Missing the target: Characterization of bullous pemphigoid patients who are negative using the BP180 enzyme-linked immunosorbant assay

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Background: Bullous pemphigoid (BP) is an autoimmune blistering disease characterized by autoantibodies specific for the 180-kd BP antigen-2 (BP180) (also termed "type XVII collagen") protein. The BP180 enzyme-linked immunosorbent assay (ELISA) is specific for the immunodominant NC16A domain of the protein. However, we and others have observed patients whose reactivity to BP180 is exclusive of the NC16A domain (referred to henceforth as non-NC16A BP).

Objective: We sought to determine the incidence of non-NC16A BP and identify regions of reactivity within the BP180 protein.

Methods: Sera from 51 patients who met the clinical and histologic criteria for BP were screened for NC16A reactivity by ELISA. Sera that were negative by ELISA were screened for IgG reactivity to an epidermal extract, recombinant BP180 protein, and subregions of BP180, by immunoblot. Demographic and clinical data were also collected on all patients.

Results: Four sera (7.8%) were negative using the BP180 ELISA but positive for IgG reactivity to the extracellular domain of BP180. Further mapping identified 4 regions outside of NC16A recognized by these sera: amino acid (AA) 1280 to 1315, AA 1080 to 1107, AA 1331 to 1404, and AA 1365 to 1413. One of these sera also had IgE specific for NC16A. One patient had an atypical presentation with lesions limited to the lower aspect of the legs and scarring of the nail beds.

Limitations: The small total number of patients with non-NC16A BP limits the identification of demographic or clinical correlates.

Conclusion: It is significant that 7.8% of sera from patients with new BP react to regions of BP180 exclusively outside of NC16A and, thus, would not be identified using the currently available BP180 ELISA. (J Am Acad Dermatol 2013;68:395-403.)

Key words: autoantibody; blister; bullous pemphigoid; enzyme-linked immunosorbent assay; immunobullous.

Bullous pemphigoid (BP) is the most common autoimmune blistering disease and is characterized by autoantibodies directed against the hemidesmosomal proteins, 230-kd BP antigen-1 (BP230) and 180-kd BP antigen-2 (BP180) (also termed "type XVII collagen").[1,2] Experimentally, it

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is well established that BP180-specific autoantibodies play a primary role in the pathogenesis of BP, as adoptive transfer of these antibodies reproduces many of the features typical of BP.[3-5] Moreover, disease activity directly correlates with circulating levels of BP180-specific autoantibodies.[6-9] The primary pathogenic significance of the BP230-specific autoantibodies remains unclear.[10,11]

BP180 is a type II transmembrane protein composed of a globular cytoplasmic domain, a single transmembrane domain, and a long extracellular region that is composed of 15 interrupted collagenous domains.[12] Approximately 90% of BP sera target the extracellular noncollagenous 16A region (NC16A) of the protein.[1,13-15] The pathologic importance of the NC16A region was demonstrated in BP180-humanized mice where absorption of NC16A-specific antibodies from BP sera, before subcutaneous injection, resulted in a significant reduction in blistering.[4]

A diagnosis of BP is based on the presence of serum autoantibodies that have been historically evaluated using indirect immunofluorescence (IF) on intact or salt-split skin and, more recently, by enzyme-linked immunosorbent assay (ELISA). The currently available ELISA for detection of BP180-specific IgG-class autoantibodies targets the NC16A region of the protein with a sensitivity of 84% and a specificity of 99%.[12] The clinical usefulness of this ELISA is well established[14,16,17]; however, we have noted a subset of patients who meet the clinical and histologic criteria for BP[18] but do not recognize the NC16A region targeted in this assay. One such patient was previously described in our epitope mapping study.[19] In studies examining the epitope spreading phenomenon in patients with BP, Di Zenzo et al[20] also identified 3 patients with autoantibodies that reacted exclusively with non-NC16A sites. The purpose of this study was to determine the incidence of non-NC16A BP at an early stage of the disease, to identify regions of reactivity within the BP180 protein, and to ascertain if these patients with non-NC16A BP had any clinical features that differed from patients in whom the immunodominant NC16A region is targeted.

CAPSULE SUMMARY

- Most patients with bullous pemphigoid (BP) have autoantibodies targeting the NC16A region of 180-kd BP antigen-2; however, occasional patients are observed whose sera do not react to this region.
- Herein, 7% to 10% of BP sera react to regions of 180-kd BP antigen-2 exclusively outside the NC16A domain.
- This study indicates that: (1) use of the enzyme-linked immunosorbent assay for confirmation of BP, rather than indirect immunofluorescence, will not identify this patient subset; and (2) antibodies targeting areas other than NC16A can be pathogenic.

METHODS

Patients

Patients with BP (N = 54) were enrolled in this study at the University of Iowa Hospitals and Clinics between January 1, 2007, and May 1, 2011, after giving written informed consent. This study was approved by the Institutional Review Board at the University of Iowa and the Veterans Affairs Medical Center in Iowa City, IA, and was performed in adherence to the Declaration of Helsinki Guidelines. BP was confirmed by clinical (cutaneous blistering, erythematous/urticarial plaques), histologic (subepidermal blistering on skin biopsy specimen), and IF (linear IgG and/or C3 at the basement membrane zone [BMZ]) criteria[18] using standard procedures. Data regarding age, sex, ethnic background, medications, and other medical history were also acquired.

ELISA for BP180 IgG and BP230 IgG, collagen VII IgG, NC16A IgE, and total IgE

In all cases, antibody levels were evaluated in patient serum by ELISA. IgG specific for BP180 and BP230 was evaluated using commercial assays (MBL International, Nagoya, Japan). To rule out epidermolysis bullosa (EBA), autoantibodies against full-length collagen VII and the first noncollagenous (NC1) domain of collagen VII were evaluated.[21] The NC16A IgE ELISA was performed as described.[22] Total IgE levels were quantitated using electrochemiluminescence performed by the pathology laboratory services at the University of Iowa.

Indirect IF

Circulating autoantibodies specific for proteins of the BMZ were detected by indirect IF using 1 mol/L sodium chloride-split skin as a substrate.[23] Sera were diluted 1:10 and were used as a primary antibody, and antihuman IgG-fluorescein isothiocyanate (fitc) was used as a secondary antibody. The localization of antibody to the roof or base of the split skin was detected with epifluorescent microscopy.