Desmoglein 1 and Desmoglein 3 Are the Target Autoantigens in Herpetiform Pemphigus

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Objective: To determine the cell surface autoimmune target of herpetiform pemphigus (HP).

Design: Serum samples of HP were examined by immunoblot studies with human epidermal extracts, enzyme-linked immunosorbent assay with baculovirus-expressed recombinant desmoglein (rDsg) 1 and rDsg3, and immunoadsorption assay with rDsg.

Patients: Twenty serum samples were obtained from patients with HP who have typical clinical and histological features. All serum samples showed positive staining against keratinocyte cell surfaces by indirect immunofluorescence studies with healthy human skin.

Results: Immunoblot results showed that of 17 HP serum samples, only 5 reacted with a 160-kd band and 1 reacted with a 130-kd band. Results of enzyme-linked immunosorbent assays with rDsg1 and rDsg3 demonstrated that of 20 HP serum samples, 16 were positive against Dsg1 and 4 were positive against Dsg3. No serum samples reacted with both. Furthermore, in 19 of 20 HP serum samples, immunoreactivity against keratinocyte cell surfaces was completely removed by preincubation with rDsg1 and rDsg3 as shown by indirect immunofluorescence, excluding a possibility that these HP sera contain autoantibodies against other cell surface molecules.

Conclusions: Dsg1 and Dsg3 are the major cell surface target molecules of HP, suggesting that most cases of HP are clinical variants of pemphigus foliaceus and that the rest might be variants of pemphigus vulgaris.

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EMPHIGUS IS A group of antibody-mediated autoimmune bullous diseases. Classic pemphigus consists of 2 major subtypes—pemphigus foliaceus (PF) and pemphigus vulgaris (PV). By immunoblotting and immunoprecipitation, autoantigens of PF and PV have been demonstrated to be 160- and 130-kd glycoproteins, respectively. Results of complementary DNA cloning of both molecules revealed that the target molecules of PF and PV are desmoglein (Dsg) 1 and Dsg3, respectively, which locate in desmosomes.

Herpetiform pemphigus (HP) was first introduced by Jablonska and colleagues as a variant of pemphigus for a patient with clinical features of dermatitis herpetiformis and immunologic features of pemphigus. Various terms have been used to describe this condition, such as acantholytic herpetiform dermatitis, pemphigus controlled by sulfapyridine, and mixed bullous disease. Clinical manifestations consist of erythematous, urticarial plaques and vesicles that present in herpetiform arrangement. Histological findings of HP are variable and include eosinophilic spongiosis and subcorneal pustules with minimal or no apparent acantholysis. Immunofluorescence findings show that most patients with HP have IgG antibodies against keratinocyte cell surfaces. Its position in the pemphigus group remains controversial. Based on its clinical features, some researchers reported the disease as a distinct entity and consider it to be different from classic pemphigus because of its clinical peculiarity and benign course. However, others described it as a variant of PF or PV.

Several sporadic studies characterize the target antigens of HP by immunohistochemical analyses such as immunoblotting or immunoprecipitation. Some studies claimed that HP sera recognized the 160-kd Dsg1, and another claimed that HP serum recognized the 130-kd Dsg3. Some authors found that HP sera did not react with any peptide in epidermal extracts by immunoblotting, although immunoblot does not detect conformational epitopes. Therefore, the target molecules of HP remain unclear.
MATERIALS AND METHODS

SERUM SAMPLES

Serum samples were obtained from 20 patients with HP whose clinical manifestations resemble those of dermatitis herpetiformis in the form of the eruption, its symmetrical distribution, and pruritus. It consists of erythematous papules, urticarial-like plaques, or vesicles. Typically, annular erythematous plaques with vesicles and erosions on their margins were seen on the trunk and extremities (Figure 1). Oral membrane lesions were involved in some patients. The Nikolsky sign was negative. Results of histological examination showed eosinophilic spongiosis and subcorneal pustules with minimal or no apparent acantholysis. Direct immunofluorescence stainings revealed the deposition of IgG on keratinocyte cell surfaces in all samples. We selected serum samples that showed positive staining against keratinocyte cell surfaces by indirect immunofluorescence testing using healthy human skin as a substrate.

IMMUNOBLOT ANALYSIS

Immunoblotting was performed as previously reported. EDTA-separated healthy human epidermal extracts were used as the source of antigens.

ELISA USING rDsg1 AND rDsg3

Ishii et al previously developed an ELISA system using baculovirus-expressed rDsg1 and rDsg3. Scores on ELISA against Dsg1 and Dsg3 were obtained as previously reported with a slight modification. Briefly, 201-fold diluted serum samples underwent reaction for 1 hour, and then peroxidase-conjugated antihuman IgG antibodies (Medical and Biological Laboratories, Nagoya, Japan) were used as a second antibody. Color development was achieved with the tetramethylbenzidine solution for 30 minutes and stopped by adding 2N sulfuric acid. The absorbance was measured at 450 nm by an ELISA reader (Bio-Rad Laboratories, Hercules, Calif).

