

Post-IID 2008 International Meeting on Autoimmune Bullous Diseases

Masayuki Amagai¹, Takashi Hashimoto² and Yasuo Kitajima³

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After the International Investigative Dermatology (IID) meeting in Kyoto, Japan, the Post-IID International Meeting on Autoimmune Bullous Diseases was held (Figure 1).^{*} Although many new findings had been presented at the IID meeting, additional discussion time was needed to explore selected topics in greater detail. At the Post-IID meeting, each speaker was allotted 10 minutes to present and 5 minutes to discuss, followed by 30–40 minutes of combined discussion for each session. Slides related to previous questions were shown again during the discussion. The more than 140 experts who attended enjoyed considerable debate on various topics in autoimmune bullous disease; in fact, in all of the sessions, so many hands were raised that the moderators found it difficult to accommodate everyone who wanted to speak. Even in such a setting, it was not possible to address all points of view in this active and developing field. However, the meeting successfully established a common base from which to proceed with further investigation into the pathophysiological and immunological mechanisms of autoimmune bullous diseases.

Desmosome dynamics in health and disease

This session, moderated by Kathleen J. Green (Northwestern University, Chicago, IL), addressed recent breakthroughs in characterizing desmosomal

components in normal and diseased conditions. Peter J. Koch (University of Colorado, Denver) reported on a conditional knockout mouse for desmocollin-3, specifically within stratified epithelia, using the Cre-loxP system. Interestingly, these mice demonstrated intraepidermal blister formation with suprabasilar acantholysis that was indistinguishable from that observed in pemphigus vulgaris. This study provided insight into the *in vivo* adhesive role of desmocollin-3 in the epidermis. Green provided evidence that the cytoplasmic domain of desmoglein 1 (Dsg1) has additional functions that regulate epidermal morphogenesis. She reported differential effects of RNAi inhibition and chronic cleavage of Dsg1 by exfoliative toxin on organotypic raft culture. A co-immunoprecipitation study revealed a complex composed of Dsg1 and cortactin, an Src-family kinase substrate, that governed actin dynamics during cell motility. Green proposed a novel, adhesion-independent role for Dsg1 in the localization and phosphorylation of cortactin, which may in turn facilitate the actin reorganization required for epidermal stratification and morphogenesis.

Using fluorescence imaging, Andrew Kowalczyk (Emory University School of Medicine, Atlanta, GA) demonstrated that desmosome disassembly occurs in three phases in living keratinocytes: Dsg3 endocytosis, disruption of desmosome organization, and loss of cell–cell

adhesion strength. Dsg3 endocytosis occurs primarily from nondesmosomal pools, followed by a second phase of disassembly characterized by the rearrangement of Dsg3, desmoplakins, and other desmosomal components from the cell–cell interface into the cytoplasm.

Autoantibody responses in pemphigus, pemphigoid, and epidermolysis bullosa acquisita

For 14 years, Luis A. Diaz (University of North Carolina, Chapel Hill) and his colleagues in Brazil have been surveying the region of Limao Verde, one of the foci of Brazilian endemic pemphigus foliaceus, also known as “fogo selvagem.” They have found that in this area anti-Dsg1 autoantibodies are commonly detected, even in healthy individuals. IgM anti-Dsg1 autoantibodies have also been detected and may prove to be a distinctive marker of fogo selvagem. It is interesting to note that these patients demonstrated a persistent IgM response, which typically occurs only transiently in early phases of an immune reaction. Using antibody phage display, Aimee S. Payne (University of Pennsylvania, Philadelphia) isolated anti-Dsg monoclonal antibodies from patients with pemphigus vulgaris and pemphigus foliaceus. Antibody gene sequence analysis indicated that a limited number of antibody variable-region genes had been used in the generation of pathogenic and nonpathogenic antibodies. More interestingly, Payne and

¹Department of Dermatology, Keio University School of Medicine, Tokyo, Japan; ²Department of Dermatology, Kurume University School of Medicine, Kurume, Japan; and ³Department of Dermatology, Gifu University School of Medicine, Gifu, Japan

President: Yasuo Kitajima; Secretary General: Takashi Hashimoto; Program Committee: Masayuki Amagai (Chair), Kathleen J Green, Michael Hertl, John R. Stanley, Kim B. Yancey, and Detlef Zillikens; Local Secretary Chair: Yumi Aoyama

Correspondence: Dr Masayuki Amagai, Department of Dermatology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: amagai@sc.itc.keio.ac.jp

John R. Stanley (also at the University of Pennsylvania, and the moderator of this session) reported that pathogenic monoclonal antibodies from humans and mice share a consensus motif (D/E-X-X-W) in the antigen-binding complementarity-determining region 3 of heavy chains, which provides a valuable opportunity to target pathogenic pemphigus antibodies therapeutically.

