CONCISE REPORT

Anti-NXP2 autoantibodies in adult patients with idiopathic inflammatory myopathies: possible association with malignancy

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ABSTRACT

Objectives Myositis-specific autoantibodies (MSAs) are useful tools for identifying clinically homogeneous subsets and predicting prognosis of patients with idiopathic inflammatory myopathies (IIM) including polymyositis (PM) and dermatomyositis (DM). Recent studies have shown that anti-NXP2 antibody (Ab) is a major MSA in juvenile dermatomyositis (JDM). In this study the frequencies and clinical associations of anti-NXP2 Ab were evaluated in adult patients with IIM.

Methods Clinical data and serum samples were collected from 507 adult Japanese patients with IIM (445 with DM and 62 with PM). Eleven patients with JDM, 108 with systemic lupus erythematosus, 433 with systemic sclerosis and 124 with idiopathic pulmonary fibrosis were assessed as disease controls. Serum was examined for anti-NXP2 Ab by immunoprecipitation and western blotting using polyclonal anti-NXP2 Ab.

Results Seven patients (1.6%) with adult DM and one (1.6%) with adult PM were positive for anti-NXP2 Ab. Except for two patients with JDM, none of the disease controls were positive for this autoantibody. Among eight adult patients with IIM, three had internal malignancies within 3 years of diagnosis of IIM. Another patient with DM also had a metastatic cancer at the diagnosis. All of the carcinomas were at an advanced stage (stage IIIb–IV).

Conclusions While less common than in juvenile IIM, anti-NXP2 Ab was found in adult IIM. Anti-NXP2 Ab may be associated with adult IIM with malignancy.

INTRODUCTION

Idiopathic inflammatory myopathies (IIM), including polymyositis (PM) and dermatomyositis (DM), are characterised by chronic inflammation of skeletal muscles and/or skin eruptions.[1] Although the aetiology remains unclear, they are considered as autoimmune diseases. The presence of disease-specific autoantibodies (autoAbs), known as myositis-specific autoAbs (MSAs), is a prominent feature. Moreover, MSAs are strongly associated with distinct clinical phenotypes and thus classify patients into groups with more homogeneous clinical features.[2–4] These MSAs include antibodies (Abs) to aminoacyl-tRNA synthetases, the Mi-2 protein, the signal-recognition particle, transcriptional intermediary factor-1 (TIF1; anti-155/140 Ab)[5–7] and melanoma differentiation-associated gene-5 (MDA5; anti-CADM140 Ab).[8]

Oddis and colleagues first described anti-MJ Ab in a US cohort of juvenile DM (JDM),[9] and Targoff *et al* subsequently identified that the antigen of anti-MJ Ab is nuclear matrix protein NXP2 (MORC3).[10] Gunawardena *et al* and Espada *et al* have demonstrated that anti-NXP2 Ab is among the most common MSAs in JDM.[11 12] In this study we evaluated the frequencies and clinical associations of anti-NXP2 Ab in adult patients with IIM.

METHODS

Patients

Serum samples were obtained from 507 consecutive Japanese adult patients with IIM, 445 with DM and 62 with PM, who had been followed up in the Department of Dermatology, Kanazawa University Hospital and collaborating medical centres from 2003 to 2010. All patients with PM and 365 patients with DM fulfilled Bohan and Peter's criteria.[13 14] The remaining 80 did not, but fulfilled Sontheimer's criteria of clinically amyopathic DM (CADM).[15] Among the patients with DM, as disease controls, 11 with JDM, 108 with systemic lupus erythematosus, 433 with systemic sclerosis and 124 with idiopathic pulmonary fibrosis were assessed. Clinical information was collected retrospectively by reviewing their clinical medical charts.

Immunoprecipitation and western blotting

Serum (10 µl) was incubated with 2 mg protein A-Sepharose beads (Amersham Biosciences, Piscataway, New Jersey, USA) in immunoprecipitation buffer (10 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.1% Nonidet P-40) for 2 h. Beads were then mixed with ^{35}S-labelled or unlabelled K562 cell extracts derived from 10^7 cells and rotated at 4°C for 2 h. After five washes, precipitated proteins were fractionated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), followed by autoradiography or western blotting.

