A Case of Pemphigus Vulgaris Showing Reactivity with Pemphigus Antigens (Dsg1 and Dsg3) and Desmocollins

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Both pemphigus vulgaris antigen (PVA; Dsg3) and pemphigus foliaceus antigen (PFA; Dsg1) are members of the desmoglein subfamily of the cadherin supergene family. Another desmosomal cadherin, desmocollin, is occasionally recognized by certain pemphigus sera. We present a 38-year-old Japanese male who showed clinically and histopathologically typical features of pemphigus vulgaris, whose sera reacted with all PVA, PFA, and desmocollins using immunoblotting of both human epidermis and bovine snout epidermis. Studies using domain-specific fusion proteins of PFA and PVA suggested that this patient’s serum reacted with the intracellular domain of PFA and the extracellular domain of PVA, the latter of which seems to be responsible for initiating the skin lesion. The patient’s serum showed reactivity with human desmocollin and was shown to react with bovine Dsc2 fusion protein, further suggesting the significance of anti-desmocollin autoantibodies in pemphigus. These results indicate that certain pemphigus cases may produce antibodies against multiple antigen molecules, although the complex mechanism of the production of autoantibodies remains to be elucidated. Key words: cadherin/desmoglein/pemphigus foliaceus. J Invest Dermatol 104:541–544, 1995

Pemphigus is divided into two major subtypes: pemphigus vulgaris (PV) and pemphigus foliaceus (PF) [1]. Brazilian PF is considered to be an endemic form of PF seen in certain areas in South America [2]. In addition, paraneoplastic pemphigus [3] and IgA pemphigus/intercellular IgA vesiculopustular dermatosis [4] have been proposed as new disease entities of the pemphigus group. The antigens in the patients’ sera with which anti-keratinocyte cell-surface autoantibodies react have been investigated extensively. Using both immunoprecipitation [5–8] and immunoblotting [9], most PV sera detected the 160-kD PV antigen (PVA). With immunoprecipitation, most PF sera detected the 160-kD PF antigen (PFA) [7,8], which was later identified as desmoglein 1, a major desmosomal transmembrane protein [10]. In contrast, with immunoblotting using normal human epidermal extracts, only one third of PF sera detect PFA [6,9], probably because of the presence of conformation-dependent epitopes that are destroyed during the immunoblot procedure. We and other investigators showed that desmosome preparations from bovine muzzle epidermis are also useful for detecting PFA by immunoblotting [9,11]. With both immunoprecipitation [7,8] and immunoblotting of bovine desmosome preparations [9], about half of PV sera recognize PVA and PFA simultaneously. Moreover, we have recently reported that another desmosomal membrane protein, desmocollin, is also recognized by certain pemphigus sera [4,12] in immunoblotting of bovine desmosome preparations. However, the significance of the anti-desmocollin autoantibodies is not yet clear, particularly because there have been no pemphigus cases reported showing reactivity with human desmocollin [4,12].

Recent molecular cloning studies have shown that all PFA, PVA, and desmocollins belong to the cadherin supergene family [13–16]. According to a new nomenclature proposed recently by Buxton et al [17], desmosomal cadherins are divided into two groups, desmoglein (Dsg) and desmocollin (Dsc), both of which consist of multiple isoforms, i.e., Dsg1–3 and Dsc1–3. Each desmocollin isoform consists of the larger “a” form and the smaller “b” form by alternative splicing of its mRNA [16,17]. PFA is Dsg1 and PVA is Dsg3 [17].

In this report, we present a clinically and histopathologically typical case of PV in which the patient’s sera detected, in addition to PVA, both PFA and desmocollins using immunoblot analyses of both normal human epidermal extracts and bovine desmosome preparations. Studies using domain-specific fusion proteins of both bovine PFA and human PVA indicated that this patient’s serum reacted with the extracellular domain of PVA and with the intracellular domain of PFA. Furthermore, this patient’s serum was revealed to react specifically with bovine Dsc2 fusion protein. However, the significance of anti-desmocollin antibodies in the pathogenesis of pemphigus remains to be elucidated in future studies.

