Research Advances in Pemphigus

Grant J. Anhalt, MD
Luis A. Diaz, MD

Pemphigus is an autoimmune disorder, known to be caused by autoantibodies directed against critical adhesion molecules of squamous epithelial cells, the desmogleins. These autoantibodies induce blistering of skin and mucosal surfaces and lead to severe morbidity and, potentially, death. Key factors include associated major histocompatibility complex class II genes, the structure of the desmoglein antigens, and the role of autoantibody in impairing cellular adhesion. This article discusses the precise structure of the major histocompatibility complex class II gene–peptide–T-cell receptor complex involved and of the environmental and genetic factors that induce autoimmunity against desmoglein 1. Discovery of antigen-specific immunotherapy and insight into environmental factors that initiate autoimmunity in genetically susceptible individuals are needed.

JAMA. 2001;285:652-654 www.jama.com

PEMPHIGUS IS A GROUP OF ORGAN-specific autoimmune cutaneous disorders that includes pemphigus vulgaris (PV) (FIGURE), pemphigus foliaceus (PF) and its endemic form seen in Brazil known as Fogo Selvagem (FS), and paraneoplastic pemphigus (PNP). These diseases are characterized by development of blisters and erosions on skin and mucous membranes, caused by cell-cell detachment of epidermal and mucosal epithelial cells (acantholysis).[1,2] The frequency depends on prevalence of susceptibility genes in individual populations.

Although pemphigus is rare, its impact on affected individuals is devastating. In the absence of therapy, these disorders can be lethal as a result of skin loss, oropharyngeal ulcerations, debilitation, and sepsis. The use of glucocorticoids and immunosuppressive drugs has improved prognosis, although quality of life is impaired by serious adverse effects from these drugs. Mortality due to pemphigus in the United States is approximately 10% for PV and PF but approaches 95% for PNP.

In all forms of pemphigus, autoantibodies against desmosomal cadherins and CD4 T cells recognize the extracellular domain of the desmosomal cadherins: desmoglein (Dsg)1 in PF and FS and Dsg3 in PV. The events that induce autoantibody production are unknown, but may be induced by drugs such as *d*-penicillamine.[3] In some cases, the disease disappears when the drug is withdrawn, but it usually continues even after removal of the initiating agent.

Major Clinical and Research Advances

Major research advances of recent decades include (1) discovery that patients with PV possess antiepidermal autoantibodies in lesional skin and in serum[4]; (2) demonstration that autoantibodies in all forms of pemphigus are pathogenic by passive transfer into neonatal mice, ie, the animals develop blisters that mimic the human disease[5,6]; (3) demonstration that PF autoantibodies recognize a desmosomal protein, Dsg 1[7];(4) demonstration that PV autoantibodies recognize Dsg3[8]; (5) demonstration by that cloning Dsg1 and Dsg3 belong to the cadherin family of calcium-dependent cell adhesion molecules[9-11]; (6) recognition that PNP is a distinct form of the disease with associated lethal pulmonary involvement and autoantibodies that recognize Dsg1, Dsg3, and antigens of the plakin family of structural epidermal proteins[12,13]; (7) demonstration that both PV and PF are associated with certain major histocompatibility complex (MHC) II alleles, 2 rare haplotypes with PV (DRB1*0402 and DQB1*0503),[14,15] and expression of DRB1 0404, 1402, or 1406 alleles in FS[16]; (8) production of a Dsg3 knockout mouse that developed spontaneous acantholysis of oral mucous membranes[17]; and (9) development of the first active animal model of the disease, created by immunization of the Dsg3 knockout mouse with Dsg3, transfer of splenocytes to immunodeficient mice, and production of pathogenic autoantibodies by the transfused immune cells.[18]

Current Research Activities

Epidermal cell detachment, the hallmark of all forms of pemphigus, is triggered by binding of anti-Dsg autoantibodies to unique epitopes of the ectodomain of these molecules. The epitopes recognized by pathogenic anti-Dsg1 or anti-Dsg3 autoantibodies are conformational and calcium-dependent[19] but are not fully characterized. The events following this initial antigen-antibody reaction that lead to cell detachment are the subject of intense investigation. The postulated mechanisms include direct impairment of binding of Dsg molecules on one keratinocyte to those on an adjacent keratinocyte and down-regulating their adhesive function[20] or as a result of phosphorylation of these proteins[21]; antibody-triggered activation of transmembrane signaling pathways; or altering the balance of Dsg1 to Dsg3 in the targeted epidermal desmosomes.[22]

Some data suggest that induction of increased levels of plasminogen activator (PA) on the cell surface of affected

Author Affiliations: Division of Dermatoimmunology, Johns Hopkins University School of Medicine, Baltimore, Md (Dr Anhalt); Department of Dermatology, University of North Carolina, Chapel Hill (Dr Diaz).
Corresponding Author and Reprints: Grant J. Anhalt, MD, Division of Dermatoimmunology, Johns Hopkins University School of Medicine, 720 Rutland Ave, Room 771, Baltimore, MD 21205 (e-mail: GJA909@aol.com).

