Bullous Systemic Lupus Erythematosus With Autoantibodies Recognizing Multiple Skin Basement Membrane Components, Bullous Pemphigoid Antigen 1, Laminin-5, Laminin-6, and Type VII Collagen

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Background: Bullous systemic lupus erythematosus is a generalized subepidermal blistering skin eruption in patients suffering from systemic lupus erythematosus. Type VII collagen was initially identified as the target antigen.

Observation: We studied an unusual patient who had bullous systemic lupus erythematosus. The patient fulfilled the criteria of systemic lupus with an antinuclear antibody titer of 1:5120. Immunopathological testing revealed in vivo deposition of all IgG subclasses, secretory IgA1, and both light chains at the patient’s skin basement membrane. The in vivo–bound IgG and IgA were localized at the hemidesmosomes and lamina densa. The patient’s IgG and IgA circulating autoantibodies labeled both the epidermal roof and the dermal floor of salt-split skin and recognized the hemidesmosomal protein BP230 as well as the full-length native form and the recombinant noncollagenous domain 1 of type VII collagen (anchoring fibril). In addition, the patient’s IgG autoantibodies recognized the anchoring filament proteins laminin-5 and laminin-6 (α3 chain and γ2 chain).

Conclusions: We conclude that patients with bullous systemic lupus erythematosus may have autoantibodies to multiple basement membrane components critical for epidermal-dermal junctional adhesion. Possible pathogenic mechanisms in this patient’s clinical diseases include provocation of organ-specific disease (bullous disease) by systemic autoimmunity (lupus) and the “epitope spreading” immune phenomenon.
METHODS AND RESULTS

HISTOPATHOLOGICAL FINDINGS

A skin biopsy specimen from a blister fixed in formalin, processed in paraffin, and stained with hematoxylin-eosin revealed a subepidermal blister with intact epidermis. Epidermal necrosis and acantholysis were not observed. An inflammatory cell infiltrate in the papillary dermis and the blister cavity included predominantly neutrophils (99%) and trace eosinophils (1%). Moderately dense mononuclear cell perivascular infiltrates were noted on upper dermis (data not shown).

IMMUNOFLUORESCENCE STUDIES

Direct immunofluorescence microscopy was performed on 6-µm-thick cryosections of the patient's perilesional skin biopsy specimen, with fluorescein-conjugated goat anti-human IgG, IgA, IgM, C3, and fibrinogen (Immco, Buffalo, NY).16 Direct immunofluorescence microscopy was also performed with monoclonal antibodies against human IgG1, IgG2, IgG3, IgG4, IgA1, IgA secretory component (Sigma-Aldrich, St Louis, Mo), and IgA2 (Southern Biotechnology, Birmingham, Ala), followed by fluorescein-conjugated goat antimouse IgG (Kirkegaard & Perry, Gaithersburg, Md). In addition, direct immunofluorescence microscopy was performed with fluorescein-conjugated goat antihuman immunoglobulin k light chain and A light chain (Kirkegaard & Perry). The in vivo–bound immune deposits at the patient's skin BMZ consist of IgG, IgA, and C3. The subclass studies detected in vivo–bound BMZ immunoglobulins of all IgG subclasses (IgG1–4), secretory IgA1, and both light chains (k and A). IgA2 deposits were not detected (data not shown). These findings indicated that the patient's anti-BMZ autoantibodies were polyclonal in nature.

Indirect immunofluorescence microscopy was performed on EDTA-split normal human skin substrate by first incubating with the patient's serum on 6-µm-thick cryosections, followed by incubation with fluorescein-conjugated goat antihuman IgG.37 Controls included serum samples from patients with bullous pemphigoid, epidermolysis bullosa acquisita, and healthy individuals. Whereas the control IgG from the patient with bullous pemphigoid labeled the epidermal roof and the control IgG from the patient with epidermolysis bullosa acquisita labeled the dermal floor, the IgG from this patient with BSLE labeled both sides. Identical findings were observed for the patient's IgA autoantibodies (data not shown). Normal serum does not label either side.

IMMUNOELECTRON MICROSCOPIC STUDIES

Direct immunoelectron microscopy was performed on 14-µm-thick normal skin and periblistered skin specimens from the patient using a modified peroxidase-antiperoxidase method.17 IgG and IgA antibodies were detected at the hemidesmosomes and lamina densa areas of the patient's skin BMZ but not at the BMZ of the healthy skin (Figure 2).

