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H. 知的財産権の出願・登録状況

1. 特許取得　　特になし。
2. 実用新案登録　　特になし。
3. その他　　特になし。

I. 文献別冊

巻末に添付。

表 1:本研究で検討した HHD 症例のまとめ

症例	国籍	性	年齢	家族歴	発症時期	病変部	重症度	*ATP2C1*	hSPCA1 の位置
1	日本	男	66	なし	不明	陰嚢	軽症	p.Lys80X (exon 4)	Upstream stalk
2	日本	男	68	なし	不明	肛囲	軽症	c.334dupA (exon 5)	Upstream stalk
3	日本	男	57	なし	50 代	鼠径	中等症	c.360+1G>C (intron 5)	M2
4	日本	女	36	有	30 代	頚部, 腋窩	軽症	p.Cys141X(exon 7)	Actuator
5	日本	男	42	なし	20 代	腋窩, 背部, 鼠径	重症	p.Arg153X (exon 7)	Actuator
6	日本	男	47	なし	30 代	腋窩, 鼠径, 肛囲	重症	c.520delC (exon 7)	Actuator
7	日本	女	32	有	20 代	鼠径, 膝窩	軽症	p.Ile216_Ala217del(exon 8)	Actuator
8	韓国	女	62	有	40 代	腋窩, 鼠径	中等症	c.681dupA (exon 8)	Actuator
9	日本	女	52	なし	40 代	頚部	軽症	c.825delTA (exon 10)	M3
10	日本	男	44	有	20 代	腋窩, 鼠径, 膝窩	中等症	c.899+1G>T (intron 11)	M4
11	日本	男	49	なし	不明	頚部, 腋窩, 肘窩　鼠径, 膝窩	重症	p.Pro307His (exon 12)	M4
12	日本	男	58	なし	40 代	腋窩, 鼠径, 肛囲	中等症	c.956delC (exon 12)	M4
13	日本	男	42	有	30 代	腋窩, 鼠径	中等症	p.Lys351Thr(exon13)	Phosphorylation
14	日本	男	48	なし	40 代	腋窩, 鼠径, 足	中等症	c.1089delTCAC (exon13)	Phosphorylation
15	日本	男	72	なし	60 代	腋窩, 鼠径	中等症	c.1308+1G>A (intron 15)	Phosphorylation
16	日本	男	61	有	50 代	頚部, 腋窩, 肘窩　鼠径, 膝窩	重症	p.Try483X (exon 17)	Nucleotide
17	日本	男	66	なし	50 代	頚部, 腋窩, 鼠径	中等症	p.493X(exon17)	
18	日本	男	54	有	50 代	鼠径, 肛囲	中等症	p.Gln504X (exon 17)	Nucleotide
19	日本	男	80	なし	不明	全身	重症	p.Gln505X (exon 17)	Nucleotide
20	韓国	女	55	なし	20 代	頚部, 腋窩, 胸部, 鼠径, 膝窩	重症	c.1570+2T>C (intron 17)	Nucleotide
21	英国	女	不明	不明	不明	不明	不明	p.Leu541Phe(exon18)	Nucleotide
22	英国	不明	不明	不明	不明	不明	不明	c.1825-1831delCAAATAG(exon19)	Nucleotide
23	日本	男	68	なし	60 代	腋窩, 鼠径, 肛囲	中等症	p.Leu632Pro (exon 21)	Hinge
24	日本	男	40	なし	20 代	頚部, 腋窩, 鼠径	中等症	c.1906-1909dup(exon21)	Hinge
25	ドイツ	不明	28	有	不明	不明	不明	p.Asn647His (exon 21)	Hinge
26	日本	女	80	有	20 代	腋窩, 胸部, 鼠径, 四肢	重症	p. Gln706X (exon 22)	M5
27	日本	男	70	有	50 代	腋窩, 鼠径	中等症	c.2375delTTGT (exon 24)	M7
28	日本	男	34	なし	30 代	頚部, 腋窩, 鼠径	中等症	p.W795X (exon 24)	M7
29	英国	不明	不明	不明	不明	不明	不明	p.R799X (exon 24)	M8
30	日本	男	66	なし	50 代	腋窩, 鼠径, 臀部, 膝窩	中等症	N.D.	
31	日本	女	60	なし	50 代	腋窩, 鼠径	中等症	N.D.	
32	日本	男	73	なし	40 代	腋窩, 鼠径	軽症	N.D.	

下線の変異は過去に報告のない新規のものであることを示す。

表2　家族性良性慢性天疱瘡(Hailey-Hailey病)診断基準(案)

1. 診断基準項目
(1) 臨床的項目
a) 腋窩、陰股部、肛囲、項頸部などの間擦部位に小水疱と痂皮を付着するびらん性紅斑局面を形成する。
b) 青壮年期に発症。
c) 症状を反復し、慢性に経過する。
d) 常染色体優性遺伝を示す(注:本邦の約3割は孤発例)。
e) 増悪因子と合併症の存在。
高温・多湿・多汗(夏季)、機械的刺激、細菌・真菌・ウイルスによる二次感染。
f) その他の稀な症状の存在
爪甲の白色縦線条、手足の点状小陥凹や角化性小結節、口腔内～食道病変。

(2) 組織学的項目
光顕上、表皮基底層直上から表皮中上層にかけて広く棘融解を形成する。まれに異常角化細胞が出現する。

(3) 遺伝子診断
ATP2C1の遺伝子解析により変異を検出する。

2. 診断
組織学的項目を満たし，かつ臨床的項目a)、c)、d)を満たすものを確実例とする。ATP2C1の遺伝子変異が同定できた場合にも診断を確定する。

3. 鑑別診断を要する疾患
脂漏性皮膚炎、白癬・伝染性膿痂疹・ヘルペス、乳房外パジェット病、尋常性天疱瘡、増殖性天疱瘡、ダリエ病

Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

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Ⅳ．研究成果の刊行物・別刷

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CASE REPORT

Exon 87 skipping of the *COL7A1* gene in dominant dystrophic epidermolysis bullosa

