Abstracts of The National Pemphigus Foundation and The American Autoimmune Related Disease Association International Meeting: Pemphigus As a Model of Organ-Specific Humoral Autoimmune Diseases

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ORGANIZING COMMITTEE
Jean-Claude Bystryn, M.D., Grant Anhalt, M.D., Luis Diaz, M.D., John Stanley, M.D.

SPONSORS
The National Pemphigus Foundation, Atrium Plaza, Suite 203, 828 San Pablo Avenue, Albany, CA, USA. (510) 527-4970
The American Autoimmune Related Diseases Association, 22100 Gratiot Avenue, East Detroit, MI, USA. (810) 776-3900

CONTRIBUTORS
Aventis Behring, Fujisawa Healthcare, Inc., Genzyme Corp., INOVA Diagnostics, Inc.

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PURPOSE STATEMENT
Recent progress in the understanding of human autoimmunity provides a solid scientific foundation on which to base rational therapies for autoimmune diseases. Pemphigus has emerged as a classic example of an autoantibody mediated disease, and provides an excellent model to study the mechanisms of such diseases and to develop new options to treat them.

This meeting brought together scientists working on basic aspects of human autoimmunity with those working on pemphigus. Its goal was to review the current understanding of the causes and treatments of pemphigus, and identify the most important areas for future research on both pemphigus and other autoantibody mediated diseases.

The meeting focused on finding answers to the following questions:
● What is the etiology of autoimmune diseases in general, and pemphigus in particular?
● What are the factors affecting the autoimmune response characteristic of pemphigus?
● What are the most current treatments for pemphigus?
● What are the prospects for new treatments for pemphigus?
Cleavage by Granzyme B is Strongly Predictive of Autoantigen Status: Implications for Initiation of Autoimmunity

L. Casciola-Rosen, F. Andrade, D. Ullner, and A. Rosen
Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A.
Systemic autoimmune diseases are genetically complex disorders in which the immune system targets a diverse but highly specific group of intracellular autoantigens. The targets are not unified by common structure, function or distribution in control cells, but they are present in autoreactive immune cells under normal conditions. We have demonstrated that the majority of autoantigens targeted across the spectrum of human systemic autoimmune diseases are efficiently cleaved by granzyme B in vitro and during cytotoxic lymphocyte granule–induced death, generating unique fragments not observed during other forms of apoptosis. These molecules are not cleaved by caspase-8, although this protease has a very specific specificity to granzyme B. The granzyme B cleavage sites in autoantigens contain amino acids in the P2 and P3 positions that are preferred by granzyme B but are not tolerated by caspase-8. In contrast to autoantigens, nonautoantigens are either not cleaved by granzyme B or are cleaved to generate fragments identical to those formed in other types of apoptosis. This striking ability of granzyme B to cleave specific autoantigens may be a critical property of autoantigens that unifies the majority of molecules targeted in this spectrum of diseases. Several autoantigens targeted in tissue-specific autoimmunity (e.g. tyrosine kinases) are also generated by granzyme B. These results focus attention on the role of the cytotoxic lymphocyte granule–induced death pathway in the initiation and propagation of systemic autoimmunity.

Paraneoplastic Pemphigus ± Autoimmunity and Cancer

G. Anhalt
Dermatohematology, Johns Hopkins University School of Medicine, Baltimore, Maryland, U.S.A.
Paraneoplastic pemphigus (PNP) is a recently described form of pemphigus that is defined by the following: (a) the presence of mucosal ulcerations and blisters and a polymorphic skin eruption in patients with, or in the context of, an occult or known neoplasm (b) histologic findings of vacuolar interface change, loss of basal cell adhesion and plasminogen activator activity. The importance of desmoglein 3 in cellular adhesion was further demonstrated by genetically engineered mice with disruption of the desmoglein 3 gene. These mice demonstrated a defect in cutaneous barrier function and were prone to autoantibody production and development of mucosal and skin autoimmunity. The clinical, histologic, and immunohistologic features of PNP are also found in the course of bullous pemphigoid and histologically similar conditions, such as linear IgA bullous dermatosis. Studies have documented the presence of autoantibodies to desmoglein 3, amino acid residues 190–204, was predicted, verified experimentally, and recently shown to be a major autoantigen in PNP. In addition, autoantibody production to desmoglein 3 has also been documented in bullous pemphigoid, pemphigus vulgaris, and pemphigus foliaceus. The clinical features of PNP are similar to other bullous diseases and are related to the presence of autoantibodies to desmoglein 3. Treatment of patients with PNP is challenging and may include the use of immunosuppressive agents such as prednisone, cyclosporine, and azathioprine. The use of these agents in the treatment of PNP is supported by the demonstration of the efficacy of these agents in the suppression of autoantibody production and clinical improvement in patients with PNP.