©2001 American Medical Association. All rights reserved.

Downloaded From: http://jama.jamanetwork.com/ on 05/05/2015

keratinocytes may contribute to acantholysis through the generation of proteolytic enzymes such as plasmin that can degrade adhesion molecules other than Dsg. However, this mechanism does not appear to be essential. After passive transfer of antibody in vivo, blistering is not prevented by inhibition of PA by corticosteroids,[23] and PA knockout mice are as susceptible to blistering as controls.[24] Acantholytic oral lesions occur in Dsg3 knockout mice due to the isolated absence of Dsg3 expression in desmosomes. Therefore, a more direct effect on inhibition of Dsg function by autoantibody binding is favored.

Although much has been learned about how autoantibodies induce epidermal detachment, little is known about key events in the interplay of environmental and genetic factors of PN and PF host that allow sensitization to self-antigens and generation of pathogenic autoantibodies. However, epidemiological evidence suggests that FS is triggered by exposure to an environmental antigen(s).[25,26] In endemic regions of Brazil, FS affects all ethnic and racial groups.

An international collaborative study in Brazil may provide critical data on an environmental trigger for the disease. Two Amerindian settlements in Brazil exhibit a high prevalence of FS, the Xavante Reservation of Mato Grosso and the Terena Reservation of Limao Verde. The Limao Verde reservation has population of 1200 individuals with a prevalence of FS of 3.2% and an incidence of 1 to 4 new cases per year.[27] Anti-Dsg1 autoantibodies are present both in FS sera and in some healthy controls from Brazilian cities.

The percentage of enzyme-linked immunosorbent assay (ELISA)–positive sera among the healthy control population is inversely related to the distance from the endemic focus of Limao Verde. In 5 FS cases, anti-Dsg1 autoantibodies were detected in blood samples 1 to 5 years prior to the onset of the disease.[27] It appears that in an area endemic for FS, certain members of the population become sensitized to an environmental antigen(s) and produce anti-Dsg1 autoantibodies that, in turn, can lead to FS after an incubation period that may last several years. Detailed ongoing epidemiologic studies may define what environmental exposures trigger autoimmunity in genetically susceptible individuals, with potential direct relevance to PV.

In PNP a small number of hematologic malignancies can initiate and drive autoimmunity against Dsgs and intracellular proteins of the Dsg plaques, such as the desmoplakins. Ongoing studies of the interaction of the immune system and tumor may provide key insights into factors that may initiate autoimmunity in this setting.

Critical Issues

Several critical issues must be resolved to advance the understanding of pemphigus. These include the following: (1) Precise definition of the Dsg3 peptides bound by MHC molecules associated with the disease (DRB1*0402 and DQB1*0503). Although the sequence of MHC-binding peptides has been predicted based on the known structure of the DRB1*0402,[28] the structure of the actual peptide must be established, either by T-cell stimulation studies or by elution of bound peptide. These studies should also be extended to Dsg1. (2) Development of disease registries to obtain precise data on prevalence and incidence of the disease in the United States. Better epidemiologic studies are required to identify environmental factors that may be relevant to disease initiation in North American cases. (3) Development of an animal model of PV and PF by active immunization. Attempts to induce pemphigus by immunization with Dsgs have not been successful. The immunization of knockout mice by Dsg3 is promising but might not accurately reflect the mechanisms of a true autoimmune disease because the disease is induced by a primary immunization response. Further definition of the existing model and novel methods to break immune tolerance in other strains of mice are critical to provide a tool to study future immunotherapeutic agents. (4) Identification of non–HLA-associated genes that may modulate the autoimmune response in patients. (5) Development of tools to identify Dsg3- and Dsg1-specific autoreactive T cells. Researchers have successfully used MHC-peptide dimers to identify class I MHC molecules,[29] but the use of such techniques for dimers for class II MHC molecules has been more problematic. (6) Characterization of pathogenic IgG idiotypes from PV and PF. These studies will be critical in defining idiotype-anti-idiotype interactions that may be manipulated to down-regulate autoantibody production. (7) Improved technology to characterize conformational dependent epitopes. (8) Better tools to study immune tolerance.

