desmoglein depletion theory of pemphigus pathogenesis. Internalization and relocalization of Dsg3 induced by PV IgG lead to its depletion in desmosomes, what causes loss of cell-cell adhesion in the areas where no compensation is present.

POSTER 30

Circulating plasmablasts exhibit increased antibody diversity in pemphigus vulgaris

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Pemphigus vulgaris (PV) is a prototypic autoimmune blistering skin disease characterized by blister-inducing anti-Desmoglein (Dsg) autoantibodies. While the pathogenic role of autoantibodies is well established, the immunological processes leading to Dsg-directed autoimmunity are not fully understood. In this study, we addressed the question whether the immune repertoire reveals properties that might be associated with PV. We sorted 4 B cell subpopulations representing different developmental stages, i.e.naïve B cells, IgM+ and IgM- memory B cells, and plasmablasts, from 10 patients and 10 controls. Subsequently, the respective B cell receptors were analyzed by next generation sequencing. For the variable heavy chain gene regions (VHs) of patients and controls, no significant differences were found in all B cell populations with regard to VH and DH gene usage, the number of mutations per VH sequence, and the replacement to silence ratio. The overall number of clones was similar between cases and controls for the naïve and memory B cell populations. When compared to controls, a reduction in clone numbers was observed in patient-derived plasmablasts which, in addition, exhibited both significantly less diversity in their VH-CDR3 aa composition. The inter-individual variability of the VH and DH gene usages, especially in plasmablasts, was found to be significantly different between PV patients and controls. These results might be explained by a relaxed peripheral control of autoreactive B cells. This hypothesis is in line with the genetic association of PV with functionally relevant Fcγ receptor IIb and IIc polymorphisms that are possibly involved in the respective control mechanisms.

POSTER 31

Functional effects of anti-thyroid peroxidase antibodies on Pemphigus vulgaris-linked keratinocyte signaling and cell-adhesion

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Increasing evidence suggests that patients with Pemphigus vulgaris (PV) harbor autoantibodies (autoAbs) directed at targets additional to desmoglein (Dsg) 3 and 1. We have previously identified a subset of patients that express autoAbs against thyroid peroxidase (TPO), and demonstrated that commercially available anti-TPO Abs disrupt cell-cell adhesion in vitro and modulate signaling pathways relevant to blister formation. To investigate the extent to which anti-TPO autoAbs in patient sera play a role in disease pathogenesis, we evaluated how depletion of anti-TPO Abs affects the activity of PVIgG in several in vitro assays. We show that depletion of anti-TPO Abs from IgG purified from patient sera (PVIgG) dampens PVIgG-induced a) p38MAPK activation, b) increases in intracellular calcium levels, and c) keratinocyte dissociation in vitro. The effects observed after anti-TPO depletion are less dramatic compared to depletion of anti-Dsg3 autoAbs in serum containing both anti-TPO and anti-Dsg3, but are more pronounced when using PVIgG from patients harboring anti-TPO but not anti-Dsg3 or -1 autoAbs. Additionally, anti-TPO purified from patient sera can significantly induce keratinocyte dissociation. However, PVIgG from patients not possessing anti-Dsg3, -Dsg1, or -TPO Abs can still activate p38MAPK, increase intracellular calcium levels and interfere with cell-cell adhesion. Collectively, our data suggests that anti-TPO autoAbs may contribute to disease pathogenesis in PV and that, in some cases, autoAbs directed against targets additional to TPO, Dsg3, or Dsg1 may be relevant to disease. Further studies are needed to characterize the broader scope, specificity, and pathogenicity of the autoAb response in PV in vivo.

Cell biology of hemidesmosomes, disease pathways in pemphigoid

POSTER 32

IL-17A governs tissue destruction in bullous pemphigoid

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Bullous pemphigoid (BP), the most frequent autoimmune blistering skin disease, mainly affects elderly patients. Treatment requires long-term use of superpotent topical or oral corticosteroids. More specific and safer therapies are urgently needed. Autoantibodies against two hemidesmosomal proteins, BP180 and BP230, are an immunopathological hallmark of BP. Binding of anti-BP180 autoantibodies prompt secretion of inflammatory mediators, reactive oxygen species, and proteases, creating subepidermal splitting. IL-17A provokes the production of additional chemokines and cytokines, collectively causing recruitment of neutrophils. Elevated serum levels of IL-17A and IL-17-producing cells in perilesional skin of BP patients have been reported. We intended at detailing the impact of IL-17A and IL-17-related cytokines in BP patients and explore the functional relevance of IL-17 in vivo. We observed significantly higher levels of IL-17A on circulating CD4+cells in peripheral blood and higher IL-17A

mRNA expression in perilesional skin biopsies in BP patients compared to controls. In perilesional patient skin, CD3-positive-T cells were the cell type with the highest IL-17A production while mast cells were identified as major source of this cytokine. Moreover, IL-17A-deficient mice were protected from the pathogenic effect of anti-BP180 IgG and IL-17A-related cytokines were down-regulated. Application of an anti-IL-17A antibody in a prophylactic and a quasi-therapeutic setting (initiated before and after first skin lesions were seen, respectively) resulted in considerably less disease in the autoantibody-transfer-induced mouse model of BP. These data imply that IL-17A regulates crucial steps in the pathophysiology of BP and thus, IL-17A inhibition may expand the future therapeutic armamentarium for this fragile patient population.

POSTER 33

Loss of C-terminal domain induces neoepitopes on processed collagen XVII.

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Transmembrane collagen XVII (COL17/ BP180) is a hemidesmosmal linker protein between the epidermis and dermis which can be targeted by autoantibodies in autoimmune blistering disorders, including linear IgA bullous dermatosis (LAD). The ectodomain of COL17 is known to be physiologically or pathologically cleaved within the juxtamembranous noncollagenous NC16A region, which results in the 120kD (LAD-1) and the further C-terminally processed 97kD (LABD-97) ectodomains. Significantly, LAD autoantibodies preferentially target LAD-1 and LABD-97 but not full-length COL17, indicating proteolysis within the NC16A domain induces neoepitopes on the cleaved COL17 ectodomains. However, little is known about how neoepitopes are produced on processed COL17, especially in relation to C-terminal cleavage. This study shows both ectodomain shedding and the C-terminal cleavage are associated with the development of neoepitopes on processed COL17. We produced a novel monoclonal antibody (mAb) that specifically reacts with LAD-1 and LABD-97, but not with full-length COL17. To investigate the roles of C-terminal cleavage in the development of neoepitopes, we produced a recombinant COL17 (COL17-ΔC; Met1-Arg1174) with a C-terminus 323 amino acids shorter than the full length COL17 (Met1-Pro1497). Interestingly, the mAb reacted with COL17-ΔC, suggesting cleaving only the C-terminal of COL17 also induces structural changes and exposes comformational neoepitopes on the 15th collagenous domain. Notably, the LAD autoantibodies also reacted with COL17-ΔC, indicating C-terminal processing of COL17 is associated with the autoimmunity of LAD. The present study shows that proteolysis of COL17 induces dynamic structural changes on proteolyzed COL17 and conformational neoepitopes, which are targeted by LAD autoantibodies.