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Pemphigus: Drugs and Infectious Agents as Inducing Factors
V. Russo
Department of Dermatology, 2nd University of Naples, Naples, Italy

The onset of pemphigus appears to depend on the interaction between predisposing and inducing factors. Genetic predisposition is known to be associated with human leukocyte antigen (HLA) in pemphigus vulgaris (PV) and pemphigus foliaceus (PF) [1]. In PV, there is an increased frequency of HLA-DR2 in patients, but not by itself sufficient to initiate the autoimmune response, as proven by the reports of pemphigus in only one of two monozygotic twins [2] and in only two of three siblings with identical predisposing factors. Furthermore, the existence of inducing factors seems to be required to set off the full-blown disease.

Even if the criteria of patients no inducing agent can be detected (idiopathic pemphigus), in several cases a metrical clinical history discloses facilitating factors (induced or triggered) pemphigus. Induced pemphigus paper refers to a condition where excessive factors play a major role, so that the disease regress after the inducing factor is eliminated, even without treatment. In triggered pemphigus, exogenous factors are more important and the inducing factors seem to only trigger, in a casual and non-specific manner, a disturbance mechanism previously programmed and ready to be set off, so that, in spite of elimination of the inducing factor, the disease self-perpetuates [4].

Induced or triggered factor of pemphigus may be effects of certain drugs (thiols, ACE-inhibitors, phenols, NSAIDs, interferons, and other cytokines) or some viruses [herpes virus and human papilloma virus (HPV)]. The induction of an autoimmune response can result in a eruption of pemphigus is often suspected on the basis of circumstantial evidence, but sometimes it can be demonstrated with certainty [4–7].

As for certain drugs, their potential of provoking an acantholytic change has been confirmed by several experimental investigations [8]. In particular, a drug may provoke an acantholytic by interfering with the keratinocyte microfilament and/or microtubule network. In pemphigus, the increase of the desmosomal plaque proteins, including desmocollin-3 (Dsg3). Using a passive transfer animal model, we have demonstrated that purified pemphigus vulgaris (PV) IgG eluted from a 75-kDa keratinocyte protein band both stained epidermis in a PV-like pattern and in a non specific manner, a disimmune mechanism previously programmed and ready to be set off, so that, in spite of elimination of the inducing factor, the disease self-perpetuates [4].

Therefore, we conclude that pemphigus is a complex process involving several antigen systems. To investigate the molecular mechanisms leading to blister formation in pemphigus

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The Pathogenic Autoantibody Response of Animal Models in Pemphigus
M. Amagai
Department of Dermatology, Keio University School of Medicine, Tokyo, Japan

Pemphigus is a unique and interesting autoimmune disease, in which IgG autoantibodies against cell-surface proteins of keratinocytes target the T-dependent immune responses usually requires the assistance of antigen-specific T cells as well as help from B and/or an ion channel. To identify the molecular structure of keratinocyte AChR(s) targeted by PV autoimmunity. In contrast to distinct

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Do T Lymphocytes Play a Role in the Development of Pemphigus?
M.-S. Lin
Department of Dermatology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, U.S.A.

Pemphigus is a cutaneous autoimmune disease characterized by intraepidermal blisters and circulating antibodies against several desmosomal proteins (1–4). Using a passive transfer animal model, we have demonstrated that pemphigus vulgaris (PV) IgG can induce pemphigus-like lesions in mice. The recognition of pemphigus vulgaris (PV) IgG, respectively. These results indicate that anti-Dsg antibodies are directly involved in the tissue damage, which occurs in pemphigus. Since the production of antibodies in pemphigus-prone genetic background is an important role to this plaque protein in maintaining the

010

Other Antigen Recognized by Pemphigus Autoantibodies
E. Frosch
Department of Dermatology, Kumamoto University School of Medicine, Kumamoto, Japan

The major autoantigens for classic types of pemphigus are desmogleins 1/3 (Dsg1/3) and desmocollin-1 (Dsc1). However, other autoantigens are also recognized by some of pemphigus patients. In pemphigus vulgaris (PV) sera react with various plakin family proteins, including plakin, desmoplakin, BP230, envoplakin, and periplakin, as well as some non-epidermal keratinocyte proteins such as keratin 10 and 19, suggesting that loss of tolerance against Dsg3 in both B and T cells is required for the development of the autoimmune state of PV. Furthermore, we have generated pathogenic anti-Dsg3 monoclonal antibodies from PV model mice. Our model is a valuable tool to dissect cellular and molecular mechanism of autoimmune production as well as to develop novel therapeutic strategies.
The Role of Antibody Distribution in the Localization of Pemphigus Lesions

M. Mahoney
Department of Dermatology and Cutaneous Biology, Thomas Jefferson University, Philadelphia, Pennsylvania, U.S.A.