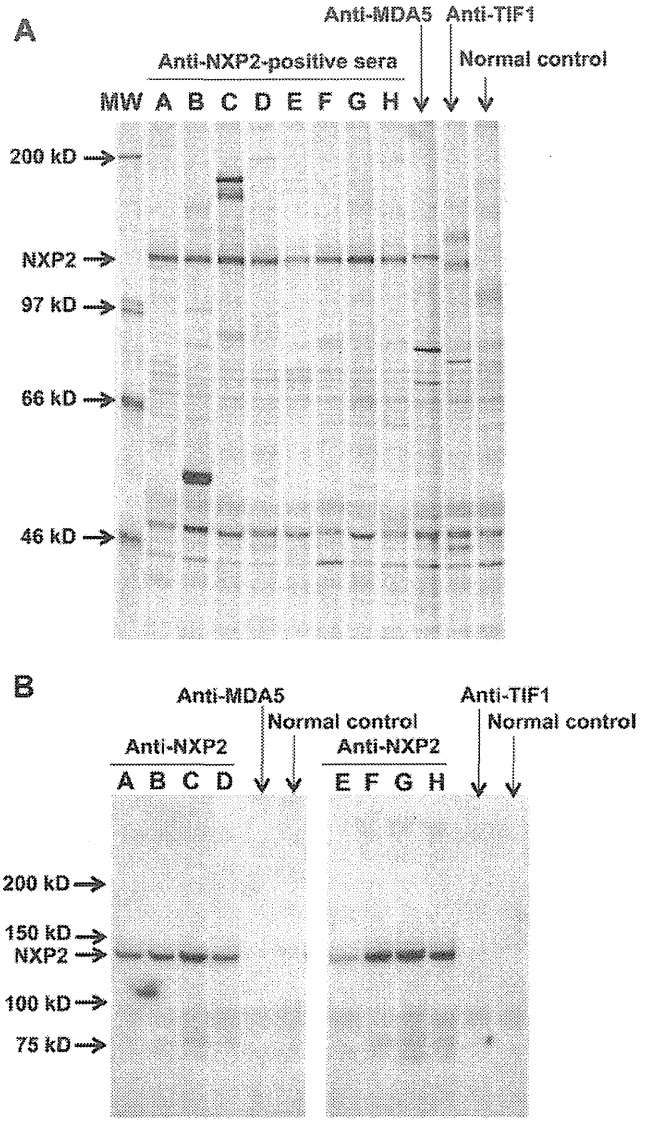

Figure 1 Detection of anti-NXP2 Ab. (A) Immunoprecipitant from ^{35}S-labelled K562 cell extract using serum samples were subjected to 7% SDS-PAGE. Lanes A–H correspond to anti-NXP2-positive patients shown in table 1. As controls, the prototype serum samples positive for anti-TIF1 Ab and anti-MDA5 Ab and normal control serum were also examined. Molecular weights (MW) are shown on the left. The position of the 140 kDa NXP2 protein is indicated by an arrow. (B) K562 cell extracts were immunoprecipitated with anti-NXP2-positive serum (patients A–H), anti-MDA5-positive serum, anti-TIF1-positive serum or normal control serum and subjected to SDS-PAGE, electro-transferred onto nitrocellulose membranes and probed with polyclonal anti-NXP2 Ab. The MW of NXP2 (140 kDa) is indicated by an arrow.

For western blotting, proteins were transferred onto a nitrocellulose membrane. After blocking, the membrane was incubated with mouse polyclonal anti-human NXP2 Ab (Abcam, Cambridge, UK) and then with horseradish peroxidase-conjugated goat antimouse IgG Ab (Thermo Scientific, Rockford, Illinois, USA). The membrane was developed using an enhanced chemiluminescence kit (Thermo Scientific).

Additional information regarding methods is available in the online supplement.

Statistical analysis

Fisher exact probability tests were used for comparison of frequencies. The Bonferroni test was examined for multiple comparisons of values following normal distribution. Values that were not normally distributed were evaluated by the Mann–Whitney U test. p Values <0.05 were considered significant.

RESULTS

Detection of anti-NXP2 Abs

Serum samples from 507 adult Japanese patients with IIM were screened for anti-NXP2 Ab. From K562 cell extracts, eight serum samples precipitated a 140 kDa protein which was different from other known autoantigens (figure 1A). Additionally, two patients with JDM were positive for this 140 kDa protein. No serum from other diseases precipitated this 140 kDa protein. On SDS-PAGE, the 140 kDa band was slightly higher than the 140 kDa band of anti-TIF1 Ab and was not accompanied by a 155 kDa band (figure 1A). While this 140 kDa protein migrated closely to MDA5, these serum samples were negative for anti-MDA5 Ab by an ELISA using recombinant MDA5 protein as antigen (data not shown). These serum samples were confirmed to react with NXP2 since precipitated proteins were recognised by polyclonal Abs to NXP2 in western blotting (figure 1B). They also reacted with recombinant human NXP2 protein by western blotting (see figure S1 in online supplement). These serum samples were negative for other MSAs and did not have other known autoAbs, except for patient B who was positive for anti-SS-A/Ro and SS-B/La Abs. In indirect immunofluorescence, five showed negative staining while three stained nuclei in speckled pattern at the maximum dilution of ×80.

Clinical and laboratory profiles of patients with IIM with anti-NXP2 Abs

Among 507 adult patients with IIM, eight were positive for anti-NXP2 Ab: seven (1.6%) in 445 adults with DM and one (1.6%) in 62 adults with PM. All patients with anti-NXP2 Ab showed strong muscle weakness and high elevation of serum creatine kinase levels. Remarkably, internal malignancies found within 3 years of the diagnosis of IIM were present in 37.5% (3/8) (table 2). All these patients had advanced disease (stage IIIb–IV). Additionally, in one patient (C), prostate cancer was found 42 months previously and it was metastatic when the diagnosis of DM was made. However, he was excluded from the statistical analysis since he did not meet the above criteria. Interstitial lung disease (ILD) was not found.

During the follow-up period which varyied from 2 to 61 months, seven of eight patients (all except patient H) were treated with systemic corticosteroid therapy. Patient A also received methotrexate and patient D also underwent intravenous immunoglobulin therapy. While the response to the treatment was favourable in all patients, two (patients C and G) died of malignancy.

Comparison with other MSAs

In addition to seven patients positive for anti-NXP2 Ab, adult patients with DM in this study included 74 patients with anti-TIF1 Ab and 15 with anti-Mi-2 Ab. They also included 51 with anti-MDA5 Ab, 26 with anti-PL-7 Ab, 18 with anti-Jo-1 Ab and 8 with anti-PL-12 Ab. Since anti-TIF1 Ab and anti-Mi-2 Ab are specifically associated with DM, the clinical features of patients with anti-NXP2 Ab were compared with those with anti-TIF1 Ab and anti-Mi-2 Ab (table 2).

Anti-NXP2 Ab was predominantly found in men. Although Gottron's sign was slightly less frequent, there was no significant difference in the frequency of each cutaneous manifestation. The frequency of fever was significantly higher than in patients

Table 1 Clinical and laboratory profile of adult patients with DM or PM positive for anti-NXP2 Ab

Patient	A	B	C	D	E	F	G	H
Age (years)	23	34	54	57	57	61	62	68
Sex	Male	Female	Male	Male	Male	Male	Memale	Male
Diagnosis	DM	DM	DM	DM	DM	DM	PM	DM
Duration (months)	11	21	10	8	23	4	2	7
Heliotrope rash	–	+	+	+	+	–	–	+
Gottron's sign	+	–	+	+	–	–	–	+
Perionychia erythema	–	–	+	+	+	+	–	–
Nailfold punctuated haemorrhage	–	–	+	–	+	+	–	+
V sign	–	–	+	+	+	–	–	–
Shawl sign	–	–	+	+	+	–	–	–
Scratch dermatitis	–	+	–	+	–	+	–	–
Erythema of extensor extremities	–	+	+	+	+	+	–	–
Calcinosis	–	–	–	–	–	–	–	–
Blistering	–	–	–	–	–	–	–	–
Ulceration	–	–	–	–	+	–	–	–
Muscle weakness	+	+	+	+	+	+	+	+
Raynaud's phenomenon	–	–	–	–	–	–	–	–
Fever	–	–	+	–	+	+	+	+
Arthralgia	–	+	–	–	–	–	–	–
Elevation of CK	+	+	+	+	+	+	+	+
Highest CK level (IU/l)	3857	1877	4927	1539	17140	26685	3713	3722
IIF titre	<40	80	<40	40	<40	<40	80	<40
IIF staining pattern	–	Sp	–	Sp	–	–	Sp	–
ILD	–	–	–	–	–	–	–	–
Malignancy	–	–	+	+	–	–	+	+
Origin	–	–	Prostate	Pancreas	–	–	Gallbladder	Lung
Histology	–	–	AC	ND	–	–	AC	SCC
Stage	–	–	IV	IVb	–	–	IVb	IIIb
Period of diagnosis*	–	–	42 months before	6 months after	–	–	Simultaneous	3 months before