POSTER 34

Contribution of IgE Autoantibodies to the Pathogenesis of Bullous Pemphigoid

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Bullous pemphigoid (BP) is an auto-immune blistering disease that has consistently been associated with IgG autoantibodies and complement activation. Additionally, the urticarial plaques frequently seen in these patients point to an involvement of IgE, corroborated by both ex- and in-vivo animal and human studies. The mechanisms by which IgE contributes to the pathogenesis of BP are however still not understood. Thus, the goal of this on-going study is to investigate the presence and pathogenicity of IgE autoantibodies in BP patients. In line with previous literature, we have detected, via ELISA, significantly higher levels of NC16a- ($p<0,0001$) and BP230-specific ($p<0,005$) IgE in BP sera comparing with healthy controls. Consistent with a class-switch phenomenon, IgG and IgE share the same dominant epitopes, as demonstrated using overlapping peptide sequences of BP180. Furthermore, we have found IgE in perilesional skin of 21 out of 32 (66 %) BP patients. This IgE was not found at the DEJ, but instead on the surface of mast cells and eosinophils, most likely bound as an immune complex. We have evidence that the high-af-

finity receptor for IgE is the primary molecule involved in this interaction and that eosinophils are expressing FcεRI in BP patients. Finally, using direct immunofluorescence we have detected dermal BP180 fragments that appear to colocalize with IgE+ eosinophils, a finding that offers a new hypothesis for IgE's mechanism of action. We have therefore confirmed the association between IgE and BP and propose a pathway of disease pathogenesis alternative to IgG and complement.

POSTER 35

Effect of a classical pathway specific inhibitor on bullous pemphigoid sera-induced complement activation

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Treatment of bullous pemphigoid (BP), the most prevalent autoimmune bullous dermatosis, involves long-term application of highly potent corticosteroids and, therefore, is associated with severe side-effects and a high relapse rate. Caused by autoantibodies against type XVII collagen, BP features activation of the classical complement pathway and dermal infiltration by inflammatory cells. To combat the pronounced medical need for novel BP treatment options, we here evaluated the effect of TNT003, a mouse monoclonal antibody that inhibits the classical pathway specific serine protease C1s, on BP mediated complement activation by employing the indirect complement activation assay. Briefly, human skin sections were incubated with BP sera to form autoimmune complexes followed by addition an exogenous complement source (normal human serum (NHS)).
When performing the assay in the presence of TNT003, indirect immunofluorescence microscopy revealed diminished deposition of C3c at the dermal-epidermal junction in a concentration-dependent manner. Moreover, generation of anaphylatoxins was prevented. Although comparable anaphylatoxin production was observed in patches sensitized with BP patient sera compared with NHS, TNT003 significantly reduced C4a and C5a formation while production of C3a remained unaffected. Furthermore, chemotactic activity of supernatants on neutrophils was diminished in samples with reduced anaphylatoxin formation due to TNT003 treatment. However, blocking complement had no effect on the release of reactive oxygen species by neutrophils.
These data provide evidence that complement activation induced by BP immune complexes can be inhibited in vitro by TNT003. Thus, a humanized version of TNT003 may prove to be an efficacious therapeutic alternative to corticosteroids for BP patients.

POSTER 36

High affinity IgE receptors (FcεRI+) bearing cells in the epidermis of bullous pemphigoid.

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Background: The role of immunoglobulin E (IgE) in the pathogenesis of bullous pemphigoid (BP) is not fully understood.
Objective: To confirm the hypothesis of the presence of high affinity IgE receptor I (FcεRI) expressing cells in the epidermis of BP skin and that Langerhans cells (LC) are equipped with FcεRI in BP and evaluate if the number of epidermal FcεRI+ cells correlates with serum IgE level and disease severity.
Methods: Skin biopsies of lesional and peri-lesional skin of 21 patients with BP, healthy controls and atopic dermatitis patients were stained for FcεRI, CD1a, CD207 and MBP. The density of FcεRI+ and CD207+ cells/mm2 was

assessed with microscopy. Total serum IgE, eosinophlia and BPDAI were assessed in BP patients.
Results: We have found FcεRI+ cells within the epidermis of BP patients and majority of these cells were CD207+ LC. FcεRI+ eosinophils are present in lesional epidermis. We have found a correlation between serum IgE and density of epidermal FcεRI+ cells in peri-lesional skin, between density of lesional FcεRI+ non-LC and severity of erythematous lesions and between density of peri-lesional non-LC cells and blood eosinophilia.
Conclusion: LC are the major population FcεRI+ epidermal cells in BP but non-LC cells such as eosinophils are also present. As we have observed the correlation of serum IgE with epidermal FcεRI+ cells in peri-lesional skin and correlation between disease severity and lesional of FcεRI+ non-LC cells therefore these cells appear to be a natural target for anti-IgE treatment strategies.

POSTER 37

Bullous pemphigoid - Increased activity of eosinophils in blister fluids and peripheral blood

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Bullous pemphigoid (BP) is an autoimmune blistering skin disease characterized by increased numbers of peripheral blood eosinophils. Our aim was to study the functional activity of eosinophils in BP. Blood eosinophils of BP patients displayed a significantly higher expression of CD69 and CD11b in comparison to eosinophils derived from healthy controls. Interestingly, the expression of CD95 in BP eosinophils was higher in addition to an increased number of apoptotic eosinophils 24 hours in culture compared to eosinophils from healthy controls.
In BP we found an almost pure accumulation of 1x10-6 eosinophils per ml of blister fluid. CCL26 levels were significantly higher in BP blister fluids as compared to serum, indicating a possible reason for eosinophil accumulation. Eosinophils in blister fluids displayed a strong expression of CD69 and CD95. Furthermore, CD95 ligand was significantly increased in BP blister fluid as compared to BP sera. Thus, we wanted to clarify whether sera and blister fluid of BP patients were able to functionally activate eosinophils from healthy donors. The expression of CD69 and CD11b was significantly upregulated in eosinophils of healthy donors incubated with BP blister fluid in comparison to BP sera, while CD95 expression remained unchanged. Further, BP blister fluid significantly inhibited apoptosis in eosinophils of healthy donors in comparison to BP upon serum stimulation. Together, our data clearly show that eosinophils in BP patients derived from the peripheral blood as well as from blister fluid are highly activated. Furthermore, BP serum can significantly activate eosinophils derived from healthy controls, underlining an important interplay in the regulation of inflammation in this autoimmune skin disease with eosinophils as main target effector cells.

