

Chapter 2

Molecular Diagnosis of Autoimmune Blistering Diseases

Daisuke Tsuruta, Teruki Dainichi, Takahiro Hamada,
Norito Ishii, and Takashi Hashimoto

Abstract

Autoimmune bullous diseases are the best-characterized autoimmune skin diseases. Molecular diagnosis of these diseases has become possible due to the identification of their target autoantigens over the past three decades. In this review, we summarize methodology for categorizing autoimmune bullous diseases by means of combinations of direct and indirect immunofluorescence techniques using normal human skin sections, rat bladder sections and COS7 cells transfected with desmocollins 1–3 encoded vectors, enzyme-linked immunosorbent assays and immunoblotting with normal human epidermal extracts, dermal extracts, purified proteins from cell cultures and recombinant proteins.

Key words: Molecular diagnosis, Autoimmune bullous disease, Immunofluorescence, COS7 cell, Immunoblot, ELISA

1. Introduction

In healthy individuals, the immune system can accurately distinguish “self” from “non-self” and attacks only the latter (1). Autoimmune diseases are caused by dysregulation of this system (1). The ligation of surface receptors on lymphocytes or the binding of antibodies to “self” epitopes can cause inflammation and tissue damage, resulting in autoimmune disease (1). Thus far, more than 80 types of autoimmune diseases have been reported. Although the causes mostly remain obscure, some diseases are known to be triggered by bacteria or viruses with epitopic similarities to body constituents (“molecular mimicry”) (2). Autoimmune diseases can be divided into two major types, systemic and tissue-specific (3). The topic of the present review is the latter, occurring in the skin.

Autoimmune skin diseases include autoimmune bullous diseases, such as pemphigus and pemphigoid, cutaneous connective tissue diseases, vasculitis, psoriasis, vitiligo, autoimmune urticaria,

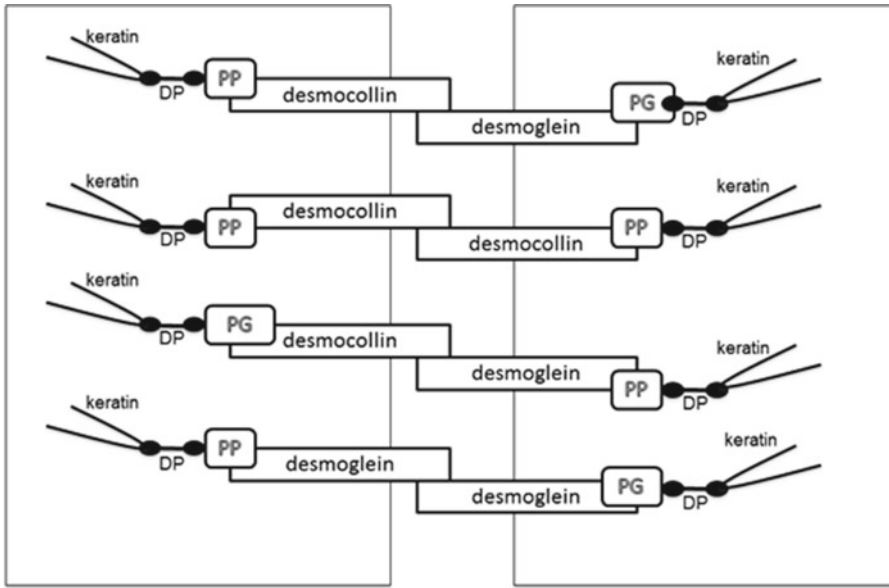


Fig. 1. Schematic diagram of the most important molecules for keratinocyte cell–cell adhesion. *PP* plakophilin, *PG* plakoglobin, *DP* desmoplakin.

and alopecia areata. In this review, we focus on autoimmune bullous diseases. Combinations of various diagnostic tools are used for the diagnosis.

2. Molecular Pathogenesis of Cell–Cell Adhesion Loss in the Epidermis

The major cell–cell adhesion moieties in keratinocytes are the desmosomes (4), the major components of which are grouped three protein families: cadherins, plakins, and armadillo proteins (Fig. 1) (4). Desmosomal cadherins are divided into two transmembrane protein families: desmogleins 1–4 and desmocollins 1–3 (4). Their cytoplasmic tails bind to armadillo family members, plakoglobin, plakophilins 1–3, and p0071 (4). Desmoplakin, a plakin family protein, tethers these molecules to keratin intermediate filaments in the cytoplasm (5).

Isoform-specific expression of desmogleins and desmocollins is observed in the epithelium and epidermis (4). Simple epithelia express only desmoglein 2 and desmocollin 2 (4). In contrast, the epidermis shows high expression of desmogleins 1 and 3, and desmocollins 1 and 3, but low expression of desmoglein 2 and desmocollin 2 (4). Desmoglein 4 is concentrated in the granular and cornified layers as well as hair follicles (4). Desmogleins are the major targets in pemphigus, the prototype of autoimmune bullous disease (6).