Pemphigus is a group of autoimmune blistering disorders of the skin and mucous membranes resulting from loss of epithelial cell-cell adhesion due to circulating pathogenic autoantibodies. These autoantibodies are directed against cell adhesion molecules, including the intracellular cadherins desmoglein 1 (Dsg1) and desmoglein 3 (Dsg3). Dsg proteins are transmembrane glycoproteins that function as adhesive components of desmosomes, discontinuous junctions that are crucial for maintaining cell-cell adhesion, tissue integrity, and organ function. The two classic forms of pemphigus are pemphigus foliaceus (PF) and pemphigus vulgaris (PV). Induction of the pemphigus disease state requires the presence of autoantibodies and desmoglein as a target. A recent study suggests that epitopes on Dsg1 and Dsg3 are shared in patients with anti-Dsg1 antibodies have been shown to bind to the entire epithelium and to the superficial epithelia of oral mucosa. In contrast, PF is restricted to Dsg3 (the bullous pemphigoid, or BP, target). The bullous pemphigoid target is acantholysis of epidermal cells as well as epithelial cells of the oral mucosa, and the blisters occurring within the basal and suprabasal layers. To correlate pemphigus antibody profiles with tissue distribution of pemphigus antigens, we assessed the distribution of anti-Dsg1 antibodies in serum from PF and PV, we used the neonatal mouse model of pemphigus by passive transfer of IgG from normal and DSG3 knock-out mice (DSG3−/−). First, we determined tissue distribution of anti-Dsg1 antibodies in serum from PF patients with pemphigus foliaceous similar to that in human tissue. Dsg1 is expressed throughout the epidermis and the oral mucosa. In contrast, Dsg3 is restricted to the superficial epidermis and the oral mucosa. To demonstrate the role of Dsg1 in limiting blister formation in PF, we injected mice with 1 mg of PF IgG. This low dosage (3/10 of the actual amount) of PF IgG caused small isolated blisters in DSG3−/− mice whereas no blisters were formed in DSG3+/- mice or in DSG3+/- mice. Thus, this annular deaf of Dsg1 are more susceptible to blister formation by anti-Dsg1 antibodies. We hypothesized that blisters occur in PF where Dsg1 is down regulated, namely on the superficial epidermis. As predicted, PF IgG only caused superficial epidermal acantholysis and no mucous membrane lesions in DSG3+/- or +/+ mice (n = 20), but in DSG3−/− mice (n = 10) in superficial and deep epidermal acantholysis and marked acantholysis of tongue mucosa (n = 7). These data also clarify the recent observation that PV patients with exclusively oral lesions have only anti-Dsg3 antibodies, while patients with skin involvement have also anti-Dsg1 antibodies, indicating that both antibodies may be necessary to interfere with both desmogleins in the deep epidermis. We confirmed the pathogenicity of the anti-Dsg1 antibodies from PV sera in deep epidermal acantholysis with deep epidermal acantholysis in PV IgG in DSG3−/− mice (n = 3). Furthermore, while PV sera containing anti-Dsg1 alone were ineffective at causing acantholysis, the combination of anti-Dsg1 and anti-Dsg3 resulted in acantholysis. These data suggest that pemphigus autoantibodies inhibit the adhesive function of desmoglein proteins and that either Dsg1 or Dsg3 alone is sufficient to maintain keratinocyte cell-cell adhesion.

Mechanism of Action of IVIg in Pemphigus

J.-C. Bystryn
Department of Dermatology, Nyu Medical Center, New York, New York, U.S.A.

Background Pemphigus vulgaris (PV) is a blistering skin disease mediated by autoantibodies to intercellular (IC) epidermal antigens. We evaluated the effectiveness of intravenous immunoglobulin (IVIg) for the control of active disease, and the mechanism of action of this agent.

Methods Six patients with active PV unresponsive to conventional therapy were treated with IVIg (400 mg/kg/day for 5 days) and concurrently given cyclophosphamide (100–150 mg/day). The five patients, aged 18–70 years, were untreated for a median of 20 days. During the IVIg therapy period, clinical activity of the disease was measured and the safety and tolerability of the treatment were monitored.

Results Within 2 weeks of initiating IVIg, the activity of the patient was controlled in all but one case and the extent of skin lesions was reduced on the average by 80%. Within 3 weeks, steroid doses were reduced on average to 50% of the initial dose. The improvement was more rapid than that reported for patients treated conventionally. Clinical improvement was associated with a rapid and selective decline in IC antibodies whose levels decreased by 72% within 1 week of initiating IVIg. The rapidity and extent of this decline were similar to that achieved by intensified plasmapheresis. The decline is not due to blocking the synthesis or the immunological activity of IC antibodies by IVIg, but to the high avidity of the IVIg for Fc receptors that it elicits in the patients.

Conclusions These results indicate that IVIg can effectively and rapidly control active PV and suggest a novel explanation for its mechanism of action. It is that it increases the catabolism of IC antibodies, and that this is achieved by the IVIg acting as a pharmacologic modulator of IC antibody production and turnover.

