Super-resolution imaging detects BP180 autoantigen in IgM pemphigoid

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Running head: IgM pemphigoid

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Abstract

Bullous pemphigoid is generally caused by IgG autoantibodies against hemidesmosomal BP180 and/or BP230. Recently, the concept of IgM pemphigoid has been proposed. A 23-year-old Japanese woman presented with a 4-month history of severely itchy papules showing subepidermal separations with mild neutrophil infiltration. Direct immunofluorescence (DIF) revealed IgM deposits at the dermal-epidermal junction, but neither IgG nor IgA deposits. Indirect immunofluorescence on 1 M NaCl-split skin demonstrated deposits on the epidermal side. The optical density (OD) value of a modified IgM ELISA for full-length BP180, but not for BP180-NC16A, was increased. The patient was diagnosed with IgM pemphigoid and was treated with diphenyl sulfone at 50 mg/day without recurrence. To confirm the precise autoantigen, we tried to obtain super-resolution imaging. The deposition pattern of IgM autoantibodies seemed to be oriented parallel to that of BP180. The detailed images detect that DIF deposits apart from BP180-NC16A staining, but are close to type VII collagen-NC1 staining. This result suggests that the IgM autoantibodies in the patient might target the C-terminus of BP180. IgM pemphigoid is still not a widely accepted concept, and the clinical course remains unknown. We will carefully follow up the patient. Super-resolution images may help to detect precise autoantigens in autoimmune blistering diseases.

Introduction

The diagnoses of autoimmune blistering diseases, including bullous pemphigoid (BP) and pemphigus, are confirmed based on a combination of characteristic clinical findings, histological features, and immunological findings. Immunological tests are classified as direct immune fluorescence (DIF) tests, which detect tissue-bound autoantibodies, and serological tests, such as indirect immunofluorescence (IIF), enzyme-linked immunosorbent assays (ELISAs), and immunoblotting. We previously investigated the tissue-bound immunoglobulin isotypes in 100 cases of BP patients and found that 17% of BP patients demonstrated IgM deposits at the dermal-epidermal junction (DEJ)[1]. However, they showed IgG deposits as well as IgM, and there were no patients with only IgM autoantibodies. This is because IgG autoantibodies are mandatory for diagnosing BP.[2]

Recently, the concept of IgM pemphigoid has been proposed[3]. We present a case showing histological subepidermal blistering associated with IgM deposits at the DEJ.

Case report

A 23-year-old Japanese woman presented with a 4-month history of severely itchy papules on her upper extremities. Despite treatment with topical corticosteroids and topical antibacterial drugs, no improvement was achieved. Clinical examination revealed papules associated with small, round erosions and pustules distributed on the lateral side of her upper limbs (Figure 1a, and b). Histopathological examination showed subepidermal separation with mild neutrophil infiltration (Figure 1c). There are necrotic epidermal keratinocytes in the blister roof. Although anti-nuclear antibody (ANA) was detected as 1:80, neither anti-dsDNA nor anti-Sm antibodies were found. She had no photosensitivity, arthralgia, or aphtha, and the absence of these suggested systemic lupus erythematosus (SLE). DIF revealed linear IgM and faint C3 deposits at the DEJ (Figure 1d),

but neither IgG nor IgA deposits. IIF on 1 M NaCl-split skin (ssIIF) demonstrated IgM deposits on the epidermal side (1:10 dilution, Figure 1e). A chemiluminescent enzyme immunoassay test showed no IgG autoantibodies against BP180-NC16A. For reference, ELISAs against full-length BP180, BP180-NC16A, and type VII collagen (COL7) were performed using HRP-conjugated anti-human IgM antibody as the secondary antibody. The optical density (OD) of full-length BP180, but not BP180-NC16A or COL7, was greater than in healthy subjects (H) and BP patients (serum dilution 1:100, 2nd Ab 1:10,000, Pt OD=0.252, H OD=0.086, BP OD=0.103). Immunoblotting using epidermal and dermal extracts showed no IgM reactivity (data not shown). Based on these results, the patient was diagnosed with IgM pemphigoid. Treatment with oral diphenyl sulfone at 50 mg/day resulted in complete remission, and 6 months have passed without recurrence.