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ABSTRACT

Dystrophic epidermolysis bullosa (DEB) is a rare, inherited, blistering disorder resulting from mutations in the *COL7A1* gene, which encodes the anchoring fibrils, type VII collagen. We herein describe a further Japanese girl diagnosed with dominant DEB (DDEB). She had blisters sporadically and erosions healed with mild scarring and milia on the knees and pretibial regions. Severe pruritus was present at this time. Direct nucleotide sequencing of genomic DNA disclosed a heterozygous same splice-site mutation c.6900G>A in the *COL7A1*, which causes in-frame exon 87 skipping. So far, five different *COL7A1* mutations leading to exon 87 skipping have been identified in rare forms of DEB: four DDEB pruriginosa and one pretibial DDEB. Therefore, a recent study suggested that exon 87 skipping in *COL7A1* was related to the phenotype of DDEB pruriginosa. When she was 18 years old, however, the blister formation and pruritus markedly decreased. Therefore, her clinical symptoms were consistent to very mild DDEB but not to DDEB pruriginosa. Taken together, in-frame exon 87 skipping through c.6900G>A mutation may account for the mild skin features, rather than DDEB pruriginosa, in the present case.

Key words: *COL7A1*, dystrophic epidermolysis bullosa, genodermatosis, genotype–phenotype correlation, pruriginosa.

INTRODUCTION

Dystrophic epidermolysis bullosa (DEB) is a rare inherited blistering disorder resulting from mutations in the *COL7A1* gene, which encodes type VII collagen, the major component of anchoring fibrils at the dermal–epidermal junction. Over 300 pathogenic mutations have been described within *COL7A1* in various clinical forms of DEB. So far, five different *COL7A1* mutations leading to exon 87 skipping have been identified in rare forms of DEB: four dominant DEB (DDEB) pruriginosa and one pretibial DDEB.[1–4] Therefore, a recent study suggested that exon 87 skipping in *COL7A1* was related to the phenotype of DDEB pruriginosa.[5] We herein describe a further DDEB patient with the same splice-site mutation c.6900G>A, but the clinical appearance of the patient was quite different from that of DDEB pruriginosa.

CASE REPORT

The patient was a Japanese girl who was the offspring of healthy unrelated parents. She presented with a history of trauma-induced skin blistering and erosions mainly on the extremities at the age of

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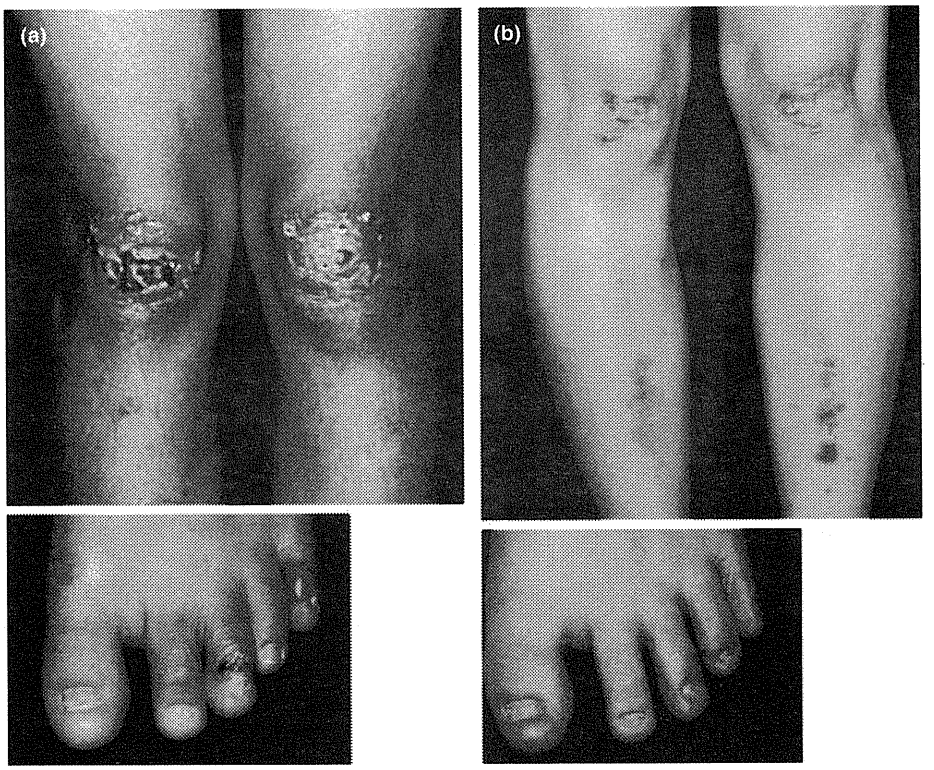

Figure 1. Clinical features of the patient. (a) Erosions healed with mild scarring on the knee, and blisters and nail dystrophy were seen on the toes at 8 years of age. (b) Symptoms were markedly improved by 18 years of age.