Novel Approaches to Therapy Based on Understanding Antibody Distribution

J. Stanley
Department of Dermatology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, U.S.A.

Understanding the pathophysiology of pemphigus leads to a rational approach to therapy. For example, in pemphigus foliaceus, the pathogenic antibodies are directed against desmoglein 3 (Dsg3), which is expressed in the epidermis and causing acantholysis. However, since in some areas there are two dsg isoforms (1 and 3) (e.g. in the deep epidermis and the oral mucosa), it is possible that the dsg 3 can be recruited by a pemphigus antibody-induced loss of function of dsg 1, preventing acantholysis. Similarly, induction of expression of dsg 3 in the superficial epidermal pemphigus PV-induced blisters.

The present therapy of pemphigus utilizes corticosteroids and various immunosuppressive drugs sometimes in combination with plasmapheresis. The rationale basis for immunosuppressive therapy and plasmapheresis is that they lower the titer of pathologic autoantibodies (along with all other antibodies). However, this cannot be the rationale for corticosteroids, because they act within days (when antibody titers may not be yet lowered) and even act locally. We hypothesize that corticosteroids might induce the transcription of dsg isoforms, providing protection from anti-dsg antibodies. Furthermore, innovative future therapies might be directed towards inducing compensating dsg isoforms.
Cooperative Clinical Trials in PV: Where Are We? V. Worth
University of Pennsylvania, Philadelphia, Pennsylvania, U.S.A.

There is a complete absence of multicenter, randomized controlled trials to evaluate the therapies used in PV. This has resulted in the lack of any standard treatment in PV from university to university and practitioner to practitioner. Reviews are based largely on case series and case reports, and there is little high quality evidence in guide therapeutic decision making. While studies, it is difficult to get insurance companies to approve potentially beneficial therapies. The difference between conventional and alternative medicine in regards to the treatment of PV is striking. To help with the application of scientific method to the analgesic on anolytes and therapies to find and treat the underlying disease processes to directing therapeutic choices. We discuss the likely need for standardized clinical studies, and it is clearly to those truly interested in the treatment of PV to develop collaborative multicenter protocols, such as those targeting autoantibodies, autoantigens, and receptor activation studies. There has been a lack of funding mechanisms for such an effort, and the cooperation between the various PV centers is quite distant from any natural of basic science research.

In 1996, the Medical Dermatology Society (MDS) was formed, and one of the goals was to serve as a coordinating body for the treatment of multicenter cooperative studies in medical dermatology, taking advantage of the collaborative spirit common to many centers. The Statisticians, and discussion with experienced trialists in the rheumatology community, that a study that had a more uniform entry population would be the best initial study. To achieve this, it was decided to study patients who were in the maintenance phase of their disease, and steroid-dependent. Such patients were often unable to taper GCs below 15-40 mg prednisone a day without experiencing new skin lesions. Preliminary studies indicate that one potential GC-sparing agent in the treatment of PV is the desire to apply the scientific method and not to rely on anecdotes and theories in the treatment of PV from university to university and practitioner to practitioner. Reviews are based largely on case series and case reports, and there is little high quality evidence in guide therapeutic decision making. While studies, it is difficult to get insurance companies to approve potentially beneficial therapies. The difference between conventional and alternative medicine in regards to the treatment of PV is striking. To help with the application of scientific method to the analgesic on anolytes and therapies to find and treat the underlying disease processes to directing therapeutic choices. We discuss the likely need for standardized clinical studies, and it is clearly to those truly interested in the treatment of PV to develop collaborative multicenter protocols, such as those targeting autoantibodies, autoantigens, and receptor activation studies. There has been a lack of funding mechanisms for such an effort, and the cooperation between the various PV centers is quite distant from any natural of basic science research.

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There is a lot of work to do. The PV trial group meets annually at the AAD meeting, is happy to involve all interested centers, and is truly looking for collaborative interactions. Funding mechanisms to link centers should reflect the necessarily collaborative nature of such initiatives.

Peptide Based Vaccine Strategies
M. Raco and J. Rasmussen

Vaccination has been used for over 160 years to specifically induce or ameliorate immune responses in human. Vaccination advantages over conventional disease treatment include that the targeting is selective, specific, and durable. In the case of pemphigus, development of a vaccine to down-regulate or eliminate pathogenic desmoglein responsive immune elements in patients, or prevent development of desmoglein specific responses in susceptible individuals would avoid the side effects for glucocorticoids and immunosuppressive regimens. The feasibility of a vaccine approach stems from our understanding of the relatively selective specificity and durability of the T cell responses. We have for the past 5 years been concentrating our efforts in our understanding of vaccine immunobiology. Current experiments relevant to autoimmune diseases focus on the generation of regulatory T cells or immune deviation via: (1) immunization with peptides or altered peptide ligands, (2) use of MHC-peptide complexes with or without a cytotoxic fusion partner; and (3) induction of high-zone tolerance using native peptide ligands. All 3 of these strategies are currently in development for other autoimmune processes including rheumatoid arthritis, rheumatoid arthritis, and multiple sclerosis. The goal is to use these model system to understand the immune responses to peptide antigens and to develop model systems that mimic the function of these cells. Immunodepletion Therapy of Refractory Pemphigus
R. Brodsky, H. Nosarz, R. Jones, and G. Anhalt
John Hopkins Hospital, Baltimore, Maryland, U.S.A.

