Pemphigus is an autoimmune bullous disorder characterized by autoantibodies to keratinocyte cell surface antigens and is divided into 2 major forms, pemphigus foliaceus and pemphigus vulgaris. Pemphigus vegetans, a variant of pemphigus vulgaris, is characterized clinically by hypertrophic vegetating skin lesions and/or pustules mainly on the intertriginous areas and histopathologically by neutrophilic and eosinophilic pustule formation in the epidermis. Pemphigus vegetans shows IgG reactivity mainly with desmoglein (Dsg) 3, but also with other autoantigens, including Dsg1 and desmocollins (Dscs).

Report of Cases

Case 1

A woman in her 80s presented with elevated skin lesions on the right inguinal region, which first developed as accumulated small pustules. The patient had no remarkable family history. One member of the patient’s family noticed the discharging tumorous skin lesion 2 weeks prior to presentation,
although the patient did not remember the onset of the lesion owing to dementia.

Physical examination revealed a slightly gray-colored vegetating plaque, with several surrounding erythemas and small pustules, on the right inguinal area (Figure 1A). Laboratory tests revealed elevations in erythrocyte sedimentation rate (29 mm/h [reference range, <15 mm/h]), presence of antihuman T-cell lymphoma/leukemia virus-1 antibodies (2048 s/co [signal to cutoff] [reference range, <16 s/co]), carcinoembryonic antigen level (7.3 ng/mL [reference range, <5.0 ng/mL]), and squamous cell carcinoma antigen level (6.5 ng/mL [reference range, <1.5]). ELISAs detected anti-Dsg1 antibodies (ELISA index value, 101 [reference range, <14]) but not anti-Dsg3 antibodies (index, <5 [reference range <14]).

Chest radiography, abdominal ultrasonography, gastrointestinal endoscopy, electrocardiogram, and vaginal examination revealed no abnormal findings. Enhanced computed tomography of the abdomen and pelvis revealed hypertrophic
figures, measuring 6 × 6 × 1 cm, for the skin lesion on the right inguinal area. In addition, edema and increased amounts of subcutaneous fat to the depth of the long adductor muscle were observed around the skin lesions. Gallium scintigraphy also revealed a strong abnormal accumulation on the right inguinal region.

Histopathological analysis of a biopsy specimen of the skin lesion revealed a reticulately arranged acanthotic epidermis with minimum acantholysis and eosinophilic spongiosis, which contained keratinous cystlike structures with eosinophilic and neutrophilic pustules (Figure 1B and C). Extensive inflammatory infiltration of eosinophils, neutrophils, and plasma cells was found in the superficial and mid-dermis (Figure 1B).

Direct immunofluorescence detected C3 deposit to keratinocyte cell surfaces (Figure 1D), without any deposits of IgG, IgA, or IgM. Findings from indirect immunofluorescence of normal human skin sections were negative for both IgG and IgA antibodies, whereas indirect immunofluorescence of monkey esophagus sections detected IgG (Figure 1E), but not IgA, antiepithelial cell surface antibodies.

Immunoblotting of normal human epidermal cell extracts revealed that IgG antibodies in a serum sample from the patient reacted with the 110-kDa and 100-kDa doublet proteins, which comigrated with a-form and b-form of Dscs, in addition to the 160-kDa Dsg1 (Figure 1F). cDNA transfection method using cDNAs of human Dsc1, Dsc2, and Dsc3 and cultured COS-7 cells was performed as described previously. This study showed that IgG, but not IgA, antibodies reacted with Dsc3 but not with either Dsc1 or Dsc2 (Figure 1G). By novel ELISAs using eukaryotic recombinant proteins of human Dsc1 through Dsc3 (Ishii et al, unpublished data; 2012), IgG antibodies reacted strongly with Dsc3 (optical density [OD], 1.803 [cutoff, 0.120]), but not with either Dsc1 (OD, 0.069 [cutoff, 0.200]) or Dsc2 (OD, 0.040 [cutoff, 0.070]).

From the typical clinical and histopathological findings, the diagnosis of pemphigus vegetans was made. We treated the patient with oral prednisolone, 20 mg/d, which successfully controlled the skin lesion, and the mass disappeared almost completely.

Case 2
A woman in her 70s presented with erosive skin lesions on the left fingers. The patient had no remarkable family history. She first noticed an erosion on the left third finger web 3 months prior to presentation. Antifungal cream, which was prescribed by an internist under the putative diagnosis of tinea interdigitale, was ineffective. The lesion increased in number and size.

Physical examination revealed erosive skin lesions with pustules and scaly erythemas on the left third and fourth fingers (Figure 2A). Laboratory tests revealed an elevated number of eosinophils (580.5/μL) and presence of antinuclear antibodies with speckled pattern (+40), with no abnormality in other test results. ELISAs did not detect antibodies to either Dsg3 or Dsg1.