1 month. She also had nail dystrophy. She was referred to our hospital when she was 8 years old. There were scattered blistering and erosions healed with mild scarring and milia on the knees and pretibial regions (Fig. 1a). Severe pruritus was initially present. However, blister formation and pruritus markedly decreased by 18 years of age (Fig. 1b). The clinical manifestations of this patient were consistent to very mild DDEB but not to DDEB pruriginosa. Direct nucleotide sequencing of genomic DNA from the patient disclosed a heterozygous G to A transition at nucleotide c.6900 that does not lead to an amino acid change in p.Gln2300 residue (Fig. 2a). Reverse transcription polymerase chain reaction (RT–PCR) across the site of the c.6900G>A transition identified two bands of 475 and 544 bp in size, compared to a single 544 bp band in the normal control (Fig. 2b). Subcloning and direct sequencing disclosed that the 475-bp band was a mutant transcript with in-frame exon 87 skipping (Fig. 2c). In contrast to the previous report by Saito *et al.*,[5] we further detected two additional aberrant transcripts with inclusion of the entire intron 86 or 87 as a new exon, leading to premature termination codon 27 or 120 bp downstream, respectively (Fig. 2c). The 544-bp band was the normal wild-type transcript.

DISCUSSION

As Saito *et al.* described,[5] it seems to be probable that exon 87 skipping is related to the phenotype of DDEB pruriginosa. However, the present case was clinically quite different from DDEB pruriginosa, although she was shown to have exon 87 skipping by RT–PCR analysis. Inter- and intrafamilial variability from the same *COL7A1* mutations has been previously described in the published work. For example, the most common *COL7A1* mutation p.Gly2043Arg has been identified in a different phenotype of DDEB.[6] It is plausible that modifying genes, epigenetic or environmental factors might influence the phenotypic variations in DEB. Therefore, genetic counseling in such cases is fraught with difficulty. It is important that families are made aware of the

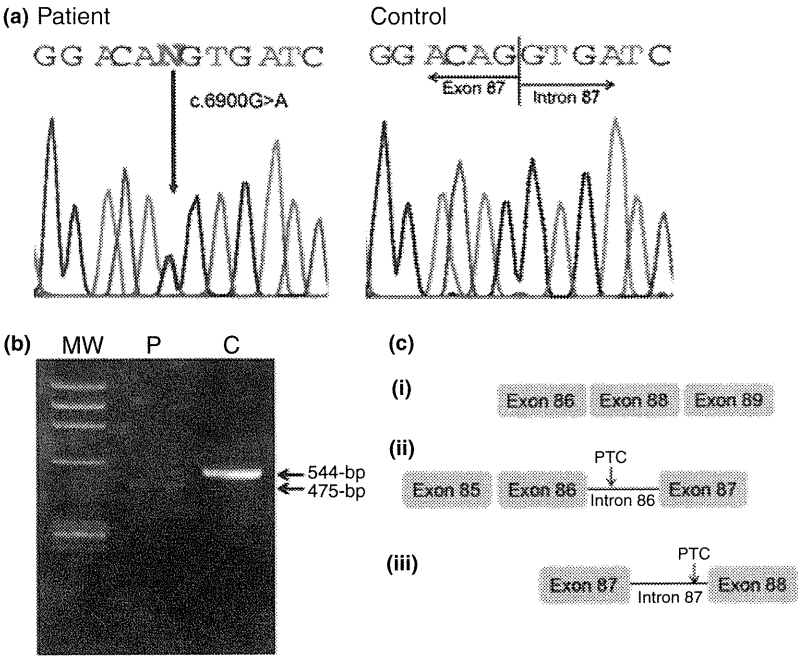

Figure 2. Molecular basis of dominant dystrophic epidermolysis bullosa (DEB) in the present patient (a) Direct nucleotide sequencing of genomic DNA from the patient disclosed a heterozygous G>A transition at nucleotide c.6900. (b) Reverse transcription polymerase chain reaction (RT–PCR) across the site of the c.6900G>A transition. In the amplified cDNA from control (lane C), a single band of 544 bp was present. By contrast, in the amplified cDNA from the patient (lane P), two different bands of 544 and 475 bp were identified. (c) Subcloning and direct sequencing revealed the band of 475 bp is a mutant transcript with in-frame exon 87 skipping. Two aberrant mutant transcripts with inclusion of the entire intron 86 or 87 were also identified, although the bands were not visible on the agarose gel, shown in (b). PTC, premature termination codon.

clinical diversity in DEB and are offered appropriate counseling.

The RT–PCR analysis in this study revealed two aberrant mutant transcripts with inclusion of the entire intron 86 or 87 as a new exon, as well as exon 87 skipping. However, the RT–PCR bands of these two mutant transcripts were not visible on the agarose gel, suggesting low mRNA expression of these mutants in the patient's skin. Taken together, in-frame exon 87 skipping through c.6900G>A mutation may account for the mild skin features, rather than DDEB pruriginosa, in the present case.

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Mucous membrane pemphigoid with autoantibodies to all the laminin 332 subunits and fatal outcome resulting from liver cirrhosis and hepatocellular carcinoma

To the Editor: Mucous membrane pemphigoid (MMP) patients rarely have autoantibodies to laminin 332 (formerly laminin 5).[1] In most cases, autoantibodies to the laminin $\alpha 3$ subunit are detected, although MMP patients with autoantibodies to the laminin $\gamma 2$ subunit alone have been reported.[2] However, MMP patients with autoantibodies to all three subunits of laminin 332 have not to our knowledge previously been reported.