ANTIBODIES

Polyclonal antihuman collagen VII antibody was prepared by immunizing rabbits with a full-length, eukaryotic-expressed, 145-kd NC1 recombinant protein.18 Human anti-α3 chain (laminin-5/laminin-6) antibody was obtained from serum of a patient with cicatricial pemphigoid.19 (Polyclonal antitrat laminin-5 antibody J-18 was supplied by J. C. R. Jones, PhD, Northwestern University, Chicago, Ill.20 Polyclonal rabbit antibody to a glutathione S-transferase fusion protein of human BP180 NC16A domain was supplied by G. Giudice, PhD, Medical College of Wisconsin, Milwaukee.21)

and sharp pain in the right side of her back. Examination revealed numerous tense bullae on her entire skin surface (Figure 1) and multiple hyperpigmented macules and patches. There were some milia in the patient's extremities, but frank scarring was not observed. Abnormal laboratory findings included hemoglobin, 86 g/L (reference range [RR], 120-160 g/L); serum urea nitrogen, 11.1 mmol/L (RR, 2.5-6.4 mmol/L), creatinine, 159 µmol/L (1.8 mg/dL) (RR, 27-97 µmol/L [0.3-1.1 mg/dL]); C3, 0.71 g/L (RR, 0.86-1.84 g/L), C4, 0.12 g/L (RR, 0.2-0.59 g/L), positive autoantibodies including antinuclear antibodies (titer 1:5120, speckled), anti-Sm, anti-SSB/La, anti-dsDNA, anti-RNP, and direct Coombs test. In addition, radiographic evidence of pleural effusion, and echocardiographic evidence of pericardial effusion were documented. The patient was successfully treated with systemic corticosteroids, azathioprine, and dapsone.

We detail the findings in a patient with BSLE whose autoantibodies recognized BPAg1, laminin-5, laminin-6, and type VII collagen. This is the first report of such a clinical case in which BMZ components other than type VII collagen have been definitively recognized by the autoantibodies of a patient with BSLE.

The phenomenon of the association of SLE, a systemic autoimmune disease, with autoantibody-mediated subepidermal blistering skin disease, an organ-specific autoimmune disease, can be explained by 2 possible immune mechanisms. The first is that the organ-specific autoimmune disease is provoked by systemic autoimmunity.25 This mechanism has been documented in a spontaneous mouse model of chronic inflammatory joint disease strikingly similar to the human disease rheumatoid arthritis.26 Crossing the nonobese diabetes mouse strain with a T-cell receptor transgenic line generated offspring that were affected by a rheumatoid arthritis–like syndrome, without the need of specific induction by external injection of joint-specific antigen.26 While our patient's clinical manifestations could conform to this scenario, it would be difficult to explain the complete pathogenesis by this mechanism. That is, this patient would need to carry T-cell receptor genes capable of recognizing 4 different BMZ components: laminin-5, laminin-6, bullous pemphigoid antigen 1, and type VII col-
PROTEIN SUBSTRATES PREPARATION

Substrates containing the full-length native form of type VII collagen were prepared by concentrating culture-conditioned medium of WISH cells. Recombinant NC1 domain of type VII collagen was prepared from culture-conditioned medium of 293 cells transfected with the full-length complementary DNA-encoding human type VII collagen NC1 domain. Substrates containing heterotrimeric of human laminin-5/laminin-6 were prepared from a primary healthy human keratinocyte culture as described. Total human epidermal cell extracts were prepared from human keratinocytes. Glutathione S-transferase fusion protein of the human BP180 NC16A domain was supplied by G. Giudice, PhD (Medical College of Wisconsin).

IMMUNOBLOT STUDIES

The above substrates containing BMZ proteins were mixed with sample buffer, loaded onto a 4% loading gel over a 4% running gel (6%, 8%, or 10%), and vertically separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis separating system (Novex, La Jolla, Calif) under reducing conditions. The separated proteins were then horizontally transferred to a supported nitrocellulose membrane (Bio-Rad, Hercules, Calif). After the transfer, efficiency was examined by a reversible Poncetan S stain (Sigma-Aldrich); the membranes were cut into strips and blocked by 5% nonfat powdered milk. The membranes were first incubated with primary antibodies overnight at 4°C, followed by incubation at room temperature for 1 hour with peroxidase-conjugated goat antibodies to rabbit IgG, human IgG γ chain, human IgA α chain, human Ig λ light chain, and human IgA light chain (Kirkegaard & Perry). The immunoreactions were visualized with peroxidase substrate 4-chloro-1-naphthol (Bio-Rad). The patient's serum revealed both IgG and IgA autoantibodies that recognized the 230-kd bullous pemphigoid antigen 1, but not the 180-kd bullous pemphigoid antigen 2 (Figure 3). Furthermore, the patient's serum did not contain IgG or IgA autoantibodies that recognized the recombinant human BP180 NC16A domain, whereas a rabbit antibody and control serum from a human patient with bullous pemphigoid labeled it (data not shown). In addition, the patient's serum contained IgG autoantibodies that recognized laminin-5 γ2 chain and α3 chain (Figure 4). The α3 chain has been identified as the major laminin-5 (and laminin-6) subunit recognized by autoantibodies from a subset of patients with cicatricial pemphigoid. Moreover, the patient's serum contained both IgG and IgA autoantibodies that recognized the full-length 290-kd native-type VII collagen and the full-length 145-kd recombinant NC1 domain (Figure 5). The autoantibodies recognizing the NC1 domain consisted of both κ and λ light chains (data not shown).