POSTER 38

Expression of IL-31 in blisters and eosinophils of patients with bullous pemphigoid

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Bullous pemphigoid is characterized by strong tissue and blood eosinophilia and intensive pruritus. Previously we have shown that the pruritogenic cytokine IL-31 is increased in patients with mastocytosis in which it also correlates with disease progression. In other inflammatory skin diseases which are also characterized with pruritus such as chronic spontaneous urticaria and atopic dermatitis, IL-31 serum levels are increased and correlate with disease

activity. Recently, we could show that peripheral blood eosinophils are able to express IL-31. In the present study, we aimed to analyze the expression of IL-31 in bullous pemphigoid.
Using immunofluorescence we analyzed the expression of IL-31 in eosinophils derived from blister fluids (n = 9) and skin (n = 5) from bullous pemphigoid patients and estimated the level of IL-31 in blister fluids and serum by ELISA (n = 13 - 22).
Blister fluids of patients with bullous pemphigoid were characterized by high levels of IL-31. These increased IL-31 levels in blister fluids were comparable to increased IL-31 serum levels of patients with mastocytosis, whereas IL-31 serum levels of bullous pemphigoid patients were as low as in healthy controls. Eosinophils from bullous pemphigoid blister fluids showed a strong IL-31 expression, which we could also assess in skin biopsies of these patients. Together, we show here for the first time that IL-31 is increased in bullous pemphigoid blister fluids and skin in which eosinophils are IL-31 positive. Thus, our data with IL-31 adds novel aspects to the pathophysiology of bullous pemphigoid which may have therapeutic implications in the future.

POSTER 39

A novel mouse model for anti-laminin 332 mucous membrane pemphigoid

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Anti-laminin 332 mucous membrane pemphigoid (MMP) is a subepidermal blistering autoimmune dermatosis characterized by autoantibodies against laminin 332, a structural protein of epidermal/epithelial basement membranes. Most patients develop autoantibodies against the alpha 3 chain of laminin 332. Until now, only little data are available about the pathophysiological mechanisms of this disease. Based on the identification of two immunodominant regions of human laminin alpha 3, we established a novel experimental model in adult C57BL/6 mice by the passive transfer of rabbit IgG raised against the murine homologues of these fragments (mLAMα3). After 12 days of repeated s.c. injection of rabbit anti-mLAMα3 IgG, erosions and crusts occurred predominantly around the snout, eyes, and ears. Interestingly, loss of up to 25% body weight was observed and histopathology revealed conjunctival lesions in about 80% of mice. Lesions in the oral mucosa cavity and oesophagus were present in 80% and 16% of mice, respectively, while stomach and colon were not affected. Direct immunofluorescence microscopy showed IgG and C3 deposits at the basal membrane zone of skin, buccal mucosa, tongue, oesophagus, colon, and conjunctiva. Fc gamma chain-deficient mice were completely protected from the pathogenic effect of anti-LAMα3 IgG and C5aR1-deficient mice developed significantly less disease compared to wildtype animals ($p<0.001$). The extensive involvement of conjunctiva and oral mucosa mirrors the human disease and clearly differentiates the novel model from previously established mouse models of bullous pemphigoid and epidermolysis bullosa acquisita. The anti-mLAMα3 IgG-induced mouse model will allow further dissecting the pathomechanisms of the disease and exploring more specific anti-inflammatory mediators for autoantibody-mediated diseases.

POSTER 40

Dipeptidyl peptidase-IV inhibitor-associated bullous pemphigoid autoantibodies preferentially target the non-NC16A extracellular domain of collagen XVII

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Bullous pemphigoid (BP) is a major autoimmune blistering disease in which autoantibodies (autoAbs) mainly target hemidesmosomal collagen XVII (COL17) and BP230. Around 80-90% of BP sera react with the non-collagenous 16A (NC16A) domain of COL17, which is known to be the major epitope of BP. Although it is still unclear why immune tolerance to COL17 can be broken, BP may develop in association with certain drugs. Recent studies suggest that dipeptidyl peptidase-IV (DPP-4) inhibitors, which are commonly used for type 2 diabetes mellitus, are associated with the onset of BP. However, the epitope profiles of DPP-4 inhibitor-associated BP have not been fully elucidated. In this study, we analyzed the precise epitopes of 10 BP cases which received DPP-4 inhibitors before the BP onset. IgG autoAbs directed against COL17 were detected in all 10 cases by ELISA using full-length COL17 as the substrate. In contrast, only 3 of the 10 sera reacted with the immune-dominant NC16A domain. Thus, DPP-4 inhibitor-associated BP has distinct autoAb profiles. To further address epitopes of autoAbs for the 7 cases without reactivity to the NC16A domain, immunoblotting was performed using various domain-specific polypeptides, including intracellular as well as extracellular COL17. All of the IgG autoAbs from the 7 cases exclusively reacted with the non-NC16A mid-portion of the COL17 ectodomain. DPP-4 inhibitor-associated BP without anti-NC16A autoAbs clinically shows a distinct phenotype, with less erythema (Izumi K, et al. J Invest Dermatol. In press.), that may be associated with unique autoAb profiles.

POSTER 41

Contribution of IgG linked sugars to autoimmunity

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IgG is an important molecule in the adaptive immune response and is a driving force in autoimmunity. On the other side, pooled IgG form healthy donors (IVIG) can be used to treat autoimmune diseases like blistering skin diseases. There is evidence that the therapeutic effect is dependent on the only conserved N-glycosylation site of the IgG-Fc part. Sialylated IgG-Fc parts have shown anti-inflammatory effects and might support the induction of tolerance, whereas Fc parts without terminal galactose and sialic acid are linked to inflammatory responses.
Here, we analyzed the regulation of IgG-Fc glycosylation in the context of different immunogenic milieus. For Ovalbumin-specific immune reactions the IgG glycosylation is dependent on the co-stimulus the antigen is presented with. In case of a high-inflammatory stimulus (e.g. Complete Freund's Adjuvant) we found antigen-specific plasma cells that express low levels of galactosyltransferase and sialyltransferase and produce low galactosylated and sialylated IgG antibodies, respectively. In INF-γR1/IL-17RA dKO mice this effect was reversed indicating an influence of the Th1 and Th17 cytokines on the Fc glycosylation pattern.
Recently, it has been shown in mice that IVIG is a beneficial therapeutic in active epidermolysis bullosa acquisita (EBA). We are currently investigating the same model in terms of plasma cell glycosyltransferase expression levels and IgG glycosylation. We assume the same T cell dependent mechanism as for Ovalbumin immunizations. This may elucidate a possible patho-mechanism in autoimmune diseases like EBA and might be a new approach for the development of future therapies as well as a predictive disease marker.

POSTER 42

Role of Human β4 in the pathogenesis of mucous membrane pemphigoid

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The purpose of this study was to demonstrate that the intracytoplasmic domain of human β4 is an antigen in mucous membrane pemphigoid (MMP). It was cloned into three fragments (IC1, IC2, IC3). MMP sera bound only to IC3.0. The antigenicity of the fragments of IC3.0 was analyzed by GENE software. Subsequently it was cloned into six smaller fragments (IC3.1, IC3.2, IC3.3, IC3.4, IC3.5 and IC3.6). Sera from patients with multiple mucosal involvement bound to IC3.6. Sera from patients with ocular cicatricial pemphigoid (OCP) bound to IC3.4. To identify epitopes for pathogenic autoantibodies, short peptides were synthesized. In smaller synthesized peptides, MMP sera bound to IC3.6.1 while OCP bound to IC3.4.1. Sera from MMP and OCP patients bound to both IC3.4.1 and IC3.6.1. This specific binding of cloned fragments and peptides to their respective sera was confirmed by immunoblotting and immunoprecipitation using appropriate controls. Disease-specific epitopes were identified. Rabbit antibodies against these cloned fragments and peptides produced subepidermal blisters in tissue culture using human oral and conjunctival mucosa and when injected neonatal mice. The most frequent MHC Class II gene associated with MMP is HLA-DQβ1*0301. Using a computer model which identifies most likely binding sites in HLA-DQβ1*0301, IC3.4.1 and IC3.6.1 would strongly bind. Hence we demonstrate that specific epitopes for MMP and OCP produce typical pathology in vitro and in vivo, bind to relevant molecular sites in HLA-DQβ1*0301. Thus they can be presented to T cells. The process by which B cells produce disease-specific autoantibodies are initiated.