A 73-year-old woman suffered from liver cirrhosis with hepatitis C virus infection for 17 years. She previously had undergone splenectomy. A hepatocellular carcinoma mass 2.5 cm in diameter persisted in the left lobule despite radiofrequency ablation and microwave coagulation therapy. A 9-year history of diabetes mellitus was currently managed with insulin injections. Three months before her visit, bullae had developed on her elbows and knees. A biopsy specimen from the left patellar skin showed subepidermal blister formation with a mild chronic inflammatory infiltrate in the papillary dermis, which is suggestive of bullous pemphigoid. Indirect immunofluorescence with the patient's serum demonstrated immunoglobulin G (IgG) antibodies to the basement membrane zone of healthy human skin sections. Indirect immunofluorescence with salt-split skin revealed IgG antibodies reactive with the dermal side (not shown). By immunoblotting using normal human dermal extract, the patient's serum reacted with neither the 290-kDa type VII collagen nor the 200-kDa laminin $\gamma 1$ subunit[3] (Fig 1, *left*). By immunoblotting using purified human laminin 332[4] as an antigen source, the patient's IgG antibodies reacted strongly with all the $\alpha 3$, $\beta 3$, and $\gamma 2$ subunits (Fig 1, *right*). We diagnosed this case as antilaminin 332 MMP.

Despite the administration of 200 mg of minocycline and 75 mg of dapsone daily together with a topical steroid, oral ulcers and blister formation spread rapidly over the body, together with the development of fever, hoarseness, and epistaxis. Oral prednisolone 30 mg daily had no effect and was tapered to 20 mg daily. Any additional steroids or immunosuppressive agents were avoided because of possible secondary infection. Advanced liver cirrhosis did not permit plasmapheresis. Intravenous immunoglobulin injection was not administered because of the poor prognosis of severe liver cirrhosis associated with cancer. Widespread painful erosions developed (Fig 2).

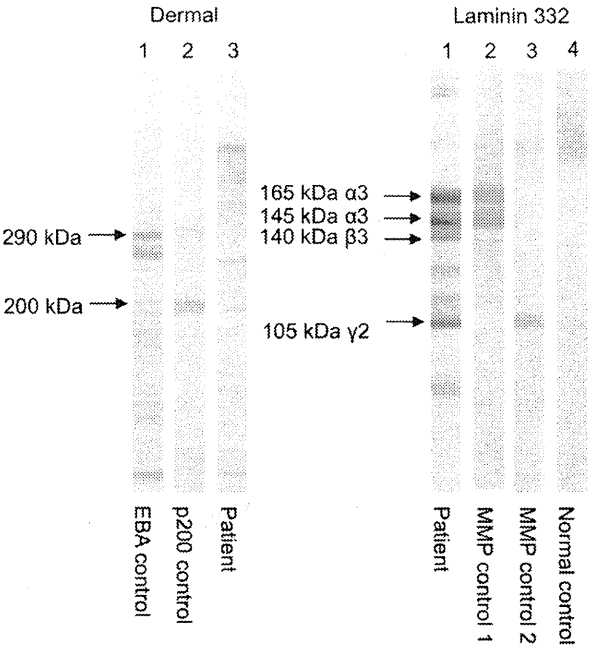

Fig 1. Immunoblot analyses. Using dermal extracts (*left column*), IgG autoantibodies of control epidermolysis bullosa acquisita serum reacted with the 290-kDa type VII collagen (lane 1), and immunoglobulin G (IgG) autoantibodies of control antilaminin $\gamma 1$ pemphigoid (formerly anti-p200 pemphigoid) serum reacted with 200-kDa laminin $\gamma 1$ (lane 2), whereas neither antigen was detected by our patient's serum (lane 3). Using purified human laminin 332 (*right column*), IgG antibodies of this patient's serum reacted with all the 165-kDa $\alpha 3$, 145-kDa $\alpha 3$, 140-kDa $\beta 3$, and 105-kDa $\gamma 2$ subunits of laminin 332 (lane 1). IgG antibodies of control MMP serum 1 reacted with the 165- and 145-kDa $\alpha 3$ subunits (lane 2), and IgG antibodies of control MMP serum 2 reacted with the 105-kDa $\gamma 2$ subunit (lane 3), while normal serum showed no reactivity (lane 4).

The patient was severely depressed and her mental and physical activities were rapidly lost. Portal vein thrombosis occurred, and the patient died of sepsis. Eye lesions were not apparent throughout her course.

To our knowledge, this is the first case of MMP with autoantibodies to all three subunits of laminin 332. This set of autoantibodies may be responsible for the extensive distribution and severity of disease in the present case. Antilaminin 332 MMP is associated with cancer, although hepatocellular carcinoma has never been reported.[5]

In an immunocompromised patient, extensive skin lesions can cause sepsis as a result of direct infection of the skin. Indirectly, however, widespread erosions can cause exacerbation of hypoalbuminemia and constipation following an extended

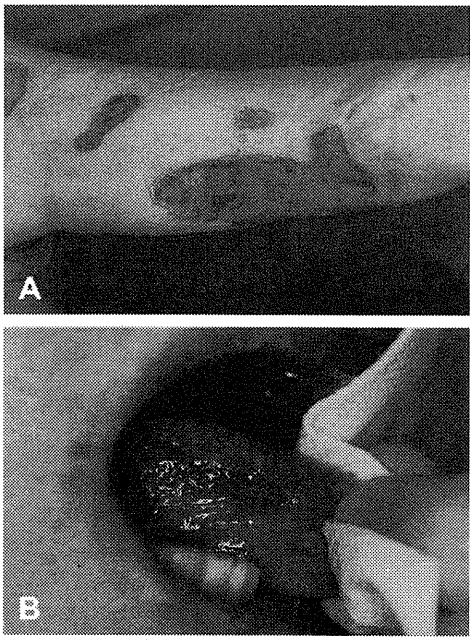

Fig 2. Clinical manifestations in the terminal stage of the present case. **A**, Fresh, large, well demarcated erosions without perilesional erythema on the left upper arm. **B**, Ulcers on the oral mucosa and tongue.

bedridden state; both can trigger portal vein thrombosis followed by spontaneous bacterial peritonitis[6] and sepsis, although a bacterial culture of ascitic fluid was not performed in the present case. Caregivers should pay attention to any infection, including spontaneous bacterial peritonitis, during the course of a severe bullous disease in immunocompromised patients with liver cirrhosis.

