From Cell Signaling to Novel Therapeutic Concepts: International Pemphigus Meeting on Advances in Pemphigus Research and Therapy

Spiro Getsios[1,6], Jens Waschke[2,6], Luca Borradori[3,6], Michael Hertl[4,6] and Eliane J. Müller[5]

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The International Pemphigus Meeting* held in Berne, Switzerland, in June 2009, was designed as a forum in which to discuss cell signaling and signaling-related therapies in pemphigus disease. Under the theme "From Cell Signaling to Novel Therapeutic Concepts," researchers and clinicians met with leaders in epidermal stem cell biology, adhesion, signal transduction, migration, and autoimmunity **(Figure 1)**. Keynote lectures on basic topics were linked to specialized sessions on pemphigus, such as cell signaling and mechanisms of acantholysis, alternative aspects of immune responses, and unconventional treatments. Workshops and interactive poster sessions terminated the day's program; the latter gave young investigators the opportunity to present their research.

Two innovative studies were honored with the Young Investigator Award sponsored by the European Academy of Dermatology and Venereology: Cory Simpson from the Department of Pathology at Northwestern University, in Chicago, for his contribution to the study on modulation of epidermal growth factor receptor signaling by desmoglein 1 (Getsios *et al.*, 2009), and Volker Spindler from the Department of Anatomy and Cell Biology, University of Würzburg, Germany, for investigations of desmocollin 3–mediated cell adhesion in pemphigus.

The congress also served as a platform for a subsidiary meeting on therapy, headed by Victoria Werth. The guest speaker, Janet Segall, was recognized for her devoted and successful work as the first secretary of the International Pemphigus and Pemphigoid Foundation.

PLENARY SESSIONS

The keynote lectures on stem cell behavior (Dennis Roop, University of Colorado, Denver: "State of the Art on Epidermal Stem Cells"; Mayumi Ito, New York University, NY: "Stem Cells and Wound Healing"; Freddy Radtke, EPFL, Lausanne, Switzerland: "Notch Signaling in Epidermal Renewal and Disease"), migration (Zena Werb, University of California, San Francisco: "State of the Art on Epithelial Adhesion and Migration"), and adhesion-mediated signaling (Kathleen J. Green, Northwestern University, Chicago, IL: "Perspective on Desmosomes") set the stage for a new paradigm in pemphigus research: the understanding that pemphigus antibodies act as triggers of outside-in signaling in keratinocytes whereas desmoglein cadherins serve as adhesion receptors and signal transmitters. However, as demonstrated by several groups after passive transfer of antibodies into neonatal mice, the hope that inhibition of epidermal signaling molecules may allow new therapeutic approaches has not yet lived up to expectations. Many potential signaling targets are key regulators of epidermal homeostasis, and their long-term inhibition can be detrimental. Hence, further effort is required to refine therapies that control pemphigus at the level of keratinocytes. New insights into the specific mode of action of pathogenic antibodies, through their cloning by phage display (Don Siegel and John Stanley, both from the University of Pennsylvania School of Medicine, Philadelphia: "State of the Art on Antibody-Mediated Immunity" and "Using Pemphigus Antibodies for Targeted Drug Delivery," respectively), might serve this purpose. Moreover, these antigen-restricted antibodies may be employed as vectors to specifically deliver drugs to the skin and its appendages. At the immune-system level, novel immunosuppressive compounds—notably rituximab (anti-CD20 antibody; Joly *et al.*, 2007)—are rising as highly promising drugs to efficiently control the disease.

The meeting concluded with a short statement from two experts in the field. Both Masayuki Amagai (University of Tokyo, Keio University School of Medicine, Japan) and Grant Anhalt (John Hopkins University, Baltimore, MD) stated that, despite considerable progress in understanding the pathogenesis of pemphigus, major effort is now required to move toward specific treatment regimens.