Figure. Trunk of an 8-Year-Old Child With Pemphigus Vulgaris

Forecast for Research Advances

Autoimmune disease is 1 of the fundamental enigmas of immunology. Many factors play a role, making it difficult to isolate individual pieces of the puzzle. However, pemphigus provides a relatively "simple" model of autoimmunity, with well-defined key components, including the antigen, the associated MHC class II genes, and the primary importance of anti-Dsg antibody–keratinocyte binding in the induction of tissue injury. Once the true MHC binding peptides are defined, the

©2001 American Medical Association. All rights reserved.

Downloaded From: http://jama.jamanetwork.com/ on 05/05/2015

key components of the MHC class II–peptide–T-cell receptor complex will be defined and can be manipulated. Therefore, pemphigus may be the first human autoimmune disease in which antigen-specific immunotherapy may be possible.

This can be accomplished by development of a peptide-based vaccine that could reinduce tolerance to Dsg3, an approach currently being explored by individual investigators and by a biotechnology company that hopes to conduct clinical trials in the very near future.[30] Alternative approaches include the development of anti-idiotypic vaccines to block the pathogenic idiotypic PV or PF autoantibodies. A serious limitation to studying immunotherapies is the small number of patients with which to conduct clinical trials. The development of a useful animal model will hasten testing of these agents.

During the next 2 decades it is likely that determination of the environmental etiology of FS will provide an opportunity to identify susceptible individuals in endemic areas, modify their risk, and potentially prevent the development of this devastating disease.

Until specific immunotherapies are developed, improvements of conventional treatment will improve, perhaps including expanded use of intensive short-term immune suppression to attempt to modify the disease and induce true remissions. High-dose cyclophosphamide, without the need for bone marrow transplantation or stem cell rescue, shows promise in inducing manageable aplasia, followed by prolonged remission of autoimmune disease,[31] including pemphigus.[32]

The pace of new discovery in pemphigus is accelerating and with continued effort will produce new approaches to the prevention and treatment of this disorder.

Funding/Support: The Albert and Mary Lasker Foundation provided honoraria to Drs Anhalt and Diaz for preparation of this article.