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Cancrum oris in a boy with Down syndrome

To the Editor: A 17-year-old Italian male with Down syndrome presented to the dermatology department with a 2-cm ulcerative lesion on the right tip of his tongue (Fig 1). The onset was about 7 days earlier with mild swelling. The patient's parents described a persistent vesicular eruption in the same area during the previous 2 months. They attributed it to biting and self-inflicted injuries and treated it with multiple but inefficacious drugs (nystatin, metisoprinol, diflucortolone, and chloramphenicol). The physical examination revealed that the right submandibular lymph nodes were slightly enlarged and tender. Fever and malaise were not reported, and neither were any other symptoms. Routine hematologic parameters were normal, apart from a chronic low leukocyte count. These findings were similar to his previous laboratory examinations. His medical history, as reported by the parents, was negative for any kind of recurrent infection. He was otherwise in good health and had never been institutionalized. An HIV test was negative. An oral swab revealed significant growth of multiple anaerobic bacteria. Cancrum oris was strongly suspected, and a 3-mm punch biopsy of the lesion was performed. Histologic examination revealed a nonspecific acute process characterized by a heavy inflammatory infiltrate mainly comprised of neutrophilic abscesses and blood vessel endothelial swelling, suggestive of bacterial infection. As indicated by microorganism culture and sensitivity tests, an intramuscular dose of lyncomicin 600 mg (2 mL)

It is important to note that Hoste *et al.* (2011) have not proposed that caspase-14 is the only protease that cleaves filaggrin. Rather, their report shows that, in the absence of caspase-14, other enzymes can initiate proteolysis of filaggrin. In addition, proteases such as calpain 1 and bleomycin hydrolase (Kamata *et al.*, 2009) are required to complete the degradation of filaggrin. Therefore, to understand the regulation of filaggrin degradation, it is necessary to determine the interplay of caspase-14 with these other proteases as well as the order in which the proteolytic cuts occur.

Concluding remarks

New evidence demonstrates that not only mutations in the filaggrin gene but also alterations in filaggrin processing may result in skin barrier defects and that caspase-14 takes part in this process. Hoste *et al.* (2011) provide a basis for improving strategies to diagnose filaggrin-associated skin disorders and to modulate caspase-14-dependent barrier function of the stratum corneum.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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See related article on pg 2271

Demonstration of Epitope Spreading in Bullous Pemphigoid: Results of a Prospective Multicenter Study

Takashi Hashimoto[1,2], Daisuke Tsuruta[1,2], Teruki Dainichi[1,2], Takahiro Hamada[1,2], Minao Furumura[1,2] and Norito Ishii[1,2]

Di Zenzo and colleagues have undertaken a multicenter prospective study to clarify the epitope profile for IgG anti-BP180 and BP230 antibodies in 35 patients with bullous pemphigoid (BP). Both intra- and intermolecular epitope spreading events were observed, in which epitopes shifted exclusively from extracellular to intracellular domains. The presence of IgG antibodies to the BP180 C-terminal domain and BP230, in addition to the BP180-NC16A domain, correlated with disease severity and activity, suggesting specific pathogenic relevance for anti-BP230 antibodies. Epitope spreading was found in both T- and B-cell recognition. IgA anti LAD-1 antibodies are frequently found in patients with BP; these antibodies appear to follow the development of IgG antibodies to BP180 and BP230 by epitope spreading. These observations provide direction for future studies of the pathogenesis of and treatments for BP.

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Epitope spreading plays an important role in the development of autoimmune diseases

Various autoimmune diseases develop and progress via epitope spreading (ES). In ES, inflammation induced by autoimmunity to an initial epitope damages target tissue, which subsequently induces antibodies to secondary epitopes on the same or different antigens (Chan *et al.*, 1998). Intra- or intermolecular ES is not simply an epiphenomenon, because it is important for the development of each disease.

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Clinical Implications

- Intra- and intermolecular epitope spreading (ES) is observed in bullous pemphigoid (BP); epitopes shift from extracellular to intracellular domains.
- The presence of IgG antibodies to the BP180 C-terminal domain and BP230, in addition to the BP180-NC16A domain, correlates with disease severity and activity. ES is found for both T- and B-cell recognition in BP.
- Analysis of ES may provide clues for developing novel therapies.

Previous studies in bullous pemphigoid (BP), using both human materials and mouse models, have clarified the progress and relevance of ES (Di Zenzo *et al.*, 2008, 2010). A retrospective study of BP sera by enzyme-linked immunosorbent assays (ELISA) that used recombinant proteins of BP180 and BP230 suggested the occurrence of ES in patients with BP (Yoshida *et al.*, 2006). However, the precise development and progress of ES and its correlation to BP disease activity have not been elucidated.

Di Zenzo *et al.* (2011, this issue) performed a multicenter prospective epitope profile study of IgG antibodies to both BP180 and BP230 during the clinical course of 35 patients with BP. ES occurred in about half of the patients. Intramolecular ES in BP180 occurred first in the extracellular domain and then spread to the intracellular domain. Intermolecular ES also occurred and spread from BP180 to BP230. Finally, reactivity, both with extracellular BP180 epitopes and with intracellular BP230 epitopes, correlated with disease severity and activity, suggesting pathogenic significance for the anti-BP230 autoantibodies.

ES occurred in patients with BP

Over a 12-month period, ES was found in 17 (49%) of 35 patients with BP, whereas 18 (51%) showed no epitope shifts. All 17 patients showing ES reacted with BP180 epitopes, and 12 reacted with BP230 epitopes at the time of diagnosis. Twenty-four different ES events were observed, and ES from extracellular BP180 epitopes to intracellular epitopes of BP180 and BP230 occurred most frequently. ES also occurred before the development of overt clinical manifestations of BP.