Below, Spiro Getsios and Jens Waschke summarize discussions they chaired in Workshop I, on cell signaling. Luca Borradorri and Michael Hertl, who chaired Workshop II, on treatment, summarize the discussion on scoring systems and conventional as well as novel therapeutic approaches.

[1]*Department of Dermatology, Northwestern University Feinberg School of Medicine, Chicago, Illinois, USA;* [2]*Institute for Anatomy and Cell Biology, University of Würzburg, Würzburg, Germany;* [3]*Department of Dermatology, University Hospital Berne, Berne, Switzerland;* [4]*Department of Dermatology and Allergology, Philipps University, Marburg, Germany and* [5]*Molecular Dermatology, Institute of Animal Pathology and DermFocus, Vetsuisse Faculty, University of Berne, Berne, Switzerland.* [6]*These authors are workshop chairs and contributed equally.*

Correspondence: Eliane J. Müller, Molecular Dermatology, Institute of Animal Pathology, University of Berne, Länggasstrasse 122, Postfach, Berne CH-3001, Switzerland. E-mail: eliane.mueller@itpa.unibe.ch

Figure 1. Organizing committee (first row from left): Luca Borradori, Maja Suter, Eliane Müller, all University of Bern, Switzerland; John Stanley, University of Pennsylvania, Philadelphia USA (missing: Thomas Hunziker, University of Bern, Switzerland)

WORKSHOP I:

"The Signaling Network in Pemphigus Pathogenesis and Potential Therapeutic Application" (Chairs: Spiro Getsios of Northwestern University, Chicago, IL, and Jens Waschke of the University of Würzburg, Germany)

The textbook explanation for the pathophysiology of pemphigus is relatively simple: autoantibodies sterically hinder desmoglein 3 and/or desmoglein 1 to cause acantholysis in areas where these adhesion proteins are most abundant. However, an increasing body of evidence suggests that desmogleins are also targeted outside of desmosomes and that a variety of intracellular signaling pathways are involved in the loss of adhesion. The goal of the workshop was to examine the relationship between keratinocyte adhesion and signaling pathways and to evaluate critically the contribution of adhesion-signaling networks to pemphigus pathogenesis. The concept that pemphigus involves more than steric hindrance of adhesion proteins by auto-antibodies emerged from the animated sessions; the complexity of pemphigus-associated adhesion and signaling events presents challenges for future investigation while at the same time affording opportunities for novel therapeutic intervention.

The workshop began by revisiting ultrastructural findings in patients and animal models of pemphigus, which reveal two seemingly conflicting pictures: (i) split desmosomes and frank acantholysis (loss of cell–cell contact) and (ii) intact desmosomes with intervening spongiosis (widening of intercellular spaces between desmosomes). These snapshots may reflect different moments in the spectrum of a dynamic blistering disease but also provide insight into different models of pemphigus pathogenesis. In particular, split desmosomes are consistent with the steric hindrance model, whereas the observation of intact desmosomes with widened intercellular spaces lends itself to alternative pathomechanisms, such as impaired desmoglein trafficking, activation of desmoglein-dependent or -independent signaling pathways, and/or molecular targets outside of the desmosomes. The discussants agreed that these explanations are not necessarily mutually exclusive and that more studies are required to identify which of these events are primary to the disease.

Although antidesmoglein antibodies are present in the majority of patients with pemphigus, and a monoclonal antibody (i.e., AK23) that interferes with the putative desmoglein 3 adhesive interface is sufficient to induce epidermal blistering, nonjunctional pools of desmogleins as well as other proteins appear also to be important pathogenic targets. Enhanced internalization of desmogleins and their trafficking into and out of desmosomes was considered a means of disrupting keratinocyte adhesion by specifically depleting desmoglein 3 from desmosomes, without changes in other junctional proteins. It was noted, however, that the skin in mucosal-type pemphigus vulgaris, which contains considerable amounts of anti–desmoglein 3 antibodies, would also be expected to blister if these antibodies were simply shifting the balance of desmoglein assembly and disassembly. The presence of other desmosomal cadherins (i.e., desmoglein 1) or the differentiated state of keratinocytes might therefore influence susceptibility to anti–desmoglein 3 antibodies. The group emphasized that the choice of cell culture or animal model systems will impact the steady-state dynamics of the keratinocyte adhesive apparatus and should be taken into consideration when interpreting the effects of antidesmoglein antibodies on trafficking events. The merits and limitations of various model systems and experimental tools were tabled for future discussion.