The epidermal-side IgM deposits as shown by ssIIF and the elevated OD as determined by the modified full-length BP180 ELISA suggested IgM autoantibodies against BP180. However, the result was for reference, because the ELISA is not an established one. To confirm the precise autoantigen, we performed super-resolution imaging by using a Nikon N-SIM[4] and super-resolution radial fluctuations (SRRF), which processed pictures taken by Keyence fluorescent microscopy. Briefly, a DIF sample was co-stained with the anti-BP180-NC16A domain (primary Ab: TS39-3[5], secondary Ab: TRITC-conjugated anti-mouse IgG) or the anti-COL7-NC1 domain (primary Ab: vWFA2[6], secondary Ab: TRITC-conjugated anti-rabbit IgG). In N-SIM images, the deposition pattern of IgM autoantibodies (green) seems to be oriented parallel to that of BP180 (red) (Figure 2a). In contrast, COL7 staining (red) shows linear staining at the DEJ and not oriented parallel to IgM deposits (green) (Figure 2b). Three-dimensional images are shown in supplementary files. In addition, SRRF images show BP180-NC16A (red) to be separated from

DIF deposits (green) (Figure 2c). In contrast, COL7-NC1 (red) is close to DIF deposits (green) (Figure 2d). Schematics of double staining in DIF and BP180/COL7 are shown in the inserts of the figures. This result also suggested that the IgM autoantibodies of the patient might have targeted the C-terminus of BP180.

Discussion

The clinical manifestations in the present case were characterized by predominant papules with no obvious blisters. However, the several small, round erosions might suggest blister formation. A histological examination demonstrated subepidermal blisters associated with neutrophil infiltrates. Based on these findings, we initially considered several differential diagnoses, such as dermatitis herpetiformis (DH), bullous SLE (BSLE), linear IgA bullous dermatosis (LAD), and epidermolysis bullosa acquisita (EBA) (Table 1)**.** Of these, DH and LAD were excluded due to the absence of IgA deposits in DIF[2]. The DIF in DH is characterized by granular IgA deposits within the dermal papillae or at the DEJ[7]. In addition, IgA tissue transglutaminase (tTG) and IgA epidermal transglutaminase (eTG) antibodies are often elevated in DH. This patient showed no elevated tTG or eTG IgA by ELISA (data not shown). BSLE may demonstrate IgM deposits at the DEJ as in our case, in the so-called lupus band test. However, the autoantigen for BSLE is the same as that for EBA, i.e., COL7[8]. Therefore, BSLE and EBA show dermal-side deposits of autoantibodies by ssIIF[8]. The present patient showed epidermal-side deposits by ssIIF, but BSLE and EBA were ruled out. The final diagnosis of IgM pemphigoid was made.

Several IgM-mediated pemphigoid diseases have been reported, such as IgM pemphigoid, IgM EBA, and IgM mucous membrane pemphigoid[3,9,10]. However, significance of IgM-mediated pemphigoid diseases remains disputed. Recently, three cases of IgM pemphigoid were reported[3],

but the clinical features differed in histology and treatment from our case. The clinical manifestations in reported cases differed slightly from those of conventional BP. They showed no obvious tense blisters, but demonstrated erythema and erythematous papules associated with itch. Even histological examination did not reveal subepidermal separation in the previous three cases. The dermal infiltrates with lymphocytes and eosinophils were major findings. In our case, we observed severe edematous changes accompanied by necrotic keratinocytes. These findings differ from those of our case. However, they clearly demonstrate deposits of IgM, but not of IgG or IgA, at the DEJ by DIF. Serum titer levels determined by ssIIF were not so high, but they successfully identified the autoantigens by immunoblotting using recombinant BP180 protein. Immunoblotting revealed specific IgM autoantibodies that reacted against the NC16A domain in 1 out of 3 cases and against the ectodomain in 3 out of 3 cases. In our patient, immunoblotting using epidermal extracts did not detect autoantigens. Instead of immunoblotting, we tried an ELISA using anti-human IgM antibody as the secondary antibody. The OD value was higher in the full-length BP180 ELISA than in the healthy control or in conventional BP patients. The COL7 and BP180-NC16A ELISAs found no elevated OD values. This result suggests that our patient had IgM autoantibodies to BP180, especially to the ectodomain but not to the NC16A domain. Interestingly, Boch et al. tried to demonstrate the pathogenicity of IgM autoantibodies and found that patient sera induced the internalization of BP180, which suggests a pathogenic antibody[3]. In our case, we were unable to obtain enough serum to investigate the pathogenicity.

To identity the autoantigen, we performed the double staining of DIF and BP180/COL7 and made observations by super-resolution imaging. It is known that high-magnification DIF may be able to distinguish the pattern of BP180 and COL7. BP180 and COL7 are observed in a u-serrated and an n-serrated pattern, respectively[11]. In our case, it was hard to identify a u- or n-serrated pattern.

