A case of antilaminin 332 mucous membrane pemphigoid showing a blister on the bulbar conjunctiva and a unique epitope on the α3 subunit

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MADAM, A 57-year-old Japanese woman developed severe erosive lesions on the oral mucosae including buccal mucosae, gingivae, hard palate and soft palate, as well as bleeding and crust formation on the nasal mucosae in August 2007. She also showed bloody blisters and crusted erosions 2–4 cm in size scattered on the trunk and extremities. Oral minocycline 200 mg daily was given. However, the erosive lesions on the oral and nasal mucosae continued to develop and a clear blister 5 × 8 mm in size appeared on the left bulbar conjunctiva (Fig. 1). Therefore, minocycline was replaced by oral prednisolone 40 mg daily, but the mucosal and skin lesions still continued. Then, a combination therapy of oral prednisolone 40 mg daily and dapsone 75 mg daily was initiated. The oral and nasal mucosal lesions healed without any scarring. The blister on the bulbar conjunctiva disappeared without any scarring or sight disturbance. The skin lesions also healed leaving slight scarring. Then, the dose of prednisolone was tapered gradually without lowering the dose of dapsone, but no mucosal or skin lesions recurred. In March 2008, the patient was free from any mucosal or skin lesions on a combination therapy of prednisolone 5 mg daily and dapsone 75 mg daily.

Histopathology of a skin biopsy specimen from the back showed a subepidermal bulla with massive neutrophil infiltration and scattered eosinophils. Direct immunofluorescence did not show positive results, probably because of damage to the basement membrane zone of the biopsy specimen. Indirect immunofluorescence using normal human skin detected IgG, but not IgA, antibasement membrane zone antibodies at a titre of 1 : 40, which reacted exclusively with the dermal side of 1 mol L^{-1} NaCl-split skin. Immunoblot analysis using purified human laminin 332 as a substrate was performed as described previously.[1,2] In this study, IgG antibodies of a representative control patient with antilaminin 332 mucous membrane pemphigoid (MMP) reacted with both the 165-kDa form and the 145-kDa form of the α3 subunit, the 140-kDa β3 subunit and the 105-kDa γ2 subunit of laminin 332 (Fig. 2, lane 1). IgG antibodies of the present case reacted clearly and exclusively with the 165-kDa form of the α3 subunit (Fig. 2, lane 2). From these results, the diagnosis of antilaminin 332 MMP was confirmed in this patient.

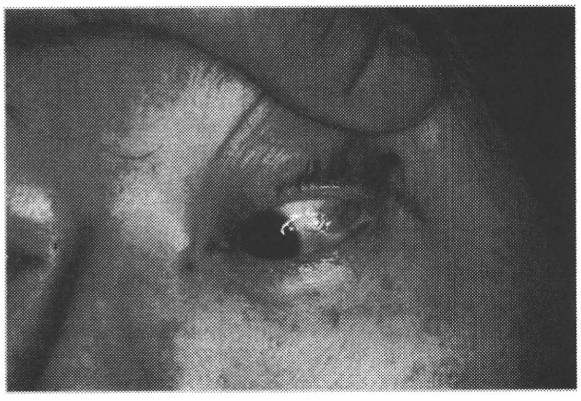

Fig 1. Clinical features of the ocular mucosa. A clear blister was seen on the bulbar conjunctiva of the left eye.

Cicatricial pemphigoid shows blisters and erosive lesions mainly on the mucous membranes, such as the oral, ocular, nasal, laryngeal, pharyngeal and genital mucosae, and skin lesions appear occasionally. These lesions heal with scar formation. However, because this subset of autoimmune bullous disease mainly shows mucosal lesions, and oral mucosal lesions usually heal without scarring, the term 'MMP' is now commonly used, following a consensus meeting.[3] The members of the consensus meeting agreed that dapsone or tetracycline (or minocycline) may be effective when the lesions are localized to the oral mucosae. However, stronger therapies have to be selected when progressive lesions are seen on the ocular or laryngeal mucosae, which may lead to blindness or dyspnoea, respectively.

MMP is highly heterogeneous, but there are at least three major subtypes: anti-BP180 MMP, antilaminin 332 MMP and ocular MMP. About 80% of cases of MMP are anti-BP180

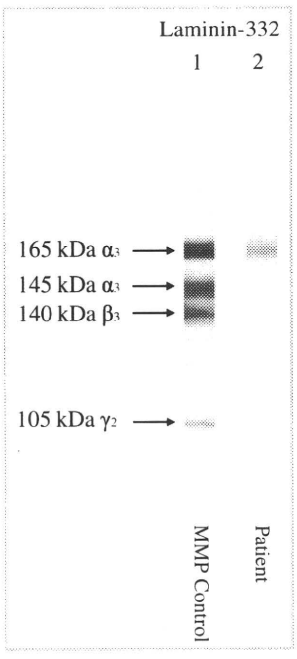

Fig 2. The result of immunoblot analysis using purified human laminin 332. A control antilaminin 332 mucus membrane pemphigoid (MMP) serum reacted with all the subunits of laminin 332, including the 165-kDa and 145-kDa forms of the α3 subunit (lane 1). The serum of the present case reacted clearly and exclusively with the 165-kDa form of the α3 subunit (lane 2). The position of each subunit is shown on the left.

MMP, which shows IgG and IgA antibodies to the carboxyl-terminus of BP180.[4] A further 10–20% of cases of MMP are antilaminin 332 MMP, which shows IgG antibodies to laminin 332 (previously called epiligrin or laminin 5).[5,6] Ocular MMP shows exclusive ocular mucosal lesions, although the autoantigen for this group has not been clearly identified.

Although ocular lesions are commonly seen in MMP, they are usually hyperaemia or erosions which result in symblepharon or epithelialization over the cornea. A clear blister on the bulbar conjunctiva is rarely seen. In our case, a clear solitary blister appeared on the bulbar conjunctiva of the left eye without apparent hyperaemia or erosion. This blister quickly disappeared without any scar formation after the treatment of prednisolone and dapsone was initiated. In addition, a unique clinical feature of our case was the excellent effectiveness of dapsone on all the mucosal and skin lesions.

Another interesting result for this case was the unique reactivity in immunoblot analysis using purified laminin 332. We have shown that the IgG antibodies in patients with antilaminin 332 MMP react with the three subunits of laminin 332, i.e. the α3 subunit, β3 subunit and γ2 subunit, in various patterns.[2] In particular, most patient sera react with both the 165-kDa and 145-kDa forms of the processed α3 subunit, but not with the 200-kDa unprocessed α3 subunit. In our preparation of laminin 332, the 200-kDa unprocessed α3 subunit is not present. The 145-kDa protein is considered to be a degradation product from the 165-kDa processed α3 subunit, although it is not known where the digested 20-kDa fragment resides in the 165-kDa processed α3 subunit. In the previous study,[2] most patient sera reacted with both the 165-kDa and 145-kDa proteins, indicating that the epitopes for these sera are present on the domain common for both proteins. In contrast, the serum of the present case reacted only with the 165-kDa protein, but not with the 145-kDa protein, indicating that the epitope for this serum is present on the 20-kDa fragment which is digested by some protease. Therefore, it is worthwhile identifying the position of the 20-kDa fragment within the 165-kDa form of the processed α3 subunit. From the results of our previous reports,[1,7] the 20-kDa fragment is assumed to correspond to the domain IIIa region of the 165-kDa form of the processed α3 subunit. A study using recombinant proteins of the α3 subunit is now going on to confirm this speculation.

Department of Dermatology, Kurume University School of Medicine, 67 Asahimachi, Kurume, Fukuoka 830-0011, Japan
**Section of Dermatology, Fukuokaken Saiseikai Futsukaichi Hospital, Fukuoka, Japan*
†Department of Dermatology, Fukuoka University School of Medicine, Fukuoka, Japan
‡Shiseido Research Center, Yokohama, Japan
§Tokyo University of Agriculture and Technology, Tokyo, Japan
E-mail: hashimot@med.kurume-u.ac.jp

T. HASHIMOTO
T. DAINICHI
B. OHYAMA
T. HAMADA
N. ISHII
N. SATO*
O. TANIGAWA†
J. NAKAYAMA†
S. AMANO‡
T. NISHIYAMA§
T. KARASHIMA
T. NAKAMA
S. YASUMOTO

References

1 Amano S, Scott IC, Takahara K *et al.* Bone morphogenetic protein 1 is an extracellular processing enzyme of the laminin 5 gamma 2 chain. *J Biol Chem* 2000; **275**:22728–35.
2 Hisamatsu Y, Nishiyama T, Amano S *et al.* Usefulness of immunoblotting using purified laminin 5 in the diagnosis of anti-laminin 5 cicatricial pemphigoid. *J Dermatol Sci* 2003; **33**:113–19.
3 Chan LS, Ahmed R, Anhalt GJ *et al.* The first international consensus on mucous membrane pemphigoid: definition, diagnostic criteria, pathogenic factors, medical treatment, and prognostic factors. *Arch Dermatol* 2002; **138**:370–9.
4 Nie Z, Hashimoto T. IgA antibodies of cicatricial pemphigoid sera specifically react with C-terminus of BP180. *J Invest Dermatol* 1999; **112**:254–5.
5 Lazarova Z, Salato VK, Lanschuetzer CM *et al.* IgG anti-laminin-332 autoantibodies are present in a subset of patients with mucous membrane, but not bullous, pemphigoid. *J Am Acad Dermatol* 2008; **58**:951–8.
6 Hashimoto T, Murakami H, Senboshi Y *et al.* Antiepiligrin cicatricial pemphigoid: the first case report from Japan. *J Am Acad Dermatol* 1996; **34**:940–2.
7 Ogura Y, Matsunaga Y, Nishiyama T, Amano S. Plasmin induces degradation and dysfunction of laminin 332 (laminin 5) and impaired assembly of basement membrane at the dermal–epidermal junction. *Br J Dermatol* 2008; **159**:49–60.

Key words: *bulbar conjunctiva, epitope, laminin 332, mucous membrane pemphigoid*

Conflicts of interest: none declared.

Fine mapping of the human *AR/EDA2R* locus in androgenetic alopecia

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MADAM, Male-pattern baldness (androgenetic alopecia, AGA) is the most common form of hair loss among humans and affects up to 80% of men by the age of 80 years.[1] Hamilton was the first to describe the two essential aetiological factors underlying the development of AGA: genetic predisposition and hormone dependency.[1] Several studies[2–8] have reported that the X-chromosomal locus containing the genes for the androgen receptor (*AR*) and the ectodysplasin A2 receptor (*EDA2R*) is the major genetic susceptibility locus for AGA. However, the association signals in these studies have been inconsistent, and the causative variant or gene has not yet been unequivocally identified. In the present study, we aimed to resolve these inconsistent data through systematic fine mapping of the *AR/EDA2R* locus in the largest sample of patients with AGA investigated to date.

