IgG Anti-LABD97 Antibodies in Bullous Pemphigoid Patients’ Sera React with the Mid-Portion of the BPAg2 Ectodomain

To the Editor

The 180 kDa bullous pemphigoid antigen, BPAg2, is a transmembrane protein that is important in basement membrane cohesion. Circulating antibodies in bullous pemphigoid (BP) patients’ sera have been shown to target epitopes that are tightly clustered in the sixteenth noncollagenous domain (NC16A) of the extracellular portion BPAg2 (Zillikens et al, 1997). We have previously shown that some BP patients’ sera contain IgG antibodies reactive with the LABD97 antigen which is structurally identical to a portion of the extracellular domain of BPAg2 (Zone et al, 1998; Egan et al, 1999). These IgG anti-LABD97 antibodies cross-reacted with BPAg2 but did not recognize epitopes in its NC16A domain. In this study, affinity-purified IgG anti-LABD97 antibodies from bullous pemphigoid patients’ sera were reacted with three protein constructs that together encode almost the entire extracellular domain of BPAg2.

Construct EC2 (amino acids 527–1187) in pCEP-Pu vector was obtained from Dr. Peter Mueller, University of Lubeck, Germany (Areida et al, 1999). Another BPAg2 ectodomain construct, EC3 coding for amino acids 482–616, was generated using the sense primer 5’-GCTTCTTCGCCCTGAATTGCTCT-3’ and the antisense primer 5’-CATGCCCCACTTCCTTCACGCT-3’. The Nhe I-Not I digested PCR product was cloned into a modified pCEP-Pu vector that additionally coded for an eight amino acid FLAG peptide that preceded the APLA residues in frame, thereby resulting in a FLAG-tagged EC3 polypeptide. The resulting peptide migrated with a molecular weight of approximately 30 kDa.

These two ectodomain constructs were expressed using the HEK 293 EBNAP mammalian expression system and selected using 5 μg per ml of the antibiotic puromycin (Sigma, St. Louis, MO). The EC3 protein was purified from conditioned media using an anti-FLAG affinity column (Sigma, St. Louis, MO), while the EC2 protein was obtained by concentrating conditioned media using a Centricon filter (Amicon, Beverly, MA) and then used for western blotting.

GST-BP-915, a GST fusion protein, contains 305 amino acids (1193–1497) of the distal region of the BPAg2 extracellular domain as reported previously (Nie and Hashimoto, 1999). To produce this recombinant protein, an appropriate cDNA fragment of BPAg2 was obtained by PCR of a KU8 cDNA library and put into a pGST-2T vector for its production. To decrease the degradation of the protein, BL12 was used as the host bacteria and the culture temperature was adjusted to 14°C. The fusion protein was induced by IPTG and purified from bacterial lysates by a glutathione-agarose affinity column.

IgG antibodies specific for the LABD97 antigen were affinity purified from sera by elution of antibodies from LABD97 bound to nitrocellulose strips as described by Olmsted (1981) and modified by Smith and Fisher (1984). Protein in sample buffer was applied to a 10% polyacrylamide gel, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed (Laemmli, 1970). Proteins were transferred electrophotochemically onto nitrocellulose, and the nitrocellulose strips were incubated with sera or eluates for 2 h at room temperature (Towbin et al, 1979). Immunoblots were developed using a biotin labeled antihuman γ-chain antibody and an avidin-biotin-peroxidase complex. Reaction times for all lanes of individual blots were identical.

In this study, 12 BP patients’ sera were used (Fig. 1). All had a circulating IgG antibody titer of at least 40 binding to the epidermal side of 1 M NaCl split skin on indirect immunofluorescence microscopy. Antibody eluates from nine of these BP sera contained IgG anti-LABD97 specific antibodies, all of which reacted with the EC2 construct containing 660 amino acids from the mid-portion of the BPAg2 ectodomain. Eluates from three patients’ sera with IgG antibodies reactive with BPAg2 but not LABD97 on western blot, or eluates from negative control sera, subjected to identical affinity purification procedures, did not react with this construct. Only one patient’s affinity-purified IgG anti-LABD97 antibodies reacted with the EC3 construct containing 134 amino acids (including the entire NC16A domain and parts of the transmembrane region and part of the fifteenth collagenous domain). Eluates from all 12 affinity-purified BP patient’s sera failed to react with the GST-BP-915 bacterial fusion protein containing the carboxy-terminal 304 amino acids of BPAg2.