John J. Zone (University of Utah, Salt Lake City) described an important role for IgE antibodies in the pathogenesis of bullous pemphigoid. IgE monoclonal antibodies against LABD97, a portion of the shed extracellular domain of BP180 (ColXVII), induced blisters with mast cell degranulation and eosinophil infiltration in human skin grafts. LABD97 from basement membrane zone degradation initiated IgE cross-linking and mast cell degranulation with eosinophil infiltration and subsequent basement membrane zone vesiculation. Detlef Zillikens (University of Lübeck, Germany) presented two models of epidermolysis bullosa acquisita: a passive transfer mouse model with rabbit anti-type VII collagen (ColVII) antibodies and an active disease mouse model immunized with a recombinant ColVII NC1 domain. These models should be useful in analyzing autoimmune responses to ColVII and in developing more specific immunomodulatory therapies for epidermolysis bullosa acquisita.

Mysterious target antigens in autoimmune bullous diseases

Anti-p200 pemphigoid is a subepidermal autoimmune bullous disease characterized by IgG antibodies directed against a previously unknown 200-kDa antigen. Using two-dimensional gel electrophoresis of dermal extracts and mass spectrometry, Teruki Dainichi (Kurume University School of Medicine, Japan) and his colleagues identified this antigen as laminin- γ 1. After sera were collected from 20 patients with anti-p200 pemphigoid, immunoblotting was used to demonstrate that 75–85% of the samples reacted with various affinity-purified laminins containing laminin- γ 1. The major epitopes of laminin- γ 1 resided in the C-terminal region. Immunoprecipitation-immunoblotting assays with anti-laminin- γ 1 monoclonal antibody

and sera from anti-p200 pemphigoid patients confirmed the reactivity. This is a major advance in characterizing pathogenic mechanisms of anti-p200 pemphigoid, as well as in addressing questions related to structural biology, particularly in terms of laminin-integrin interaction.

Sergei A. Grando (University of California at Irvine) discussed non-desmoglein targets of pemphigus antibodies, including acetylcholine receptors α 3, α 9, pemphaxin, the α -chain of the high-affinity IgE receptor, and neuronal voltage-gated K⁺ channels. The significance of these non-desmoglein antibodies was discussed intensively, including the fundamental question of whether they fulfill the immunological version of Koch's postulates. A direct role for these antibodies in the pathogenesis of pemphigus remains uncertain.

The session was moderated by Takashi Hashimoto (Kurume University School of Medicine, Japan).

Plenary lectures

The first of two plenary lectures was delivered by Mikio Furuse (Kobe University Graduate School of Medicine, Japan), who discussed the role of tight junctions in epithelial barrier function, with special emphasis on the tight-junction-associated membrane protein claudin. The heterogeneity in claudin content creates functional diversity in the barrier properties of tight junctions. Claudin 1-deficient mice died within 1 day of birth with extremely dehydrated skin, indicating that tight junctions in the stratum granulosum are crucial for maintaining barrier function in mammalian stratified squamous epithelia. The human claudin 1 gene was identified as the causal gene for a syndrome associating ichthyosis and neonatal sclerosing cholangitis (NISCH syndrome). In the second plenary session, Akihiro Kusumi (Institute of Frontier Medical Sciences, Kyoto University, Japan) described a novel technique of single-molecule tracking in living cell membranes. Application of this technique will broaden our understanding of molecular interactions in cell membranes.

Signal transduction: primary or secondary for acantholysis?

Signal transduction was one of the hottest topics discussed at the meeting.

Yasuo Kitajima (Gifu University School of Medicine, Japan)—the session's moderator and a pioneer who in the early 1990s proposed an important role for signal transduction in the process of acantholysis using cultured keratinocytes—sent out questionnaires to speakers and other attendees to determine a common basis for discussion. The participants were asked, for example, whether antidesmoglein 3 (Dsg3) or Dsg1 antibodies are essential and specific in the pathogenesis of pemphigus acantholysis (seven said yes, one said no) and whether the depletion of Dsg3 or Dsg1 is a specific characteristic of keratinocytes in pemphigus (six “yes,” two “no”). David S. Rubenstein (University of North Carolina, Chapel Hill) demonstrated that MAPK p38 is activated by pemphigus IgG and that the blockade of p38 prevented blister formation in a passive transfer model using neonatal mice. Jens Waschke (Institute of Anatomy and Cell Biology, University of Würzburg, Germany), sharing data obtained via atomic force microscopy and laser tweezer experiments, proposed that anti-Dsg3 IgG in pemphigus vulgaris may fulfill a different role in the loss of cell-cell adhesion than anti-Dsg1 IgG in pemphigus foliaceus. Eliane J. Müller (Institute of Animal Pathology, University of Bern, Switzerland) emphasized the importance of plakoglobin in acantholysis and its downstream effects on keratinocyte proliferation and differentiation. Yumi Aoyama (Gifu University School of Medicine, Japan) demonstrated the involvement of p120-catenin in pro-acantholytic signaling. The availability of pathogenic and nonpathogenic monoclonal antibodies from pemphigus model mice (AK mAbs), as well as single-chain fragment variants from patients (rather than serum-derived polyclonal IgG), has facilitated progress in this field and will continue to do so.