We defined the associated *AR/EDA2R* locus as the region in which single-nucleotide polymorphisms (SNPs) had P-values of < 0·01, on the basis of the findings of our previous genome-wide association study (GWAS).[7] To maximize the efficiency of the study, we used the tagger algorithm of the Haploview software (http://www.broadinstitute.org/mpg/

The findings reported by Jones *et al.* are of importance when considering the optimal therapeutic strategy to initiate in a given patient with CTCL. Because methylation of the Fas promoter is not observed in all patients with SzS, the use of hypomethylating drugs may not be equally effective in restoring sensitivity to Fas-mediated apoptosis in all patients. Thus, not only is the existence of potent hypomethylating agents important, but their use will require a "personalized" medical approach in which these agents are employed in patients having tumors with positional methylation of the Fas CpG island.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Batty N, Malouf GG, Issa JP (2009) Histone deacetylase inhibitors as anti-neoplastic agents. *Cancer Lett* 280:192–200

Bird AP (1986) CpG-rich islands and the function of DNA methylation. *Nature* 321:209–13

Braun FK, Fecker LF, Schwarz C *et al.* (2007) Blockade of death receptor-mediated pathways early in the signaling cascade coincides with distinct apoptosis resistance in cutaneous T-cell lymphoma cells. *J Invest Dermatol* 127:2425–37

Bullani RR, Wehrli P, Viard-Leveugle I *et al.* (2002) Frequent downregulation of Fas (CD95) expression and function in melanoma. *Melanoma Res* 12:263–70

Contassot E, Kerl K, Roques S *et al.* (2008) Resistance to FasL and tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis in Sezary syndrome T-cells associated with impaired death receptor and FLICE-inhibitory protein expression. *Blood* 111:4780–7

Costello JF, Fruhwald MC, Smiraglia DJ *et al.* (2000) Aberrant CpG-island methylation has non-random and tumour-type-specific patterns. *Nat Genet* 24:132–8

Dereure O, Portales P, Clot J *et al.* (2000) Decreased expression of Fas (APO-1/CD95) on peripheral blood CD4+ T lymphocytes in cutaneous T-cell lymphomas. *Br J Dermatol* 143:1205–10

Dereure O, Levi E, Vonderheid EC *et al.* (2002) Infrequent Fas mutations but no Bax or p53 mutations in early mycosis fungoides: a possible mechanism for the accumulation of malignant T lymphocytes in the skin. *J Invest Dermatol* 118:949–56

Esteller M (2007) Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 8:286–98

Fenaux P, Raza A, Mufti GJ *et al.* (2007) A multicenter phase 2 study of the farnesyltransferase inhibitor tipifarnib in intermediate- to high-risk myelodysplastic syndrome. *Blood* 109:4158–63

Gardiner-Garden M, Frommer M (1987) CpG islands in vertebrate genomes. *J Mol Biol* 196:261–82

Gazin C, Wajapeyee N, Gobeil S *et al.* (2007) An elaborate pathway required for Ras-mediated epigenetic silencing. *Nature* 449:1073–7

Holliday R, Grigg GW (1993) DNA methylation and mutation. *Mutat Res* 285:61–7

Jones CL, Wain EM, Chu C-C *et al.* (2010) Downregulation of Fas gene expression in Sézary syndrome is associated with promoter hypermethylation. *J Invest Dermatol* 130:1116–25

Nagasawa T, Takakuwa T, Takayama H *et al.* (2004) Fas gene mutations in mycosis fungoides: analysis of laser capture-microdissected specimens from cutaneous lesions. *Oncology* 67:130–4

Olsen EA, Kim YH, Kuzel TM *et al.* (2007) Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol* 25:3109–15

Petak I, Danam RP, Tillman DM *et al.* (2003) Hypermethylation of the gene promoter and enhancer region can regulate Fas expression and sensitivity in colon carcinoma. *Cell Death Differ* 10:211–7

Piekarz RL, Frye R, Turner M *et al.* (2009) Phase II multi-institutional trial of the histone deacetylase inhibitor romidepsin as monotherapy for patients with cutaneous t-cell lymphoma. *J Clin Oncol* 27:5410–7

Rieux-Laucat F, Le Deist F, Hivroz C *et al.* (1995) Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* 268:1347–9

Scarisbrick JJ, Woolford AJ, Calonje E *et al.* (2002) Frequent abnormalities of the p15 and p16 genes in mycosis fungoides and Sezary syndrome. *J Invest Dermatol* 118:493–9

van Doorn R, Dijkman R, Vermeer MH *et al.* (2002) A novel splice variant of the Fas gene in patients with cutaneous T-cell lymphoma. *Cancer Res* 62:5389–92

Wu J, Nihal M, Siddiqui J *et al.* (2009) Low FAS/CD95 expression by CTCL correlates with reduced sensitivity to apoptosis that can be restored by FAS upregulation. *J Invest Dermatol* 129:1165–73

Zhang CL, Kamarashev J, Qin JZ *et al.* (2003) Expression of apoptosis regulators in cutaneous T-cell lymphoma (CTCL) cells. *J Pathol* 200:249–54

Zoi-Toli O, Vermeer MH, De Vries E *et al.* (2000) Expression of Fas and Fas-ligand in primary cutaneous T-cell lymphoma (CTCL): association between lack of Fas expression and aggressive types of CTCL. *Br J Dermatol* 143:313–9

See related article on pg 1040

How Does Intramolecular Epitope Spreading Occur in BPAG2 (BP180)?

Takashi Hashimoto[1], Takahiro Hamada[1], Teruki Dainichi[1], Norito Ishii[1], Tadashi Karashima[1], Takekuni Nakama[1] and Shinichiro Yasumoto[1]

Several studies have suggested that autoantibodies directed against multiple epitopes occur via epitope spreading in autoimmune bullous skin diseases. However, the precise sequence of events in epitope spreading has not been elucidated for any of the epidermal autoantigens. In this issue, using a transgenic mouse model, Di Zenzo *et al.* report that intramolecular epitope spreading does occur for human BPAG2.

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In order to investigate the mechanism of epitope spreading for BPAG2 (BP180 or type XVII collagen), Di Zenzo *et al.* (2010, this issue) performed a sophisticated set of experiments using transgenic mice harboring human BPAG2. To immunize mice with human BPAG2, skin samples from transgenic mice that expressed human BPAG2 were grafted onto syngeneic mice. Sequential serum samples were then obtained from the immunized mice, and antibodies against human BPAG2 were detected by an enzyme-linked immunosorbent assay using recombinant proteins for four intracellular domains (ICDs) and three extracellular domains (ECDs) of human BPAG2. Most grafted mice

[1]*Department of Dermatology, Kurume University School of Medicine, Fukuoka, Japan*

Correspondence: Takashi Hashimoto, Department of Dermatology, Kurume University School of Medicine, 67 Asahimachi, Kurume, Fukuoka 830-0011, Japan. E-mail: hashmot@med.kurume-u.ac.jp

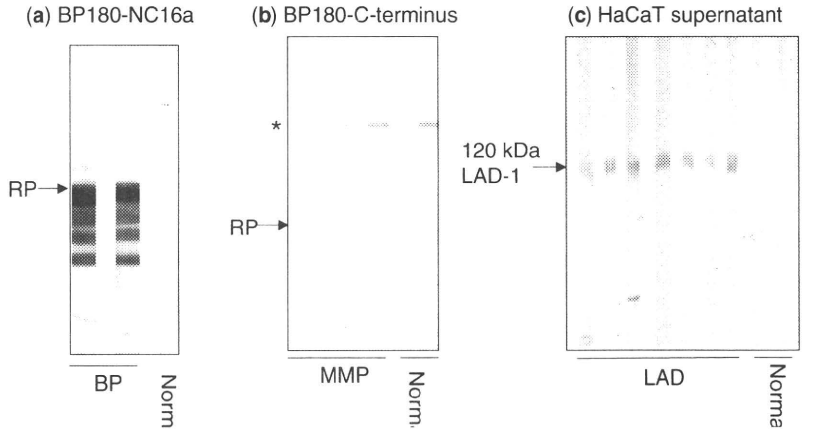

Figure 1. Representative results of immunoblot analyses for BPAG2 (BP180) using three different antigen sources. (**a**) Bacterial recombinant protein of the NC16a domain of BPAG2. IgG antibodies in bullous pemphigoid (BP) sera reacted with this recombinant protein (RP). (**b**) Bacterial recombinant protein of C-terminal domain of BPAG2. IgG antibodies in anti-BP180-type mucous membrane pemphigoid (MMP) sera reacted with this RP. The upper protein band marked with an asterisk indicates nonspecific reactivity because it is also shown by normal controls. (**c**) Concentrated supernatant sample from cultured HaCaT cells. IgA antibodies in lamina lucida type of linear IgA bullous dermatosis (LAD) sera reacted with the 120-kDa LAD-1 antigen.

initially developed anti-BPAG2 antibodies directed against ECD epitopes. Subsequently, some of the mice developed antibodies to additional ECD epitopes and to ICD epitopes. In general, the titers of antibodies against the ECD epitopes were high, whereas antibodies against the ICD epitopes were low, and they were detectable for shorter periods of time. An interesting observation was that the development of antibodies against ICD epitopes correlated with graft loss, but rejection occurred by an unknown mechanism. Thus, Di Zenzo *et al.* confirmed successfully and directly that epitope spreading does occur in this animal model of an autoimmune bullous skin disease.

Multiple epitopes occur in the autoantigens that characterize autoimmune bullous diseases

Epitope spreading has been shown to occur in several autoimmune bullous skin diseases (Chan *et al.*, 1998). The multiple epitopes on desmoglein 1 (Dsg1) or Dsg3 are targets of antibodies found in sera from patients with pemphigus vulgaris, pemphigus foliaceus, and paraneoplastic pemphigus as determined by ELISA assays using domain-swapped molecules between human Dsg1 and Dsg3 (Futei *et al.*, 2003). Recently, these results were confirmed in an ELISA assay using newly elaborated domain-swapped molecules of human Dsg1 and Dsg3 against the human Dsg2 backbone (Chan *et al.*, in press; B Ohyama *et al.*, personal communication). Previously, we demonstrated that paraneoplastic pemphigus sera had autoantibodies against multiple epitopes in human envoplakin and periplakin, two major autoantigens found in paraneoplastic pemphigus, as demonstrated by an ELISA assay, using bacterial recombinant proteins from various domains of envoplakin and periplakin (Nagata *et al.*, 2001). We showed that bullous pemphigoid sera had autoantibodies against multiple epitopes in the various domains of human BPAG1 (BP230), particularly to the C-terminal globular domain, by immunoblot analysis using bacterial recombinant proteins of various domains of human BPAG1 (Hamada *et al.*, 2001). We also showed that anti-basement membrane zone antibodies in the sera of patients with epidermolysis bullosa acquisita reacted with distinct epitopes in the NC1 domain, the central collagenous domain, and the NC2 domain by immunoblot analysis using bacterial recombinant proteins from selected domains of human type VII collagen and immunoelectron microscopy (Ishii *et al.*, 2004).

These studies strongly suggest that intramolecular epitope spreading occurs in several autoimmune bullous skin diseases. Although the previous studies detected autoantibodies against multiple epitopes, no study detailed the sequential development of autoantibodies to different epitopes over time. Di Zenzo *et al.* (2010) have now shown that epitope spreading actually takes place. They found that mice immunized against human BPAG2 occasionally developed autoantibodies against some epitopes present in the ICD of BPAG2, although such autoantibodies appeared late and were less persistent. Interestingly, we found that pemphigus patients sometimes have autoantibodies that react against the ICD of Dsg1 and Dsg3 (Ohata *et al.*, 2001). Thus, the study by Di Zenzo *et al.* also confirms that antibodies against the ICD of transmembranous antigens can occur in autoimmune bullous skin diseases, probably also by epitope-spreading mechanisms. The mechanism by which such antibodies develop remains unknown.

BPAG2 is the most suitable autoantigen for studying mechanisms of epitope spreading

Autoantibodies to distinct epitopes within BPAG2 develop in a variety of autoimmune subepidermal bullous skin diseases. First, anti-basement membrane zone autoantibodies in both bullous pemphigoid and herpes gestationis were reported to preferentially react with the NC16a domain of BPAG2 (Matsumura *et al.*, 1996). In addition, IgG and IgA antibodies in anti-BP180 type mucous membrane pemphigoid have been shown to react with the C-terminal domain of BPAG2 (Nie and Hashimoto, 1999). Furthermore, we showed that IgA antibodies in lamina lucida–type linear IgA bullous dermatosis reacted with epitope(s) within the fifteenth collagenous domain of BPAG2, which is hidden in the intact 180-kDa BPAG2 molecule (Nie *et al.*, 2000). For these reasons, BPAG2 is considered the most suitable antigen with which to elucidate mechanisms of epitope spreading in autoimmune bullous skin diseases. Figure 1 shows representative immunoblot analyses for three antigen sources used routinely in our laboratory.

Clinical Implications

- Epitope spreading is the sequential development of new antibodies against seemingly less accessible regions of target proteins in autoimmunity.
- The identification of mechanisms of epitope spreading in the immunobullous diseases may lead to novel therapies that limit the process of spreading.
- Because of accessibility, the analysis of epitope spreading in skin disease may provide insight into pathogenic mechanisms in systemic autoimmune diseases and transplantation immunity.