Additional mechanisms by which desmosomal adhesion is destabilized beyond steric hindrance were debated, including the extent to which proteolysis contributes to pemphigus. It was emphasized that proteases, such as matrix metalloproteinases and caspases, have multiple cellular targets that extend well beyond desmogleins and that this fact should be considered when accounting for the apparent specificity of desmoglein disruption in pemphigus.

The group also examined the potential contribution of nondesmoglein targets such as the desmocollins. Desmocollin 3 collaborates with desmogleins to form the adhesive core of desmosomes and might serve as a bona fide target in pemphigus. Moreover, its genetic ablation leads to more severe blistering than the related desmoglein 3. Interestingly, homozygous null desmocollin 3 embryos die prior to implantation or desmosome formation, implicating signaling mechanisms in the normal function of this protein.

Day 2 focused on signaling events in pemphigus. Emerging data from several groups suggest that desmogleins are both adhesion and signaling proteins. Widening of intercellular spaces and loss of desmosomes were considered as

different stages of a continuous process finally leading to acantholysis. The group evaluated whether widening of intercellular spaces prior to the loss of desmosomes suggested that nonjunctional desmogleins or nondesmoglein antigens might be the initial targets of pathogenic antibodies. Indeed, most anti–desmoglein 3–specific antibodies appear to bind to Triton-soluble pools of desmoglein 3, indicating that adhesive defects may originate outside of desmosomes. On the other hand, ultrastructural observations from lethal acantholytic epidermolysis bullosa suggest that there is more to the story. Here, mutations in desmoplakin—the plaque protein required for keratin filament anchorage in desmosomes—also lead to acantholysis, suggesting that the desmosome itself is ultimately the target of disease. Given that desmogleins are found outside desmosomes, widened interdesmosomal spaces themselves do not rule out desmogleins as primary targets in autoantibody binding.

The group considered the plethora of signaling events linked to pemphigus and the question of what accounts for the specificity of signaling pathways to desmoglein internalization. Although this specificity may be defined by desmogleins themselves, recent results suggest that the spatiotemporal distribution of key factors in the keratinocyte adhesion-signaling network, such as p120 catenin binding to desmogleins, may contribute to signaling specificity. It should be emphasized, however, that not all signaling mechanisms should be regarded as primary events; rather, they can also be triggered later in the process of acantholysis and may not rely on desmoglein endocytosis. They may impair desmoglein-mediated adhesion more indirectly, for example, by affecting the cytoskeletal anchorage of desmogleins or the cytoskeleton-mediated transport of desmosomal components to the sites of cell–cell contact. Evidence exists that cytoskeletal components beyond keratin become reorganized during acantholysis but the relevance of these changes remains unclear. The question was raised whether the target of antibody binding or the hierarchy of signaling cascades matters for patients, because the modulation of signaling pathways can ultimately prevent blistering.

Participants sought to explain how hyperproliferation, which is observed in pemphigus patients, can lead to acantholysis. The participants agreed that there is no simple paradigm at present to explain how blocking proliferation leads to the restoration of adhesion. On the other hand, proliferation-associated signaling pathways may reflect a modified wound healing program that is also associated with decreased cell–cell adhesion, possibly rendering keratinocytes more vulnerable to pathogenic antibodies.

Finally, apoptosis and the recent apoptolysis model of pemphigus pathology was a topic of considerable discussion. The group agreed that apoptotic cell death is not a hallmark of pemphigus but that the apoptotic signaling machinery appears to be activated in response to the autoantibodies in pemphigus lesions. It was mentioned that some apoptotic signaling effectors, including caspases, also participate in keratinocyte differentiation. Importantly, inhibition of caspase signaling has been found to block acantholysis in response to pemphigus autoantibodies in some studies but not in others, leaving the relevance of these cell death-related mechanisms uncertain.