POSTER 43

Role of α6 integrin subunit in pathogenesis of oral pemphigoid

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A subset of mucous membrane pemphigoid is limited to the oral cavity. No other mucosa or skin is involved. It is referred to as "Oral Pemphigoid" (OP). The aim of this study was to determine the role of α6 in the pathogenesis of OP.

Protein sequences of α6 were analyzed for antigenicity, flexibility and β turn by PC GENE software. The OP sera bound only to the extracellular (EC) domain. The EC domain was cloned into two fragments, A and B. OP sera bound only to fragment A. It was sub-cloned into two fragments, A1 (23-131aa) and A2 (217-462aa). DU145 cell lysate was used as substrate. GoH3 was positive control. Using Western blot OP sera bound to A2 fragment only. The PCGENE software analysis showed two antigenic domains in A2 labeled as A2.1 (290-305aa) and A2.2 (302-330aa). All the OP sera bound to A2.2. Blocking experiments and absorption studies demonstrated binding specificity of A2.2. Immunoperoxidase staining showed binding of antibodies produced in rabbits to α6, fragment A and subfragment A2.2 and OP sera bind to the basement membrane zone (BMZ) of normal oral mucosa. The same antibodies incubated with normal oral mucosa in tissue culture demonstrated subepithelial separation. DQβ1*0301 is in linkage disequilibrium in patients with oral pemphigoid. Using computer model to determine potential binding sites it was observed that there is a specific site for fragment A2.2. Thus we hypothesize that α6 binds to DQβ*0301, which stimulates T cells, that proceed to activate B cells to produce pathogenic autoantibody.

POSTER 44

Reduced skin blistering in experimental epidermolysis bullosa acquisita after anti-TNF treatment

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Epidermolysis bullosa acquisita (EBA) is a difficult-to-treat subepidermal autoimmune blistering skin disease (AIBD) with circulating and tissue-bound anti-type VII collagen antibodies. Different reports have indicated an increased concentration of tumor necrosis factor alpha (TNF) in the serum and blister fluid of patients with subepidermal AIBDs. Furthermore, successful anti-TNF treatment has been reported for individual patients with AIBDs. Here, we show that in mice, induction of experimental EBA by repeated injections of rabbit-anti mouse type VII collagen antibodies led to increased expression of TNF in skin, as determined by real-time PCR and immunohistochemistry. To investigate if the increased TNF expression is of functional relevance in experimental EBA, we inhibited TNF function using the soluble TNF receptor fusion protein etanercept (Enbrel®) or a monoclonal antibody to murine TNF. Interestingly, mice receiving either of these two treatments showed significantly milder disease progression than controls. In addition, immunohistochemical staining demonstrated reduced numbers of macrophages in lesional skin in mice treated with TNF inhibitors compared to controls. Furthermore, etanercept treatment significantly reduced the disease progression in immunization-induced EBA. In conclusion, the increased expression of TNF in experimental EBA is of functional relevance, as both the prophylactic blockade of TNF and the therapeutic use of etanercept impaired the induction and progression of experimental EBA. Thus, TNF is likely to serve as a new therapeutic target for EBA and AIBDs with a similar pathogenesis.

POSTER 45

T cell receptor repertoire analysis indicates a bifunction role of T cells in Epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) is an organ-specific autoimmune disease and associated with skin lesions and subepidermal blisters. In the experimental mouse model, the disease progression is characterized by the production of complement-activating autoantibodies, targeting type VII collagen (mCol7). Antigen-specific CD4 T cells were identified as key mediators for autoantibody production. However, a possible disease promoting role of CD4 T cells accumulating within in the skin remains unclear.
In the present study, we focus on the identification of CD4 T cell mediated factors promoting blister formation at affected skin sites. To address the question, if the T cell receptor (TCR) repertoire is shared between the autoantibody promoting T cells and those in affected skin lesions, we identified the TCR beta CDR3 sequences by next generation sequencing. Comparing the TCR beta CDR3 sequences of lesional skins and of T cell zones (TCZ) of draining lymph nodes, we found over 60% to be shared between both compartments. Moreover, 10% of the lesion-derived TCR beta sequences could also be detected in corresponding germinal centres (GC). Most interestingly, among those 10% shared TCR beta sequences 70% appear at a high abundance in both compartments (skin lesi-

on and GC). Further analysing the gene expression in lesional skin, we identified an elevated expression of the T cell chemokines CXCL9/CXCL10 and the Th1 cytokine IFN gamma. Since only activated antigen-specific T cells, which bind p:MHCII complexes with highest avidity, are able to migrate into germinal centres indicates that the accumulation of T cells in the skin is not a random but rather antigen-driven process. Thus, we propose that CD4 T cells play a bifunctional role in the pathogenesis of EBA: 1) induction of Ig class switching in GCs by providing B cell help and 2) promotion of lesion formation by expression of the pro-inflammatory cytokine IFN gamma in the skin in an auto-antigen driven manner. It will be important in further studies to dissect the role of antigen-specific T cells in EBA skin lesions and whether disease severity can be ameliorated by blockage of T cell skin homing.

POSTER 46

SYK is a key regulator of autoantibody-induced skin inflammation

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Because of the morbidity and limited therapeutic options of autoimmune diseases, there is a high, and thus far, unmet medical need for development of novel treatments. Pemphigid diseases (PD) are prototypical autoimmune diseases that are caused by autoantibodies targeting structural proteins of the skin, leading to inflammation, mediated by myeloid cells. To identify novel treatment targets, we performed cutaneous genome-wide mRNA expression profiling in almost 200 outbred mice after PD induction. Comparison of genome-wide mRNA expression profiles in diseased and healthy mice and construction of a co-expression network identified SYK as a major hub-gene. Aligned, pharmacological SYK inhibition protected mice from experimental PD. Using cell lineage-specific SYK-deficient mice, we identified SYK expression on myeloid cells as an absolute requirement to induce PD. The in vitro use of specific inhibitors confirmed the central role of SYK. Within the predicted co-expression network, interactions of SYK with several partners (e.g., Tlr13, Jdp2 and Nfkbid) were validated by curated databases. Additionally, novel gene-interaction partners of SYK were experimentally validated. Collectively, our results identify SYK expression in myeloid cells as a requirement to promote inflammation in autoantibody-driven pathologies. This should encourage exploitation of SYK and SYK-regulated genes as potential therapeutic targets for autoantibody-mediated diseases.