*Period of diagnosis indicates when the diagnosis of malignancy was made before or after the onset of idiopathic inflammatory myopathies.
AC, adenocarcinoma; CK, creatine kinase; DM, dermatomyositis; IIF, indirect immunofluorescence; ILD, interstitial lung disease; ND, not done; PM, polymyositis; Sp, speckled; SSC, squamous cell carcinoma.

with anti-TIF1-positive DM. The highest creatine kinase level was similar to that in patients with anti-Mi-2 Ab-positive DM and significantly higher than in patients with anti-TIF1 Ab. The frequency of internal malignancy in those with anti-NXP2 Ab was lower than in those with anti-TIF1 Ab and higher than in patients with anti-Mi-2 Ab, although the differences were not statistically significant.

DISCUSSION

Two studies have recently detected anti-NXP2 Ab in 23% and 25% of patients with JDM in the UK and Argentina, respectively.[11 12] While the number of patients was small, anti-NXP2 Ab was detected in 18% in the control JDM population in this study. Anti-NXP2 Ab is therefore likely to be a major MSA in JDM across racial groups as well as anti-TIF1 Ab, which is detected in 23–29% of patients with JDM.[16] In addition to JDM, Espada *et al* reported that two (28%) of seven patients with juvenile PM were also positive for anti-NXP2 Ab.[12] In this study we identified eight anti-NXP2 Ab-positive adult patients in a Japanese cohort of IIM. The frequencies were 1.6% in both adult DM and adult PM. Therefore, while the population sizes of DM and PM are substantially different, anti-NXP2 Ab has been found at similar frequencies both in juvenile and adult IIM.[12] In contrast, in a preliminary report in a UK cohort, Betteridge *et al* detected anti-NXP2 Ab in 13 (5%) patients with DM but not in patients with PM.[17]

Intriguingly, 37.5% of adult IIM patients positive for anti-NXP2 Ab had malignancy which was found within 3 years, in addition to a patient with metastatic prostate cancer found 42 months previously. Moreover, all of these carcinomas were at an advanced stage. Anti-TIF1 Ab also has a strong association with internal malignancy in adult DM, as it is found in 50–70% of patients with cancer-associated DM.[5 6 18] Therefore, while anti-NXP2 Ab may not be restricted to DM, anti-TIF1 Ab and anti-NXP2 Ab may have a shared property in that they represent two clinical subsets of cancer-associated adult DM and JDM. However, in contrast to our study, Betteridge *et al* reported that anti-NXP2 Ab was not associated with malignancy but with ILD.[17] This may be due to ethnical differences. It may also have resulted from a different distribution of patients between dermatology and rheumatology clinics. Since both studies had relatively small numbers of patients, more studies are needed to evaluate more precisely the clinical relevance of anti-NXP2 Ab in patients with IIM.

Among adult patients with DM, anti-NXP2 Ab appeared to have strong muscle involvement while we could not find any particular cutaneous manifestations related to anti-NXP2 Ab. Unlike anti-NXP2-positive JDM,[11] no adults with DM with anti-NXP2 Ab had cutaneous calcinosis. This may be due to the relatively low incidence of calcinosis in adult DM compared with JDM.

In summary, this study shows that anti-NXP2 Ab occurs in adult patients with IIM and suggests that anti-NXP2 Ab may be correlated with cancer-associated myositis. NXP2 is involved in the activation and localisation of a tumour suppressor gene,

Table 2 Comparison of clinical and laboratory profile of adult patients with dermatomyositis with anti-NXP2 Ab, anti-TIF1 Ab and anti-Mi-2 Ab*

				p Values	
	Anti-NXP2-positive	**Anti-TIF1-positive**	**Anti-Mi-2-positive**	**vs Anti-TIF1**	**vs Anti-Mi-2**
Number	7	74	15		
Age at onset, mean (range)	57 (23–68)	59 (27–89)	50 (16–67)	NS	NS
Sex (male:female)	6:1	24:34	8:7	0.033	NS
Skin eruptions					
Heliotrope rash	71	62	60	NS	NS
Gottron's sign	57	81	87	NS	NS
Perionychia erythema	57	57	60	NS	NS
Nailfold punctuated haemorrhage	57	40	73	NS	NS
Trunk erythema	57	62	53	NS	NS
Calcinosis	0	2	7	NS	NS
Ulceration	14	7	0	NS	NS
Clinical features					
Muscle weakness	100	69	93	NS	NS
Raynaud's phenomenon	0	9	0	NS	NS
Arthritis	14	3	7	NS	NS
Fever	57	16	20	0.025	NS
Organ involvement					
Interstitial lung disease	0	13	13	NS	NS
Internal malignancy within 3 years	29	66	7	NS	NS
Laboratory findings					
Elevated CK, %	100	63	100	NS	NS
Highest CK level, IU/l, mean (range)	3857 (1539–26685)	425 (40–8670)	3934 (401–10000)	<0.001	NS

*Unless noted otherwise, values are percentages.
CK, creatine kinase.

p53.[19] TIF1 proteins also have a functional relationship with p53.[20] Since these two autoAbs may share similar clinical characteristics, especially the association with cancer, they may develop during antitumour immune responses.

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Competing interests None.