Pemphigus encompasses a group of autoimmune mucocutaneous blistering diseases characterized by two features: a) disruption of the cell-cell adhesion of stratified squamous epithelia and (b) the presence of pathogenic IgG autoantibodies reacting against desmosomal adhesion molecules. The three major subsets of the disease are pemphigus vulgaris (PV), pemphigus foliaceus (PF), and paraneoplastic pemphigus (PNP). We have previously demonstrated that high-dose cyclophosphamide (Cy) without anti-thymocyte globulin (ATG) can induce durable complete remissions in a variety of severe autoimmune disorders including aplastic anemia, systemic lupus erythematosus, and autoimmune hemolytic anemias. We now report the results of high-dose Cy (30 mg/kg/day for 4 consecutive days) in 6 patients with refractory PV. Eligible patients had persistent disease activity despite treatment with mycophenolate mofetil (MMF) and/or azathioprine (az) and were dependent on corticosteroids. Patients had a median age of 33.5 years (range, 27–47) years, a median disease duration of 3.5 years (range, 2–5) years, and a median prednisone dosage of 50 mg (range, 30–65) mg/day. All patients were refractory to az, 5 were refractory to MMF. High-dose Cy was well tolerated; common toxicities included reversible alopecia, transient nausea/vomiting, febrile neutropenia (2 patients). The median time to a neutrophil count of 0.5x10^9/L was 11.5 days (range, 11–18) days; the median number of packed red cell transfusions was 2 (range, 0–4 units and the median number of platelet transfusions was 1 (range, 0–21). Complete remission (CR) was defined as: resolution of all skin lesions and reduction of circulating pemphigus antibody levels to ≤ 1: 20. Partial remission (PR) was defined as: improvement in skin lesion and reduction of pemphigus antibody levels to ≤ 1: 80. Four patients have achieved a CR and 2 patients are in a PR with a median follow-up of 6 (range, 1–30) months. The median prednisone dosage after therapy in 2.5 (range 2.5–10) mg/day. Additional follow-up and a larger number of patients are needed; however, these preliminary data suggest that high-dose Cy can produce durable complete remissions in patients with refractory PV.

Gene Therapy to Treat Autoimmune Diseases
C. Fathman
Department of Medicine, Division of Immunology and Rheumatology, Stanford University School of Medicine, Stanford, California, U.S.A.

CD4+ T Cells have been implicated in the pathogenesis of several autoimmune diseases such as rheumatoid arthritis (RA), type 1 diabetes (Mellis) and TID (IDDM). Autoreactive-specific T Cells have specific homing properties, suggesting that these cells may be ideal vehicles for the local delivery of “immunoregulatory” products. We have used this homing model of autoimmune disease by using autogenous-specific CD4+ T Cells expressing, following gene transfer, as vehicles to deliver “immune-regulatory proteins” for the treatment autoimmune models of human autoimmune disease. Autoreactive-specific T Cells were transduced to express the interleukin (IL)–12 antagonist, IL-12p40, using retroviral vectors encoding IL-12p40 cDNA. Transfer of relevant autoreactive-specific IL-12p40 producing CD4+ T Cells after immunization significantly prolonged the development of collagen-induced arthritis (CIA) or EAE, while cells transduced with the vector control had no effect. Additionally, i.d. specific T Cells, transduced to express IL-12p40 blocked the adoptive transfer of CIA or EAE. The relevance of these findings to the development of CIA and EAE was not clear. The goal is to use these model system to understand the immune responses to peptide antigens and to develop model systems that mimic the function of these cells.

A tolerance using native peptide ligands. All 3 of these strategies are currently in development for other autoimmune processes including rheumatoid arthritis, rheumatoid arthritis, and multiple sclerosis. The goal is to use these model system to understand the immune responses to peptide antigens and to develop model systems that mimic the function of these cells.

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P001

Pemphigus Vulgaris (PV): The Cleveland Clinic Experience 1987–2000

C. Cannis and W. Kurz

Cleveland Clinic Foundation (CCF), Department of Dermatology, Cleveland, Ohio, U.S.A.

The purpose of the study was to assess the clinical and histological profile and treatment outcomes in a consecutive group of patients with PV followed at one center. A retrospective chart analysis with telephone follow-up on 36 PV patients seen at the CCF between 1987 and 2000 was performed. The gender of patients was 66% female and the mean age of onset of PV was 48 years. The areas of involvement: 56% oral, 38% (67.9%) skin, 16% (26.6%) genital, 15% (26.6%) nasal, < 25% pyrargyral, oculair, laryngal, anal, or esophageal involvement (in descending order). 63% (96.9%) of 63 biopsies in 53 patients were consistent with PV. 43% (81.1%) of 53 direct immunofluorescence tests performed in 46 patients were consistent with PV. 45% (90.0%) of the 50 indirect immunofluorescence tests performed were positive for PV. 44% (78.6%) achieved complete remission (CR) with a median duration of 17 months. The medications used at onset of CR were prednisone in 30 (68.2%), dapsone in 10 (22.7%), azathioprine in 8 (18.2%), and no treatment in 5 (11.4%). topical corticosteroid, cyclophosphamide, oral methyl prednisolone, tetracycline, niacinamide, and mycophenolate (in descending order). The median time needed to achieve CR after the first visit was 9 months. The majority of PV patients were females. Middle age was the time at which the disease most often presented. The oral cavity was involved in all cases over the course of the disease. Histologic examination of biopsy material confirmed the diagnosis of PV most reliably. CR was achieved in the majority of patients using combinations of medications. Drugs associated with CR most frequently were prednisone, dapsone and azathioprine either alone or in combination.