POSTER 47

Drug re-purposing identifies B-cell modulatory compounds within a commercially available chemical library; potential therapeutics in autoimmune disorders.

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Self-reactive antibodies produced by B-cell derived plasma cells play a crucial role in the pathogenesis of autoimmune disorders (ADs). By targeting the source of these pathogenic antibodies, B-cell modulation is one treatment option. Here, using a drug library consisting of 1,200 commercially available, off-patent compounds, we investigated a hypothesis that some drugs, which are already used for other treatment purposes, might possess unknown B-cell modulatory effects and they could be therapeutics in ADs.
Initial in vitro cell proliferation screening assay, evaluated by 5-Bromo-2´-Deoxyuridine (BrdU) ELISA, showed 48 out of 1,200 drugs inhibited IL-21/ anti-CD40 antibody stimulated human B-cell proliferation by 50% at the concentration of 1µM. Following dose-dependency and cytotoxicity assays narrowed down the candidate drug number from forty-eight to six. In addition to the effect on proliferation, these compounds prevented B-cell differentiation into plasmablasts that was accompanied with decreased immunoglobulin production. Subsequent in vivo investigation showed that a prophylactic treatment with the candidate drugs delayed disease development in an experimental animal model of epidermolysis bullosa acquisita (EBA). Furthermore, drug-treated mice had fewer numbers of total and antigen-specific B-cells and plasma cells in the draining lymph nodes, and lower titers of antigen-specific IgG in the circulation, indicating in vivo drug effects on B-cell and adaptive immunity.
Present results suggest potentials of old drugs as new therapeutic agents in the treatment of ADs, and these drug screening methods would save time and be more cost efficient than the conventional approach.

POSTER 48

Blocking the activating Fc gamma RIV enhances neutrophil extravasation into the skin in autoantibody-induced cutaneous inflammation

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Epidermolysis bullosa acquisita (EBA) is a prototypical autoimmune bullous dermatosis where autoantibodies target type VII collagen (COL7), an integral part of anchoring fibrils. In an EBA mouse model, we established visualization of autoantibodies, target tissue and myeloid effector cells in vivo and identified new checkpoints for antibody-induced cutaneous inflammation. Following up these findings, we aimed to unravel the molecular control of these checkpoints. Therefore, we evaluated the impact of Fc gamma receptor IV (FcgRIV) inhibition on neutrophil recruitment to the dermal-epidermal junction (DEJ). Fluorescently labeled anti-COL7 IgG was injected into LysM-eGFP mice (eGFP transgenic mice under the lysozyme M promotor) in the presence of either blocking or isotype antibody. Using multiphoton microscopy, we studied extravasation and antibody binding of neutrophils and recruitment to the DEJ in vivo following a defined time course (days 1, 3 and 8 after the initial anti-COL7 IgG injection). Unexpectedly, at all time points, we observed a significant extravasation of LysM-eGFP+ cells into the skin with numbers significantly higher in mice treated with the FcgRIV-blocking antibody compared to control. In contrast, localization of LysM-eGFP+ cells to the target antigen, located along the DEJ was, except of day 8, significantly reduced in anti-FcgRIV antibody-treated mice. In addition, ROS release data underscore the dose dependent inhibition of neutrophils via anti-FcgRIV blockade. These findings are in sharp contrast to the previously reported role of activating FcgRs in experimental EBA and underscore the importance of advanced in vivo imaging techniques to fully understand the complexity of antibody-mediated neutrophil-dependent inflammation.

POSTER 49

Effects of lymphocyte-derived IL-10 on the effector phase of experimental epidermolysis bullosa acquisita

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Epidermolysis bullosa acquisita (EBA) belongs to the group of autoimmune blistering skin diseases, called pemphigoid diseases (PD). These diseases are all mediated by autoantibodies directed against components of the anchoring complex of the dermal-epidermal junction of the skin and mucous membranes. Thereby, EBA is characterized by autoantibodies targeting collagen type VII (Col7) and by neutrophil infiltration into the skin.
B cells not only modulate autoimmunity via T cells or innate immune cells, but also by their provision of costimulatory molecules and cytokines. In fact, autoreactive plasma cells significantly contribute to the development of PD via their production of autoantibodies but are also able to slow down the progression of various autoimmune diseases by the provision of interleukin-10 (IL-10).
Our group just recently demonstrated that terminal differentiated B cells (plasma cells) produce IL-10 and are able to completely inhibit EBA inflammation. After polyclonal B cell activation, a plasmacytosis was induced and led to a massive IL-10 production by plasma cells. This in turn had the ability to significantly reduce the skin disease in the experimental EBA model for at least another 3-4 weeks connected to a strong suppression of neutrophil migration into the inflamed skin. Thereby, plasma cell-derived IL-10 acts either directly or indirectly via CD4+ T cell-derived IL-10.
Based on our recent data, we expect that B cell/plasma cell IL-10 can control the onset and the severity of the EBA skin disease. So far, we don't know if IL-10 displays the same effects during the natural course of EBA without an additional B cell activation but we have the indication that IL-10 is a key modulator of the EBA effector phase. For our study, we want to use the Col7 immunization-induced and the anti-Col7 autoantibody transfer-induced EBA mice model.

Outcome measures, novel treatments and case reports

POSTER 50

Increased Relapses and Complications in Pemphigus Patients Treated by the Same Physicians in a Public Safety Net Versus a Private University Healthcare System

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Access to healthcare and its relationship with socioeconomic status has been documented for different diseases. Information on how different healthcare systems, which vary widely in terms of incentives for doctors, affect the care of patients with complex diseases is lacking.
The aim of this study was to determine whether any patient care disparities existed when patients with pemphigus,

a severe mucocutaneous autoimmune blistering disease requiring on-going immunosuppression, are treated by the same physicians in two different healthcare systems, i.e., a county-funded safety net hospital system (Safety Net System) and a private university hospital system (Private System).
We performed a retrospective chart review study of 65 patients with pemphigus vulgaris and foliaceus who were managed in the Safety Net System (n=34) and Private System (n=31) between July 2001 and May 2015.
Patients in the two systems did not differ considerably with regards to applied treatments and achievement of clinical or immunological remission. Safety Net System patients, however, experienced more disease relapses with a shorter recurrence-free survival time after achieving remission and more infectious adverse events. The greater rate of medication non-compliance observed in the Safety Net System patients likely played a role in creating these disparities. Other potential contributory factors are ethnicity, language, and/or socioeconomic status.
Understanding the etiologies of the divergent outcomes within the Safety Net System and the Private System may allow health professionals to optimize the treatment of patients with complex disorders and to discover system-related processes that are responsible for disease relapses and increased health care costs.