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REFERENCES

1. **Zong M**, Lundberg IE. Pathogenesis, classification and treatment of inflammatory myopathies. *Nat Rev Rheumatol* 2011;**7**:297–306.
2. **Targoff IN**. Myositis specific autoantibodies. *Curr Rheumatol Rep* 2006;**8**:196–203.
3. **Gunawardena H**, Betteridge ZE, McHugh NJ. Myositis-specific autoantibodies: their clinical and pathogenic significance in disease expression. *Rheumatology (Oxford)* 2009;**48**:607–12.
4. **Hamaguchi Y**, Kuwana M, Hoshino K, *et al*. Clinical correlations with dermatomyositis-specific autoantibodies in adult Japanese patients with dermatomyositis: a multicenter cross-sectional study. *Arch Dermatol* 2011;**147**:391–8.
5. **Targoff IN**, Mamyrova G, Trieu EP, *et al*. A novel autoantibody to a 155-kd protein is associated with dermatomyositis. *Arthritis Rheum* 2006;**54**:3682–9.
6. **Kaji K**, Fujimoto M, Hasegawa M, *et al*. Identification of a novel autoantibody reactive with 155 and 140 kDa nuclear proteins in patients with dermatomyositis: an association with malignancy. *Rheumatology (Oxford)* 2007;**46**:25–8.
7. **Fujimoto M**, Hamaguchi Y, Kaji K, *et al*. Myositis-specific anti-155/140 autoantibodies target transcriptional intermediary factor 1 family proteins. *Arthritis Rheum*. Published Online First: 10 Oct 2011. doi:10.1002/art.33403.
8. **Sato S**, Hoshino K, Satoh T, *et al*. RNA helicase encoded by melanoma differentiation-associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: association with rapidly progressive interstitial lung disease. *Arthritis Rheum* 2009;**60**:2193–200.
9. **Oddis CV**, Fertig N, Goel A, *et al*. Clinical and serological characterisation of the anti-MJ antibody in childhood myositis. *Arthritis Rheum* 1997;**40**:S139.
10. **Targoff IN**, Trieu EP, Levy-Neto M, *et al*. Sera with autoantibodies to the MJ antigen react with NXP2. *Arthritis Rheum* 2007; **56**:S787.
11. **Gunawardena H**, Wedderburn LR, Chinoy H, *et al*. Autoantibodies to a 140-kd protein in juvenile dermatomyositis are associated with calcinosis. *Arthritis Rheum* 2009;**60**:1807–14.
12. **Espada G**, Maldonado Cocco JA, Fertig N, *et al*. Clinical and serologic characterization of an Argentine pediatric myositis cohort: identification of a novel autoantibody (anti-MJ) to a 142-kDa protein. *J Rheumatol* 2009;**36**:2547–51.
13. **Bohan A**, Peter JB. Polymyositis and dermatomyositis (second of two parts). *N Engl J Med* 1975;**292**:403–7.
14. **Bohan A**, Peter JB. Polymyositis and dermatomyositis (first of two parts). *N Engl J Med* 1975;**292**:344–7.
15. **Sontheimer RD**. Would a new name hasten the acceptance of amyopathic dermatomyositis (dermatomyositis siné myositis) as a distinctive subset within the idiopathic inflammatory dermatomyopathies spectrum of clinical illness? *J Am Acad Dermatol* 2002;**46**:626–36.
16. **Gunawardena H**, Wedderburn LR, North J, *et al*. Clinical associations of autoantibodies to a p155/140 kDa doublet protein in juvenile dermatomyositis. *Rheumatology (Oxford)* 2008;**47**:324–8.
17. **Betteridge Z**, Gunawardena H, Chinoy H, *et al*. Clinical associations of anti-p140 autoantibodies in adult myositis. *Ann Rheum Dis* 2010;**69**(Suppl 3):127.
18. **Chinoy H**, Fertig N, Oddis CV, *et al*. The diagnostic utility of myositis autoantibody testing for predicting the risk of cancer-associated myositis. *Ann Rheum Dis* 2007;**66**:1345–9.
19. **Takahashi K**, Yoshida N, Murakami N, *et al*. Dynamic regulation of p53 subnuclear localization and senescence by MORC3. *Mol Biol Cell* 2007;**18**:1701–9.
20. **Jain AK**, Barton MC. Regulation of p53: TRIM24 enters the RING. *Cell Cycle* 2009;**8**:3668–74.

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Five Japanese cases of antidesmoglein 1 antibody-positive and antidesmoglein 3 antibody-negative pemphigus with oral lesions

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Summary

Background Oral mucosal lesions develop in pemphigus vulgaris, but not in pemphigus foliaceus. This clinical phenomenon is explained by the 'desmoglein (Dsg) compensation theory'. Dsg3 and Dsg1 are major autoantigens for pemphigus vulgaris and pemphigus foliaceus, respectively. Dsg3 is overexpressed and Dsg1 is weakly expressed on the oral mucosa. Thus, on the oral mucosa, suppression of Dsg3 function by anti-Dsg3 autoantibodies is not compensated by weakly expressed Dsg1 in pemphigus vulgaris, while suppression of Dsg1 function by anti-Dsg1 autoantibodies is perfectly compensated by richly expressed Dsg3 in pemphigus foliaceus.

Objectives We present five Japanese patients with pemphigus who deviate from this theory, i.e. all patients showed oral lesions (three also had cutaneous lesions) and reacted only with Dsg1, but not with Dsg3, by enzyme-linked immunosorbent assay.

Methods To confirm whether the unique clinical phenotypes in our patients were due to a different immunological profile from that in classical pemphigus, we examined the reactivity of the patient sera by immunoprecipitation-immunoblotting analysis using five Dsg1/Dsg2 domain-swapped molecules.

Results The sera of two patients who had only oral lesions tended to react with the extracellular (EC) 5 domain of Dsg1, the domain that is considered nonpathogenic in classical pemphigus foliaceus. Sera of three patients with mucocutaneous lesions reacted with EC1 domain or with both EC1 and EC2 domains of Dsg1, like classical pemphigus foliaceus.

Conclusions These results indicate that antigenic diversity of anti-Dsg1 antibodies in these patients may cause the unique oral mucosal and cutaneous lesions, although further studies are required to elucidate the pathomechanisms.

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Conflicts of interest
None declared.

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Pemphigus foliaceus (PF) is an autoimmune bullous disease, which clinically shows superficial blisters on the skin, but no oral mucosal lesions. PF is characterized immunologically by the presence of IgG autoantibodies reacting with desmoglein (Dsg) 1, a cell adhesion molecule of keratinocytes. In contrast, oral lesions occur frequently in patients with pemphigus vulgaris (PV) who have anti-Dsg3 antibodies. The difference in clinical phenotype between PF and PV is often explained using the 'Dsg compensation theory'.[1–3] According to this theory, anti-Dsg3 antibodies, but not anti-Dsg1 antibodies, cause oral mucosal lesions, as Dsg1 is only weakly expressed and Dsg3 is overexpressed on the oral mucosa.