ES always spread from extracellular to intracellular domains

Three BP patients showed ES from extracellular BP180 epitopes to intracellular epitopes of BP180 and BP230, whereas the opposite was not observed. In addition, three patients showed intermolecular ES from BP180 epitopes to BP230 epitopes, whereas the opposite did not occur. This single direction of ES events should provide important insights into the mechanism of ES.

This study indicated clinical significance of ES events in BP

ES events occurred most frequently in the first 3 months after BP diagnosis, suggesting that ES is an early event in the course of the disease. Accordingly, ES was observed rarely during relapses of the disease. Perhaps immunosuppressive therapy impairs T- and B-cell activation, reducing subsequent ES events.

Severity and activity of disease were correlated with reactivity with both the NC16A and the C-terminal domains of BP180, but not with the N-terminal intracellular domain. Notably, two patients with active disease showed exclusive reactivity with the BP180 C-terminal domain. These results indicate that reactivity with the C-terminal domain of BP180 is pathogenic not only in mucous membrane pemphigoid but also in BP.

Importantly, ES was clearly related to disease severity and activity at the time of diagnosis. It is interesting that reactivity with epitopes occurred later via ES decreased rapidly. IgG reactivity with BP180 extracellular domains and with BP230 intracellular domains was clearly related to disease severity and activity, whereas reactivity with BP180 intracellular domains was not. These results suggest that not only antibodies to BP180 extracellular domains but also antibodies to BP230 are pathogenic, whereas reactivity with BP180 intracellular domains is an epiphenomenon.

Anti-BP230 autoantibodies were suggested to be pathogenic

Three patients without BP180-NC16A reactivity nonetheless reacted with other BP180 and BP230 epitopes. As mentioned above, IgG reactivity with BP230 was clearly related to disease severity and activity, suggesting that anti-BP230 antibodies are pathogenic. Although reactivity with BP230 has generally been considered a nonpathogenic epiphenomenon, previous evidence suggests a pathogenic role for anti-BP230 antibodies. Anti-BP230 antibodies induced skin lesions in a mouse model, and BP230 titers by immunoblotting and ELISA correlated with disease activity (Hamada *et al.*, 2001; Yoshida *et al.*, 2006). Recently, sera we collected from 35 patients with active BP showed only anti-BP230 antibodies by ELISA, reacted only with intracellular sites of hemidesmosomes by immunoelectron microscopy, and showed a unique ES profile (Fujihara *et al.*, 2011). This combination of evidence suggests pathogenic significance for BP230 antibodies in BP and would thus indicate that autoantibodies bind to intracellular antigens by unknown mechanisms.

Nonpathogenic IgA anti-LAD-1 antibodies were found frequently in BP

Di Zenzo *et al.* (2011) detected IgA reactivity with LAD-1 in 11 of their 35 patients. This result complements that of our study, in which a significant number of BP sera possessed IgA antibodies reactive with recombinant protein of LAD-1 by ELISA (Csorba *et al.*, 2011). In four patients in the present study, IgG reactivity with BP180 and BP230 spread to IgA reactivity with LAD-1. However, because IgA anti-LAD-1 antibodies showed no association with disease severity and activity, disease duration, mucosal involvement, or ES, they were considered to have a limited pathogenic role.

Nevertheless, because this finding may provide insight into development of pathogenic IgA anti-LAD-1 antibodies in linear IgA bullous dermatosis, further experiments should explore the mechanisms of class switching between IgG and IgA.

ES also occurred in T-cell recognition

ES in T-cell reactivity was examined in nine of the patients with BP, and a proliferative T-cell response to BP180 and/or BP230 was observed in five. Two of these five patients showed ES events in T-cell recognition. One showed a shift from the extracellular BP180 domain to the BP230 C-terminal domain, and another showed a shift from the BP180-NC16A domain to the BP180 C-terminal domain. In general, autoreactive B and T cells showed similar epitope profiles throughout the course of the disease.

Perspectives

In this report of their prospective study, Di Zenzo *et al.* reveal many novel and interesting results. In future studies, the pathogenic role in BP should be confirmed for IgG antibodies to the BP230 and the BP180 C-terminal domain, as well as for IgA anti-LAD-1 antibodies. Novel ES mechanisms may be discovered in the production of pathogenic IgG anti-BP230 antibodies and IgA anti-LAD-1 antibodies.

Di Zenzo and co-workers' results also give us insight into potential new treatments, including the use of decoy peptides, tolerance-inducing peptides, and antigen-specific immunoabsorption. In particular, the rapid decrease of autoantibodies against ES-induced late epitopes should yield clues for pursuing novel therapeutic approaches.

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENTS
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IL-6 and IL-8 synthesis in tumor cells in our assay. To prove this assumption, MC supernatants were pre-incubated with a neutralising antibody against TNF-α or isotype control antibody before stimulation of the cancer cells. When TNF-α was neutralised, MC–mediated augmentation of IL-8 mRNA expression was almost completely lost and IL-8 concentration decreased to basal levels in Mel-1, SCL-1, SCC-12 and SCC-13 cells (Fig. 2a–d grey bars). Regarding IL-6 mRNA expression, blockade of TNF-α in MC supernatants also abrogated the increase in expression in SCL-1 and SCC-12 cells, but led to an only 50 percent inhibition in SCC-13 cells (Fig. 1d–f). Thus, regulation of IL-6 in SCC-13 cells seems not only processed through TNF-α but probably also through other factors like histamine or tryptase. Similar to our results obtained in SCC-13 cells, TNF-α seemed not responsible for IL-8 synthesis in Mel-4 cells. (Fig. 2e). However, stimulation of Mel-4 cells with histamine, another MC mediator, led to increased gene expression of IL-8 (Fig. 2f). Inhibition experiments with H1- or H2-receptor antagonists desloratadine or famotidine confirmed that up-regulation of IL-8 synthesis in Mel-4 cells was histamine dependent and mediated via the H2 receptor (Fig. 2f).