Perspectives

Although Di Zenzo *et al.* (2010) demonstrated convincingly that intramolecular epitope spreading occurs in BPAG2, many questions remain. The first is why patients with bullous pemphigoid preferentially develop IgG autoantibodies to epitopes on the NC16a domain of BPAG2. Second, why do autoantibodies in bullous pemphigoid react with epitopes in the NC16a domain of BPAG2, whereas autoantibodies in anti-BP180–type mucous membrane pemphigoid react with epitopes in the C-terminal domain? More important, how do the antibodies directed against these distinct domains of BPAG2 result in different clinical features (i.e., large, tense skin blisters in bullous pemphigoid and predominant erosive mucosal lesions in anti-BP180-type mucous membrane pemphigoid)? Why do IgA antibodies in lamina lucida–type linear IgA bullous dermatosis react with specific epitopes in 120- and 97-kDa linear IgA bullous dermatosis (LAD)-1 antigens produced from 180-kDa intact BPAG2 by proteolytic processing (Nie *et al.*, 2000)? Future studies should unravel the mechanisms by which the hidden epitope in intact 180-kDa molecule (intact BPAG2) is exposed in linear IgA bullous dermatosis to autoantibodies against the 120- and 97-kDa LAD-1 antigens.

Finally, and perhaps most important, we do not know yet why the development of antibodies against ICD epitopes in human BPAG2 correlated with skin-graft loss. The relevance of this phenomenon to autoimmune bullous diseases remains to be determined.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Chan LS, Vanderlugt CJ, Cooper KD *et al.* (1998) Epitope spreading: lessons from autoimmune skin diseases. *J Invest Dermatol* 110:103–9

Chan PT, Ohyama B, Nishifuji K *et al.* Immune response towards amino-terminus of desmoglein 1 prevails across different activity stages in nonendemic pemphigus foliaceus. *Br J Dermatol* (in press)

Di Zenzo G, Calabresi V, Olasz EB *et al.* (2010) Sequential intramolecular epitope spreading of humoral responses to human BPAG2 in a transgenic model. *J Invest Dermatol* 130:1040–7

Futei Y, Amagai M, Hashimoto T *et al.* (2003) Conformational epitope mapping and IgG subclass distribution of desmoglein 3 in paraneoplastic pemphigus. *J Am Acad Dermatol* 49:1023–8

Hamada T, Nagata Y, Tomita M *et al.* (2001) Bullous pemphigoid sera react specifically with various domains of BP230, most frequently with C-terminal domain, by immunoblot analyses using bacterial recombinant proteins covering the entire molecule. *Exp Dermatol* 10:256–63

Ishii N, Yoshida M, Hisamatsu Y *et al.* (2004) Epidermolysis bullosa acquisita sera react with distinct epitopes on the NC1 and NC2 domains of type VII collagen: study using immunoblotting of domain-specific recombinant proteins and postembedding immunoelectron microscopy. *Br J Dermatol* 150:843–51

Matsumura K, Amagai M, Nishikawa T *et al.* (1996) The majority of bullous pemphigoid and herpes gestationis serum samples react with NC16a domain of the 180-kDa bullous pemphigoid antigen. *Arch Dermatol Res* 288:507–9

Nagata Y, Karashima T, Watt FM *et al.* (2001) Paraneoplastic pemphigus sera react strongly with multiple epitopes on the various regions of envoplakin and periplakin, except for C-terminal homologous domain of periplakin. *J Invest Dermatol* 116:556–63

Nie Z, Hashimoto T (1999) IgA antibodies of cicatricial pemphigoid sera specifically react with C-terminus of BP180. *J Invest Dermatol* 112:254–5

Nie Z, Nagata Y, Joubeh S *et al.* (2000) IgA antibodies of linear IgA bullous dermatosis recognize the 15th collagenous domain of BP180. *J Invest Dermatol* 115:1164–6

Ohata Y, Amagai M, Ishii K *et al.* (2001) Immunoreactivity against intracellular domains of desmogleins in pemphigus. *J Dermatol Sci* 25:64–71

See related article on pg 1126

More or Less: Copy Number Alterations in Mycosis Fungoides

William M. Lin[1] and Michael Girardi[1]

Mycosis fungoides (MF) is the most common form of cutaneous T-cell lymphoma (CTCL), a heterogeneous group of non-Hodgkin's lymphomas of skin-homing T cells. MF may vary from limited patchy skin disease to extensive cutaneous plaque and tumor involvement to extracutaneous compartments of blood, lymph nodes, and viscera. Advances in genomic technologies have enabled the increasing characterization of genetic alterations in this malignancy; using this technology, investigators hope to understand MF's variable behavior and pathogenesis. In this issue, Salgado *et al.* identify regions of genomic DNA alterations from 41 MF samples and report associations with prognosis.

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In recognition that cancer is fundamentally dependent on genetic alterations (Vogelstein and Kinzler, 2004), the number of genomic

[1]*Department of Dermatology, Yale University School of Medicine, New Haven, Connecticut, USA*

Correspondence: Michael Girardi, Department of Dermatology, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06520, USA. E-mail: girardi@yale.edu

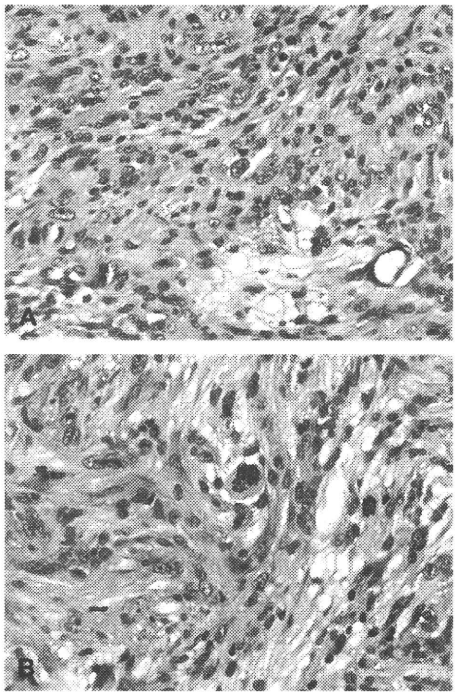

Fig 2. Myxoinflammatory fibroblastic sarcoma. High-power view showing large Reed-Sternberg–like cells (**A**) and mitotic figures (**B**).

We would like to thank Christopher Fletcher, MD, at Harvard Medical School for assistance with the histopathological diagnosis.

Kim Bui, MS,[a] Sarah Glorioso, MD,[b] Thomas Nicotri, Jr, MD,[b] and Jeffrey Frederic, MD[b]

Louisiana State University Health Sciences Center School of Medicine[a] and the Department of Dermatology,[b] Louisiana State University Health Sciences Center in New Orleans

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Reprint requests: Thomas Nicotri, Jr, MD, Department of Dermatology, Louisiana State University Health Sciences Center in New Orleans, PO Box 1713, Mandeville, LA 70470

E-mail: thomasnicotrijr@bellsouth.net

REFERENCES

1. Meis-Kindblom JM, Kindblom LG. Acral myxoinflammatory fibroblastic sarcoma: a low-grade tumor of the hand and feet. Am J Surg Pathol 1998;22:911-4.
2. Montgomery EA, Devaney KO, Giordano TJ, Weiss S. Inflammatory myxohyaline tumor of distal extremities with virocyte or Reed-Sternberg–like cells: a distinctive lesion with features simulating inflammatory conditions, Hodgkin's disease, and various sarcomas. Mod Pathol 1998;11:384-91.
3. Lang JE, Dodd L, Salutario M, Brigman BE. Case reports: acral myxoinflammatory fibroblastic sarcoma: a report of five cases and literature review. Clin Orthop Relat Res 2006;445:254-60.
4. Jurcic V, Zidar A, Montiel M, Frkovic-Grazio S, Nayler SJ, Cooper K, et al. Myxoinflammatory fibroblastic sarcoma: a tumor not restricted to acral sites. Ann Diagn Pathol 2002;6:272-80.
5. Sakaki M, Hirokawa M, Wakatsuki S, Sano T, Endo K, Fujii Y, et al. Acral myxoinflammatory fibroblastic sarcoma: a report of five cases and review of the literature. Vi, chows Arch 2003;442:25-30.
6. Kovarik CL, Barrett T, Auerbach A, Cassarino DS. Acral myxoinflammatory fibroblastic sarcoma: case series and immunohistochemical analysis. J Cutan Pathol 2008;35:192-6.

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Refractory oral ulcers with multiple immunoglobulin G/immunoglobulin A autoantibodies without skin lesions

To the Editor: Current molecular diagnostics have characterized many new autoimmune bullous diseases that traditional descriptive dermatology would not have been able to define. Herein we report a case of refractory oral ulcers with immunoblots that were confusing, causing difficulty in making a definitive diagnosis.

Oral ulcers suddenly appeared in a 76-year-old male and were protracted over 8 months. He had been suffering from diabetes mellitus and hypertension, but had no history of malignancy. On the patient's first visit, very painful shallow ulcers and blisters with red halos were present over the palate, gingiva, labiogingival groove, buccal mucosa, and larynx (Fig 1, *A*). The conjunctivae, vermilion border of lips, glans penis, and anus were unaffected. There were no cutaneous lesions. A biopsy specimen of the buccal mucosa showed subepithelial blister formation with slight acanthosis and copious infiltration of lymphocytes and plasma cells in the submucosa, in which capillaries were dilated. There was no apparent acantholysis (Fig 1, *B*). Direct immunofluorescence examination showed linear deposition of both immunoglobulin G (IgG; Fig 1, *C*) and immunoglobulin A (IgA; Fig 1, *D*) antibodies along the basement membrane zone. Indirect immunofluorescence with the patient's serum using 1 M sodium chloride split-skin sections demonstrated IgG antibodies reactive with both epidermal and dermal sides (Fig 1, *E*) and IgA antibodies reactive with the epidermal side (Fig 1, *F*). The following antibodies were detected on immunoblot analysis: IgG and IgA to the BP180 NC16a domain (Fig 2, *A*), IgG and IgA to the 120-kDa linear IgA dermatosis antigen, LAD-1,[1,2] an ectodomain of the BP180 molecule (Fig 2, *C*), and

