Detection of IgA Autoantibodies to Desmogleins by an Enzyme-Linked Immunosorbent Assay

The Presence of New Minor Subtypes of IgA Pemphigus

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Objective: To examine the frequency of antidesmoglein 1 (Dsg1) and antidesmoglein 3 (Dsg3) IgA autoantibodies in IgA pemphigus.

Design: We developed an enzyme-linked immunosorbent assay against recombinant Dsg1 and Dsg3 to detect IgA autoantibodies.

Patients: Twenty-two patients with IgA pemphigus were studied. Among them, 10 patients had subcorneal pustular dermatosis type, 9 patients had intraepidermal neutrophilic IgA dermatosis type, and 3 patients had pemphigus foliaceus–like clinical features.

Results: Of the 22 cases of IgA pemphigus, 3 cases were positive for anti-Dsg1 IgA antibodies and only 1 case was positive for anti-Dsg3 IgA antibodies. In those 4 cases, there were no IgA autoantibodies against other components of the keratinocyte cell surfaces because preincubation with the respective recombinant desmogleins removed the immunoreactivity on immunofluorescence. All 10 patients with subcorneal pustular dermatosis type IgA pemphigus were positive against desmocollin 1 expressed on COS-7 cells. No target antigen was detected in the other 8 cases.

Conclusions: Desmogleins were recognized by IgA antibodies of a few patients with IgA pemphigus. Considering that subcorneal pustular dermatosis type IgA pemphigus recognizes desmocollin 1, autoimmune targets of IgA pemphigus are more heterogeneous than previously considered.

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DESOMOSOMAL cadherins are of 2 types, desmoglein (Dsg) and desmocollin (Dsc), both of which occur as 3 isoforms, Dsg1, Dsg2, and Dsg3 and Dsc1, Dsc2, and Dsc3.1,2 Classic pemphigus showing IgG antikeratinocyte cell surface autoantibodies are divided into pemphigus foliaceus (PF) and pemphigus vulgaris, that react with Dsg1 and Dsg3.3-7 We have developed a novel enzyme-linked immunosorbent assay (ELISA) using recombinant proteins of Dsg1 and Dsg3 produced by baculovirus expression.8-10 This ELISA has been shown to be a highly sensitive and specific assay to detect autoantibodies in the serum samples of patients with PF and pemphigus vulgaris.

However, patients with vesiculopustular skin lesions that have anti–cell surface antibodies of the IgA class exclusively have been identified in patients and are commonly called “IgA pemphigus.”11,12 IgA pemphigus is also divided into 2 major subtypes, the intraepidermal neutrophilic IgA dermatosis (IEN)13,14 and the subcorneal pustular dermatosis (SPD).15,16 The results of previous studies of the target antigens for IgA pemphigus were controversial.11,12 Standard immunoblotting showed no convincing reactivity with any known epidermal component proteins, probably because the epitopes for IgA pemphigus are conformation dependent and cannot be detected by immunoblotting. In addition, conventional immunoprecipitation cannot be used to detect antigens for IgA antibodies. Therefore, in a previous study,17 we developed a novel assay using complementary DNA transfection to COS-7 cells to identify conformation-dependent epitopes for IgA antibodies. Using the assay, we showed that the antigen for the SPD type IgA pemphigus is Dsc1,17 although the antigen for the IEN type IgA pemphigus was not identified.

Our aim in this study was to examine whether IgA antibodies in IgA pemphigus serum samples react with Dsg1 and Dsg3, ie, classic pemphigus antigens. To achieve this we modified the previously established ELISA of Dsg1 and Dsg3 baculoproteins to develop a new ELISA for de-
PATIENTS AND METHODS

PATIENTS

We collected serum samples from 22 patients with confirmed IgA pemphigus showing anti-keratinocyte cell surface antibodies of the IgA class exclusively. Ten and 9 patients showed typical clinical and histopathologic features of the SPD type and the IEN type, respectively. Three patients had clinical and pathologic features resembling PF and were diagnosed as PF-like IgA pemphigus or IgA herpetiform pemphigus, although no IgG anti–cell surface antibodies were detected. Some of the cases tested in this study have been reported as a single case report.13-16,18-20 Serum samples obtained from 50 normal volunteers were used as controls. Immunofluorescence of normal human skin sections was performed using a standard method.15 Preparation of mammalian cell expression constructs of human Dsc1, 2, and 3, transfection into COS-7 cells, and the detection of IgA antibodies to desmocollins were described previously.17

METHODS

ELISA of Dsg1 and Dsg3 Baculoproteins for Detecting IgA Antibodies

Enzyme-linked immunosorbent assay to detect IgA antibodies against Dsg1 and Dsg3 was developed by modifying the established ELISA for IgG antibodies.6,9 Briefly, secreted forms of recombinant Dsg1 and Dsg3 were produced in High Five (Invitrogen, San Diego, Calif) cells by infecting recombinant baculoviruses, purified on TALON-affinity metal resin (CLONTECH Laboratories, Palo Alto, Calif), and 10 µg/mL of the recombinant protein was coated on 96-well microtiter plates, as described previously.6 All antibodies against Dsg1. None of the 22 IgA pemphigus serum samples was recognized by IgA antibodies of a few patients with IgA pemphigus. As previously reported,17 complementary DNA transfection assay for IgA antibodies with Dsc1, 2, and 3 showed that all of the serum samples from the 10 patients with SPD type IgA pemphigus reacted clearly with Dsc1-expressing COS-7 cells, while none of the serum samples from patients with other types of IgA pemphigus reacted with any of the 3 Dscs (data not shown).