P003

Immunological Follow-Up of Patients with Pemphigus Vulgaris

M. Hered, A. Stauber, R. Eising, R. Sperath, and R. Kirschner

Department of Dermatology, University of Innsbruck, Austria

There is major interest in defining immunological markers that can be applied to monitor the activity of pemphigus vulgaris (PV). We thus sought to correlate the clinical activity of PV with T helper (Th) cell and auto-antibody (Ab) reactivity against desmoglein 3 (Dsg3), the major autoantigen of PV.

Desmoglein 3 (Dsg3) Ab subtypes against Dsg3 was analysed by immunoblot and ELISA analysis utilizing baculovirus-derived recombinant Dsg3. The cell reactivity against Dsg3 was quantitated by EILSPOT analysis. A total of 41 patients with PV were examined by immunoblot analysis. In active PV, Dsg3-reactive IgG1 was detected in 28/36 (60%), IgG in 29/36 (80.5%), IgA in 21/33 (63.6%) and IgD in 4/33 (12.1%) sera. Sera from patients with remittent PV contained Dsg3-reactive IgG in 6/ 8 (75%) and IgG4 (5/8) activity, but not Dsg3-reactive IgA and IgD. By ELISA, sera from patients with active disease contained titers of Dsg3-reactive IgG4 that exceeded those of IgG1 and IgA by a factor of at least 2. By EILSPOT assay, three patients with active oral PV had 3.8 ± 6.6 reactive Th1 cells/10^5 PBL while 0/3 PV patients in remission had detectable autoreactive Th1 cells/10^5 PBL. Our study suggests that, in quantitating total IgG reactivity to Dsg3, the titers of Ab subtypes may be helpful therapeutic and/or prognostic markers in PV. Quantification of autoreactive Th1 and Th2 cells may represent an additional highly sensitive parameter of disease activity and may provide further insight into the role that autoreactive Th2 cells play in the pathogenesis of PV.

P004

Pemphigus Erythematosus: An Attempt to Redefine the Clinical and Immunopathological Features

K. Shiragawa, R. Shigutky, M. Magaji, B. Volic-Platerz, R. Kimbauer, and G. Tingl

University of Vienna Medical School, Vienna Austria and Kero University School of Medicine, Tokyo, Japan

Diagnostic criteria for Pemphigus erythematosus (PE) remain to be defined. PE was originally described as a variant of pemphigus with additional features of lupus erythematosus. Other associated diseases included thyroiditis, myasthenia gravis and rheumatoid arthritis. By direct immunofluorescence (DIF) PE typically shows intercellular substance (ICS) and basement membrane zone (BMZ) staining. Its diagnosis is often based on the localization of eruptions in seborrheic and/or malar areas and currently PE is frequently regarded a localized early form of pemphigus foliaceus (PF).

In this report we describe two patients with erythematosus erosive skin lesions without mucosal involvement. The first patient with 11 years' medical history of seropositive rheumatoid arthritis showed classical localized PE whereas the second patient with an unremarkable medical history displayed a single erosive lesion with crust confined to the forehead. Lesional biopsies showed superficial acantholytic; DIF testing revealed an ICS staining pattern with IgG and C3 in both patients. Indirect immunofluorescence (IEF) performed on monkey esophagus showed circulating anti-ICS antibodies with a titer of 320 and 80, respectively. Using normal human "salt split skin" as a substrate both patients' sera contained circulating anti-BMZ IgG antibodies that bound to the epidermal side. By ELISA and by Western blot desmoglein-1 (Dsg-1) specific antibodies were detected in the first patient's serum. In contrast, desmoglein-3 (Dsg-3) specific antibodies were present in the serum of the second patient. In addition, both sera contained antibodies targeting a 250-kDa band comigrating with the bullous pemphigoid antigen-1 (BPAg-1). Laboratory studies revealed a positive rhammoccid factor and elevated antinuclear antibodies in the former but not in the latter patient. Despite the differences in the extent of skin involvement, in autoantibody reactivity against desmogleins and in associated diseases, we believe that both patients qualify as PE localized form of PF. They both show similar superficial primary lesions without involvement of mucous membranes and both display a combined ICS and BMZ staining pattern by DIF.