MATERIALS AND METHODS

Presentation of a Case A 38-year-old Japanese man presented with a 3-year history of flaccid bullae and erosions predominantly on the trunk,

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extremities, and oral mucosa. Histopathology exhibited suprabasal acantholytic blisters in the epidermis. Direct immunofluorescence showed IgG and C3 deposition at the cell surface in the epidermis, and indirect immunofluorescence of normal human skin sections revealed anti-cell-surface antibodies at a titer of 1:2560. From these results, PV was diagnosed, and the condition was well controlled by combination therapy with oral prednisolone and cyclosporin A.

**Sera** We obtained sera from this patient, as well as from six PV patients, six PF patients, and six normal volunteers as controls. Anti-desmoglein monoclonal antibody 32-2B [18] and anti-desmocollin monoclonal antibody 52-3D [16] were also used. All the pemphigus sera contained circulating anti-cell-surface antibodies at a titer of greater than 1:640, as determined by indirect immunofluorescence of both normal human skin and bovine muzzle sections.

**Immunoblot Analysis of Normal Human Epidermal Extracts and Bovine Desmososome Preparations** Preparation of extracts of normal human epidermis separated with dispase treatment, partial purification of desmosome from bovine snout epidermis, and the immunoblot procedure were described previously [9].

**Production of Domain-Specific Fusion Proteins of Human PVA** Fusion proteins for extracellular EC1-2 and EC3-5 domains of human PVA with cro-f-galactosidase were obtained by the same method using pUEX1 expression plasmid vector (Amerham Corp., Arlington Heights, IL), as described previously [15]. In addition, we obtained a fusion protein for the intracellular domain of human PVA by a similar method. Briefly, a cDNA insert representing the entire intracellular domain was excised from the full-length form of human PVA cDNA by polymerase chain reaction amplification using primers containing the appropriate restriction sites and subcloned into the pUEX1 expression plasmid vector.

**Production of Full-Length Fusion Proteins of Bovine Dsc1b, Dsc2b, and Dsc3b** We also obtained full-length fusion proteins of bovine Dsc1b, Dsc2b, and Dsc3b. To obtain Dsc1b fusion protein, the XbaI-digested 2.77-kilobase pair cDNA fragment obtained from the bovine cDNA clone CN35 [12,16] was blunt-ended by DNA polymerase (Klenow fragment) and subcloned into SmaI-digested pGEX-3X expression vector. To obtain Dsc2b fusion protein, the cDNA clone BMDCT2-DC4.1 (a generous gift from Dr. Peter J. Koch and Dr. Werner W. Franke, Division of Cell Biology, German Cancer Research Center, Heidelberg, Germany) [13], and pGEX-2T expression vector (Pharmacia, Uppsala, Sweden).

**Production of Domain-Specific Fusion Proteins of Bovine Dsc1b, Dsc2b, and Dsc3b** We also obtained full-length fusion proteins of bovine Dsc1b, Dsc2b, and Dsc3b. To obtain Dsc1b fusion protein, the XbaI-digested 2.77-kilobase pair cDNA fragment obtained from the bovine cDNA clone CN35 [12,16] was blunt-ended by DNA polymerase (Klenow fragment) and subcloned into SmaI-digested pGEX-3X expression vector. To obtain Dsc2b fusion protein, the cDNA clone BMDCT2-DC4.1 (a generous gift from Dr. Peter J. Koch and Dr. Werner W. Franke) [13] was first digested with SacI, blunt-ended by Klenow fragment, and then digested with Xhol. The resultant 3.6-kilobase pair fragment was subcloned into Sma1/Xhol-digested pGEX-4T-3 expression vector. Dsc3b fusion protein was obtained as described previously [21]. Briefly, cDNA fragments encoding partial sequences of bovine Dsc3 were obtained using randomly primed cDNA synthesized from 1 μg total RNA obtained from bovine nasal epidermis and Dsc3-specific primers. Primers YS8 and YA2 incorporated the BamHI restriction site at the 5' end. After we joined the fragments together, the resultant construct was subcloned into pGEX-2T at the BamHI site. Fusion proteins with glutathione-S-transferase were obtained as described previously [19]. Immunoblotting was performed as mentioned previously [19], except that alkaline-phosphatase-conjugated second antibodies and the alkaline phosphatase substrate kit IV BCIP/NBT were used.