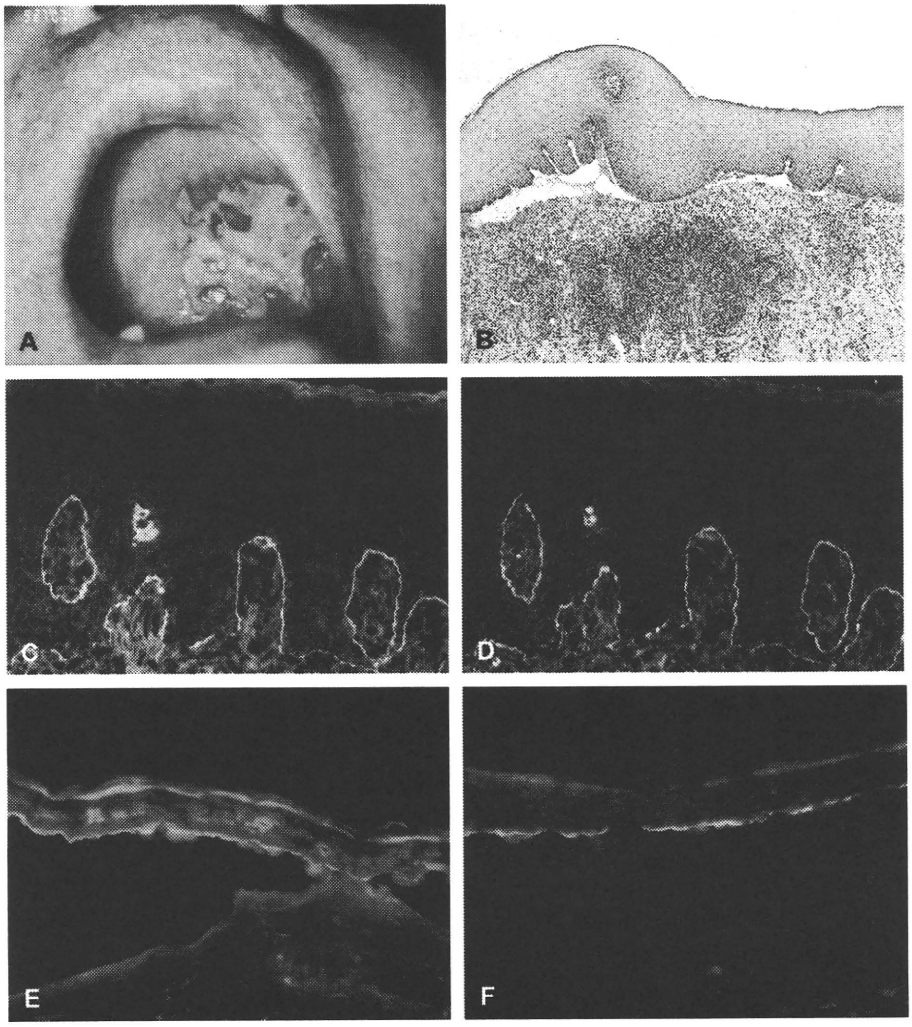
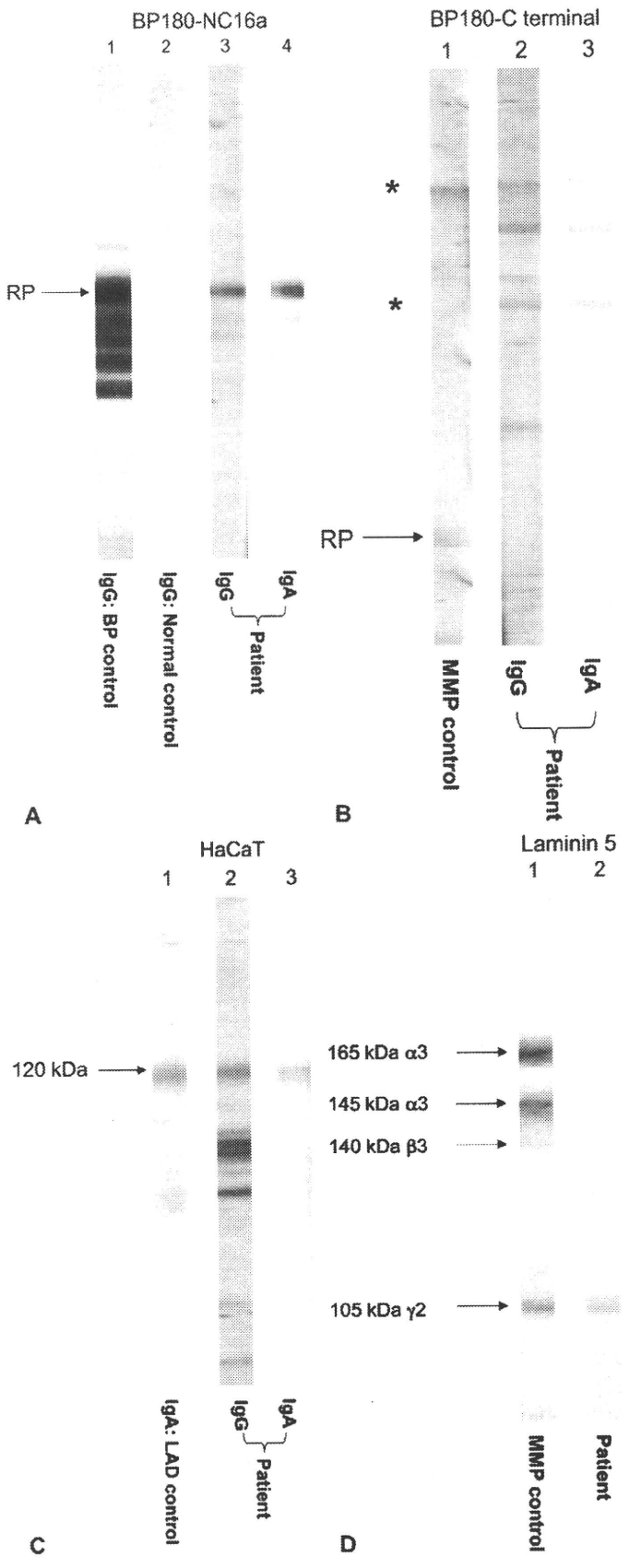


Fig 1. Oral lesions in the present case. **A**, Ulcers distributed on the palate. Histologic features of a biopsy specimen obtained from a lesion on the buccal mucosa, stained with hematoxylin–eosin (scale bar, 100 μm). **B**, The specimen showed subepithelial blister formation with infiltration of lymphocytes and plasma cells in the lamina propria. Direct immunofluorescence showed linear deposition of both (**C**) immunoglobulin G and (**D**) immunoglobulin A antibodies along the basement membrane zone. Indirect immunofluorescence with the patient's serum using 1M sodium chloride split-skin sections demonstrated (**E**) immunoglobulin G antibodies reactive with both epidermal and dermal sides and (**F**) immunoglobulin A antibodies reactive with the epidermal side.

IgG to the γ2 subunit of laminin 332 (Fig 2, *D*).[3] No IgG or IgA autoantibodies against BP230/BP180, 130-kDa desmoglein (Dsg) 3, 160-kDa Dsg 1, 210-kDa envoplakin, or 190-kDa periplakin were detected on immunoblot analysis using epidermal extracts (data not shown). By enzyme-linked immunosorbent assay, the index value of anti- BP180 NC16a domain of IgG antibody was 27.69 (normal range, <15). We ultimately diagnosed this case as mucous membrane pemphigoid complicated with linear IgA/IgG bullous dermatosis.[4] Daily administration of tetracycline hydrochloride 500 mg and nicotinamide 200 mg, relatively low doses because of the patient's advanced age, was partially effective, and lesions have been confined to the oral mucosa for 6 months. Systemic steroid administration was avoided because of his preexisting diabetes mellitus.

This is an unusual case of mucous membrane pemphigoid complicated with linear IgA/IgG bullous dermatosis. Extraoral involvement is common in patients with mucous membrane pemphigoid.[5,6]


BP180-NC16a
1 2 3 4
RP
IgG: BP control
IgG: Normal control
IgG
IgA
Patient
A
BP180-C terminal
1 2 3
*
*
RP
MMP control
IgG
IgA
Patient
B
HaCaT
1 2 3
120 kDa
IgA: LAD control
IgG
IgA
Patient
C
Laminin 5
1 2
165 kDa α3
145 kDa α3
140 kDa β3
105 kDa γ2
MMP control
Patient
D

Most patients with linear IgA bullous dermatosis show mainly cutaneous lesions, although mucous membrane lesions are predominant in a few cases.[7,8] Therefore, the present case cannot be simply explained as a confluence of various autoimmune bullous diseases with different characteristic autoantibodies. The patient should be monitored to determine whether extraoral lesions develop.

Even the current advanced molecular diagnostic technology cannot perfectly solve the highly complicated pathogenesis of cases such as ours. This rare case provides useful information for further investigations of unclassified autoimmune bullous diseases.

Teruki Dainichi, MD,[a] Bungo Ohyama, MD,[a] Norito Ishii, MD,[a] Zenichi Yamaguchi, MD,[b] Shinichiro Yasumoto, MD,[a] and Takashi Hashimoto, MD[a]

Department of Dermatology,[a] Kurume University School of Medicine, Fukuoka, and the Division of Dermatology,[b] Department of Medicine, Nippon Dental University School of Life Dentistry, Tokyo, Japan

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Correspondence to: Teruki Dainichi, MD, Department of Dermatology, Kurume University School of Medicine, 67 Asahimachi, Kurume, Fukuoka 830-0011, Japan

E-mail: dainichi@med.kurume-u.ac.jp

REFERENCES

1. Roh JY, Yee C, Lazarova Z, Hall RP, Yancey KB. The 120-kDa soluble ectodomain of type XVII collagen is recognized by autoantibodies in patients with pemphigoid and linear IgA dermatosis. Br J Dermatol 2000;143:104-11.
2. Schumann H, Baetge J, Tasanen K, Wojnarowska F, Schäcke H, Zillikens D, et al. The shed ectodomain of collagen XVII/BP180 is targeted by autoantibodies in different blistering skin diseases. Am J Pathol 2000;156:685-95.
3. Hisamatsu Y, Nishiyama T, Amano S, Matsui C, Ghohestani R, Hashimoto T. Usefulness of immunoblotting using purified laminin 5 in the diagnosis of anti-laminin 5 cicatricial pemphigoid. J Dermatol Sci 2003;33:113-9.
4. Hertl M, Büdinger L, Christophoridis S, Yancey KB, Borradori L. IgG and IgA antibodies in linear IgA/IgG bullous dermatosis target the ectodomain of bullous pemphigoid antigen 2. Br J Dermatol 1999;140:750-2.
5. Chan LS, Ahmed AR, Anhalt GJ, Bernauer W, Cooper KD, Elder MJ, et al. The first international consensus on mucous membrane pemphigoid: definition, diagnostic criteria, pathogenic factors, medical treatment, and prognostic indicators. Arch Dermatol 2002;138:370-9.
6. Thorne JE, Anhalt GJ, Jabs DA. Mucous membrane pemphigoid and pseudopemphigoid. Ophthalmology 2004;111:45-52.
7. Wojnarowska F, Marsden RA, Bhogal B, Black MM. Chronic bullous disease of childhood, childhood cicatricial pemphigoid, and linear IgA disease of adults. A comparative study demonstrating clinical and immunopathologic overlap. J Am Acad Dermatol 1988;19:792-805.
8. Leonard JN, Wright P, Williams DM, Gilkes JJ, Haffenden GP, McMinn RM, et al. The relationship between linear IgA disease and benign mucous membrane pemphigoid. Br J Dermatol 1984;110:307-14.

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Acquired combined nutritional deficiency presenting as psoriasiform dermatitis

To the Editor: Nutritional deficiency, a prevalent problem worldwide, remains rare in developed countries. In the United States, acquired nutritional deficiencies have been reported in patients with anorexia nervosa, malabsorption syndromes, those on long-term parenteral nutrition, and patients with food allergies.[1-4] Given the significant morbidity and sometimes mortality associated with certain nutritional deficiencies, their prompt recognition, diagnosis, and treatment by clinicians is of great importance.[1] Many nutritional deficiencies have classic cutaneous presentations that are valuable clinical diagnostic tools.[5] However, combined nutritional deficiencies often put forth a mixed clinical

Fig 2. Immunoblot analyses. **A**, Immunoglobulin G (IgG) antibodies of control bullous pemphigoid serum reacted with the recombinant protein (RP) of the BP180 NC16a domain (*arrow*; lane 1), while normal control serum did not react (lane 2). IgG (lane 3) and immunoglobulin A (IGA; lane 4) antibodies of the serum of the present case reacted with this recombinant protein. **B**, IgG antibodies of control anti-BP180 type mucous membrane pemphigoid serum reacted with the recombinant protein of BP180 C-terminus (*arrow*; lane 1), while IgG (lane 2) and IgA (lane 3) antibodies of the serum of the present case did not react with this recombinant protein. *Asterisks* show the nonspecific protein bands. **C**, IgA antibodies of control linear IgA bullous dermatosis serum reacted with the 120-kDa linear IgA dermatosis antigen LAD-1 (*arrow*) in cultured HaCaT cell supernatant. Both IgG (lane 2) and IgA (lane 3) antibodies of the serum of the present case also reacted with the LAD-1. **D**, IgG antibodies of control antilaminin 5 mucous membrane pemphigoid serum reacted with all of the 165-kDa α3, 145-kDa α3, 140-kDa β3, and 105 kDa γ2 subunits of laminin 332 (formerly laminin 5) in purified laminin 332 (lane 1). IgG antibodies of the serum of the present case reacted with the 105 kDa γ2 subunit (lane 2).

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INVITED ARTICLE

Epidermolysis bullosa acquisita: What's new?