ELISA OF RECOMBINANT Dsg1 AND Dsg3 FOR DETECTING IgA ANTIBODIES

Of 9 cases of IEN type IgA pemphigus, one case reacted with Dsg1 and another case reacted relatively weakly with Dsg3 (Figure 1). None of the serum samples from the 10 patients with SPD type IgA pemphigus reacted with either Dsg1 or Dsg3. Interestingly, 2 of the 3 cases diagnosed as PF-like IgA pemphigus showed IgA antibodies against Dsg1. None of the 22 IgA pemphigus serum samples showed IgG antibodies with either Dsg1 or Dsg3 using the established ELISA for IgG antibodies.

IMMUNOADSORPTION ASSAY

We further performed the immunoadsorption assay with the 4 serum samples containing anti-Dsg1 or anti-Dsg3
IgA autoantibodies (Table and Figure 2). The immunoreactivity against cell surfaces in all 3 cases with IgA antibodies to Dsg1 was removed by preincubation with recombinant Dsg1, but not with recombinant Dsg3. The reactivity in the case with anti-Dsg3 antibodies was absorbed with recombinant Dsg3, but not with Dsg1. These results indicate that these serum samples did not contain IgA autoantibodies against other components of keratinocyte cell membranes.

**COMMENT**

Although there are a few single case reports of IEN type IgA pemphigus reactive with either Dsg3 or Dsg1, findings from our previous immunoblotting studies for a series of IgA pemphigus serum samples did not show any reactivity with either Dsg. Our previous study using a complementary DNA transfection assay indicated that IgA antibodies in the serum samples of SPD type IgA pemphigus react exclusively with conformation-dependent epitopes on human Dsc1. Therefore, it is conceivable that IgA pemphigus may contain IgA antibodies reactive with such conformation-dependent epitopes on Dsg1 and Dsg3.

Using this new ELISA for IgA antibodies, we found that 4 serum samples from 22 patients with IgA pemphigus reacted with either Dsg1 or Dsg3. Interestingly, 2 of 3 patients who were diagnosed as having PF-like IgA pemphigus had IgA antibodies to Dsg1. One of the 2 Dsg1-positive cases of PF-like IgA pemphigus has previously been reported as IgA pemphigus foliaceus. This may indicate that this group is a distinct subtype of IgA pemphigus, although it is necessary to define the cases of PF-like IgA pemphigus (or IgA PF) by accumulating more cases.

Furthermore, one serum sample from each of the 9 patients with IEN type IgA pemphigus reacted with Dsg1 and Dsg3, the autoantigens for classic IgG type pemphigus. In this study, to detect antigens for IgA antibodies, we modified the ELISA of Dsg1 and Dsg3 baculoproteins, which is a highly sensitive and specific assay for IgG autoantibodies.

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**Figure 1.** The results of enzyme-linked immunosorbent assay for IgA and IgG autoantibodies against desmoglein 1 (A) and desmoglein 3 (B) in various types of IgA pemphigus, which were subdivided into intraepidermal neutrophilic dermatosis IgA (IEN) type, subcorneal pustular dermatosis (SPD) type, pemphigus foliaceus–like (PF-like), and normal control subjects. The dashed line is the cutoff value (optical density 450 nm=0.15).

**Figure 2.** Immunoadsorption assay revealed that IgA immunoreactivity against keratinocyte cell surfaces in an IgA pemphigus serum sample (1:40 dilution) was removed by preincubation with culture supernatant containing recombinant desmoglein 1 (B), while it was not removed by preincubation with uninfected culture supernatant (A).

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ent from desmosomal proteins. Further studies should reveal this autoantigen.

In contrast, none of the serum samples from the 10 patients with SPD type IgA pemphigus had IgA antibodies to either Dsg1 or Dsg3. This is consistent with the results that, in indirect immunofluorescence, IgA antibodies in SPD type IgA pemphigus react exclusively with the cell surface in the uppermost epidermis, which is different from the distribution of either Dsg1 (the whole epidermis, being stronger in the upper epidermis) or Dsg3 (the lower epidermis).11,17

Although found in very few cases, the presence of IgA antibodies against Dsg1 and Dsg3 indicated in this study made the understanding of commonly called IgA pemphigus more complicated. Future studies of more cases should clarify the clinical and immunopathologic characteristics of this interesting condition.

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