Conclusions

Using a novel model to study the communication between human MCs and human tumor cells derived from the same tissue site, we have been able to show that normal human MCs communicate with tumor cells and that TNF-α and histamine are key players in this process. Surprisingly, melanoma and squamous carcinoma cells differed in their response to MC-derived TNF-α: while IL-8 expression and release were up-regulated in melanoma and SCC cells, IL-6 was unaltered in melanoma cell lines, but up-regulated in SCC cell lines. In the SCC lines, MCs further increased constitutive IL-6 and IL-8 mRNA generation, whereas in the melanoma cell lines, Mel-1, Mel-2 and Mel-4, which exhibit an only marginal constitutive IL-8 production, IL-8 synthesis was enhanced by MC-derived TNF-α and/or histamine, far exceeding baseline levels.

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Conflict of interest

The authors have no conflicting interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Determination of TNF-α protein levels in co-culture supernatants.

Figure S2. Induction of IL-6 and IL-8 mRNA expression in mast cell/cancer cell co-cultures.

Appendix S1. Material and Methods.

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Letter to the Editor

Lesional Th17 cells and regulatory T cells in bullous pemphigoid

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Abstract: Th17 cells play crucial roles in the pathogenesis of autoimmune diseases. We previously reported that Th17 cells are recruited to the lesional skin in pemphigus vulgaris (PV) and pemphigus foliaceus (PF). The aim of this study was to evaluate

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lesional Th17 cells and Treg cells in bullous pemphigoid (BP). Correlations between these cells and disease severity of BP were also evaluated. Immunohistochemical studies showed that both IL-17+ and Foxp3+ cells were present in higher numbers in BP lesions, compared with control skin. IL-17/CD4 ratio in BP was significantly higher than that in PF. Foxp3/CD4 ratio in BP was significantly less than that in either PV or PF. There were no obvious correlations between these cells and disease severity of BP. This study suggests that, compared with pemphigus, BP shows more Th17 cell-related inflammation and less Treg-related regulation.

Key words: bullous pemphigoid – IL-17 – pemphigus foliaceus – pemphigus vulgaris – Th17 – Treg

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Background

Th17 cells, characterized by interleukin-17 (IL-17) production, play crucial roles in the pathogenesis of autoimmune diseases (1). We previously reported that Th17 cells are recruited to the lesional skin in pemphigus vulgaris (PV) and pemphigus foliaceus (PF) (2).

Questions addressed

The aim of the present study was to evaluate the status of lesional Th17 cells and regulatory T cells (Treg) in bullous pemphigoid (BP) by an immunohistochemical approach.

Experimental design

Twenty-five patients (15 men and 10 women, 32–92 years old, average 71.84 years) were allocated to this study. The data on PV and PF were retrieved from our previous study (2). All patients were diagnosed by histological subepidermal blister, linear IgG deposition at the basement membrane zone and detection of circulating autoantibodies to BP antigens by an enzyme-linked immunosorbent assay (ELISA). The severity indexes in the 24 BP cases (unknown in one case), ranged from 2 to 10 (average: 5.875); they were scored according to the diagnostic criteria of the Specified Disease Treatment Research Program by the Japanese Ministry of Health, Labour and Welfare.

The lesional skin biopsy specimens were obtained before treatment. The control skin was taken from three healthy people. One half of each specimen was used for histological analyses. The rest of the specimens were used for immunohistochemical studies; goat anti-human IL-17 antibody (R&D, Minneapolis, MN, USA), mouse anti-human Foxp3 (Abcam, Cambridge, UK) and mouse anti-human CD4 antibodies (Nichirei, Tokyo, Japan) were used as primary antibodies. The counts of IL-17+, possibly Th17 cells (IL-17); Foxp3+, possibly Treg cells (Foxp3) and CD4+ cells (CD4) in two sections were evaluated. The ratio of IL-17+ or Foxp3+ count to CD4+ count (IL17/CD4, Foxp3/CD4, respectively) was also evaluated. IL-17 levels in BP patient sera and control sera were evaluated by ELISA.

We analysed correlations between two groups of data as follows: (a) IL-17/CD4 vs CD4, (b) Foxp3/CD4 vs CD4, (c) IL-17/CD4 vs age at onset (d) Foxp3/CD4 vs age of onset, (e) IL-17/CD4 vs maximal dose of prednisolone (PSLmax: as the index for disease severity), (f) Foxp3/CD4 vs PSLmax, (g) IL-17/CD4 vs BP180 ELISA index (BP180), (h) Foxp3/CD4 vs BP180, (i) IL-17/CD4 vs severity index, (j) Foxp3/CD4 vs severity index, (k) number of eosinophils (Eos) vs IL-17/CD4, (l) Eos vs Foxp3/CD4 and (m) Eos vs severity index.