REFERENCES

1. Stanley JR. Pemphigus and pemphigoid as paradigms of organ-specific, autoantibody-mediated diseases. *J Clin Invest*. 1989;83:1443-1448.
2. Nousari HC, Anhalt GJ. Pemphigus and pemphigoid. *Lancet*. 1999;354:667-672.
3. Korman NJ, Eyre RW, Zone J, et al. Drug-induced pemphigus. *J Invest Dermatol*. 1991;96:273-276.
4. Beutner EH, Jordon RE. Demonstration of skin antibodies in sera of pemphigus vulgaris patients by direct immunofluorescent staining. *Proc Soc Exp Biol Med*. 1964;117:505-510.
5. Anhalt GJ, Labib RS, Voorhees JJ, Beals TF, Diaz LA. Induction of pemphigus in neonatal mice by passive transfer of IgG from patients with the disease. *N Engl J Med*. 1982;306:1189-1196.
6. Roscoe JT, Diaz L, Sampaio SA, et al. Brazilian pemphigus foliaceus autoantibodies are pathogenic to BALB/c mice by passive transfer. *J Invest Dermatol*. 1985;85:538-541.
7. Eyre RW, Stanley JR. Human autoantibodies against a desmosomal protein complex with a calcium sensitive epitope are characteristic of pemphigus foliaceus patients. *J Exp Med*. 1987;160:1719-1724.
8. Eyre RW, Stanley JR. Identification of pemphigus vulgaris antigen extracted from normal human epidermis and comparison with pemphigus foliaceus antigen. *J Clin Invest*. 1988;81:807-812.
9. Amagai M, Klaus-Kovtun V, Stanley J. Autoantibodies against a novel epithelial cadherin in pemphigus vulgaris, a disease of cell adhesion. *Cell*. 1991;67:869-877.
10. Koch PJ, Walsh MJ, Schmelz M, Goldschmidt MD, Zimbelmann R, Franke WW. Identification of desmoglein, a constitutive desmosomal glycoprotein, as a member of the cadherin family of cell adhesion molecules. *Eur J Cell Biol*. 1990;53:1-12.
11. Buxton RS, Cowin P, Franke WW, et al. Nomenclature of the desmosomal cadherins. *J Cell Biol*. 1993; 121:481-483.
12. Anhalt GJ, Kim SC, Stanley JR, et al. Paraneoplastic pemphigus. *N Engl J Med*. 1990;323:1729-1735.
13. Nousari HC, Deterding R, Wojtczack H, et al. The mechanism of respiratory failure in paraneoplastic pemphigus. *N Engl J Med*. 1999;340:1406-1410.
14. Sinha AA, Brautbar C, Szafer F, et al. A newly characterized HLA DQ allele associated with pemphigus vulgaris. *Science*. 1988;239:1026-1029.
15. Ahmed AR, Wagner R, Khatri K, et al. Major histocompatibility complex haplotypes and class II genes in non-Jewish patients with pemphigus vulgaris. *Proc Natl Acad Sci U S A*. 1991;88:7658-7662.
16. Moraes ME, Fernandez-Viña M, Lazaro A, et al. An epitope in the third hypervariable region of the *DRB1* gene is involved in the susceptibility to endemic pemphigus foliaceus ("fogo selvagem") in three different Brazilian populations. *Tissue Antigens*. 1997;49:35-40.
17. Koch PJ, Mahoney MG, Ishikawa H, et al. Targeted disruption of the pemphigus vulgaris antigen (desmoglein 3) gene in mice causes loss of keratinocyte cell adhesion with a phenotype similar to pemphigus vulgaris. *J Cell Biol*. 1997;137:1091-1102.
18. Amagai M, Tsunoda K, Suzuki H, Nishifuji K, Koyasu S, Nishikawa T. Use of autoantigen-knockout mice in developing an active autoimmune disease model for pemphigus. *J Clin Invest*. 2000;105:625-631.
19. Labib RS, Rock B, Robledo MA, Anhalt GJ. The calcium sensitive epitope of pemphigus foliaceus antigen is present on a murine tryptic fragment and constitutes a major antigenic region for human autoantibodies. *J Invest Dermatol*. 1991;96:144-147.
20. Amagai M, Karpati S, Prussick R, Klaus-Kovtun V, Stanley J. Autoantibodies against the amino-terminal cadherin-like binding domain of pemphigus vulgaris antigen are pathogenic. *J Clin Invest*. 1992;90:919-926.
21. Aoyama Y, Owada MK, Kitajima Y. A pathogenic autoantibody, pemphigus vulgaris-IgG, induces phosphorylation of desmoglein 3, and its dissociation from plakoglobin in cultured keratinocytes. *Int J Immunol*. 1999;29:2233-2240.
22. Mahoney MG, Wang Z, Rothenberger K, Koch PJ, Amagai M, Stanley JR. Explanations for the clinical and microscopic localization of lesions in pemphigus foliaceus and vulgaris. *J Clin Invest*. 1999;193:461-468.
23. Anhalt GJ, Patel HP, Labib RS, Diaz LA, Proud D. Dexamethasone inhibits plasminogen activator activity in experimental pemphigus in vivo but does not block acantholysis. *J Immunol*. 1986;136:113-117.
24. Mahoney MG, Wang ZH, Stanley JR. Pemphigus vulgaris and pemphigus foliaceus antibodies are pathogenic in plaminogen activator knockout mice. *J Invest Dermatol*. 1999;113:22-25.
25. Diaz LA, Sampaio SA, Rivitti EA, et al. Endemic pemphigus foliaceus (fogo selvagem), I: clinical features and immunopathology. *J Am Acad Dermatol*. 1989;20:657-669.
26. Diaz LA, Sampaio SA, Rivitti EA, et al. Endemic pemphigus foliaceus (fogo selvagem), II: current and historic epidemiologic studies. *J Invest Dermatol*.1989; 92:4-12.
27. Warren SJ, Lin M-S, Giudice GJ, et al. The prevalence of antibodies against desmoglein 1 in endemic pemphigus foliaceus in Brazil. *N Engl J Med*. In press.
28. Wucherpfennig KW, Yu B, Bhol K, et al. Structural basis for major histocompatibility complex (MHC)-linked susceptibility to autoimmunity. *Proc Natl Acad Sci U S A*. 1995;92:11935-11939.
29. Slansky JE, Rattis FN, Boyd LF, et al. Enhanced antigen-specific antitumor immunity with altered peptide ligands that stablize the MHC-peptide-TCR complex. *Immunity*. 2000;13:529-538.
30. Warren KG, Catz I, Wucherpfennig KW. Tolerance induction to myelin basic protein by intravenous synthetic peptides containing epitope P85 VVHFFKNIVTP96 in chronic progressive multiple sclerosis. *J Neurol Sci*. 1997;152:31-38.
31. Brodsky RA, Petri M, Smith BD, et al. Immunoablative high-dose cyclophosphamide without stem cell rescue for refractory severe autoimmune disease. *Ann Intern Med*. 1998;129:1031-1035.
32. Nousari HC, Brodsky RA, Jones RJ, Grever MR, Anhalt GJ. Immunoablative high-dose cyclophosphamide without stem cell rescue in paraneoplastic pemphigus. *J Am Acad Dermatol*. 1999;40:750-754.

©2001 American Medical Association. All rights reserved.

Downloaded From: http://jama.jamanetwork.com/ on 05/05/2015