POSTER 51

Chinese version of the treatment of autoimmune bullous disease quality of life (TABQOL) questionnaire: reliability and validity

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Background: Treatments for autoimmune blistering disease (AIBD) carry significant risks of medical complications and can affect the patient's quality of life (QOL). Recently the treatment of autoimmune bullous disease quality of life (TABQOL) questionnaires was developed in Australia.
Objective: To evaluate the reliability and validity of the Chinese version of the treatment autoimmune bullous disease quality of life (TABQOL) measurement instrument in Chinese patients with autoimmune blistering diseases (AIBD).
Methods: The Chinese version of the TABQOL questionnaire was produced by forward- backward translation and cross-cultural adaptation of the original English version. A total of 86 AIBD patients self-administered the Chinese TABQOL questionnaire, the Dermatology Life Quality Index (DLQI), and the 36-item Short Form Health Survey (SF-36). Reliability of the Chinese TABQOL was evaluated using internal consistency and test-retest (day 0 and day 7) methods. Validity was analyzed by face, content, construct, convergent, and discriminant validity measures.
Results: Cronbach's α coefficient (internal consistency) was 0.883 and the intraclass correlation coefficient (test-retest reliability) was 0.871. Face and content validity were satisfactory. Convergent validity testing revealed correlation coefficients of 0.664 for the TABQOL and DLQI and –0.571 for the TABQOL and SF-36. With respect to discriminant validity, no significant differences were observed in the TABQOL scores of males and females ($t=-0.938$, $p=0.352$), inpatients and outpatients ($t=0.134$, $p=0.894$), patients on steroids and steroid-sparing medications ($t=0.672$, $p=0.325$), and patients with different AIBD subtypes ($F=0.030$, $p=0.971$).
Conclusions: The Chinese version of the TABQOL questionnaire is a reliable and valid instrument to measure treatment burden and to serve as an endpoint in clinical trials in Chinese AIBD patients.
Keywords: Chinese version, autoimmune bullous disease, treatment quality of life, reliability, validity

POSTER 52

No response to rituximab in a case of mucosal-dominant to and fro mucocutaneous shifting pemphigus vulgaris previously treated with a range of more traditional anti-pemphigus therapies: clinical hint relevant to pemphigus pathogenesis

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A male in his thirties with mucosal-dominant to and fro mucocutaneous shifting pemphigus vulgaris (PV) is described. PV was suspected four years ago after presentation with oral/inguinal/hand periunguinal lesions, weight loss and halitosis with anti-DSG-1/3 IgG ELISA, but DIF of oral mucosa was equivocal. He received high dose of intravenous/oral methylprednisolone (mp) with oral cyclophosphamide. After the discontinuation of cyclophosphamide due to its malignancy-inducing worries, there was a flare of mucocutaneous PV. Next treatments were: plasmapheresis, mp/dapsone, intravenous/oral cyclosporine, IVIG, mp/azathioprine. Vertebral compression fractures and acute respiratory distress with radiology features (ground-glass opacities) suggesting pulmonary sarcoidosis were serious side-effects. In 2016 patient received rituximab, purchased by our hospital despite not being registered for using in autoimmune blistering dermatoses in our country as its manufacturer declined repetitive calls for applying for such a registration, 2g intravenously in two equally divided doses at fortnight interval combined with low dose oral mp. In a 4-month follow-up, there were particularly stubborn gum erosions and halitosis, what may suggest limited response to rituximab in a patient previously receiving a wide range of traditional therapies. Still, comparative studies needed may conclude that rituximab as an initial/first-line modality does produce longer lasting clinical-laboratory remission despite experimental data that low-memory B-cells can escape/proliferate after rituximab cessation. According to limited clinical data initial treatment with anti-CD20 biologics may lead to better outcomes as delay of their administration deteriorates prognosis, so anti-CD20 compounds, despite their limitations, should be regarded as drugs widening the choice of therapies for multifaceted PV.

POSTER 53

IgG4-dominant bullous pemphigoid without complement activation

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IgG4 comes from the predominant IgG subclass of autoantibodies in autoimmune bullous diseases. However, IgG4 has not been considered to mediate tissue damage in pemphigoid diseases, because IgG4 has a very limited ability to bind to the Fc receptor or to activate complement. Here, we report two pemphigoid cases with circulating anti-BP180 IgG4 antibodies, with no complement activation linearly at the basement membrane zone (BMZ) of epidermis, which is a hallmark of bullous pemphigoid (BP). Both cases developed with itchy, eczematous skin lesions without urticarial erythemas that precede blister formation in common BP. Oral steroids were effective in both cases whereas topical steroids were not. To investigate the mechanism, we tested the patients' sera for the ability to fix complement in situ by using complement indirect immunofluorescence techniques. Neither serum samples from these patients nor healthy control serum fixed complement at the BMZ, whereas BP serum did. Furthermore, we carried out the complement fixation test in vitro using human full-length BP180 and found that circulating autoantibodies against BP180 from either case did not fix complement, whereas sera from common BP cases did. These results suggest that autoantibodies may lack the ability to fix complement, and that there is a complement-independent mechanism in blister formation in these cases. Our extraordinary cases suggest that the balance between the contribution of complement-dependent mechanism with IgG1 autoantibodies and that of complement-independent mechanism with IgG4 autoantibodies in blister formation partly explain the clinical diversity in BP.

POSTER 54

Evaluation of a clinical score for the newly established mouse model of anti-laminin-332 mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is an immunobullous disease with autoantibodies against components of the dermal-epidermal-junction and a predominant mucosal involvement. One third of affected MMP patients show reactivity against laminin-332 and 25% of these patients develop a malignant tumor. Recently, an antibody transfer-induced mouse model for anti-laminin-332 MMP has been established in adult mice, which reflects major clinical and immunopathological characteristics of human MMP. As the currently used scoring systems for models of other pemphigoid diseases in adult mice only mirror the extent of skin lesions, the aim of this project was to evaluate a novel scoring system that also includes conjunctival, oral and gastrointestinal involvement determined by histopathology, endoscopy and body weight, respectively. By the transfer of samples with different antibody concentrations, a variety of disease activities was generated and analyzed with different weighting factors. For these experiments, also C5aR1- and/or Fcγ-deficient mice were employed to extend the possible spectrum of disease activity. The highest distinction between diseased and control mice was obtained for conjunctival involvement by using a score based on the length of subepithelial splitting ($p<0.001$), for oral lesions by counting affected oral areas ($p<0.001$), and for body weight when a linear score was exerted ($p=0.053$). Interestingly, a high correlation was found between the extent of oral lesions by endoscopy and weight loss ($r=0.7$). In summary, the new scoring system allows to quantify disease activity of the passive, antibody transfer-induced mouse model of anti-laminin-332 MMP equally weighting skin, conjunctival, and oral/gastrointestinal involvement (as expressed by body weight loss). The extended scoring system will be valuable to explore novel anti-inflammatory mediators that may exert different effects on skin, conjunctival and oral lesions.