Amagai et al previously produced recombinant Dsg1 (rDsg1) and rDsg3 by baculovirus expression system, which properly reflects native conformational epitopes. Using these recombinant antigens, Ishii et al developed a sensitive and highly specific enzyme-linked immunosorbent assay (ELISA) system for detection of autoantibodies against Dsg1 and Dsg3. Amagai et al also developed an immunoadsorption assay using these recombinant proteins to determine whether there are antibodies against molecules other than Dsg1 or Dsg3.

The purpose of this study was to characterize the autoantigen of HP. As a target antigen, we focused on Dsg1 and Dsg3, which are the autoantigens of PF and PV, respectively. Serum samples from patients with HP were analyzed by immunoblotting, ELISA with rDsg1 and rDsg3, and immunoadsorption assay. We demonstrated that HP serum samples contain IgG against either Dsg1 or Dsg3 and that in most HP serum samples there were no antibodies directed against cell surface molecules other than Dsg1 or Dsg3.

RESULTS

ANALYSIS BY IMMUNOBLOTTING STUDIES

Seventeen HP serum samples were examined by immunoblotting studies using healthy human epidermal extracts. Five serum samples recognized a 160-kd polypeptide, which comigrated with the band recognized by PF sera. Only 1 serum sample recognized a 130-kd polypeptide, which comigrated with the band recognized by PV sera (Table). No antibodies directed against cell surface molecules other than Dsg1 or Dsg3.

ANALYSIS BY rDsg1 AND rDsg3 ELISAs

In immunoblotting, the antigen sources are denatured by heating and sodium dodecyl sulfate. Therefore, im-
munoblotting cannot detect autoantibodies against the conformationally sensitive epitope. In contrast, the ELISA system with rDsg1 and rDsg3 expressed by baculovirus detects autoantibodies against conformational epitopes on Dsg1 or Dsg3. Of 20 HP serum samples tested, 16 showed positive reactivity against Dsg1 ELISA, 4 showed positive reactivity against Dsg3 ELISA, and none reacted with both (Table).

Regarding IgA-class anti-Dsg1 and anti-Dsg3 antibodies, we analyzed these serum samples by ELISA. Four HP serum samples showed slightly higher OD values compared with those of control serum samples (samples 5 and 6 for Dsg1 ELISA and samples 18 and 19 for Dsg3 ELISA). However, the meaning of reactivities against Dsg1 and Dsg3 was unclear because indirect immunofluorescence showed no staining on keratinocyte cell surfaces for IgA antibodies (data not shown).

**HP SERA DO NOT CONTAIN IgG AGAINST CELL SURFACE MOLECULES OTHER THAN Dsg1 OR Dsg3**

To further determine whether HP serum samples possess antibodies against other components of keratinocyte cell surfaces, an immunoadsorption assay was performed. In 19 of 20 HP serum samples examined, immunoreactivity against keratinocyte cell surfaces was completely removed by preincubation with a mixture of rDsg3 and rDsg1 baculoproteins, whereas control medium did not change the immunoreactivity (Table and Figure 2). This finding indicates that most HP serum samples possess only antibodies against the extracellular domain of Dsg1 or Dsg3 and no other antibodies against components of keratinocyte cell surfaces other than Dsg1 or Dsg3.

**COMMENT**

Herpetiform pemphigus is a unique form of pemphigus that clinically resembles dermatitis herpetiformis—an IgA-mediated disease—and immunologically has a feature of pemphigus—circulating and in vivo bound IgG autoantibodies against keratinocyte cell surfaces. To date, sporadic reports indicate that patients with HP have circulating autoantibodies against Dsg1 or Dsg3, as determined

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**Table: Immunologic Characteristics of Sera of Patients With Herpetiform Pemphigus**

<table>
<thead>
<tr>
<th>Serum Sample No.</th>
<th>Immunoblot†</th>
<th>ELISA (IgG)‡</th>
<th>IIF After Immunoadsorption§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>160 kd (Dsg1)</td>
<td>130 kd (Dsg3)</td>
<td>Dsg1 (Index)</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>–</td>
<td>125.8</td>
</tr>
<tr>
<td>2</td>
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</tr>
<tr>
<td>3</td>
<td>+</td>
<td>–</td>
<td>107.5</td>
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<tr>
<td>4</td>
<td>+</td>
<td>–</td>
<td>98.0</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
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</tr>
<tr>
<td>6</td>
<td>+</td>
<td>–</td>
<td>88.3</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>ND</td>
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</tr>
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<td>–</td>
<td>–</td>
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<tr>
<td>20</td>
<td>–</td>
<td>–</td>
<td>9.7</td>
</tr>
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</table>