In this report, we describe five Japanese patients with pemphigus who deviate from this theory. Three patients had both oral mucosal and cutaneous lesions, and two patients had only oral lesions. Enzyme-linked immunosorbent assay (ELISA) showed only anti-Dsg1 antibodies. Novel immunoprecipitation-immunoblotting (IP-IB) methods using Dsg1/Dsg2 domain-swapped molecules demonstrated diverse antigenic sites in our cases.

Materials and methods

Patient background

The patient backgrounds are summarized in Table 1. Of the five patients, two had only oral lesions and three had both oral and cutaneous lesions. Biopsy specimens were taken from oral mucosa in patient 1 and from skin in patients 3 and 4. We examined ELISA indices of Dsg1 and Dsg3 in all patients at regular intervals and at exacerbation of their symptoms using commercially available Mesacup DSG-1/DSG-3 Test (MBL Co. Ltd, Nagoya, Japan) according to the protocol recommended by the supplier. ELISA detected only anti-Dsg1 antibodies, but not anti-Dsg3 antibodies, in all patients throughout our observation period.

Patient 1 was a 70-year-old Japanese woman who had shown erosive lesions on the tongue and buccal mucosa without any cutaneous lesions for 8 years (Fig. 1a). Histopathological examination of a buccal mucosal lesion revealed acantholytic suprabasal clefting (Fig. 1c). Direct immunofluorescence for the biopsied buccal mucosal lesion demonstrated IgG deposits on the cell surfaces of the epithelium (Fig. 1d), whereas no IgA and IgM deposits were seen. Combination therapy of tetracycline and nicotinamide was effective, but some extent of oral lesions continued. Three years after her first visit, she was diagnosed as having breast cancer, for which she underwent surgery. Her oral mucosal symptoms did not improve even after resection of cancer.

Patient 2 was a 54-year-old Japanese man with a 1-year history of erosions in the oral cavity, particularly on the tongue and pharynx, without any skin lesions (Fig. 1b). Oral prednisolone 20 mg daily successfully improved the lesions, and was thereafter tapered off with no relapse. We followed him for 18 months but no cutaneous lesions appeared.

Patient 3 was a 57-year-old Japanese man who had had oral lesions for 5 months and developed cutaneous lesions 10 days before his first visit. Histopathological examininations of a chest lesion showed acantholysis. Direct immunofluorescence demonstrated deposits of IgG and IgA on the cell surfaces of the epidermis. Dsg1 ELISAs for both IgG and IgA antibodies were positive. Dapsone 50 mg daily partly improved his symptoms.

Patient 4 was a 62-year-old Japanese woman with oral and cutaneous lesions. Histopathological examination of a trunk lesion showed a suprabasilar acantholytic blistering. Oral prednisolone controlled skin lesions, while oral mucosal lesions continued. Five years after her first visit, she complained of

Table 1 Summary of the present cases

Patient	Age (years)/ sex	Observation period (months)	Lesions: oral/skin	ELISA indices		Epitope on Dsg1	Histopathology (location of biopsy)	DIF and IIF	Complications	Others
				Dsg1 (cut off < 14)	Dsg3 (cut off < 7)					
1	70/F	60	+/−	46	<5	EC3 EC5 (EC4)	Suprabasal acantholytic cleft (oral mucosa)	DIF: IgG CS+ IIF: −	Breast cancer	
2	54/M	18	+/−	21	<5	EC5	Not performed	DIF: not performed IIF: −		
3	57/M	9	+/+	146	<5	EC1 EC2	Acantholysis on stratum spinosum (skin)	DIF: IgG CS+, IgA CS+ IIF: IgG CS x 160		IgA ELISA: OD for Dsg1 0·693 (cut off < 0·15)
4	62/F	144	+/+	157	<5	EC1 EC2	Acantholysis on stratum spinosum (skin)	DIF: IgG CS+ IIF: IgG CS × 160	Thymoma (AchR Ab+), dermatomyositis	IgG Dsc3 Ab (+)
5	58/F	2	+/+	81	<5	EC1	Not performed	DIF: not performed IIF: IgG CS × 40		

Ab, antibody; AchR, acetylcholine receptor; CS, cell surface; DIF, direct immunofluorescence; Dsg, desmoglein; EC, extracellular; ELISA, enzyme-linked immunosorbent assay; IIF, indirect immunofluorescence; OD, optical density.

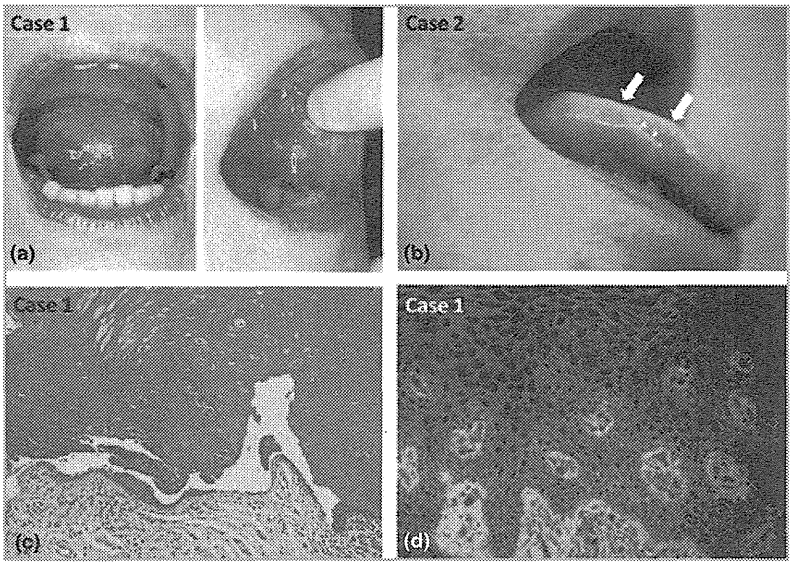

Fig 1. (a, b) Clinical appearance of patient 1 and patient 2. Erosions on the tongue and the buccal mucosa in patient 1 (a) and erosions on the tongue in patient 2 (b, arrows). (c) Histopathological findings for buccal mucosal lesion in patient 1, showing acantholytic suprabasal cleft (haematoxylin and eosin; original magnification × 100). (d) Direct immunofluorescence in patient 1, demonstrating IgG deposits in the cell surfaces of the epithelium.

myalgia with serological elevation of creatinine kinase and aldolase, and was diagnosed as having dermatomyositis. Antinuclear antibodies were positive with no evidence of Jo-1 antibodies. One and a half years later, thymoma was detected and resected. However, the oral mucosal lesions still continued.