ENZYME-LINKED IMMUNOSORBENT ASSAY

Enzyme-linked immunosorbent assay testing of the patient's serum on purified recombinant human type VII collagen NC1 domain was carried out. The patient's IgG antibodies specifically reacted with NC1 domain of type VII collagen in a titratable manner (data not shown).

IMMUNOPRECIPITATION

Immunoprecipitation studies were performed with conditioned media from sulfur 35 (35S) methionine/cysteine metabolically labeled normal human keratinocyte cultures containing laminin-5 and laminin-6 proteins. Control antibodies included normal human serum and polyclonal antibodies to laminin-5. The patient's serum contained IgG autoantibodies that coprecipitated laminin-5 proteins with polyclonal anti–laminin-5 antibody. Normal human serum does not precipitate these proteins (data not shown).

It would also be difficult to explain how the immune system can recognize bullous pemphigoid antigen 1, an intracellular protein, but not bullous pemphigoid antigen 2, an extracellular protein that has been documented to be pathogenic in a passive transfer mouse model. However, the exclusion of bullous pemphigoid antigen 2 as a target antigen of this patient's autoantibodies was based solely on detectability of her circulating autoantibodies. It is possible that the patient generated anti-BP180 autoantibodies that were all bound to skin BMZ and were not available in her circulation for detection, as only a small percentage of patients with bullous pemphigoid have circulating antibodies against BP180 detectable by immunoblotting and immunoprecipitation studies. A recent study reported that the skin-infiltrating, predominantly CD4+ T cells from patients with SLE recognize a relatively limited epitope and are likely expanded by antigen-driven stimulation, thus suggesting a potential role of these T cells in inducing organ-specific immunobullous diseases against skin BMZ components.

The second possible mechanism may involve an epitope-spreading phenomenon. Epitope spreading describes an immunologic event in which a primary autoimmune or inflammatory process causes tissue injury, releasing previously “sequestered” antigenic epitopes, and leading to a secondary autoimmune response to the “new” antigenic epitope. There are many antibody-mediated blistering skin diseases in which epitope spreading may play a role in the initiation or progression of the disease. Chen et al recently found that the NC1 domain of type VII collagen forms binding with the β3 chain of laminin-5. One could envision that an inflammatory process initially involving the NC1 domain of type VII collagen can easily cause injury to the adjacent component laminin-5. Thus, by the mechanism of epitope spreading, the primary autoimmune reaction against type VII collagen can lead to secondary autoimmune reactions against laminin-5 and other adjacent BMZ components. In this patient, the history suggested that the systemic autoimmune (SLE) component and the organ-specific blistering skin disease developed simultaneously. The concurrent development of systemic and organ-specific diseases, at first glance, may not lend strong support for an essential role of epitope spreading. This phenomenon requires a sequence of events that involves injuries in-
duced by chronic inflammation, release of “sequestered” antigen, exposure of “new” antigen to antigen-presenting cells and helper T cells and B cells, and activation of autoreactive T cells and B cells. Nevertheless, it is certainly possible that this patient had a long-standing subclinical SLE, which caused tissue injury and exposed BMZ components to autoreactive lymphocytes prior to her clinical manifestations of blistering skin disease.

A third explanation is the involvement of both of the above mechanisms. Following the primary autoimmune reaction against type VII collagen as a result of provocation by SLE, epitope spreading plays a role in the induction of secondary autoimmune reactions against laminin-5, laminin-6, and BPAg1. Regardless of the actual mechanism involved in the initiation and progression of the organ-specific autoimmune subepi-

Figure 1. The clinical manifestations of bullous systemic lupus erythematosus. The patient exhibited large bullae on her chest (top) and face (bottom).

Figure 2. The ultrastructural localization of in vivo–bound immunoglobulins to hemidesmosomes and lamina densa. The in vivo–bound IgG (top) and IgA (bottom) autoantibodies in the patient’s skin were detected both in the hemidesmosomes and in the lamina densa and sublamina densa areas by direct immunoelectron microscopy using a peroxidase-antiperoxidase method. The bar indicates 1 µm and applies to both panels.
dermal blistering skin disease, this unusual case of BSLE demonstrates the dynamic interplay between systemic autoimmunity and organ-specific skin diseases.

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