Norito ISHII,[1] Takahiro HAMADA,[1] Teruki DAINICHI,[1] Tadashi KARASHIMA,[1] Takekuni NAKAMA,[1] Shinichiro YASUMOTO,[1] Detlef ZILLIKENS,[2] Takashi HASHIMOTO[1]

[1]Department of Dermatology, Kurume University School of Medicine, Fukuoka, Japan, and [2]Department of Dermatology, University of Lübeck, Lübeck, Germany

ABSTRACT

Type VII collagen is an adhesion molecule of the extracellular matrix in epithelial basement membranes, and the main constituent of anchoring fibrils at the dermal–epidermal junction (DEJ). Autoimmunity against this protein is causing the rare organ-specific epidermolysis bullosa acquisita (EBA). EBA is a rare acquired, heterogeneous, chronic blistering disease of skin disease of skin and mucous membranes characterized by subepidermal blisters and tissue-bound as well as circulating autoantibodies to the DEJ. EBA has several distinct clinical presentations with other subepidermal bullous diseases, such as mainly dystrophic epidermolysis bullosa or bullous pemphigoid. The circulating immunoglobulin G autoantibodies for EBA react with a 290-kDa dermal protein, type VII collagen, as detected by immunoblot analysis using dermal extracts. The pathogenicity of these autoantibodies has been demonstrated by experimental animal models, in which anti-type VII collagen antibodies injected into a mouse produced an EBA-like blistering disease in the animal. EBA cases often require high doses of systemic corticosteroids and a variety of immunosuppressants. Although treatment for EBA is frequently difficult and unsatisfactory, some therapeutic success has been reported with colchicine, dapsone, infliximab and i.v. immunoglobulin. In this review, we will focus on recent progress in our understanding of the clinical manifestations, the etiopathogenesis as well as the management of EBA.

Key words: clinical manifestations, epidermolysis bullosa acquisita, pathogenesis, therapy.

INTRODUCTION

Autoimmune blistering skin diseases are a group of severe, potentially long and life-threatening diseases, clinically characterized by blisters and erosions of skin and/or mucous membranes. Autoimmune blistering skin diseases develop autoantibodies reactive with the epidermal keratinocyte cell surfaces or the epidermal basement membrane zone, which in turn induce separation between epidermal keratinocytes or at the dermal–epidermal junction.[1,2] Based on histopathological, immunological and clinical criteria, autoimmune bullous diseases are classified into two major groups associated with autoantibodies to desmosomal (pemphigus group) or hemidesmosomal proteins (subepidermal blistering diseases, e.g. pemphigoid diseases and epidermolysis bullosa acquisita [EBA]).[3–5]

The term "epidermolysis bullosa acquisita" was proposed as a descriptive clinical diagnosis for patients with adult onset and features resemble those of hereditary dystrophic epidermolysis bullosa were reported by Elliott in 1904.[6] In 1971, Roenigk *et al.*[7] was the first to distinguish, on the basis of distinctive clinical and histological features, EBA from other bullous diseases, suggesting the first diagnostic criteria

Correspondence: Takashi Hashimoto, M.D., Department of Dermatology, Kurume University School of Medicine, 67 Asahimachi, Kurume, Fukuoka 830-0011, Japan. Email: hashimot@med.kurume-u.ac.jp

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for EBA. After that, during the 1970s and 1980s, characteristic clinical, histological, immunohistological and serological features of EBA were categorized.[8–10]

Epidermolysis bullosa acquisita is a chronic blistering disease of skin and mucous membranes characterized by subepidermal blisters and tissue-bound as well as circulating autoantibodies to the dermal–epidermal junction.[11–13] The circulating immunoglobulin (Ig)G antibodies in EBA react with a 290-kDa dermal protein, type VII collagen, which is the main constituent of anchoring fibrils located at the dermal–epidermal junction, an adhesion molecule of the extracellular matrix in epithelial basement membranes. EBA is a rare disease with a prevalence of approximately 0.2/million people.[14,15] There is no sex and racial predilection known, although several studies have reported an increased occurrence of the human leukocyte antigen (HLA)-DR2 allele in patients with EBA and bullous systemic lupus erythematosus (SLE).[16,17] This HLA phenotype has been associated with hyper-immunity which suggests an autoimmune etiology for EBA. The significance of certain HLA-DR2 molecules in the pathogenesis of EBA needs to be demonstrated in the context of specific autoantigens in future laboratory investigations.[16,17]

Although EBA represents the rare autoimmune blistering disease in general, therapy for patients with EBA remains unsatisfactory, and mainly relies on immunosuppressive agents such as methotrexate, azathioprine or cyclophosphamide.[18–21] Thus, there is a need for the identification of safe and effective alternatives for treatment of EBA. In this review, we will focus on recent progress in our understanding of the pathogenesis of EBA, the characterization of clinical manifestations, and their role in maintenance.

THE AUTOANTIGENS OF EBA

Type VII collagen, the main constituent of anchoring fibrils, was identified as the autoantigen of EBA.[22,23] Anchoring fibrils are thought to anchor the epidermis and its underlying basement membrane zone to the papillary dermis.[24] Type VII collagen is composed of three identical α-chains, each consisting of a 145-kDa central collagenous triple helical portion, flanked by a large 145-kDa amino terminal non-collagenous domain (NC1), and a smaller 34-kDa carboxy-terminal non-collagenous domain (NC2). In the extracellular space, type VII collagen molecules form anti-parallel tail-to-tail dimers stabilized by disulfide bonding through a small carboxy-terminal overlap (NC2), while a fragment of the NC2 domain is proteolytically removed.[25] Several dimers aggregate laterally to form the unique cross-banded structure, namely, anchoring fibrils, which comprise anti-parallel dimers and contain NC1 domains at both ends, locating in the lamina densa and forming semicircular loops visible by electron microscope.[26] Previous studies have established that the major antigenic epitopes of type VII collagen are located within the NC1 domain of type VII collagen.[27–30]

The autoimmune nature of EBA and the pathogenic relevance of antibodies against type VII collagen are supported by the following compelling evidence. Patients' autoantibodies to type VII collagen were shown to recruit and activate leukocytes *ex vivo* resulting in dermal–epidermal separation in cryosections of human skin.[31,32] Recently, two different animal models of EBA were established: The disease can be induced in mice by injection of autoantibodies against type VII collagen into mice, when passively transferred into mice.[33,34] In this "passive" EBA model, skin lesions develop in all strains of mice investigated so far.[33] Subepidermal blisters can also be induced in mice by immunization with a recombinant fragment of the murine NC1 domain (GST-mCOL7C). Disease development in this "active" model is restricted to certain strains of mice; for example, SJL.[35,36] Both models duplicate the clinical, histological and immunological features seen in patients with EBA. Furthermore, complement activation and infiltration of granulocytes into the skin are required for blister formation in experimental EBA.[37,38] Although mechanisms of tissue damage and blister formation in EBA are not fully understood, mechanisms by which EBA autoantibodies are thought to be initiated by the binding of the autoantibodies to antigenic sites, most commonly located within the NC1 domain of type VII collagen.[27–30] Subsequently, complement is activated by the Fc-portion of autoantibodies,[37] leading to the recruitment of neutrophils,[38] which release reactive oxygen species,[32,38] ultimately resulting in subepidermal blister formation. EBA patients have a decrease in normally functioning anchoring fibrils secondary to an abnormality in their

immune system in which they produce "pathogenic" autoantibodies against type VII collagen.[13]

CLINICAL PRESENTATION

Cutaneous manifestations in EBA are heterogeneous and may mimic other bullous diseases. Although the clinical spectrum of EBA is still being defined, EBA patients have two major clinical subtypes: an inflammatory and a non-inflammatory phenotype (Fig. 1).[9,10] The mechanobullous, non-inflammatory form of EBA, so-called classic EBA, comprising the majority of cases, is characterized by the appearance of skin fragility and tense blisters, vesicles or bullae, with some being hemorrhagic or erosions localized to the extensor skin surface. The lesions heal with scarring and milia formation.[7] In general, lesions may appear on any mucocutaneous surface and located primarily on anatomic areas subjected to repetitive minor trauma, such as the extensor upper extremities, for example, elbows, knees, buttocks, dorsal feet, dorsal hands and toes. A group of patients with predominant mucosal disease who have autoantibodies to type VII collagen have been reclassified as mucous membrane pemphigoid patients in a recent consensus meeting.[39] Post-inflammatory hyper- and hypopigmentation are also commonly observed as well as nail dystrophy. EBA patients with the mechanobullous classic form may resemble hereditary dystrophic epidermolysis bullosa clinically, such as scarring, loss of hair on the scalp, loss of nails, and esophageal stenosis or esophageal involvement.

In addition to the mechanobullous classic variant, several inflammatory subtypes of EBA were described, clinically mimicking bullous pemphigoid, linear IgA disease, mucous membrane pemphigoid or Brunsting–Perry pemphigoid.[40–48]

Although the original diagnostic criteria for EBA stated that disease onset should be in adulthood, several childhood cases have been documented. Interestingly, several previous reports described that some childhood EBA patients with reactivity to the triple-helical collagenous domain, as well as the NC1 and NC2 domains, were of the inflammatory type.[49–52] To date, although the relationship between the epitope profile and the clinical features (particularly classical non-inflammatory vs inflammatory EBA) remains to be elucidated at the present, suggesting that reactivity with a different epitope, such as the NC1 domain and other domains, leads to the different clinical phenotypes. One possible mechanism is that, when the patient's serum react with the region other than NC1 domain, a clinical phenotype of inflammatory type presents, further suggesting the possible causative role of such autoantibodies on complement activation and inflammatory infiltrates, which in turn develops the inflammatory type of EBA. Further studies on a large number of patients with EBA should characterize the epitope specificity of EBA autoantibodies and their correlation with clinical features, such as age at disease onset, extent of skin lesions and clinical course.

Autoantibodies against type VII collagen are also responsible for bullous SLE.[53–55] EBA associated with systemic diseases have been also often reported, including rheumatoid arthritis and diabetes mellitus, as well as cryoglobulinemia and psoriasis.[56–60] An association between EBA and inflammatory bowel disease (IBD) has been extensively documented; in particular, Crohn's disease has been described in approximately 30% of EBA patients.[61–65] However, to date, their relevance for the pathogenesis of both IBD and EBA is still unclear.

HISTOPATHOLOGICAL AND IMMUNOPATHOLOGICAL FEATURES

The histological picture of lesional EBA skin typically shows subepidermal blister accompanied by various degrees of dermal inflammatory infiltrate.[66] In detail, classic EBA usually presents with a non- or pauci-inflammatory subepidermal blister, whereas the inflammatory forms of EBA are associated with a neutrophil-rich infiltrate with variable numbers of eosinophils and mononuclear cells (Fig 2a).[67] As an ultrastructural observation, in general, it has been reported that blister formation in conditions occurs beneath the region of the lamina densa by electron microscope (Fig. 3a).[22,68] These studies have been carried out using skin from patients with active EBA, obtained by biopsy. In some EBA patients, the split localizes to the lamina lucida of the dermal–epidermal junction.[69] Direct immunoelectron microscopic studies reveal immunoreactants within the lamina densa, and/or sub-lamina densa of the basement membrane