Patient 5 was a 58-year-old Japanese woman with erosions in the oral cavity and on the back, suggesting mucocutaneous type PV. However, ELISA detected only anti-Dsg1 antibodies, but not anti-Dsg3 antibodies. She was treated with oral prednisolone 20 mg daily, but her symptoms did not improve.

Production of recombinant proteins

We have previously constructed recombinant swapped molecules containing one each of extracellular (EC) 1–5 domains of Dsg1 on the backbone of Dsg2.[4] Recombinant proteins were produced in baculovirus-infected insect cells.[4–6] The reactivities of patients with nonepidemic PF were 88%, 50%, 13%, 22% and 0% with EC1–5, respectively.[4]

Immunoprecipitation-immunoblotting analysis

IP was carried out by mixing a 20 μL bed volume of Protein G Sepharose 4 Fast Flow (GE Healthcare, Uppsala, Sweden), 500 μL of culture medium containing baculoproteins and 2 μL anti-E tag monoclonal antibody (mAb) (Amersham Bioscience, Piscataway, NJ, U.S.A.) or 15 μL of patient sera. The mixture was incubated at 4°C overnight with rotation, and then washed three times by Tris-buffered saline with 0·5 mmol L^{-1} $CaCl_2$. The immunoprecipitated proteins were then resuspended in sodium dodecyl sulphate (SDS) sample buffer with 5% 2-mercaptoethanol and incubated for 3 min at 96°C. They were fractionated by 10% SDS–polyacrylamide gel electrophoresis, and then transferred to a polyvinylidene difluoride membrane (Invitrogen, Carlsbad, CA, U.S.A.). To visualize the protein, anti-E tag mAb at 1 : 5000 dilution was used as primary antibody, and then alkaline phosphatase-conjugated antimouse IgG antibody (Zymed, San Francisco, CA, U.S.A.) at 1 : 4000 dilution was used as secondary antibody. Precipitated proteins were visualized by using 1-Step NBT/BCIP (Thermo Fisher Scientific, Rockford, IL, U.S.A.). The concentrations of baculoproteins were adjusted to show similar density by a preliminary IP-IB experiment using anti-E tag mAb for immunoprecipitation (Fig. 2, inset).

Desmocollin cDNA transfection study

A cDNA transfection method to detect IgA or IgG antidesmocollin (Dsc) antibodies was performed using eukaryotic expression cDNA clones of human Dsc1–3 and cultured COS-7 cells, as described previously.[7]

Results

We analysed sera from five patients having oral lesions with anti-Dsg1 antibodies and without anti-Dsg3 antibodies. For initial studies of IP-IB and cDNA transfection, we used the sera from the patients on their first visit, except for patient 1, whose serum was taken at the exacerbation of her symptoms, 3 years after her first visit. In IP-IB studies, the sera from three patients (patients 3–5 with both oral mucosal and cutaneous lesions) reacted with EC1 domain or with both EC1 and EC2 domains of Dsg1 (Fig. 2). In contrast, the serum from patient 1 reacted with EC3 and EC5 domains, and slightly with EC4 domain of Dsg1 (Fig. 2). The serum from patient 2 reacted exclusively with EC5 domain of Dsg1 (Fig. 2). We also examined sera at different time points in all five patients throughout our observation period of 2 months to 12 years, when clinical symptoms were milder. However, Dsg1 ELISA indices were still high, and domain profiles of Dsg1 were unchanged in all cases (data not shown).

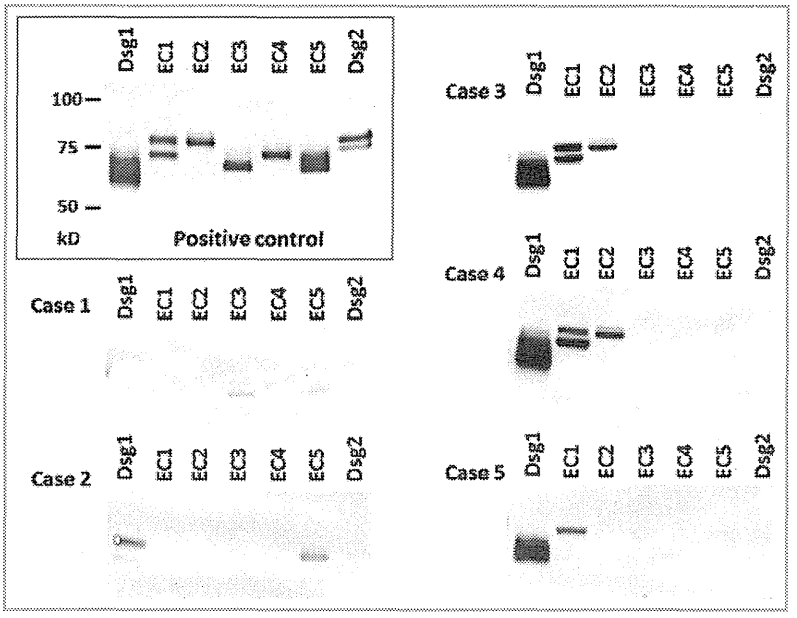

Fig 2. Immunoprecipitation-immunoblotting analysis. Patient 1 sera reacted with the extracellular (EC) 3 and EC5 domains of desmoglein (Dsg) 1 (weakly with EC4 domain), and patient 2 sera reacted exclusively with EC5 domain. Sera from patients 3 and 4 reacted with EC1 and EC2 domains, and patient sera 5 reacted only with EC1 domain. Inset, upper left: preliminary control immunoprecipitation-immunoblotting study using anti-E tag monoclonal antibody for the first immunoprecipitation step, instead of patient sera.