There are several methods to get super-resolution imaging, such as N-SIM and SRRF[12,13]. The resolutions of N-SIM and SRRF are approximately 200 nm and 70 nm, respectively. At the basement membrane zone, the length of BP180 is more than 200 nm, and the NC16A domain is only 100 nm from the C-terminus[14]. In addition, hemidesmosomes and anchoring fibrils are approximately 100 nm apart[15]. This means that BP180 and COL7 could be distinguished using super-resolution imaging. Figure 2 (a, b) and reconstructed 3D images (supplementary file) suggest that the DIF staining pattern of IgM is oriented parallel to that of BP180 compared to COL7. Furthermore, SRRF images (Figure 2c, d) suggest that IgM autoantibodies might target the C-terminus of BP180. These results indicate that super-resolution imaging may help to detect the precise autoantigens in autoimmune blistering diseases.

In conclusion, the data suggest a case of IgM pemphigoid, a rare autoimmune subepidermal blistering disease. IgM pemphigoid is not still widely accepted, and the clinical course remains unknown. We will pay attention to recurrence, including immunoglobulin class switching, and will follow up the patient.

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Figure legends

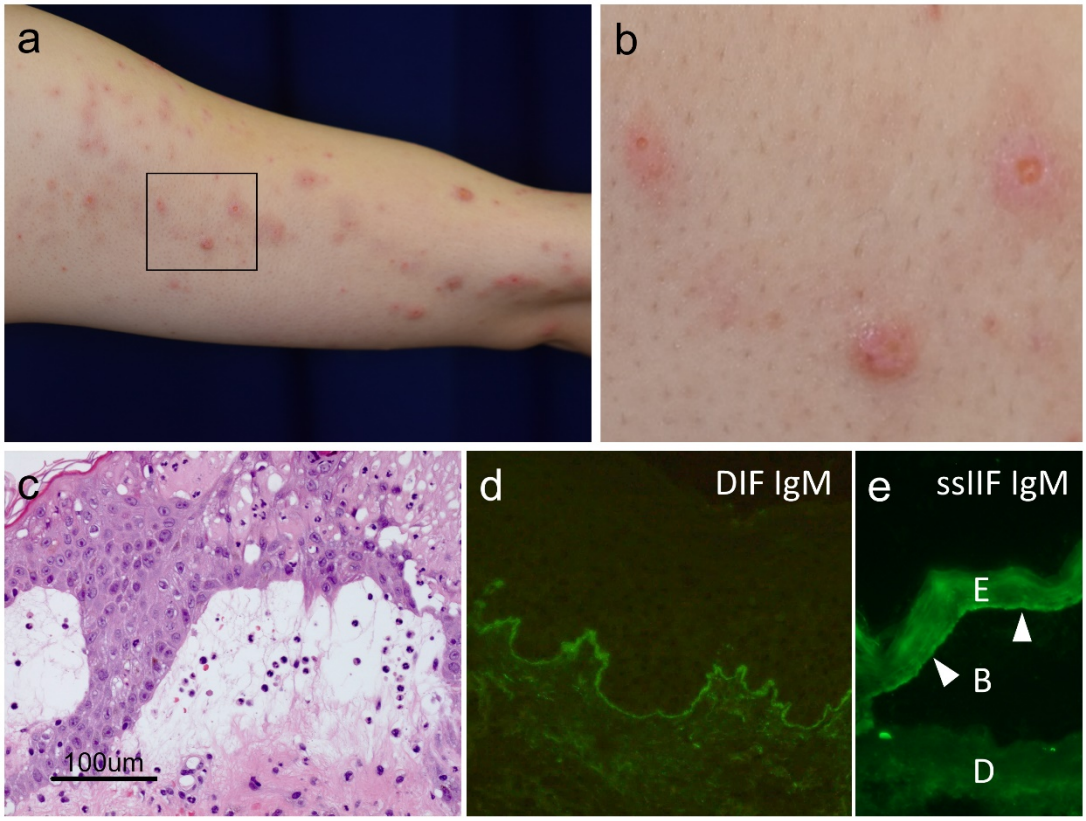

Figure 1 Clinical, histological, and immunological findings

a) Severe itchy eruptions on the lateral side of the upper limbs. b) Small, round erosions at the center of papules (close view of the square from a). c) Subepidermal separations with necrotic keratinocytes accompanied by neutrophil and lymphocyte infiltrates. d) IgM deposits at the DEJ observed by DIF. e) IgM deposits at the epidermal side in 1M NaCl-split skin IIF (ssIIF). E: epidermis, B: blister, D: dermis.