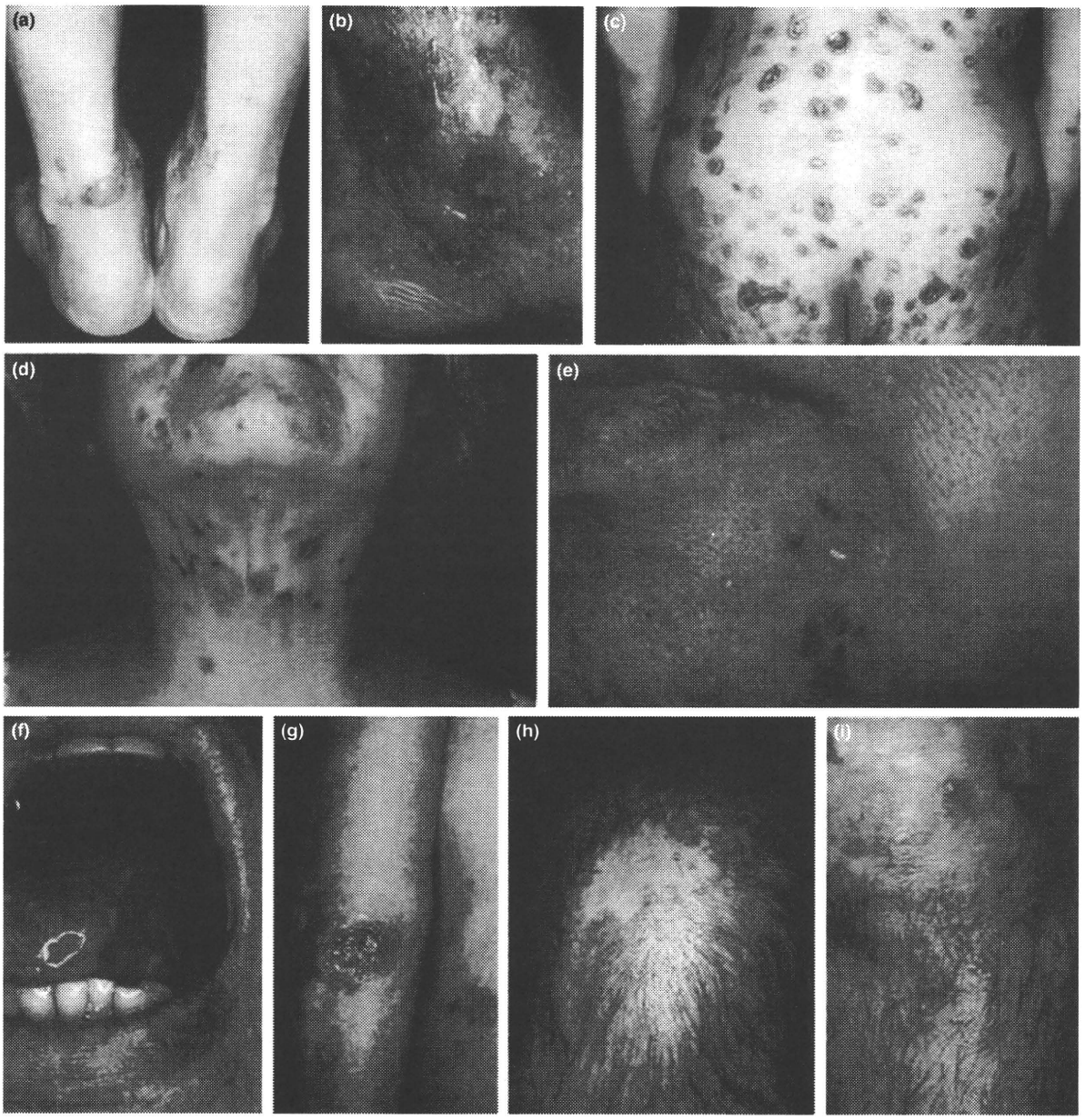

Figure 1. Clinical appearance of epidermolysis bullosa acquisita (EBA). Skin fragility and tense blisters on the heel and ankles, located primarily on anatomic areas subjected to repetitive minor trauma (a,b). Erosive lesions on the back and buttock (c). Blisters and erosions with erythematous atrophic plaques on the neck (d) and face (e). EBA also present with mucosal involvements (f). The lesions heal with scar (g), scarring alopecia (h) and milia formation (i), following blister.

zone.[70] Post-embedding indirect immunoelectron microscopic study revealed that most EBA sera showed a broad immunoreactivity within the lamina densa, whereas some sera located in the dermis below the lamina densa (Fig. 3b).[71–73]

IMMUNOFLUORESCENCE STUDIES

Direct immunofluorescence microscopy of perilesional skin biopsies from patients with EBA demonstrates linear deposits of IgG and/or C3 at the

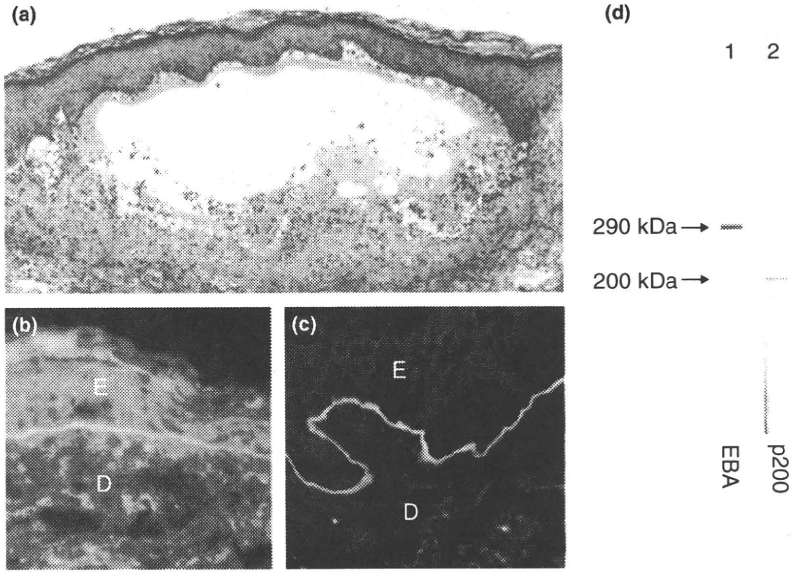

Figure 2. Histopathological and immunopathological features of epidermolysis bullosa acquisita (EBA). Histopathological findings of a perilesional skin biopsy reveals a subepidermal blister with numerous neutrophils in the upper dermis and blister cavity (a). Indirect immunofluorescence microscopy using serum from a patient with EBA shows linear deposits of immunoglobulin (Ig)G along the basement membrane zone (b). Indirect immunofluorescence microscopy on NaCl-split normal human skin demonstrates circulating IgG autoantibodies binding to the dermal side of the split (c). Immunoblot reactivity patterns of sera from patients with EBA and anti-p200 pemphigoid. Lane 1, serum of a patient with epidermolysis bullosa acquisita (EBA) reacts with 290-kDa full-length type VII collagen. Lane 2, serum of a patient with anti-p200 pemphigoid recognizes a 200-kDa protein in dermal extract. Migration of molecular weight markers is shown on the left. E, epidermis; D, dermis.

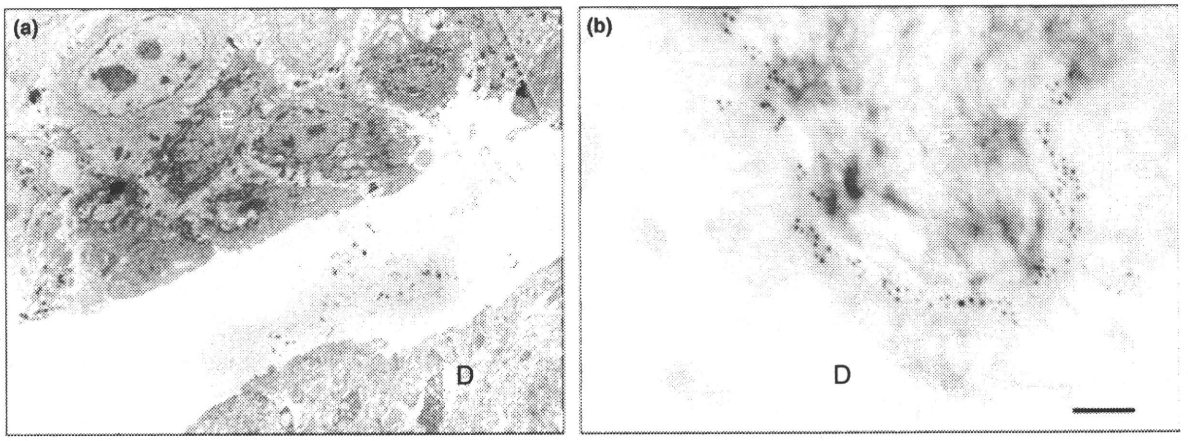

Figure 3. Electron microscopic studies of epidermolysis bullosa acquisita (EBA). Under transmission electron microscopy, lesional skin obtained from a patient with EBA shows a separation below the lamina densa (a). Immunoelectron microscopy using normal human skin reveals immunoreactivity within/below the lamina densa (b). Bars = 200 nm.

dermal–epidermal junction.[68] In rare cases, an additional staining for IgA was described.[44–46,74] Indirect immunofluorescence microscopy using 1 mol/L NaCl-split normal human skin as a substrate demonstrates circulating IgG autoantibodies binding to the dermal side of the artificial split in serum of EBA patients (Fig. 2b,c), which label the sublamina densa zone by indirect immunoelectron

microscopy.[72,75,76] Tissue-bound and circulating antibodies in EBA patients mainly belong to the IgG1 and IgG4 subclasses.[77–80] Wozniak and Kowalewski demonstrated prominent invaginations of the lamina densa and vertically-oriented clumps of anchoring fibrils at and below the dermal–epidermal junction using laser scanning confocal microscopy.[81]

IMMUNOBLOT ANALYSIS

Sera from patients with EBA recognize the 290-kDa protein, or its immunodominant region, the NC1 domain, by immunoblotting with normal human dermal extracts.[22,72] Immunoblot analysis with extracts of human epidermis or cultured keratinocytes and fibroblasts is usually negative (Fig. 2d).[82] A sensitive enzyme-linked immunosorbent assay for the detection of autoantibodies to type VII collagen using recombinant protein is also available.[83]

Some cases of a subepidermal blistering disease with autoantibodies against more than two antigens have been reported. EBA also sometimes complicates other subepidermal autoimmune bullous diseases, for example, against anti-laminin 332, anti-bullous pemphigoid and anti-p-200 antigen.[84–88] At present, clinical features, and histological and immunofluorescence findings are not useful to distinguish between each other. Immunoblot analysis and other molecular biological studies are necessary to further characterize these complicated subepidermal autoimmune bullous diseases. Moreover, the relationship between the antigenic reactivity of these autoantibodies and their prognostic significance needs to be elucidated by more precise analyses.

CLINICAL COURSE AND TREATMENT OPTIONS

Treatment of EBA can often be challenging and primarily consists of systemic corticosteroids, while it remains unsatisfactory, and mainly relies on immunosuppressive agents such as methotrexate, azathioprine or cyclophosphamide.[18–21] Overall, treatment of EBA is difficult, despite the use of corticosteroids combined with other immunosuppressants. Furthermore, long-term immunosuppression has been shown to be associated with increased morbidity and mortality. This includes systemic infections, gastrointestinal disorders, hypertension, osteoporosis, hyperlipidemia, psychiatric disorders, moon face, diabetes mellitus and obesity. Hence, there is a need for the identification of safe and effective alternatives for the treatment modalities of EBA. If required, colchicine or other adjuvants can be added. Some cases of EBA have been identified in which colchicine treatment may be beneficial.[89] This is often used as a first-line management because its side-effects are relatively benign compared with other therapeutic choices. Diarrhea is a common side-effect of colchicine, however, which makes it difficult for many patients to achieve a high enough dose to control the disease. Dapsone has been used in some EBA patients, especially when neutrophils are present in their dermal infiltrate. Recently, i.v. immunoglobulin (IVIG) is one potential promising therapy for patients with EBA, as evidence of its effectiveness and safety is increasing. A number of autoimmune bullous skin diseases have been identified in which IVIG treatment may be beneficial. A review of published work revealed that more than 10 patients with extensive treatment-resistant EBA have – in most cases successfully – been treated.[90–96] Recommended doses are 2 g/kg IVIG monthly until clinical improvement is achieved and no lesions are developed. Because of the limited duration of response retreatment with IVIG (several cycles) is necessary. However, experience with IVIG in patients with autoimmune skin blistering disease is limited. Thus, IVIG is recommended as second-line therapy in autoimmune bullous skin diseases, or for patients not responding to conventional therapy. The mode of action of IVIG in autoimmune diseases including bullous disease, is far from being completely understood. In addition, the most novel treatment is the anti-CD20 monoclonal antibody, rituximab, which is a monoclonal humanized antibody directed against the B-cell-specific cell surface antigen CD20. CD20 is expressed on the cell surfaces of pre-B cells and mature B cells. Rituximab is a chimeric monoclonal anti-CD20 antibody that abolishes these cells through complement- and antibody-dependent cytotoxicity and apoptosis. Thus, rituximab significantly reduces circulating B cells and antibody-producing plasma cells. Rituximab had a dramatic effect on EBA patient in a