All patients underwent detection of both IgG and IgA antibodies using COS-7 cells transfected with cDNAs of human Dsc1, Dsc2 and Dsc3. The results revealed that IgG antibodies, but not IgA antibodies, in patient 4 reacted only with Dsc3. Other patients showed no anti-Dsc reactivity for either IgG or IgA antibodies. In patient 3 the optical density value of IgA anti-Dsg1 antibodies was elevated in IgA ELISA performed as described previously.[8]

Discussion

In this study, we examined the precise epitope profile of anti-Dsg1 antibodies in five Japanese patients who showed oral lesions, but not anti-Dsg3 antibodies. Patients 1 and 2 showed only oral mucosal lesions, while patients 3–5 had both oral mucosal and cutaneous lesions. Patients 3–5 reacted with EC1 domain and/or EC2 domain of Dsg1, major target domains in classical PF.[4] Strikingly, patients 1 and 2 showed distinct patterns: patient 1 reacted with EC3 and EC5 domains, and patient 2 reacted only with EC5 domain.

Regarding the immune mechanisms for oral lesions in our patients, we suggest the following possibilities: (i) anti-Dsg1 antibodies caused oral mucosal lesions, (ii) patients had circulating anti-Dsg3 antibodies at an undetectable level, (iii) patients had paraneoplastic pemphigus, as patients 1 and 4 had associated internal tumours,[9,10] and (iv) antibodies to non-Dsg molecules targeted the oral mucosal epithelium.

The first possibility may be supported by the fact that detection of anti-Dsg1 antibodies in normal sera is quite rare and cannot be accidental.[11,12] In this context, it is intriguing that patients 1 and 2 showing only oral lesions reacted with EC3–5 domains, that are considered nonpathogenic in patients with ordinary PF. Such unique anti-Dsg1 antibodies may overcome the 'Dsg compensation theory' and produce oral lesions in our patients. The second possibility is unlikely because the sensitivity of Dsg3 ELISA is quite high and false-negatives are rare.[11] In addition, we performed Dsg3 ELISA at several points throughout our observation period of 2 months to 12 years, and the results were always negative.

The third possibility is also unlikely, because the patient sera did not react with plakins in our IB study using normal human epidermal extracts, and indirect immunofluorescence using rat bladder cryosections showed negative results. Moreover, oral lesions in our patients were much milder than those in patients with typical paraneoplastic pemphigus. However, paraneoplastic pemphigus may be a T cell-mediated disease, in which autoantibodies are not detected, and cytotoxic T cells mediate oral lesions.[13] The fourth speculation is possible. Previous studies reported the existence of autoantibodies in pemphigus sera against acetylcholine receptor,[14] mitochondria,[15] plakoglobin,[16] envoplakin, periplakin[17] and E-cadherin.[18] Moreover, anti-Dsc antibody is a possible causative antibody for oral mucosal lesions, because anti-Dsc3 antibodies were reported to cause loss of cell adhesion by *in vivo* and *in vitro* studies,[19,20] and because conditional Dsc3 knockout mice developed blisters on the oral mucosa and on the skin.[21] From these results, oral lesions in patient 4 could be produced by anti-Dsc3 antibodies. In patient 3, IgA anti-Dsg1 antibodies may play some pathogenic role. IgA autoantibodies in linear IgA bullous dermatosis and dermatitis herpetiformis induce inflammatory reaction by activating complement and granulocytes.[22] Histopathological examination in patient 3 actually showed neutrophilic infiltration. Therefore, the first and fourth possibilities might explain the oral lesions in our patients.

Patients 3–5, but not patients 1 and 2, showed skin lesions. The autoantibodies against EC1 and EC2 domains of Dsg1 are known to be pathogenic for skin blister formation in several

experiments.[23,24] On the other hand, no blister formation was induced in mice passively transferred with anti-EC5 domain antibodies, which were prepared from endemic PF sera. In addition, anti-EC5 domain antibodies show no reactivity by indirect immunofluorescence,[23] which is in agreement with negative indirect immunofluorescence results in our patients 1 and 2. These results may confirm the findings in our study that anti-Dsg1 EC1 and EC2 domain antibodies in patients 3–5, but not anti-Dsg1 EC3 and EC5 domain antibodies in patients 1 and 2, were pathogenic in blister formation in the skin.

In conclusion, the results in this study provide us with several interesting questions, which will be answered by further studies in accumulated similar cases.

What's already known about this topic?

- In autoimmune bullous diseases, autoantigens known to play a causative role in oral lesions are laminin 332, type VII collagen, integrin α6, desmoglein (Dsg) 3 and desmocollin (Dsc) 3, but not Dsg1 and Dsc1.

What does this study add?

- We report five atypical pemphigus cases that cannot be explained by the 'Dsg compensation theory'.