Thus, PE can occur as a generalized as well as a localized disease and does not represent simply a localized variant of PF. In addition, we propose that PE is not necessarily associated with other autoimmune diseases and the PE sera not only target Dsg-1 but can also target Dsg-3. The significance of the anti-BPAg-1 antibodies in both patients' sera escapes us at the present time; while they may represent an innocent bystander phenomenon it is tempting to speculate that they serve as a trigger of the anti-desmoglein mediated disease. Larger studies are warranted to investigate the presence of anti-BP-Ag-1 antibodies in is regular occurrence in PE.

P005

IgA Pemphigus. Report of Five Cases with Different Therapy Approach

B. Marinovic and J. Lipozencic

Department of Dermatology and Venerology, Zagreb University Hospital Center, Zagreb, Croatia

We previously described a silent single nucleotide polymorphism (SNP) of the desmoglein 1 gene which consists of a T to C transition at position 809. To investigate the role of genetic background in pemphigus foliaceus and to ask whether PF, like other autoimmune diseases, is expressed as a complex trait, we simultaneously examined the role of major histocompatibility complex (MHC) class II polymorphism and SNP. Fifteen PF patients were included in the study. Thirty-one Caucasian French patients and 84 healthy Caucasian French controls were studied by PCR-REFLP for SNP(S809) genotyping and by PCR-SNO and PCR-SSP for DRB1 and DQBI typing.

This analysis confirmed involvement of DRB1*04 (p = 0.01) and DRB1*14 (p = 0.04) genotypes in disease susceptibility and individualized DRB1*0402 (p = 0.04), DRB1*0402 (p = 0.02), DRB1*0401 (p = 0.03), DRB1*1404 (p = 0.03), and DRB1*1404 (p = 0.03). Antigenic profile of PF patients. Homozygous C/C(S809) genotype was also found associated with the disease (p = 0.03). Furthermore, patients with both DRB1*04 and C/C(S809) had a very significant risk to develop PF (p = 0.0001). In conclusion, patients with PF carrying both DRB1*04 and C/C(S809) were more likely to develop PF than patients carrying other combinations of genetic background. This may provide a new opportunity to understand disease susceptibility for PF. Detection of other polymorphisms may be of interest in further studies for genetic susceptibility to pemphigus foliaceus.

P006

Pemphigus Foliaceus as a Model of Polygenic Disease

P. Martel, D. Gilbert, M. Busson, L. Drouot, P. Joly, D. Charron, and F. Tron

INSERM U519, Rouen, France, Hospital Saint Louis, Paris, France

We previously described a single nucleotide polymorphism (SNP) of the desmoglein 1 gene which consists of a T to C transition at position 809. To investigate the role of genetic background in pemphigus foliaceus and to ask whether PF, like other autoimmune diseases, is expressed as a complex trait, we simultaneously examined the role of major histocompatibility complex (MHC) class II polymorphism and SNP. Fifteen PF patients were included in the study. Thirty-one Caucasian French patients and 84 healthy Caucasian French controls were studied by PCR-REFLP for SNP(S809) genotyping and by PCR-SNO and PCR-SSP for DRB1 and DQBI typing.

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P007
The Pathogenic Study of EC1-2 of Pemphigus Vulgaris Antigen
M. Pazi, J. Zhen, X. D. Kang, W. P. Li, and F. Xue
Ruijin Hospital affiliated to Shanghai Second Medical University, Dermatology, Shanghai, China

Purpose Pemphigus Vulgaris autoantigen (PV A) is a transmembrane glycoprotein, which is called desmoglein 3 (Dsg3). It has five extracellular domains (EC1 to EC5). The purpose is to test whether EC1-2 is immunogenic domain and whether its antibody is pathogenic by establishing neonatal mouse model of PV.

Methods A segment of the human Dsg3 cDNA (about 1300 bp in the amino terminus, including EC1-2) was subcloned into glutathione S-transferase (GST) gene by pGEX-4T-1 expression vector, and the recombinant vector was used to generate GST fusion protein (FP) containing the EC1-2 segment of Dsg3. The FP was expressed in E. coli JM109 and was purified by glutathione affinity chromatography. It was confirmed by nucleotide sequence analysis and immunoblotting analysis with purified EC1-2 FP. New Zealand white rabbits were immunized with the EC1-2 FP. IgG fraction was got from the antiserum of rabbit by precipitation with caprylic acid, followed by DEAE-S2 ion-exchange chromatography. Then it dialyzed against phosphate-buffered saline (PBS, pH 7.4) and concentrated with Polyethylene glycol 20000 to 40mg/mL. Purified IgG fraction was injected subcutaneously into the upper back skin of neonatal BALB/c mice (<24h of age). The IgG dose was 200µg each time, two doses 5h apart, about 10mg/g body weight. Neonatal mice were examined 16h after injection. Skin was studied by light microscopy, electron microscopy and direct immunofluorescence (DIF). Serum was assayed by indirect immunofluorescence (IF).