This study demonstrates that BP patients’ sera that contain IgG antibodies reactive with LABD97 react with an epitope or epitopes contained in a 571 amino acid stretch of the mid-portion of the BPAg2 ectodomain. It confirms the findings of our previous study by showing that nearly all of the affinity-purified IgG anti-LABD97 specific antibodies fail to react with the NC16A domain of BPAg2 (Egan et al, 1999). One patient’s affinity-purified anti-LABD97 antibodies did react with the EC3 construct. We postulate that these antibodies reacted either with a post-translationally modified epitope not contained in the bacterial fusion protein used previously, or that they recognized an epitope contained in the amino-terminal region of the fifteenth collagenous domain.

It is not surprising that none of the patients’ affinity-purified antibodies reacted with the bacterial fusion protein containing the carboxy-terminal region of BPAg2. When LABD97 was partially sequenced, no homologous amino acid sequences were detected in the carboxy-terminal 237 amino acids of BPAg2, suggesting that the carboxy-termius of LABD97 lies in this region of BPAg2 (Zone et al, 1998). Therefore, any antibodies in the test patients’ sera reactive with epitopes in this region of BPAg2 would not bind to LABD97 and thus would not be affinity purified.

The fact that the IgG anti-LABD97 antibodies cross-react with BPAg2 is notable. IgA antibodies targeting LABD97 do not cross-react with BPAg2 (Zone et al, 1990; Dmochowski et al, 1993). Therefore, it seems likely that both antibody isotypes recognize different epitopes on LABD97. The finding of antibodies targeting epitopes other than the NC16A domain of BPAg2 in BP patients’...
sera is well documented (Perriard et al., 1999; Egan et al., 1999; Schumann et al., 2000). This may just represent the phenomenon of epitope spreading (Chan et al., 1998). The pathogenic relevance of these IgG antibodies to LABD97 in BP is unknown, however. In a mouse model of BP, using rabbit IgG anti-BPAg2 antibodies, Liu et al. (1995) have demonstrated that pathogenic antimurine BPAg2 antibodies bind to a site within the murine NC14A domain, which is equivalent to the NC16A domain of human BPAg2. These antibodies bind to neonatal mouse skin basement membrane and induce complement activation leading to an inflammatory infiltrate with neutrophils leading to subepidermal separation with rapid sloughing of epidermis. We are currently developing an animal model of BP using human skin engrafted immunodeficient mice. Preliminary results suggest that passive transfer of mouse IgG class monoclonal antibodies to LABD97 in BP is unknown, however. In a mouse model of BP, using rabbit IgG anti-BPAg2 antibodies, Liu et al. (1995) have demonstrated that pathogenic antimurine BPAg2 antibodies bind to a site within the murine NC14A domain, which is equivalent to the NC16A domain of human BPAg2. These antibodies bind to neonatal mouse skin basement membrane and induce complement activation leading to an inflammatory infiltrate with neutrophils leading to subepidermal separation with rapid sloughing of epidermis. We are currently developing an animal model of BP using human skin engrafted immunodeficient mice. Preliminary results suggest that passive transfer of mouse IgG class monoclonal antibodies to LABD97 induces subepidermal graft separation with an inflammatory infiltrate of eosinophils, mirroring the disease process in humans.1 This suggests that antibodies to the LABD97 antigen region of BPAg2 (i.e., antibodies targeting epitopes outside the NC16A domain) may be important in the pathogenesis of BP.

