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.

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Bullous Systemic Lupus Erythematosus (BSLE) is an autoantibody-mediated blistering skin disease occurring in patients with systemic lupus erythematosus (SLE). In addition to having clinical and laboratory findings that fulfill the American Rheumatism Association diagnostic criteria of SLE, patients with BSLE have a generalized subepidermal blistering skin disease with immunoglobulin deposition along the skin basement membrane zone (BMZ). Some patients had detectable IgG circulating autoantibodies that labeled the dermal floor of chemically separated normal skin substrate, indicating that the target antigen was located below the middle of the lamina lucida. Some patients with BSLE had autoantibodies that recognized the major anchoring fibril component type VII collagen. The antigenic domains recognized by BSLE autoantibodies were within the noncollagenous (NC1) domain, identical to the antigenic domains recognized by autoantibodies from patients with epidermolysis bullosa acquisita. More recently, other investigators have reported indirect immunofluorescence findings suggesting that type VII collagen may not be the exclusive target antigen in BSLE. In this article, we report an unusual patient with BSLE whose autoantibodies labeled both the epidermal roof and the dermal floor of chemically separated normal skin substrate. We took the opportunity to delineate the multiple skin BMZ components recognized by this patient’s autoantibodies and to discuss the possible mechanisms responsible for such unusual immune responses.

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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 monocellular 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 anti-human immunoglobulin κ light chain and λ 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 (κ and λ). 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.17 Controls included serum samples from patients with bullous pemphigoid, epidermolysis bullosa acquista, 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 acquista 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 perilesional skin specimens from the patient using a modified peroxidase-anti-peroxidase method.18 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).

ANTIBODES

Polyclonal antihuman collagen VII antibody was prepared by immunizing rabbits with a full-length, eukaryotic-expressed, 145-kd NC1 recombinant protein.19 Human anti-α3 chain (laminin-5/laminin-6) antibody was obtained from serum of a patient with cicatricial pemphigoid.19 (Polyclonal antirat laminin-5 antibody J-18 was supplied by J. C. R. Jones, PhD, Northwestern University, Chicago, Ill.) 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.20

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 autoimmune antibodies including anti-nuclear 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 clini-
ligen. 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, which suggests a potential role of these T cells in inducing organ-specific immunobullosus 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 immuno-electron microscopy using a peroxidase-antiperoxidase method. The bar indicates 1 µm and applies to both panels.

Figure 3. The patient’s serum contains both IgG and IgA autoantibodies that recognize the bullous pemphigoid antigen 1 (BP230) but not BP180. Cultured human keratinocyte extracts separated by a 6% sodium dodecyl sulfate–polyacrylamide gel electrophoresis separating system and transferred to nitrocellulose membranes were incubated with positive bullous pemphigoid control serum samples (lanes 1 and 2), serum of our patient with bullous systemic lupus erythematosus (lanes 3 and 5), or normal human serum (lanes 4 and 6), followed by incubation with peroxidase-conjugated goat antibodies to human IgG γ chain (lanes 1-4) or IgA α chain (lanes 5 and 6). M indicates molecular-weight standards.

Figure 4. The patient’s serum contains IgG autoantibodies that recognize laminin-5 and laminin-6 α3 and γ2 chains. Human keratinocyte matrix preparations were separated by an 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis separating system under reducing conditions, transferred to nitrocellulose paper, then reacted with polyclonal rabbit antibody to laminin-5 J-18 (lane 1), a human autoantibody that recognized α3 chain of laminin-5 and laminin-6 (lane 2), our patient’s serum (lanes 3 and 6), the serum of a patient with epidermolysis bullosa acquisita (lane 4), and normal human serum (lanes 5 and 7), followed by incubation with peroxidase-conjugated goat antibodies to rabbit IgG (lane 1), human IgG γ chain (lanes 2-5), and human IgA α chain (lanes 6-7). M indicates molecular-weight standards.
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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REFERENCES