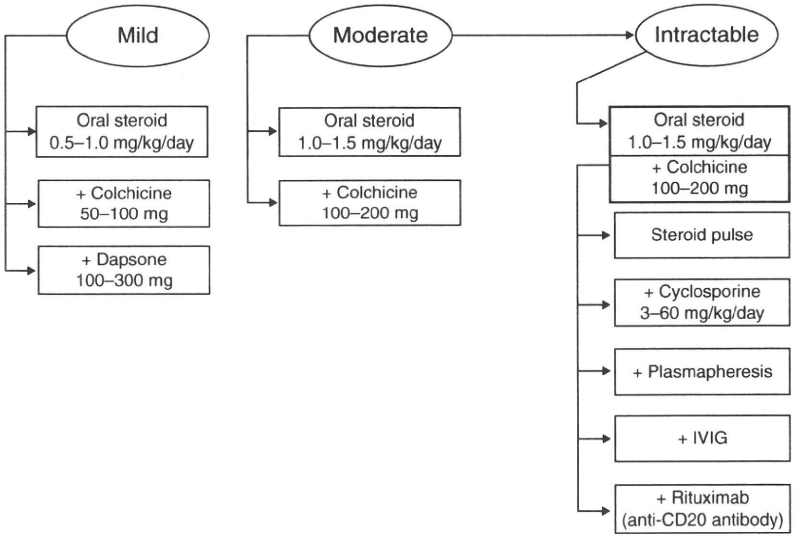

Figure 4. The algorithm for the practice to treat patients with epidermolysis bullosa acquisita (EBA). For the management of EBA, the first-line is of course the use of systemic steroid therapy. Systemic steroid therapy may be a sole treatment in some patients with relatively mild EBA; however, many cases do not respond to this regimen. At present, we fortunately have a variety of other adjuvant regimens available. Among the adjuvant therapies, colchicine is the first choice of treatment; the steroid pulse therapy is the second line of adjuvant therapy. In addition, various immunosuppressive agents may be used in addition to systemic steroid therapy. The effectiveness of these immunosuppressive agents is varied among patients. Intravenous immunoglobulin (IVIG) is considered to be an ideal treatment because it is the only treatment that does not suppress the normal immune activity. The most novel treatment is the anti-CD20 monoclonal antibody rituximab. In the most intractable cases, the combination therapies of these adjuvant treatments may be used with intensive care for severe and possibly fatal infections. Future therapeutic attempts may include the use of monoclonal antibodies able to modulate the immune response (by targeting for example B and/or T cells) or the induction of immunological tolerance by application of peptides or peptidomimetics.

life-threatening situation. In some patients with severe widespread EBA resistant to conventional therapies were successfully treated with rituximab as adjuvant therapy.[97,98] Rituximab is the newest potent therapy in severe and refractory EBA patients. Now, the regimens for these therapies are being examined worldwide. Further data and challenge are needed to establish the real potential of new treatment in EBA. In addition, there are several anti-tumor necrosis factor-α (anti-TNF-α) inhibitors in the class of biological agents (such as infliximab, an anti-TNF-α chimeric monoclonal antibody) that are being considered for use in the treatment of patients with EBA. The algorithm for the practice to treat patients with EBA is shown in Figure 4.

CONCLUSIONS AND PERSPECTIVES

Considerable progress has been made in the last years regarding our understanding of the pathogenesis of EBA. The availability of animal models of EBA provides an important tool to gain further insight into the pathophysiology of the disease. Recently, several new therapeutic agents and modalities have been reported and show promise in the treatment of patients with EBA. The multidisciplinary approach to understanding the mechanisms of central and peripheral tolerance as well as the inflammatory cascade, induced by binding of autoantibodies to type VII collagen, is leading to the more specific therapeutic strategies that counteract the chronic morbidity and mortality of this autoimmune disorder.

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REFERENCES

1 Robinson ND, Hashimoto T, Amagai M *et al.* Continuing medical education: the new pemphigus variants. *J Am Acad Dermatol* 1999; **40**: 649–671.
2 Hashimoto T. Skin diseases related to abnormality in desmosomes and hemidesmosomes. *J Dermatol Sci* 1999; **20**: 81–84.
3 Sitaru C, Goebeler M, Zillikens D. [Bullous autoimmune dermatoses (I): pathogenesis and diagnosis]. *J Dtsch Dermatol Ges* 2004; **2**: 123–128.
4 Bystryn JC, Rudolph JL. Pemphigus. *Lancet* 2005; **366**: 61–73.
5 Stanley JR, Amagai M. Pemphigus, bullous impetigo, and the staphylococcal scalded-skin syndrome. *N Engl J Med* 2006; **355**: 1800–1810.
6 Elliott GT. Two cases of epidermolysis bullosa. *J Cutan Genitourin Dis* 1895; **13**: 10.
7 Roenigk HH, Ryan JG, Bergfeld WF. Epidermolysis bullosa acquisita: report of three cases and review of all published cases. *Arch Dermatol* 1971; **103**: 1–10.
8 Kushniruk W. The immunopathology of epidermolysis bullosa acquisita. *Can Med Assoc J* 1973; **108**: 1143–1146.
9 Richter BJ, McNutt NS. The spectrum of epidermolysis bullosa acquisita. *Arch Dermatol* 1979; **115**: 1325–1328.
10 Palestine RF, Kossard S, Dicken CH. Epidermolysis bullosa acquisita: a heterogeneous disease. *J Am Acad Dermatol* 1981; **5**: 43–53.
11 Zillikens D. Acquired skin disease of hemidesmosomes. *J Dermatol Sci* 1999; **20**: 134–154.
12 Mihai S, Sitaru C. Immunopathology and molecular diagnosis of autoimmune bullous diseases. *J Cell Mol Med* 2007; **11**: 462–481.
13 Woodley DT, Remington J, Chen M. Autoimmunity to type VII collagen: epidermolysis bullosa acquisita. *Clin Rev Allergy Immunol* 2007; **33**: 78–84.
14 Zhu XJ, Niimi Y, Bystryn JC. Epidermolysis bullosa acquisita. Incidence in patients with basement membrane zone antibodies. *Arch Dermatol* 1990; **126**: 171–174.
15 Bernard P, Vaillant L, Labeille B *et al.* Incidence and distribution of subepidermal autoimmune bullous skin diseases in three French regions. Bullous Diseases French Study Group. *Arch Dermatol* 1995; **131**: 48–52.
16 Gammon WR, Heise ER, Burke WA *et al.* Increased frequency of HLA DR2 in patients with autoantibodies to EBA antigen: evidence that the expression of autoimmunity to type VII collagen is HLA class II allele associated. *J Invest Dermatol* 1988; **91**: 228–232.
17 Lee CW, Kim SC, Han H. Distribution of HLA class II alleles in Korean patients with epidermolysis bullosa acquisita. *Dermatology* 1996; **193**: 328–329.
18 Hallel-Halevy D, Nadelman C, Chen M *et al.* Epidermolysis bullosa acquisita: update and review. *Clin Dermatol* 2001; **19**: 712–718.
19 Engineer L, Ahmed AR. Emerging treatment for epidermolysis bullosa acquisita. *J Am Acad Dermatol* 2001; **44**: 818–828.
20 Mutasim DF. Management of autoimmune bullous diseases: Pharmacology and therapeutics. *J Am Acad Dermatol* 2004; **51**: 859–877.
21 Kirtschig G, Murrell D, Wojnarowska F *et al.* Interventions for mucous membrane pemphigoid/cicatricial pemphigoid and epidermolysis bullosa acquisita: a systematic literature review. *Arch Dermatol* 2002; **138**: 380–384.
22 Woodley DT, Briggaman RA, O'Keefe EJ *et al.* Identification of the skin basement- membrane autoantigen in epidermolysis bullosa acquisita. *N Engl J Med* 1984; **310**: 1007–1013.
23 Woodley DT, Burgeson RE, Lunstrum G *et al.* Epidermolysis bullosa acquisita antigen is the globular carboxyl terminus of type VII procollagen. *J Clin Invest* 1988; **81**: 683–687.
24 Briggaman RA, Wheeler CE Jr. The epidermal–dermal junction. *J Invest Dermatol* 1975; **65**: 71–84.
25 Keene DR, Sakai LY, Lunstrum GP *et al.* Type VII collagen forms an extended network of anchoring fibrils. *J Cell Biol* 1987; **104**: 611–621.
26 Shimizu H, Ishiko A, Masunaga T *et al.* Most anchoring fibrils in human skin originate and terminate in the lamina densa. *Lab Invest* 1997; **76**: 753–763.
27 Lapiere JC, Woodley DT, Parente MG *et al.* Epitope mapping of type VII collagen. Identification of discrete peptide sequences recognized by sera from patients with acquired epidermolysis bullosa. *J Clin Invest* 1993; **92**: 1831–1839.
28 Gammon WR, Murrell DF, Jenison MW *et al.* Autoantibodies to type VII collagen recognize epitopes in a fibronectin-like region of the noncollagenous (NC1) domain. *J Invest Dermatol* 1993; **100**: 618–622.
29 Tanaka T, Furukawa F, Imamura S. Epitope mapping for epidermolysis bullosa acquisita autoantibody by molecularly cloned cDNA for type VII collagen. *J Invest Dermatol* 1994; **102**: 706–709.

30 Jones DA, Hunt SW III, Prisayanh PS *et al.* Immunodominant autoepitopes of type VII collagen are short, paired peptide sequences within the fibronectin type III homology region of the non-collagenous (NC1) domain. *J Invest Dermatol* 1995; **104**: 231–235.
31 Sitaru C, Kromminga A, Hashimoto T *et al.* Autoantibodies to type VII collagen mediate Fcgamma-dependent granulocyte activation and induce dermal–epidermal separation in cryosections of human skin. *Am J Pathol* 2002; **161**: 301–311.
32 Shimanovich I, Mihai S, Oostingh GJ *et al.* Granulocyte-derived elastase and gelatinase B are required for dermal–epidermal separation induced by autoantibodies from patients with epidermolysis bullosa acquisita and bullous pemphigoid. *J Pathol* 2004; **204**: 519–527.
33 Sitaru C, Mihai S, Otto C *et al.* Induction of dermal–epidermal separation in mice by passive transfer of antibodies to type VII collagen. *J Clin Invest* 2005; **115**: 870–878.
34 Woodley DT, Chang C, Saadat P *et al.* Evidence that anti-type VII collagen antibodies are pathogenic and responsible for the clinical, histological, and immunological features of epidermolysis bullosa acquisita. *J Invest Dermatol* 2005; **124**: 958–964.
35 Sitaru C, Chiriac MT, Mihai S *et al.* Induction of complement-fixing autoantibodies against type VII collagen results in subepidermal blistering in mice. *J Immunol* 2006; **177**: 3461–3468.
36 Sitaru C. Experimental models of epidermolysis bullosa acquisita. *Exp Dermatol* 2007; **16**: 520–531.
37 Mihai S, Chiriac MT, Takahashi K *et al.* The alternative pathway of complement activation is critical for blister induction in experimental epidermolysis bullosa acquisita. *J Immunol* 2007; **178**: 6514–6521.
38 Chiriac MT, Roesler J, Sindrilaru A *et al.* NADPH oxidase is required for neutrophil-dependent autoantibody-induced tissue damage. *J Pathol* 2007; **212**: 56–65.
39 Chan LS, Ahmed AR, Anhalt GJ *et al.* The first international consensus on mucous membrane pemphigoid: definition, diagnostic criteria, pathogenic factors, medical treatment, and prognostic indicators. *Arch Dermatol* 2002; **138**: 370–379.
40 Gammon WR, Briggaman RA, Woodley DT *et al.* Epidermolysis bullosa acquisita: a pemphigoid- like disease. *J Am Acad Dermatol* 1984; **11**: 820–832.
41 Dahl MG. Epidermolysis bullosa acquisita: a sign of cicatricial pemphigoid? *Br J Dermatol* 1979; **101**: 475–484.
42 Gammon WR, Briggaman RA, Wheeler CE Jr. Epidermolysis bullosa acquisita presenting as an inflammatory bullous disease. *J Am Acad Dermatol* 1982; **7**: 382–387.
43 Kurzhals G, Stolz W, Meurer M *et al.* Acquired epidermolysis bullosa with the clinical features of Brunsting-Perry cicatricial bullous pemphigoid. *Arch Dermatol* 1991; **127**: 391–395.
44 Hashimoto T, Ishiko A, Shimizu H *et al.* A case of linear IgA bullous dermatosis with IgA anti-type VII collagen autoantibodies. *Br J Dermatol* 1996; **134**: 336–339.
45 Lee CW. Serum IgA autoantibodies in patients with epidermolysis bullosa acquisita: a high frequency of detection. *Dermatology* 2000; **200**: 83–84.
46 Cox NH, Bearn MA, Herold J *et al.* Blindness due to the IgA variant of epidermolysis bullosa acquisita, and treatment with osteo-odonto-keratoprosthesis. *Br J Dermatol* 2007; **156**: 775–777.
47 Mutasim DF, Bilic M, Hawayek LH *et al.* Immunobullous diseases. *J Am Acad Dermatol* 2005; **52**: 1029–1043.
48 Tanaka N, Dainichi T, Ohyama B *et al.* A case of epidermolysis bullosa acquisita with clinical features of Brunsting-Perry pemphigoid showing an excellent response to colchicine. *J Am Acad Dermatol* 2009; **61**: 715–719.
49 Tanaka H, Ishida-Yamamoto A, Hashimoto T *et al.* A novel variant of acquired epidermolysis bullosa with autoantibodies against the central triple-helical domain of type VII collagen. *Lab Invest* 1997; **77**: 623–632.
50 Schmidt E, Hopfner B, Chen C *et al.* Childhood epidermolysis bullosa acquisita: a novel variant with reactivity to all three structural domains of type VII collagen. *Br J Dermatol* 2002; **147**: 592–597.
51 Fukumoto T, Umemura T, Higuchi M *et al.* Childhood epidermolysis bullosa acquisita with autoantibodies against all 3 structural domains of type VII collagen. *J Am Acad Dermatol* 2004; **50**: 480–482.
52 Mayuzumi M, Akiyama M, Nishie W *et al.* Childhood epidermolysis bullosa acquisita with autoantibodies against the noncollagenous 1 and 2 domains of type VII collagen: case report and review of the literature. *Br J Dermatol* 2006; **155**: 1048–1052.
53 Chan LS, Lapiere JC, Chen M *et al.* Bullous systemic lupus erythematosus with autoantibodies recognizing multiple skin basement membrane components, bullous pemphigoid antigen 1, laminin-5, laminin-6, and type VII collagen. *Arch Dermatol* 1999; **135**: 569–573.
54 Herrero-González JE, Mascaró JMJ, Herrero C *et al.* Autoantibodies from patients with BSLE inducing recruitment of leukocytes to the dermoepidermal junction and subepidermal splits in cryosections of human skin. *Arch Dermatol* 2006; **142**: 1513–1516.
55 Shirahama S, Furukawa F, Yagi H *et al.* Bullous systemic lupus erythematosus: detection of antibodies against noncollagenous domain of type VII collagen. *J Am Acad Dermatol* 1998; **38**: 844–848.
56 Chan L, Woodley D. Epidermolysis bullosa acquisita in the elderly. *J Geriatr Dermatol* 1995; **4**: 47–52.
57 Krivo JM, Miller F. Immunopathology of epidermolysis bullosa acquisita. Association with mixed cryoglobulinemia. *Arch Dermatol* 1978; **114**: 1218–1220.