Figure 1. Affinity-purified IgG anti-LABD97 antibodies from BP patients’ sera recognize the mid-portion of the BPAg2 ectodomain. The top panels are immunoblots using BP patient sera that were affinity-purified using the LABD97 antigen. In each case western blots were performed using the recombinant constructs illustrated in the lower panel. The order of BP patients’ affinity-purified IgG antibodies is the same in all three blots (lanes 1–12). (EC3) Western immunoblot showing reactivity of LABD97 affinity-purified IgG antibodies from one patient’s serum with construct EC3 (lane 9). Affinity-purified negative control sera (lanes 13, 14) demonstrated no reactivity with this construct. Two BP patients’ sera, known to contain antibodies to the NC16A domain, reacted with the construct (lanes 15, 16). (EC2) Western immunoblot showing reactivity of LABD97 affinity-purified IgG antibodies from nine BP patients’ sera reacting with construct EC2 (lanes 1–9). Eluates from three BP patients’ sera with IgG antibodies reactive with BPAg2 but not to LABD97, did not react with this construct (lanes 10–12). Affinity-purified negative control sera (lanes 15, 16) and an antisera with no primary antibody (lane 13) demonstrated no reactivity with this construct. A positive control rabbit antibody specific for BPAg2 reacted with the construct (lane 14). (915) Western immunoblot showing no reactivity of any affinity-purified IgG antibodies with bacterial fusion protein GST-BP-915 from BP sera (lanes 1–12) or from negative control sera (lanes 13, 14). Two cicatricial pemphigoid patients’ sera known to react with this fusion protein were used as positive controls (lanes 15, 16). (Lower panel) Diagram showing the structure of BPAg2. The amino-terminus of LABD97 is illustrated. The three protein constructs are outlined above the corresponding part of the BPAg2 ectodomain that they represent. Arrows point from each construct to its western immunoblot with the affinity-purified IgG antibodies to LABD97.

In summary, we have characterized the binding specificity of anti-LABD97 IgG class antibodies in BP patients’ sera and demonstrated that they selectively bind to a region in the mid-portion of the BPAg2 ectodomain that is potentially different from the epitope recognized by IgA antibodies in linear IgA bullous dermatosis. Moreover, this region is distinct from antibody reactive sites previously described on the BPAg2 ectodomain in BP patients’ sera.

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Fcgamma Represents the Fc Receptor gamma Chain Whereas FcgammaR Means the Fc Receptor for IgG

To the Editor:

We read with great interest the article titled “Chemical activation of innate and specific immunity in contact dermatitis” in the recent issue of the Journal of Investigative Dermatology (Zhang and Tinkle, 2000).

The cell surface receptors for the Fc portion of immunoglobulins are termed as Fc receptors (FcR). FcR are defined by their specificity for immunoglobulin isotypes. Fc receptors for IgG are referred as FcγR, for IgE as FcεR, for IgA as FcαR, for IgM as FcμR, etc. (Kinet, 1989; Ravetch and Kinet, 1991).

It has been demonstrated that some FcR are multimeric complexes and share identical subunits. The high-affinity FcεR (FcεRI) is composed of one α chain (FcεRIα), one β chain (FcεRIβ), and two γ chains (FcεRIγ). The low-affinity FcγR (FcγRIII) shares the identical γ chain with FcεR (Wang et al., 1992; Kinet, 1999). Loss of the γ chain results in the loss of FcεRI and FcγRIII expression on cells where those receptors are normally present. Targeted disruption of the γ chain results in immunocompromised mice. The innate immunity is impaired in such mice. Defects in the natural killer cell-mediated antibody-dependent cytotoxicity (ADCC) and mast cell-mediated allergic responses are evident in mutant mice deficient in FcγR γ chain (FcγRγ−) (Takai et al., 1994).

Zhang and Tinkle investigated the effect of deficiency in FcγR γ chain on oxazolone-induced contact hypersensitivity and irritant responses in mice. Such studies are very interesting; however, the authors employed the abbreviation FcγR for the FcγR γ subunit, and thus FcγR KO mice represented FcγR γ chain KO mice. This leads to a misunderstanding. Actually, FcγR KO mice should represent KO mice deficient in Fc receptors for IgG rather than deficient in FcγR γ chain. Therefore, we would suggest that FcγR, rather than FcγR, KO mice should be used for the gene-targeted mutant mice deficient in Fc receptor γ chain.

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REFERENCES