Results Immunoblotting analysis of EC1-2 FP with 22 PV patients sera demonstrated that 17 PV patients were positive. By DIF, the skin of mice showed IgG antisera deposition on the cell surface of acantholytic cells. By IF, using normal neonatal mouse skin as substrate, the titer of IgG was 40-80. Histologically, we can see stratum corneum detached and acantholysis, but no blisters in the epidermis by light microscopy. Electronic microscopy examination confirmed that wider intercellular spaces (10§) occurred in the regions of the cell surface. Conclusions These results show that EC1-2 in the amino terminals of Dsg3 is immunogenic in PV and its antisera is pathogenic. The antibody of EC1-2 FP can induce a neonatal mouse model of PV which gives a tool of studying autoimmune disease.

P008
Direct Immunofluorescence of Skin, Oral Mucosae and Esophagus in Pemphigus Vulgaris Patients in Remission
M. Pulido-Galvan, F. Barzallo-Viterri, and G. Leon-Dorantes
Hospital General de Mexico, Mexico City, Mexico

Objective To compare the results of skin, oral mucosae and esophagus direct immunofluorescence (DIF) tests of patients with pemphigus vulgaris in the remission stage of treatment.


Methods Female or male adult patients with history of confirmed pemphigus vulgaris in remission stage (absence of skin or mucosal lesions for more than 6 months, with <20 rashes/prednisone /day), with informed consent had three biopsies: skin, oral mucose, and esophageal mucose. Esophagus biopsies were obtained by endoscopic means. Tissues were tested with a standard method of direct immunofluorescence.

Results 8 female and 2 male patients, 46 (3.1-12-2) years of mean age and 11 (5.6-2) months mean time under remission were included. 4 patients were not taking already prednisone. Nine had objective esophageal abnormalities when endoscopy was performed. DIF was positive in 7 esophagitis biopsies, 4 oral mucose biopsies and only 3 skin biopsies. Sensitivities of DIF skin test and of DIF oral mucose test were 43% and 57% as compared to DIF esophagitis mucose test. Conclusions A negative DIF esophageal mucose test may be useful when the decision to stop treatment becomes an issue.

P009
Kininogens-Kallikreins-Kinin System in Plasma of Brazilian Patients with Pemphigus Foliateux
T. Roselli, R. Jovilliano, M. Reiz, A. Roselino, and E. Donadi
School of Medicine of Ribeirao Preto-USP, 2. School of Pharmaceutical Sciences of Ribeirao Preto-USP, Brazil

Objective Pemphigus foliaceus (PF) is an autoimmune bullous disease affecting the skin caused by autoantibodies against desmoglein. Considering that there are few studies involved kallikrein-kinin system, we evaluated the activation of kininogens in plasma of PF patients.

Methods Fifteen patients (11 men and 4 women) presenting active PF with nikolsky sign were included. The concentration of total kininogen (TKg), low molecular weight kininogen(LKg) and was determined by ELISA(J Reumathol., 25:1-4, 1988).The activity of plasma kallikrein and tissue kallikrein were evaluated upon selective substrates.

Results The results are shown in the table and indicate the median values of kininogens and substrates.

Conclusion The impaired levels of kallikrein and a decreased kininogens suggested a possible role of this system in PF, we evaluated activation of kininogens in this condition.

P010
Dendritic Cells in Endemic Pemphigus Foliaceus (EPF)
M.P. Chiossi, R.S. Costa, and A.M. Roselino
University of Sao Paulo, Faculty of Medicine of Ribeirao Preto, Brazil

To elucidate the pathophysiology of EPF, dendritic cells (DC) were measured in skin biopsies (lesional skin) from 22 EPF patients and in normal thoracic sun-protected skin of a non-epidermal area in 13 of them. Controls consisted of normal thoracic skin from 8 cadavers and from 12 women submitted to breast plastic surgery. DC were identified with anti-CD1a and quantified by morphometry. Epidermal DC numbers in lesion [60±18 DC/mm², 5.0±0.6 DC/mm basement membrane (BM)], 3.55 DC/mm stratum corneum (SC) and normal skin (28.45 DC/mm², 2.50 DC/mmBM, 2.87 DC/mmSC) of EPF patients were similar to those for the plastic surgery (72.35 DC/mm², 4.53 DC/mmBM, 4.42 DC/mmSC) and cadaver controls (47.15 CD/mm², 2.53 CD/mmBM, 2.42 CD/mmSC). Dermal DC number in lesions (0.98 DC/mmBM) of EPF patients was similar to the plastic surgery control (0.48 DC/mmBM), but higher than in the cadaver controls (0.13 DC/mmBM, p < 0.05). The epidermal DC/mmBM/dermal DC/mmBM ratio was lower in lesional EPF skin (5.72) than in controls (9.22, p < 0.05), confirming that dermal DC could play an important role in EPF pathogenesis. We may propose that DC may be in transit through the dermis towards the regional lymph nodes, stimulating T lymphocytes to produce autoantibodies.