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References

1 Amagai M. The molecular logic of pemphigus and impetigo: the desmoglein story. *Vet Dermatol* 2009; **20**:308–12.
2 Udey MC, Stanley JR. Pemphigus – disease of antidesmosomal autoimmunity. *JAMA* 1999; **282**:572–6.
3 Mahoney MG, Wang Z, Rothenberger K *et al.* Explanations for the clinical and microscopic localization of lesions in pemphigus foliaceus and vulgaris. *J Clin Invest* 1999; **103**:167–70.
4 Chan PT, Ohyama B, Nishifuji K *et al.* Immune response towards the amino-terminus of desmoglein 1 prevails across different activity stages in nonendemic pemphigus foliaceus. *Br J Dermatol* 2010; **162**:1242–50.
5 Amagai M, Hashimoto T, Green KJ *et al.* Antigen-specific immunoadsorption of pathogenic autoantibodies in pemphigus foliaceus. *J Invest Dermatol* 1995; **104**:895–901.
6 Tsunoda K, Ota T, Aoki M *et al.* Induction of pemphigus phenotype by a mouse monoclonal antibody against the amino-terminal adhesive interface of desmoglein 3. *J Immunol* 2003; **170**:2170–8.
7 Hashimoto T, Kiyokawa C, Mori O *et al.* Human desmocollin 1 (Dsc1) is an autoantigen for subcorneal pustular dermatosis type of IgA pemphigus. *J Invest Dermatol* 1997; **109**:127–31.
8 Hashimoto T, Komai A, Futei Y *et al.* Detection of IgA autoantibodies to desmogleins by an enzyme-linked immunosorbent assay: the presence of new minor subtypes of IgA pemphigus. *Arch Dermatol* 2001; **137**:735–8.
9 Anhalt GJ, Kim SC, Stanley JR *et al.* Paraneoplastic pemphigus. An autoimmune mucocutaneous disease associated with neoplasia. *N Engl J Med* 1990; **323**:1729–35.
10 Zhu X, Zhang B. Paraneoplastic pemphigus. *J Dermatol* 2007; **34**:503–11.
11 Amagai M, Komai A, Hashimoto T *et al.* Usefulness of enzyme-linked immunosorbent assay recombinant desmogleins 1 and 3 for serodiagnosis of pemphigus. *Br J Dermatol* 1999; **140**:351–7.
12 Ishii K, Amagai M, Hall RP *et al.* Characterization of autoantibodies in pemphigus using antigen-specific enzyme-linked immunosorbent assays with baculovirus-expressed recombinant desmogleins. *J Immunol* 1997; **159**:2010–17.
13 Cummins DL, Mimouni D, Tzu J *et al.* Lichenoid paraneoplastic pemphigus in the absence of detectable antibodies. *J Am Acad Dermatol* 2007; **56**:153–9.
14 Grando SA, Pittelkow MR, Shultz LD *et al.* Pemphigus: an unfolding story. *J Invest Dermatol* 2001; **117**:990–5.
15 Marchenko S, Chemyavsky AI, Arredondo J *et al.* Antimitochondrial autoantibodies in pemphigus vulgaris: a missing link in disease pathophysiology. *J Biol Chem* 2010; **285**:3695–704.
16 Ishii M, Izumi J, Fujiwara H *et al.* Immunoblotting detection of gamma-catenin (plakoglobin) antibody in the serum of a patient with paraneoplastic pemphigus. *Br J Dermatol* 2001; **144**:377–9.
17 Huang Y, Li J, Zhu X. Detection of anti-envoplakin and anti-periplakin autoantibodies by ELISA in patients with paraneoplastic pemphigus. *Arch Dermatol Res* 2009; **301**:703–9.
18 Evangelista F, Dasher DA, Diaz LA *et al.* E-cadherin is an additional immunological target for pemphigus autoantibodies. *J Invest Dermatol* 2008; **128**:1710–18.
19 Mao X, Nagler AR, Farber SA *et al.* Autoimmunity to desmocollin 3 in pemphigus vulgaris. *Am J Pathol* 2010; **177**:2724–30.
20 Rafei D, Muller R, Ishii N *et al.* IgG autoantibodies against desmocollin 3 in pemphigus sera induce loss of keratinocyte adhesion. *Am J Pathol* 2011; **178**:718–23.
21 Chen J, Den Z, Koch PJ. Loss of desmocollin 3 in mice leads to epidermal blistering. *J Cell Sci* 2008; **121**:2844–9.
22 Sitaru C, Zillikens D. Mechanism of blister induction by autoantibodies. *Exp Dermatol* 2005; **14**:861–75.
23 Li N, Aoki V, Hans-Filho G *et al.* The role of intramolecular epitope spreading in the pathogenesis of endemic pemphigus foliaceus (fogo selvagem). *J Exp Med* 2003; **197**:1501–10.
24 Sekiguchi M, Futei Y, Fujii Y *et al.* Dominant autoimmune epitopes recognized by pemphigus antibodies map to the N-terminal adhesive region of desmogleins. *J Immunol* 2001; **167**:5439–48.

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RESEARCH ARTICLE Open Access

Prevalence of collagen VII-specific autoantibodies in patients with autoimmune and inflammatory diseases

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Abstract

Background: Autoimmunity to collagen VII is typically associated with the skin blistering disease epidermolysis bullosa acquisita (EBA), but also occurs occasionally in patients with systemic lupus erythematosus or inflammatory bowel disease. The aim of our present study was to develop an accurate immunoassay for assessing the presence of autoantibodies against collagen VII in large cohorts of patients and healthy donors.

Methods: Based on *in silico* antigenic analysis and previous wetlab epitope mapping data, we designed a chimeric collagen VII construct containing all collagen VII epitopes with higher antigenicity. ELISA was performed with sera from patients with EBA (n = 50), Crohn's disease (CD, n = 50), ulcerative colitis (UC, n = 50), bullous pemphigoid (BP, n = 76), and pemphigus vulgaris (PV, n = 42) and healthy donors (n = 245).

Results: By ELISA, the receiver operating characteristics analysis yielded an area under the curve of 0.98 (95% CI: 0.9638-1.005), allowing to set the cut-off at 0.32 OD at a calculated specificity of 98% and a sensitivity of 94%. Running the optimized test showed that serum IgG autoantibodies from 47 EBA (94%; 95% CI: 87.41%-100%), 2 CD (4%; 95% CI: 0%-9.43%), 8 UC (16%; 95% CI: 5.8%-26%), 2 BP (2.63%; 95% CI: 0%-6.23%), and 4 PV (9.52%; 95% CI: 0%-18.4%) patients as well as from 4 (1.63%; 95% CI: 0%-3.21%) healthy donors reacted with the chimeric protein. Further analysis revealed that in 34%, 37%, 16% and 100% of sera autoantibodies of IgG1, IgG2, IgG3, and IgG4 isotype, respectively, recognized the recombinant autoantigen.

Conclusions: Using a chimeric protein, we developed a new sensitive and specific ELISA to detect collagen specific antibodies. Our results show a low prevalence of collagen VII-specific autoantibodies in inflammatory bowel disease, pemphigus and bullous pemphigoid. Furthermore, we show that the autoimmune response against collagen VII is dominated by IgG4 autoantibodies. The new immunoassay should prove a useful tool for clinical and translational research and should improve the routine diagnosis and disease monitoring in diseases associated with collagen VII-specific autoimmunity.

Background

An immune response against collagen VII is typically associated with epidermolysis bullosa acquisita (EBA) and bullous systemic lupus erythematosus, but may occur in other conditions, including inflammatory bowel disease (IBD) and dystrophic epidermolysis bullosa [1,2]. EBA is an acquired subepidermal blistering disease of the skin and mucous membranes associated with an autoimmune response to collagen VII [3,4]. EBA is characterized by bound and circulating IgG autoantibodies which label the dermal side of split skin by direct and indirect immunofluorescence (IF) microscopy, respectively [5-7]. Accumulating clinical and experimental evidence demonstrates that collagen VII-specific IgG autoantibodies are pathogenic. Transient skin blistering was reported in a newborn from a mother with EBA showing the transplacental

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