Finally, suggestions for the format of future workshops included the following: (i) a single workshop session after all podium talks to enable a more comprehensive discussion of the new findings presented at the meeting, (ii) encouraging young investigators to participate in and lead the discussion, and (iii) focusing on a key topic area, such as the advantages and limitations of model systems for studying the mechanisms of pemphigus acantholysis.

WORKSHOP II:

"Current Knowledge in Pemphigus Treatment"

"Clinical Course, Activity, Prognosis, and Standard Therapeutic Options" (Chair: Luca Borradori, University of Berne, Switzerland). After two years of collaborative effort, a consensus statement on the definitions of disease, end points, and therapeutic responses for pemphigus has been published (Murrell *et al.*, 2008). Aiming at consistent reporting of outcomes, the pemphigus community highly recommends worldwide application of the statement.

Two new measurement instruments are currently being evaluated as reliable scoring systems to grade disease activity and standardize the practical management of patients: the pemphigus disease area index and the autoimmune bullous skin disorder intensity score (Rosenbach *et al.*, 2009). Preliminary findings indicate that the former is more reproducible and correlates better with physicians' overall impression of a patient.

Prospective multicenter studies are urged in order to estimate the reliability of these instruments in larger groups of patients (including those with severe disease) and their ability to capture variability in disease activity during the disease course. So far, the extent of disease, delayed responses to therapy and the presence of mucosal (including anogenital) involvement have emerged as negative predictive factors. Despite the development of highly sensitive and specific ELISAs, their practical usefulness for monitoring disease severity and their superiority in indirect immunofluorescence microscopy, for example, have not been validated. Current evidence indicates that desmoglein 1 ELISA values correlate more closely with the course of disease than do desmoglein 3 ELISA values (Abasq *et al.*, 2009). Desmoglein 3 ELISA values remain high in a significant percentage of patients despite remission. Finally, no prospective studies have addressed the value of markers useful for either deciding when to stop therapy or predicting clinical relapses. Positive direct immunofluorescence studies at cessation of therapy, a significant increase of desmoglein 1 ELISA values, or very high desmoglein 3 values may constitute fair predictors of further relapse.

Clinical interventions must be standardized, and the need for high-quality prospective clinical studies was underlined. Systemic glucocorticoid therapy has significantly improved the overall prognosis of affected patients; however, optimal glucocorticoid doses and the role of adjuvant immunosuppressive therapy (such as azathioprine, mycophenolate mofetil, and cyclophosphamide) remain unclear (Martin *et al.*, 2009). A recent double-blind trial from Japan suggests that patients relatively resistant to steroids benefit from intravenous

immunoglobulins based on a novel, but somehow ill-defined end point: time to escape from protocol. The length of the period ending when a patient had been on the protocol without any additional treatment was significantly longer in patients who received a cycle of intravenous immunoglobulin compared with controls (Amagai *et al.*, 2009).

Regarding the course and prognosis of pemphigus, a follow-up study of 155 patients in Israel reported an overall mortality rate of 10%; none of the deaths could be directly associated with pemphigus. Patients who were young at diagnosis (<40 years), had mucosal involvement, or were of a distinct ethnic origin (such as Sephardic Jews) appear to have a poorer prognosis.