**RESULTS**

To characterize further the antigen molecules in the present case, we performed immunoblot analysis using normal human epidermal extracts and bovine desmosome preparations. With immunoblotting of epidermal extracts (Fig 1, left), all of the six control PV sera reacted exclusively with the 130-kD human PVA, whereas two of the six PF sera reacted with the 160-kD human PFA. The serum of the present case reacted strongly with both antigens. Furthermore, it also reacted weakly but clearly with a doublet of 110-kD and 100-kD proteins, which showed the same migration as those recognized by the anti-desmocollin monoclonal antibody 52-3D. None of other sera reacted with these proteins.

With immunoblotting of bovine desmosome preparations (Fig 1, right), four of the six control PF sera reacted with the 160-kD bovine PVA, whereas the three PV sera reacted with PFA and weakly with the 135-kD bovine PVA, and one PV serum reacted only with the PVA. This patient’s serum strongly reacted with PFA and also with a doublet of 115-kD and 105-kD proteins that showed the same migration as the 52-3D monoclonal antibody, with slightly different intensities.

The patient’s serum reacted at almost the same intensity with both PVA and PFA in human epidermal extracts and only with PFA in bovine desmosomal preparations, although the patient had a clinically and histopathologically typical case of PV. Therefore, to elucidate the possible mechanism for blister formation induced by these antibodies, we performed immunoblot analysis of fusion proteins of both PVA and PFA (Fig 2). The EC1-2 domain of human PVA was recognized by this patient’s serum and by all of the six PV sera, but not by any PF or normal sera. The EC3-5 domain of PVA was recognized by two of six PV sera but not by this patient’s serum. The intracellular domain of PVA was strongly recognized by the 32-2B monoclonal antibody, confirming the specificity of this fusion protein. However, this domain was not detected by any sera used in this study, including the patient’s serum.

In contrast, the EC1-5 domain of bovine PFA was recognized by two of the four PF sera reactive with PFA in the bovine desmosome preparations, but not by any other sera. Conversely, the intracellular domain of bovine PFA was clearly recognized by the patient’s serum and by the three PV control sera reactive with PFA in the bovine desmosome preparations, as well as by one PF serum.

Furthermore, the patient’s serum labeled specifically bovine Dsc2b fusion protein in a manner similar to that of the 52-3D monoclonal antibody (Fig 3). The patient’s serum reacted with
Figure 2. The patient's serum reacted with the EC1-2 domain of PVA and the intracellular domain of PFA with immunoblotting of PVA and PFA fusion proteins. Left (DG1) shows the results of the EC1-5 domain (EC) and the intracellular domain (IC) of bovine PFA; right (PV A) shows the results of the EC1-2 domain, the EC3-5 domain, and the intracellular domain (IC) of human PVA. Results show the reactivity of this patient's serum (lane P1), a control PV serum (lane P1'), a control PF serum (lane PF), and anti-desmoglein monoclonal antibody 32-2B (lane DG). Lane GAL in PVA shows the reactivity of anti-β-galactosidase polyclonal antibodies. Arrowheads indicate the positions of the fusion protein.

This case is interesting for at least two reasons. First, the serum of this patient recognized both PVA and PFA simultaneously with immunoblotting of normal human epidermal extracts. Second, this patient showed antibodies reactive with human desmocollin.