58 Hoshina D, Sawamura D, Nomura T *et al.* Epidermolysis bullosa acquisita associated with psoriasis vulgaris. *Clin Exp Dermatol* 2007; **32**: 516–518.
59 Endo Y, Tamura A, Ishikawa O *et al.* Psoriasis vulgaris coexistent with epidermolysis bullosa acquisita. *Br J Dermatol* 1997; **137**: 783–786.
60 Kirtschig G, Chow ET, Venning VA *et al.* Acquired subepidermal bullous diseases associated with psoriasis: a clinical, immunopathological and immunogenetic study. *Br J Dermatol* 1996; **135**: 738–745.
61 Sherry F, Dothridge MD. Case for diagnosis: epidermolysis bullosa acquisita. *Proc R Soc Med* 1962; **55**: 409.
62 Ray TL, Levine JB, Weiss W *et al.* Epidermolysis bullosa acquisita and inflammatory bowel disease. *J Am Acad Dermatol* 1982; **6**: 242–252.
63 Hughes BR, Horne J. Epidermolysis bullosa acquisita and total ulcerative colitis. *J R Soc Med* 1988; **81**: 473–475.
64 Labeille B, Gineston J-L, Denoeux JP *et al.* Epidermolysis bullosa acquisita and Crohn's disease. *Arch Intern Med* 1988; **148**: 1457–1459.
65 Chen M, O'Toole EA, Sanghavi J *et al.* The epidermolysis bullosa acquisita antigen (type VII collagen) is present in human colon and patients with crohn's disease have autoantibodies to type VII collagen. *J Invest Dermatol* 2002; **118**: 1059–1064.
66 Woodley DT, Gammon WR, Briggaman RA. Epidermolysis bullosa acquisita. In: Freedberg IM, Eisen AZ, Wolff K, Austen KF, Goldsmith LA, Katz SI, Fitzpatrick TB, eds. *Fitzpatrick's Dermatology in General Medicine*, 4th edn. New York: McGraw-Hill, 1999; 702–709.
67 Woodley DT, Briggaman RA, Gammon WR. Acquired epidermolysis bullosa. A bullous disease associated with autoimmunity to type VII (anchoring fibril) collagen. *Dermatol Clin* 1990; **8**: 717–726.
68 Yaoita H, Briggaman RA, Lawley TJ, Provost TT, Katz SI. Epidermolysis bullosa acquisita: ultrastructural and immunological studies. *J Invest Dermatol* 1981; **76**: 288–292.
69 Fine JD, Tyring S, Gammon WR. The presence of intralamina lucida blister formation in epidermolysis bullosa acquisita: possible role of leukocytes. *J Invest Dermatol* 1989; **92**: 27–32.
70 Nieboer C, Boorsma DM, Woerdeman MJ *et al.* Epidermolysis bullosa acquisita. Immunofluorescence, electron microscopic and immunoelectron microscopic studies in four patients. *Br J Dermatol* 1980; **102**: 383–392.
71 McMillan JR, Matsumura T, Hashimoto T *et al.* Immunomapping of EBA sera to multiple epitopes on collagen VII: further evidence that anchoring fibrils originate and terminate in the lamina densa. *Exp Dermatol* 2003; **12**: 261–267.
72 Ishii N, Yoshida M, Hisamatsu Y *et al.* Epidermolysis bullosa acquisita sera react with distinct epitopes on the NC1 and NC2 domains of type VII collagen: study using immunoblotting of domain-specific recombinant proteins and postembedding immunoelectron microscopy. *Br J Dermatol* 2004; **150**: 843–851.
73 Ishii N, Yoshida M, Ishida-Yamamoto A *et al.* Some epidermolysis bullosa acquisita sera react with epitopes within the triple-helical collagenous domain as indicated by immunoelectron microscopy. *Br J Dermatol* 2009; **160**: 1090–1093.
74 Vodegel RM, de Jong MC, Pas HH *et al.* IgA-mediated epidermolysis bullosa acquisita: two cases and review of the literature. *J Am Acad Dermatol* 2002; **47**: 919–925.
75 Gammon WR, Briggaman RA, Inman AO *et al.* Differentiating anti-lamina lucida and anti-sublamina densa anti-BMZ antibodies by indirect immunofluorescence on 1.0 M sodium chloride-separated skin. *J Invest Dermatol* 1984; **82**: 139–144.
76 Gammon WR, Fine JD, Forbes M *et al.* Immunofluorescence on split skin for the detection and differentiation of basement membrane zone autoantibodies. *J Am Acad Dermatol* 1992; **27**: 79–87.
77 Bernard P, Prost C, Aucouturier P *et al.* The subclass distribution of IgG autoantibodies in cicatricial pemphigoid and epidermolysis bullosa acquisita. *J Invest Dermatol* 1991; **97**: 259–263.
78 Cho HJ, Lee IJ, Kim SC. Complement-fixing abilities and IgG subclasses of autoantibodies in epidermolysis bullosa acquisita. *Yonsei Med J* 1988; **39**: 339–344.
79 Gandhi K, Chen M, Aasi S *et al.* Autoantibodies to type VII collagen have heterogenous subclass and light chain compositions and their complement- activating capacities do not correlate with the inflammatory clinical phenotype. *J Clin Immunol* 2000; **20**: 416–423.
80 Mooney E, Gammon WR. Heavy and light chain isotypes of immunoglobulin in epidermolysis bullosa acquisita. *J Invest Dermatol* 1990; **95**: 317–319.
81 Wozniak K, Kowalewski C. Alterations of basement membrane zone in autoimmune subepidermal bullous diseases. *J Dermatol Sci* 2005; **40**: 169–175.
82 Stanley JR, Rubinstein N, Klaus-Kovtun V. Epidermolysis bullosa acquisita antigen is synthesized by both human keratinocytes and human dermal fibroblasts. *J Invest Dermatol* 1985; **85**: 542–545.
83 Chen M, Chan LS, Cai X *et al.* Development of an ELISA for rapid detection of anti-type VII collagen autoantibodies in epidermolysis bullosa acquisita. *J Invest Dermatol* 1997; **108**: 68–72.
84 Jonkman MF, Schuur J, Dijk F *et al.* Inflammatory variant of epidermolysis bullosa acquisita with IgG autoantibodies against type VII collagen and laminin alpha3. *Arch Dermatol* 2000; **136**: 227–231.
85 Fairley JA, Woodley DT, Chen M *et al.* A patient with both bullous pemphigoid and epidermolysis bullosa acquisita: an example of intermolecular epitope spreading. *J Am Acad Dermatol* 2004; **51**: 118–122.
86 Furukawa H, Miura T, Takahashi M *et al.* A case of anti-p200 pemphigoid with autoantibodies against both a

novel 200-kD dermal antigen and the 290-kD epidermolysis bullosa acquisita antigen. *Dermatology* 2004; **209**: 145–148.
87 Yamada T, Suzuki M, Koike Y *et al.* A case of epidermolysis bullosa acquisita with autoantibody to anti-p200 pemphigoid antigen and exfoliative esophagitis. *Dermatology* 2006; **212**: 381–384.
88 Pastar Z, Rados J, Lipozencic J *et al.* Case of concurrent epidermolysis bullosa acquisita and anti-p200 pemphigoid–how to treat it? *Int J Dermatol* 2007; **46**: 295–298.
89 Cunningham BB, Kirchmann TT, Woodley D. Colchicine for epidermolysis bullosa acquisita. *J Am Acad Dermatol* 1996; **34**: 781–784.
90 Caldwell JB, Yancey KB, Engler RJ *et al.* Epidermolysis bullosa acquisita: efficacy of high-dose intravenous immunoglobulins. *J Am Acad Dermatol* 1994; **31**: 827–828.
91 Mohr C, Sunderkotter C, Hildebrand A *et al.* Successful treatment of epidermolysis bullosa acquisita using intravenous immunoglobulins. *Br J Dermatol* 1995; **132**: 824–826.
92 Kofler H, Wambacher-Gasser B, Topar G *et al.* Intravenous immunoglobulin treatment in therapy-resistant epidermolysis bullosa acquisita. *J Am Acad Dermatol* 1997; **36**: 331–335.
93 Harman KE, Black MM. High-dose intravenous immune globulin for the treatment of autoimmune blistering diseases: an evaluation of its use in 14 cases. *Br J Dermatol* 1999; **140**: 864–874.
94 Gourgiotou K, Exadaktylou D, Aroni K *et al.* Epidermolysis bullosa acquisita: treatment with intravenous immunoglobulins. *J Eur Acad Dermatol Venereol* 2002; **16**: 77–80.
95 Campos M, Silvente C, Lecona M *et al.* Epidermolysis bullosa acquisita: diagnosis by fluorescence overlay antigen mapping and clinical response to high-dose intravenous immunoglobulin. *Clin Exp Dermatol* 2006; **31**: 71–73.
96 Segura S, Iranzo P, Martínez DE *et al.* High-dose intravenous immunoglobulins for the treatment of autoimmune mucocutaneous blistering diseases: evaluation of its use in 19 cases. *J Am Acad Dermatol* 2007; **56**: 960–967.
97 Schmidt E, Benoit S, Bröcker EB *et al.* Successful adjuvant treatment of recalcitrant epidermolysis bullosa acquisita with anti-CD20 antibody rituximab. *Arch Dermatol* 2006; **142**: 147–150.
98 Sadler E, Schafleitner B, Lanschuetzer C *et al.* Treatment-resistant classical epidermolysis bullosa acquisita responding to rituximab. *Br J Dermatol* 2007; **157**: 417–419.