Results

Both IL-17+ and Foxp3+ cells were present in higher numbers in BP lesions (Fig. 1a–c), compared to control skin (Fig. 1d–f). The IL-17/CD4 ratio in BP was 16.28%, significantly higher than that in PF (1.8%) and not significantly but relatively higher than that in PV (5.2%) (Fig. 1g). The Foxp3/CD4 ratio was 5.52%, which was significantly lower than that in either PV or PF (more than 20%) (Fig. 1h). The Th17/Foxp3 ratio in BP was significantly higher (2.95%) than those in PV and PF (Fig. 1i). The serum IL-17, assessed by ELISA, was significantly higher (3.6-fold) in BP

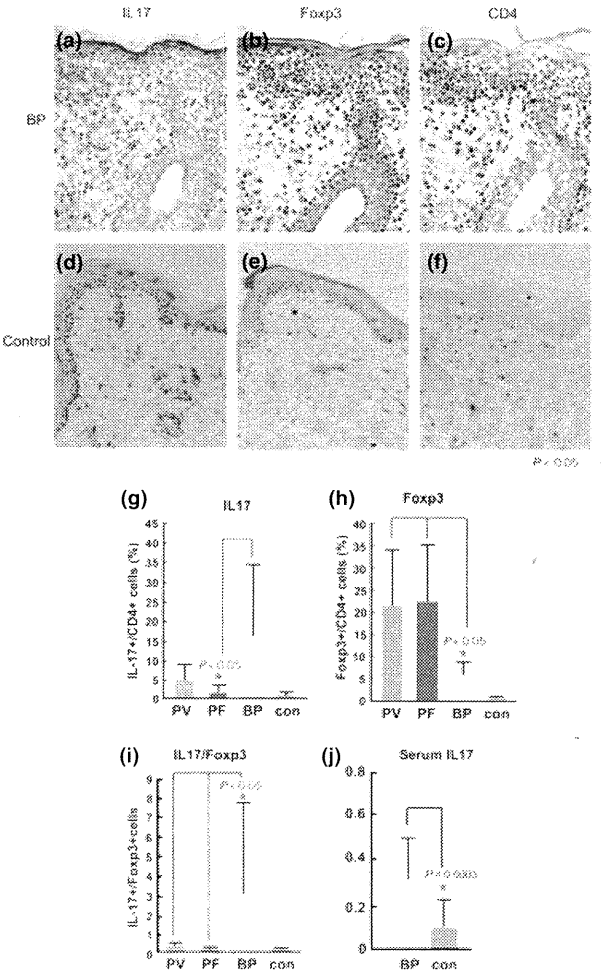

Figure 1. Immunohistochemical studies of lesional skin specimens (a–c) and control skin specimens (d–f). Positively stained lymphocytes were detected by antibodies to IL-17 (a, d), Foxp3 (b, e) and CD4 (c, f). Quantification of the number of IL-17-producing (g), Foxp3-expressing (h) and the ratios of IL-17-producing cells/Foxp3 expressing cells (i) in BP, PV, PF and normal control (con). The significance of the differences was assessed by an unpaired *t*-test. (j) Serum IL-17 levels in BP and normal control. BP, bullous pemphigoid, PV, pemphigus vulgaris, PF, pemphigus foliaceus.

serum than in control serum (Fig. 1j). Although not statistically significant as assessed by Spearman's rank correlation coefficient ($P > 0.05$), some pairs of parameters showed tendencies to correlate, i.e., negative correlations are seen in [IL-17/CD4 vs CD4] (Figure S1a), in [Foxp3/CD4 vs CD4] (Figure S1b) and in [Foxp3/CD4 vs BP180] (Figure S1h). Other pairs showed no correlation (Figure S1c–g, i–m).

Conclusions

This is the first study in which lesional IL-17+ cells and Foxp3+ cells have been quantitatively evaluated in BP. The numbers of Th17 cells were increased in BP and PV skin lesions, but not in PF lesions, while the number of Treg cells in BP lesional skin was significantly smaller than those in PV and PF lesional skin.

The first issue that should be considered is that the numbers of IL-17+ cells were increased in BP and PV, but not in PF. A potential role of Th17 cells in BP was recently suggested (1), because an increased recruitment of IL-17+ cells in the lesional tissue was observed in mucous membrane pemphigoid (MMP), another pemphigoid member (3). Although the role of Th17 cells in the pathogenesis of autoimmune diseases is unresolved, they may be the initiators of diseases (2). Alternatively, Th17 cells may possibly appear in a protective response to maintain epithelial homoeostasis (4). In fact, IL-17 production was induced by keratinocytes in an *in vitro* system (5). If the latter is the case, the more severe disruption in epithelial integrity in BP and PV, when compared with PF, may increase the number of regional Th17 cells.

The second intriguing issue is that the number of Foxp3+ cells in BP was significantly smaller than that in pemphigus groups. In fact, a decreased number or impaired functions of circulating Treg cells in several autoimmune diseases have been reported (6). Therefore, the decrease in Treg cells in BP is plausible. However, the different results between the BP and pemphigus groups cannot be explained. Treg cells were reported to be upregulated in pemphigus groups (2,7). One possibility is the difference in pathogenesis between pemphigus and BP; inflammation is more crucial for blister formation in BP than in pemphigus (8). Therefore, it is tempting to speculate that the involvement of an inflammatory milieu may decrease the number of Treg cells in BP skin lesions. In fact, it was reported that IL-6 secretion was upregulated, TGF-β secretion was downregulated, and the number of circulating $\gamma\delta$ T cells was reduced in BP (9–11). The other possibility is repression of the migration of Treg cells into the inflammatory region in BP, but it may not be the case, because Treg chemoattractants were observed in both the affected skin and the bullae in BP (12,13). Previously, another group suggested that Treg cells accumulated in BP or MMP tissue (14,15). The discrepancy between our results and theirs is unexplained, but the genetic or racial differences maybe involved.

Our studies showed clear differences in the status of lesional effector/regulatory T-cell subsets between BP and pemphigus, although this study was not conducted in a perfectly blinded fashion. The results in the present study provide clues to elucidation of the pathogenesis of BP.

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Conflict of interest

There is no conflict of interest.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Correlation of lesional lymphocytes or eosinophils with various parameters.

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