The possible involvement of apoptotic and cdk2 signaling was addressed by Carlo Pincelli (University of Modena, Italy) and Nicolla Cirillo (University of Naples, Italy), respectively. These signaling theories were challenged by an argument that pemphigus acantholysis has not been regenerated using any



Figure 1. Participants in the Post-IID 2008 International Meeting on Autoimmune Bullous Diseases.

key signaling molecule without antibodies against Dsg3 or Dsg1.

Mouse model for pemphigus and pemphigoid

Three mouse models were discussed intensively in a session moderated by Masayuki Amagai (Keio University School of Medicine, Tokyo, Japan). The first was an active disease model for pemphigus generated by the adoptive transfer of Dsg3^{-/-} lymphocytes into immunodeficient mice expressing Dsg3. This model was used to dissect the immunological mechanisms of pathogenic anti-Dsg3 IgG production and to characterize Dsg3-specific B and T cells. Hayato Takahashi (Keio University School of Medicine, Tokyo, Japan) reported that Dsg3-reactive T-cell clones show pathogenic heterogeneity in the induction of anti-Dsg3 IgG production and that IL-4 plays an important role in this process. The second mouse model involved the humanized MHC class II and the CD4 coreceptor. These mice express the HLA class II alleles, DRB1*0402/DQB1*0302, which are strongly associated with pemphigus vulgaris but lack endogenous mouse class II. Rüdiger Eming (Philipps University of Marburg, Germany) demonstrated that this model mimics, at least in part, immune responses to Dsg3 in patients and provides an excellent tool for studying human responses in mice.

The final mouse model expressed the humanized bullous pemphigoid antigen, which was generated by the transgenic

expression of human BP180 in BP180-deficient mice, as presented by Wataru Nishie (Hokkaido University School of Medicine, Japan). This model overcame the long-standing problem of the lack of cross-reactivity between the human and mouse NC16a domains, the major epitopes of BP180. The advantages and limitations of these models were discussed extensively. Although mice and humans are different, these models provide valuable tools with which to clarify unsolved mysteries in autoimmune diseases and develop novel therapeutic strategies.

Rituximab and IVIG

Rituximab and intravenous immunoglobulin (IVIG) are recently introduced approaches to treating pemphigus, and their effectiveness has been documented in various institutes. In this session, moderated by Kim B. Yancey (University of Texas Southwestern Medical Center, Dallas), speakers shared their clinical experiences using rituximab, in France (Pascal Joly, Rouen University Hospital), Germany (Enno Schmidt, University of Lübeck), and the United States (Jean-Claude Bystry, New York University). Although protocols for the use of rituximab—alone or in combination with other medications—differed among these investigators, all agreed that rituximab is an effective treatment modality against pemphigus, which shows resistance to other therapeutic options. Joly emphasized that a single cycle of rituximab is sufficient, although its use should

be limited to the most severe cases to prevent potentially severe side effects.

Koji Hashimoto (Ehime University Graduate School of Medicine, Japan) presented results of a randomized double-blind multicenter clinical trial that examined the effectiveness of IVIG in steroid-resistant pemphigus. The first such clinical trial for pemphigus, it used a novel primary end point: time to escape from the protocol. This study concluded that a single cycle of IVIG at 400 mg/kg/day for 5 consecutive days can be a safe and effective treatment for severe steroid-resistant pemphigus.

A disease activity scoring system for pemphigus

Luca Borradori (University Hospital, Geneva, Switzerland) moderated a session in which participants examined validated scoring systems for rating disease activity, which are essential in large, multicenter controlled trials. Victoria P. Werth (University of Pennsylvania, Philadelphia) and Michael Hertl (Philipps University of Marburg, Germany) discussed the reliability and validity of the Pemphigus Disease Area Index (PDAI), which was developed by the International Pemphigus Committee, and the Autoimmune Bullous Skin Disorder Intensity Score (ABSIS), which was developed by Rüdiger Eming and his colleagues at Marburg. Werth, who presented the initial comparison between PDAI and ABSIS data collected by 10 experienced dermatologists from several countries, indicated that the PDAI is more reproducible and demonstrates better correlation with the consensus opinion of a patient's condition. Further studies, including photovalidation tests, are required to confirm these results. Hertl pointed out that results of the PDAI and the ABSIS should also be compared with patients' cumulative required dose of steroid, immunosuppressive agents, and titer of antidesmoglein IgG autoantibodies.

**The Post-IID International Meeting on Autoimmune Bullous Diseases was held at the Otsu Prince Hotel, Otsu, Japan, 17–19 May 2008.*