POSTER 55

Successful treatment of a bullous pemphigoid patient with rituximab who was refractory to corticosteroid and omalizumab treatments

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Background: Bullous pemphigoid (BP) is an autoimmune blistering disorder which is usually characterized by urticarial lesions initially and then tense blisters and erosions. The pathogenic IgG antibodies are usually directed at the NC16A portion of the BP 180 antigen. However, it has recently been shown that IgE antibodies might be also pathogenic in BP. According to increasing evidence of IgE inhibition, omalizumab was suggested as a therapeutic approach for BP. Rituximab has been reported to be effective in various autoimmune diseases, including autoimmune bullous dermatoses. There is only small case series and case reports about efficacy and safety of rituximab in BP.
Aim: Here we present a young BP patient well-responded to the rituximab therapy who was refractory to conventional and omalizumab therapies although he has elevated IgE levels and eosinophilia.
Case report: Omalizumab was thought a reasonable treatment because of our patient's high IgE serum level and recent literatures about well response. But while IgE levels gradually decreased in our patient, BP180 antibody titers stayed at the same level (>200 IU) without any clinical improvement during follow-up. He was then treated with rituximab in rheumatoid arthritis protocol. Serum autoantibodies of BP180 were finally decreased with rituximab therapy as soon as clinical improvement was seen.
Conclusion: Our case supports the knowledge about the effectiveness and safety of rituximab not only in pemphigus but also in BP. On the other hand although it did not work in our case, omalizumab may be a potentially effective agent in some carefully selected patients with certain subtypes of BP.

POSTER 56

Efficacy and safety of perilesional/intralesional triamcinolone injections of oral mucous membrane pemphigoid

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Mucous membrane pemphigoid (MMP) is a heterogeneous group of autoimmune blistering disorders in a term with both clinical and immunological features. In patients with lesions confined to the oral mucosa extensive course of topical corticosteroids are applied as first line treatment. We present a series of 4 patients with oral MMP who were initially treated with topical corticosteroids, however without success and finally resolved after several injections perilesional/intralesional triamcinolone injections (PITA). We injected inclusively 5 erosions, located on the buccal mucosa or palate of a size oscillating from 2 to 5 cm in diameter. The initial amount injected at each visit ranged from 0,5 to 2 ml per lesion (concentration of PITA 4mg/ml).The injections were repeated every 4 weeks for the period varying from 2 to 18 months with complete resolution obtained in all patients. The side-effects of the therapy were minimal including slight bleeding during procedure. The patients were monitored for a period ranging from 2 to 10 months with no symptoms of recurrence.
Injections with triamcinolone are effective and safe alternative therapy for those patients with oral MMP in whom topical corticosteroids are not effective and systemic therapies are contraindicated.

POSTER 57

Novel chimeric immunoreceptors for pemphigus vulgaris (PV) therapy

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Pemphigus therapy relies on chronic immunosuppression, which causes significant morbidity and mortality. Ideally, therapy should target pathogenic autoimmune cells while sparing protective immunity. Alternatively, complete, transient B cell depletion should cure PV, since autoreactive clones do not recur upon generation of a new B cell repertoire. To achieve these goals, we investigated 2 immunotherapy strategies. For targeted therapy, we designed chimeric autoantibody receptors (CAARs), consisting of the extracellular domains of the PV autoantigen, desmoglein 3 (Dsg3), fused to T cell cytoplasmic signaling domains. CAARs direct human T cells to specifically kill B cells expressing surface anti-Dsg3 IgG both in vitro and in vivo in a PV mouse model. Circulating anti-Dsg3 IgG do not compromise CAAR-T cell function, as mAbs with slower off-rates reduce but still preserve significant CAAR-T cytotoxicity. To investigate safety in vivo, we injected CAAR-T cells in human skin xenografted mice. CAARs cause no epidermal toxicity compared to an anti-CD19 T cells. These data indicate that Dsg3 CAAR-T cells are specific and effective in targeting autoimmune B cells in PV. For complete, transient B cell depletion, we designed an anti-CD19 chimeric antigen receptor, co-expressed with an inducible caspase-9 "suicide" gene (sCAR). sCAR-T cells deplete effectively CD19+ B cells in an in vivo leukemia model. Induced activation of caspase-9 results in efficient in vivo depletion of sCAR-T cells, showing the feasibility of this strategy. In summary, we have validated 2 novel, non-redundant approaches for immunotherapy of PV, which can be applied to other autoimmune diseases.

POSTER 58

Bullous Pemphigoid development in a hemodialysis patient.

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Haemodialysis is the most frequent form of renal replacement therapy in patients with end-stage renal disorder (ESRD). Patients with ESRD frequently develop skin problem, mainly xerosis, pruritus, hyperpigmentation, porphyria or pseudoporphyria and rarely bullous pemphigoid. Bullous pemphigoid (BP) is the most common autoimmune subepidermal blistering disease and it predominantly affects the elderly people. Clinically, BP is characterized by a generalized pruritic, bullous eruptions and urticaria-like lesions. Usually, BP is an idiopathic disorder, however in some cases underlying internal disorders are present, like diabetes or neurologic disorders. Herein, we present a 33-year-old man with ESRD, maintained on hemodialysis, who developed tense blisters on the left forearm in area of fistula placement for hemodialysis, then the blisters spread out on the trunk and lower legs. Direct immunofluorescence study on patient's skin showed the presence of linear deposits of IgG and C3 along the dermal-epidermal junction and serum study revealed reaction of circulating IgG anti-BMZ antibodies with the roof of artificial blister using salt split skin and NC16a epitop of BP 180 kD antigen on BIOCHIP. The patient responded promptly to tetracycline and 0.05% clobetasol propionate lesionaly. The relationship between BP and fistula for hemodialysis remains unknown but it is possible that skin injury associated with fistula placement was responsible for alteration of the BMZ and stimulation of the immune system leading to the BP development. To explain the real role of fistula placement as a provocative factor in BP, more such cases are required for the assessment.

POSTER 59

Assessment of the quality of life of Egyptian and Tunisian autoimmune bullous diseases' patients

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The Autoimmune Bullous disease Quality Of Life (ABQOL) questionnaire and the treatment of Autoimmune Bullous Disease Quality of Life (TABQOL) proved valid and reliable tools that measure the disease and treatment burden. We aimed to assess the ABQOL and TABQOL in the Arabic population.The English questionnaires were forward-translated into the Arabic language then back-translated to English language by an independent certified translation agency. Eighty autoimmune bullous disease (AIBD) patients were included in this study. Patients were asked to answer the questions in the 2 questionnaires. After 1 week the same patients were asked to answer the same questionnaire again.The range of ages of the patients was from 19 to 81 years (mean= 46), 19 were males, 61 females.The ABQOL ranged from 0-37 (mean= 16.4±9.2). The TABQOL ranged from 2-43 (mean= 21.5±9.4). Test-retest reliability was acceptable, with a spearman correlation coefficient r=0.99 for ABQOL and r=0.98 for TABQOL. The highest ABQOL (34) and TABQOL (35) scores were in BP. Among the pemphigus group, PF patients had the highest ABQOL (mean was 19±3.7) while PV patients had the highest TABQOL score (mean was 21.6±9.6). There is significant negative correlation between the age of the patients and the TABQOL, p value was 0.04. There is significant negative correlation between the education of the patients and the TABQOL, p value was 0.007. Objective and valuable measurements such as ABQOL and TABQOL questionnaires are now available to help physicians understand their patient's distress and should be used in every patient with AIBD.