Because a considerable number of PV sera immunoprecipitate PFA from radiolabeled human epidermal extracts [7,8], it is reasonable to assume that some PV sera should also react with both PVA and PFA upon immunoblotting of human epidermal extracts. We could find such a concomitant reactivity by some PV sera in bovine desmosome preparations, but not in human epidermal extracts [9]. We have no explanation for this discrepancy. It may be due to the much lower abundance of PFA in epidermal extracts than in desmosome-enriched fractions or to the species difference between the two antigen sources. However, this patient's serum clearly showed concurrent reactivity with human PVA and PFA.

PV cases with concomitant anti-PVA and anti-PFA antibodies, including the present case, show no features characteristic of PF. There may be at least two plausible explanations for this. One possibility is that the effect of the anti-PVA antibodies predominates over the effect of the anti-PFA antibodies. In other words, because PVA is expressed predominantly in the lower epidermis and PFA in the higher regions, the PVA-mediated adhesions will be disrupted before keratinocytes pass to the upper layers, thereby masking any additional PFA dysfunction. Another possibility is that the anti-PVA antibodies have pathogenic activity but the anti-PFA antibodies in these PV sera are nonpathogenic. The results of immunoblotting of PVA and PFA fusion proteins in this study suggest the latter. The anti-PFA and anti-PVA antibodies in the present case have different types of targets, i.e., the serum reacted with the extracellular domain of PVA and with the intracellular domain of PFA. Therefore, it is conceivable that the production of antibodies against the intracellular domain of PFA in PV may be an epiphenomenon induced after the cell membrane is damaged by pathogenic antibodies against the extracellular domains of PVA. Although the intracellular domain of PVA was not detected by any sera used in this study, further study of more patients' sera will be needed to

Table I. Summary of Immunoblotting Data for the Sera and Monoclonal Antibodies Used in This Study

<table>
<thead>
<tr>
<th>Human Epidermal Extracts</th>
<th>Bovine Desmosome Preparations</th>
<th>PFA</th>
<th>PVA</th>
<th>Dsc</th>
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<td>+</td>
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<tr>
<td>32-2B</td>
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<td>52-3D</td>
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</tr>
<tr>
<td>PF sera (n = 6)</td>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Normal (n = 6)</td>
<td></td>
<td>0</td>
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<td>0</td>
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</tbody>
</table>

* Data are presented as +, positive for the protein; -, negative for the protein; or as the number of sera reactive with the protein. Dsc, desmocollin; IC, intracellular domain; ND, not done.
obtain a final conclusion. Nevertheless, this study showed that the EC1-2 domain is recognized specifically by PV sera but not by any PF sera, confirming the results of a previous study [15] and suggesting that this reactivity may be a specific marker for PV sera.

Upon repeated immunoblot studies of human epidermal extracts, this patient’s serum showed a weak but clear reactivity with doublet protein bands of 110 kD and 100 kD, which is the same reactivity as that of anti-desmocollin monoclonal antibody. Furthermore, the patient’s serum reacted specifically with bovine Dsc2b fusion protein. It is not known why only the present case can detect human desmocollin, whereas a considerable number of pemphigus sera react specifically with desmocollins in bovine desmosome preparations [4,12]. This may be because the amount of desmocollin in human epidermal extracts is too low to be detected by pemphigus sera. Another possible explanation is the species difference between the antigen sources.

It is interesting that the patient’s serum recognized only Dsc2b, and not Dsc1b or Dsc3b. One possible reason is that this serum reacted with Dsc2b-specific epitopes. This speculation should be proved or disproved by future epitope-mapping studies.

This study helps clarify the nature of anti-desmosomal cadherin autoantibodies in pemphigus. The issue of antigen recognition in various types of pemphigus seems to be much more complicated than assumed previously. However, future studies to elucidate many questions in this field should give us further insight into keratinocyte cell biology and cellular and humoral immunity in pemphigus.

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