†Dsg indicates desmoglein; ELISA, enzyme-linked immunosorbant assay; IIF, indirect immunofluorescence; rDsg, recombinant Dsg; plus sign, positive staining against keratinocyte cell surfaces; minus sign, negative staining against keratinocyte cell surface; and ND, not done.
‡Boldface results indicate positive reactivity of serum samples on each ELISA.
§Herpetiform pemphigus serum samples were incubated with the control solutions or a mixture of rDsg1 and rDsg3 baculoproteins and subjected to immunofluorescence staining on healthy human skin.
to antibodies against the cytoplasmic portion of Dsg1 or Dsg3. Alternatively, this sample may have autoantibodies against molecules other than Dsg3 in addition to antibodies and 4 had anti-Dsg3 IgG autoantibodies. Furthermore, immunoadsorption study results excluded a possibility that there are any autoantibodies reacting with molecules on keratinocyte cell surfaces other than Dsg1 or Dsg3 in 19 of 20 HP serum samples. These findings indicate that Dsg1 is the most frequently recognized autoimmune target molecule and Dsg3 is the alternative autoimmune target molecule.

There was 1 HP serum sample that had anti-Dsg3 IgG autoantibodies but whose immunoreactivity was not removed by preincubation with a mixture of rDsg1 and rDsg3. This particular serum sample may contain autoantibodies against molecules other than Dsg3 in addition to antibodies against Dsg3. Alternatively, this sample may have autoantibodies against the cytoplasmic portion of Dsg1 or Dsg3 because the recombinant proteins used for ELISA and immunoadsorption assay represent only the extracellular domains. Immunofluorescence staining of this serum showed a unique pattern with coarse dotlike staining along the cell-cell contact sites (data not shown), which favors the former possibility. However, there are no specific clinical features in this case, and therefore the pathophysiological relevance of this antibody is currently unknown.

Herpetiform pemphigus has some clinical and histological features in common with IgA pemphigus. However, HP can be differentiated from IgA pemphigus by the presence of IgG-class antibodies against keratinocyte cell surfaces because IgA pemphigus is characterized by the presence of IgA-class antibodies without the coexistence of IgG-class antibodies against keratinocyte cell surfaces.24 A more intriguing question is why the clinical presentations of HP and classic pemphigus are so different despite the common presence of anti-Dsg1 or anti-Dsg3 IgG autoantibodies. Clinical pictures of HP consist of erythematous, urticarial plaques with occasional vesicles in herpetiform arrangement. Histological pictures of HP include eosinophilic spongiosis and subcorneal pustules with minimal or no apparent acantholysis, whereas, in PF and PV, acantholysis caused by loss of cell adhesion of keratinocytes is the major histological finding. The pathogenic role of anti-Dsg1 or anti-Dsg3 IgG autoantibodies in PF and PV is established. First, IgG fractions prepared from patients with PF and PV can induce blisters in skin organ culture and in neonatal mice.25-27 Second, removal of anti-Dsg1 or anti-Dsg3 autoantibodies from PF or PV sera, respectively, abolished the ability of the sera to induce blister formation in neonatal mice.18,19 Third, Dsg1 and Dsg3 are expressed in the epidermis, where blister formation occurs in PF and PV, respectively.21 At present, the distinct pathophysiological events responsible for these differences are unknown. However, it can be speculated that the phenotype differences may be caused by differences of epitopes recognized by autoantibodies. Autoantibodies in PF and PV recognize functionally important regions on Dsg and inhibit their adhesive function, leading to the loss of cell adhesion. In contrast, autoantibodies in HP may recognize a functionally less important part of the molecule and therefore are not able to induce loss of cell adhesion. However, these antibodies may be sufficient to cause some inflammatory processes through complement or induction of cytokine release by keratinocytes, leading to intercellular edema and eosinophilic spongiosis.

Results of this study demonstrate that Dsg1 and Dsg3 are the target cell surface antigens of HP. Most HP serum samples contained the autoantibodies against Dsg1. The fact that several patients with HP show features of PF or PV in the course of the disease, or that some patients with HP evolved into having PF or PV, suggests that HP is closely related to PF and PV.14,15 Taking these together, we conclude that HP is a clinical and histological variant of PF or PV.

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REFERENCES


Clinical Implications

Future Diagnosis of Autoimmune Diseases

This study presents an evolving technology in the evaluation and diagnosis of autoimmune diseases. Enzyme-linked immunosorbent assay with specific recombinant target antigens, rather than the less specific immunofluorescence studies that most clinicians rely on to diagnose autoimmune bullous diseases, is used by these investigators to study a unique form of pemphigus. Enzyme-linked immunosorbent assay may be a more cost-effective and specific replacement for immunofluorescence in the future diagnosis of autoimmune disease.

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