POSTER 60

Mucous membrane pemphigoid with severe stricture of esophagus mediated by IgG and IgA autoantibodies to LAD-1

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Mucous membrane pemphigoid (MMP) is a heterogeneous group of blistering disorders with high predilection to mucosa involvement. For MMP cases the most characteristic target antigen is carboxyterminal fragment of BP180, but other basement membrane zone (BMZ) antigens may also be a target for circulating antibodies. However, in most of the MMP patients sera are negative.
Here, we report case of a 78-year-old woman with erosive lesions in the oral cavity and esophagus, with dysphagia. Radiographic examination of the esophagus revealed no stenosis or functional disorders at that time.
Immunoblotting allowed the final diagnosis of MMP on the basis of the reaction of both IgG and IgA autoantibodies to the LAD-1 antigen with molecular weight of 120 kDa.
In laser scanning confocal microscopy in vivo bound IgG and IgA were localized above type IV collagen and below laminin-332, characteristic of MMP. Although high doses of prednisone the patient developed severe stricture of esophagus. Seven years after the diagnosis of MMP slight scarring of the conjunctiva was also observed. In conclusion, MMP with esophageal stricture and autoantibodies both IgG and IgA exclusively to LAD-1 has not been published so far. Laser scanning confocal microscopy may be helpful in diagnosis of MMP cases in which sera are negative. Since classification of blistering disorders is still a matter of controversy, more such cases may provide better understanding of relationship between clinical features and target antigens in MMP and subsequently therapeutic implications.

POSTER 61

Quality of life in patients with autoimmune bullous diseases assessed by ABQOL and TABQOL questionnaires – the Greek validation study

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Introduction & Objectives: Autoimmune bullous diseases (AIBD) have a significant burden on patients' life. Specified QoL instruments, ABQOL (Autoimmune Bullous Diseases Quality of Life) and TABQOL (Treatment Autoimmune Bullous Diseases Quality of Life), were introduced to quantify the impact of AIBD and their treatment on patients' well being. Aim of this study was to validate ABQOL and TABQOL in Greek patients and in Greek culture.
Material and Methods: Fifty patients were recruited, 35 patients with subepidermal AIBD and 15 patients with intraepidermal AIBD. All patients completed ABQOL at 4 different time points. TABQOL was completed at 3 time points. DLQI and SF-36 were assessed at time points 4 and 2, respectively. Clinical severity was asessed by PDAI, BPDAI, ABSIS and by titres of circulating autoantibodies (DSG-1, DSG-3, BP180, BP230).
Results: In all patients ABQOL and DLQI were correlated with ABSIS ($r>0,5$, $p<0,001$).
ABQOL showed statistically significant differences at all time points. TABQOL was strongly correlated with ABQOL, DLQI and with parameters of SF-36v2. In patients with subepidermal AIBD, BPDAI was strongly correlated

with ABQOL before treatment initiation (r>0,55, p<0,001) and also with DLQI (r=0,515, p=0,006). TABQOL was not correlated with disease severity. AntiBP180 and antiBP230 titres were not correlated with QoL measures. In patients with intraepidermal AIBD, antiDSG1 titres were strongly correlated with PDAI (r=0,752, p=0,001). Both initial ABQOL and DLQI showed positive correlation with PDAI and only ABQOL showed correlation with anti DSG-1 titres (r=0,5, p=0,059) .

Conclusion: ABQOL seemed to be a more sensitive quality of life index in patients with AIBD. TABQOL score alterations may be attributed to the fact that steroid treatment affects quality of life even more than the blistering disease itself.

POSTER 62

Novel Therapies for Pemphigus Vulgaris Patients: a Signaling-based Approach

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Over the last decade, molecular studies of PV revealed that acantholytic mechanisms implicate loss of transadhesion between Dsg3 molecules coupled to altered signal transduction. Specifically, i) PV antibodies bind to Dsg3 adhesion receptors outside of desmosomes transducing outside-in signals; ii) within 30 minutes after PV antibody binding specific signaling pathways of keratinocytes are significantly altered; iii) signal activation such as of epidermal growth factor receptor (EGFR) or non-apoptotic caspase-3 are pathogenic as their inhibition prevents acantholysis in the PV mouse model. Based on PV mouse models, we also find, however, that inhibition of these signaling effectors can enhance or reduce acantholysis depending on the inhibitor dose, status of the signaling effectors and time of injection. This implies that while a signaling-based therapy can now be proposed to enter clinical trials, the patient's endogenous effector activity and degree of blistering need to be included as a parameter in the clinical design of the study.

POSTER 63

Life-threatening course of pemphigus vulgaris complicated by sepsis

› Ágnes Kinyó[1], Krisztián Schaig[1], Anna Bátor[2], Alíz Gyimesi[1], Lívia Szélig[3], Csaba Csontos[3], Zoltán Knausz[4], Csaba Gyömörei[5], Enikő Telegdy[2], Rolland Gyulai[1]

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Please place your abstract here: We report the case of a 26-year-old male with extended erosions and blisters affecting more than 60% of mucosa and skin. The patient had a history of drug abuse, had been smoking synthetic cannabinoid. The immunohistologic findings were consistent with the diagnosis of pemphigus vulgaris. Treatment with high-dose methylprednisolone (125 mg/d) and azathioprine (150 mg/d) lead to no improvement in his skin lesions. Fever and elevated C-reactive protein level developed, along with the worsening of skin symptoms, and the patient was admitted to intensive care unit. Bacterial cultures of blood, skin and conjunctives showed the presence of MRSA, Acinetobacter baumanii, Klebsiella pneumoniae, Pseudomonas aeruginosa and Stenotrophomonas maltophilia. Withdrawal of azathioprine, initiation of imipenem, vancomycin and fluconazole therapy, and antiseptic baths in general anesthesia every other day resulted in the resolution of septicaemia and improvement of skin symptoms. Severe forms of pemphigus vulgaris complicated with sepsis present a life-threatening condition with a high mortality rate. With our case report we would like emphasize the importance of comprehensive care in the management of severe, complicated forms of pemphigus vulgaris.

POSTER 64

Pemphigus erythematous

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A 68 year old female woman applied with erosion and desquamation on her body. Her lesions had started from neck, and then spread to body. There was not any disease or drug history. In dermatologic examination malar rash on face, squamous lesions on scalp, eroded lesions and few flaccid bullous lesions on trunk were observed. There was no oral involvement. Nikolsky sign was positive on the edges of eroded lesions. In laboratory examination ANA positive (2+ speckled), RF 121 (0-15) were found. There was epidermal separation on stratum granulosum level in biopsy. Widespread pericellular painting in epidermis with IgG, linear granular painting in epidermis basale membrane with C3 was observed. With these accompanying findings it is diagnosed as 'pemphigus erythematous '.
In conclusion, pemphigus erythematous, is a rare autoimmune blistering skin condition. Both pemphigus foliceous and lupus erythematous findings exist in pemphigus erythematous.

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