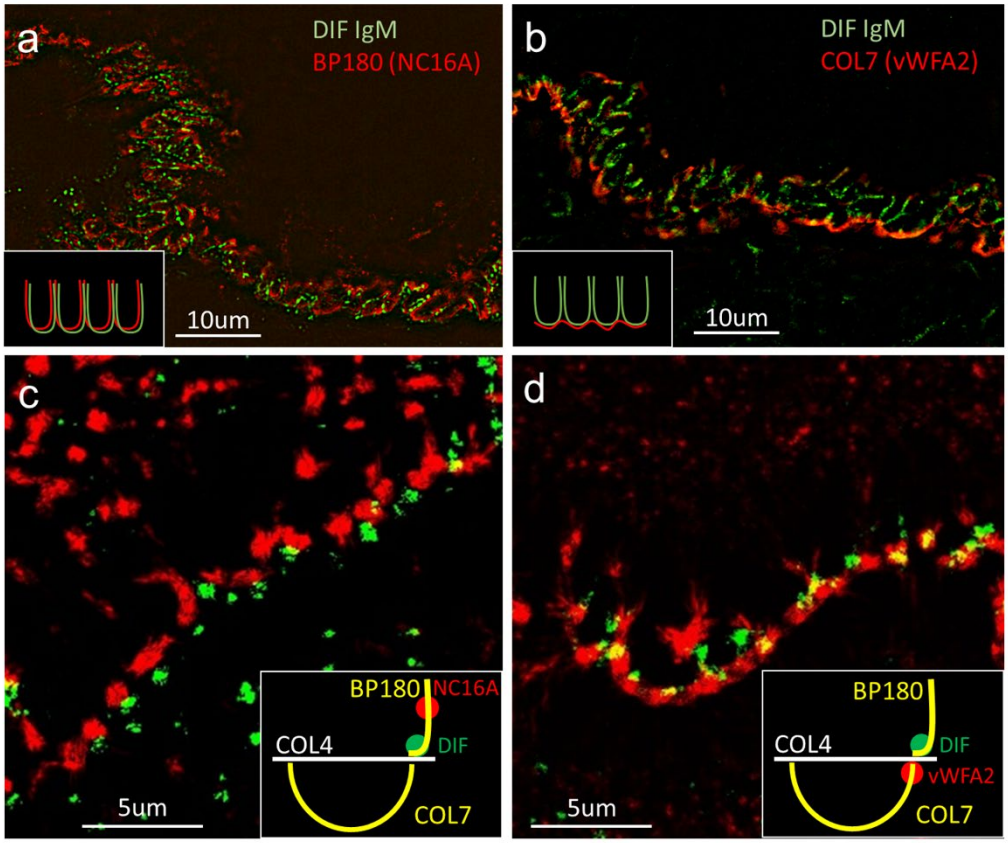

Figure 2 Super-resolution imaging

N-SIM images (a, b) and SRRF images (c, d). Double staining of DIF IgM (green), the BP180-NC16A domain (TS39-3, red, a and c) and the COL7-NC1 domain (vWFA2, red, b and d). The inset images are schematics of double staining of DIF and BP180/COL7. COL4: collagen IV

	IgM pemphigoid	DH	LAD	BSLE	EBA
Autoantigen	BP180	tTG, eTG	LAD-1, LABD97	COL7	COL7
Histology	subepidermal blister (may be absent), lymphatic or eosinophil infiltration	subepidermal blister, neutrophil infiltration, microabscess	subepidermal blister, neutrophil infiltration	subepidermal blister, neutrophil infiltration	subepidermal blister, neutrophil infiltration
DIF (isotype, staining pattern)	IgM linear along the DEJ	IgA granular within the dermal papillae or along the DEJ	IgA linear along the DEJ	IgM, IgG along the DEJ	IgG linear along the DEJ
ssIIF	epidermal side	(-)	epidermal side	dermal side	dermal side

Table 1: Differential diagnosis of IgM pemphigoid

DH: dermatitis herpetiformis, LAD: linear IgA bullous dermatoses, BSLE: bullous systemic lupus erythematosus, EBA: epidermolysis bullosa acquisita, eTG: epidermal transglutaminase, tTG: tissue transglutaminase, COL7: type VII collagen, DEJ: dermal-epidermal junction, ssIIF: indirect immunofluorescence on 1M salt-split skin