"Towards Novel Immunomodulatory Therapeutic Strategies" (Chair: Michael Hertl, Philipps University, Marburg, Germany). Based on the consensus that the immune pathogenesis of pemphigus is primarily driven by IgG (or, rarely, IgA) autoantibodies against desmosomal adhesion molecules (Amagai *et al.*, 2006), their removal by immunoadsorption (IA) or intravenous immunoglobulins (IVIG) should improve the overall disease course. Autoreactive B cells as the precursors of autoantibody-secreting plasma cells have become a major therapeutic focus because treatment with B cell–depleting agents, such as rituximab, has led to pronounced therapeutic effects. It is not yet clear, however, whether autoreactive B cells fulfill additional functions, such as antigen presentation for autoreactive T cells. Because autoreactive T cells are thought to be critical inducers and perpetuators of autoreactive B-cell responses, they may also represent a relevant therapeutic target. In pemphigus vulgaris, an imbalance toward a pathogenic T-helper type 2 phenotype is associated with a lack of autoreactive type 1 regulatory T cells (Hertl *et al.*, 2006).

IA is considered a highly efficient procedure to rapidly deplete pathogenic IgG autoantibodies in pemphigus, and it is used as an adjuvant to the standard immunosuppressive treatment. Specialized centers in Germany perform IA in refractory or extensive pemphigus with impressive therapeutic outcomes in single patients. A German multicenter prospective phase III IA trial will start in late 2010. Moreover, IA has been used successfully in combination with rituximab and/or immunoglobulins (Shimanovich *et al.*, 2008; Pfütze *et al.*, 2009).

IVIG treatment has pleiotropic effects on various autoimmune responses. Specifically, IVIG blocks Fc receptors on inflammatory cells, induces autoantibody catabolism through activation of the neonatal Fc receptor, and blocks B cell growth and survival factors such as B-cell-activating factor. Currently, IVIG is not approved for the treatment of pemphigus in most countries (Cines *et al.*, 2003). However, based on the findings of a multicenter, prospective, controlled trial, IVIG treatment of pemphigus was recently approved in Japan (Amagai *et al.*, 2009). Further controlled clinical trials are warranted to facilitate its wider use in dermatology.

Excellent results with rituximab were reported in a long-term follow-up of 21 patients with severe types of pemphigus who received a single cycle of rituximab (mean follow-up: 58 months). Eighteen patients (81%) were in complete remission or almost complete remission, three patients had persistent active disease, and two patients died (in one case, death was due to an unrelated cardiovascular cause). Among the patients in complete remission, seven (33%) and six (29%) were off therapy or on minimal therapy (less than 10 mg/day prednisone), respectively. The 2-, 3- and 5-year relapse rates were 33, 43, and 62%, respectively. Overall, the data seem to demonstrate a remarkable long-term efficacy of rituximab and good tolerance to the drug. A multicenter study will soon be initiated in France to assess the efficacy of rituximab as a first-line and steroid-sparing agent.

Several B-cell-depleting antibodies that have been tried in human autoimmune diseases (Dörner and Burmester, 2008; Nagel *et al.*, 2009) target B-cell survival factors, such as B-cell-activating factor (belimumab) and may thus act synergistically with rituximab (Nagel *et al.*, 2009). In addition to the depletion of autoreactive B cells as plasma cell precursors, rituximab reduces the frequencies of desmoglein 3–specific T cells (Eming *et al.*, 2008). Furthermore, rituximab also induces regulatory T cells as shown in systemic lupus erythematosus (Sfikakis *et al.*, 2007). Currently applied doses of rituximab are presumably higher than necessary; 1–2 g/year should be sufficient. Elimination of autoreactive plasma cells using the proteasome inhibitor bortezomib may be another promising therapeutic approach.

Finally, based on findings in animal models of pemphigus, there is evidence that autoreactive B cells require T-cell help for autoantibody production (Aoki-Ota *et al.*, 2006). A recent Peptimmune phase I trial aimed at targeting autoreactive T cells with a single immunodominant desmoglein 3–derived peptide (Anhalt *et al.*, 2005) did not lead to considerable clinical improvement. A major obstacle is potentially unexpected side effects of T-cell-based immune interventions, such as the *de novo* induction of immune-deviated T-cell responses, which have been identified in similar trials in multiple sclerosis.

*The International Pemphigus Meeting on Advances in Pemphigus Research was held at the Hotel Allegro, Berne, Switzerland, 27–29 June 2009.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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