Histopathological analysis of a biopsy specimen of the skin lesion showed acanthotic epidermis with no apparent acantholysis and extensive inflammatory infiltrates in the dermis (Figure 2B). Typical large eosinophilic pustules with a few neutrophils were also present in the epidermis (Figure 2C).

Direct immunofluorescence detected no deposits of IgG, IgA, IgM, or C3. Results of indirect immunofluorescence of normal human skin sections were negative for both IgG and IgA antibodies, while indirect immunofluorescence of monkey esophagus sections detected IgG (Figure 2D), but not IgA, antiepithelial cell surface antibodies.

Immunoblotting of normal human epidermal cell extracts revealed that IgG antibodies in a serum sample from the patient reacted with the 110-kDa a-form and the 100-kDa b-form of Dscs, as well as the 230-kDa BP230-like band and the 190-kDa periplakin-like band, but did not react with Dsgs (Figure 2E). cDNA transfection method revealed that IgG, but not IgA, antibodies reacted only with Dsc3 (Figure 2F). Novel Dsc ELISAs revealed that IgG antibodies reacted with Dsc2 (OD, 0.142 [cutoff, 0.070]) and strongly with Dsc3 (OD, 1.324 [cutoff, 0.120]), but not with Dsc1 (OD, 0.103 [cutoff, 0.200]).

From the typical clinical and histopathological findings, the diagnosis of pemphigus vegetans was made. We treated the patient first with a topical corticosteroid because the lesions were limited to the fingers. Although the treatment was effective, oral mucosal lesions and pustular skin lesions on the left inguinal area subsequently appeared. Oral prednisolone, 20 mg/d, completely controlled the lesions. We tapered the prednisolone dose to 5 mg/d without any recurrence. The clinical course was well correlated with serum eosinophil numbers.

Discussion
We extensively analyzed autoantigens for 2 cases of clinically and histopathologically typical pemphigus vegetans. Three serological tests, including immunoblotting, cDNA transfection method, and novel ELISAs for Dsc1, Dsc2, and Dsc3, indicated that case 1 had IgG anti-Dsc3 antibodies, in addition to IgG anti-Dsg1 antibodies. Case 2 also showed strong IgG reactivity with Dsc3, although lower reactivity with Dsc2 was detected by ELISA but not cDNA transfection method. Intriguingly, case 2 showed no antibodies to either Dsg3 or Dsg1.

Immunoblotting of normal human epidermal extracts is known to detect anti-Dsc antibodies in only few cases, probably because epitopes on Dscs are conformation dependent and cannot be detected by immunoblotting. Because the results of reactivity with Dsc1, Dsc2, and Dsc3 in this study were almost identical between cDNA transfection method and novel Dsc ELISAs, combination of these tests should be a reliable method to detect autoantibodies to Dscs in various types of pemphigus in the future.

In case 2, immunoblotting of normal human epidermal cell extracts showed the 190-kDa periplakin-like and 230-kDa BP230-like bands. We considered the periplakin-like band as a nonspecific reaction because it occasionally occurs even in normal control serum samples. The BP230-like band was also considered as nonspecific reaction because the patient’s serum sample did not show anti–basement membrane zone an-
Bodies in indirect immunofluorescence, and the 230-kDa band is occasionally shown in serum samples from patients with nonbullous pemphigoid.

Clinically, both case 1 and case 2 showed Hallopeau-type clinical features. Both cases showed almost identical histopathological features, characteristic of pemphigus vegetans. In addition, both cases showed strong IgG reactivity with Dsc3. Considering that clinical features of both Neuman- and Hallopeau-types concur in some cases of pemphigus vegetans, the results of the present study may suggest a common pathomechanism in the Hallopeau-type of pemphigus vegetans.

To date, autoantibodies to Dscs were identified only occasionally in patients with nonclassic types of pemphigus, in-
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Case Report/Case Series

Research

including pemphigus vegetans, pemphigus herpetiformis, para-neoplastic pemphigus, and atypical pemphigus. However, precise prevalence of IgG anti-Dsc autoantibodies in various types of pemphigus, including pemphigus vegetans, has not been fully elucidated. To answer this question, extensive studies using Dsc ELISAs in large numbers of patients with pemphigus should be performed.

The pathogenic role of anti-Dsc antibodies is not well understood in any types of pemphigus. In our study, case 2 is particularly interesting because this case showed typical clinical features of pemphigus vegetans but no anti-Dsg antibodies, supporting our previous speculation of pathogenic role of anti-Dsc autoantibodies in pemphigus vegetans.

The studies of knockout mice of Dsc1 and Dsc3 showed pemphigus-like skin fragility with defective epidermal barrier function.

These previous studies suggested that anti-Dsc antibodies play a pathogenic role by influencing keratinocyte cell adhesion. Further functional studies should be required to elucidate the pathogenic relevance of anti-Dsc antibodies in pemphigus.