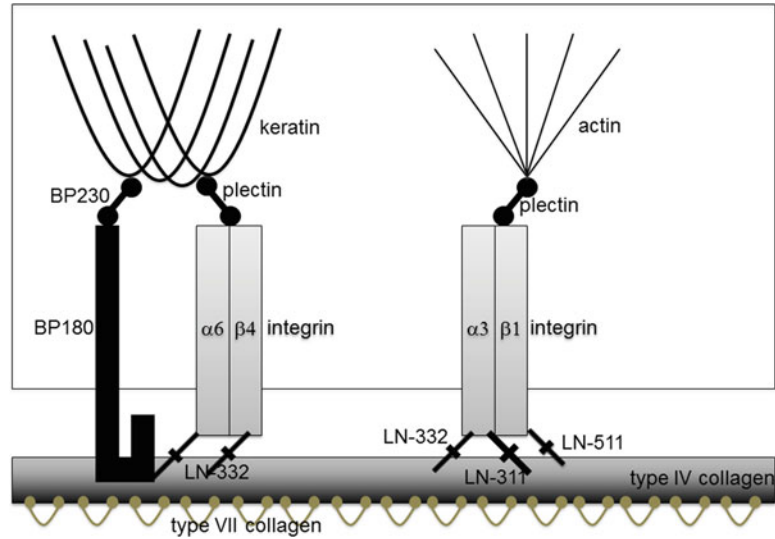


Fig. 2. Schematic diagram for the main molecules for keratinocyte–extracellular matrix adhesion. *LN* laminin.

3. Molecular Pathogenesis of Cell–Matrix Adhesion Loss in the Skin

The major structures for cell–extracellular matrix adhesion in keratinocytes are hemidesmosomes (Fig. 2) (7). Major hemidesmosomal transmembrane proteins are BP180/collagen XVII, integrin $\alpha 6$ subunit, integrin $\beta 4$ subunit, and CD151 tetraspanin (8). Both BP180 and $\alpha 6 \beta 4$ integrin interact with laminin-332 in the basement membrane zone (9). In the cytoplasm, $\alpha 6 \beta 4$ integrin associates with keratin intermediate filaments (10). The cytoplasmic tail of $\beta 4$ integrin has a unique long stretch of 1,000 amino acids (7). Through this cytoplasmic tail, $\alpha 6 \beta 4$ integrin binds to BP180, BP230, and plectin (7). The latter two proteins belong to the plakin family and mediate the indirect connection of $\alpha 6 \beta 4$ integrin not only to keratin intermediate filaments but also microtubules and actin microfilaments (10). Hemidesmosomal components are targets of autoantibodies in subepidermal autoimmune bullous diseases.

4. Autoimmune Bullous Diseases Targeting Keratinocyte Cell–Cell Adhesion

Pemphigus is an autoimmune bullous disease whose autoantigens are the desmogleins, main desmosomal constituents (6). The pemphigus disease variants and the associated autoantigens are summarized in Table 1. Histopathologically, it is characterized by acantholysis

Table 1
Classification, antibody classes and autoantigens for autoimmune bullous diseases which target keratinocyte cell–cell adhesion

Disease	Ab class	Autoantigen
Pemphigus vulgaris		
Mucosal-dominant type	IgG	Dsg3
Mucocutaneous type	IgG	Dsg3, Dsg1
Pemphigus vegetans	IgG	Dsg3, Dsg1, Dscs
Pemphigus foliaceus	IgG	Dsg1
Pemphigus erythematosus	IgG	Dsg1
Pemphigus herpetiformis	IgG	Dsg3, Dsg1, Dscs
Paraneoplastic pemphigus	IgG	Dsg3, Dsg1, Desmoplakin I, II, BP230, envoplakin, periplakin, plectin, epiplakin, Dscs, A2ML1
Drug-induced pemphigus	IgG	Multiple (mainly Dsg1)
IgA pemphigus		
SPD type	IgA	Dsc 1
IEN type	IgA	unidentified

Dsc desmocollin

and intraepidermal blister formation (11). Pemphigus is divided into two main types: pemphigus vulgaris (PV) and pemphigus foliaceus (PF) (12). Pemphigus vegetans is a rare variant of PV, and pemphigus erythematosus resembles PF (13). In addition, further very rare entities of the pemphigus group are represented by IgA pemphigus and tumor-related paraneoplastic pemphigus (13, 14).

The expression of desmogleins 1 and 3 is different in the skin and the oral mucosa (Fig. 3). Desmoglein compensation theory can explain the difference of clinical findings between PV reactive with desmoglein 3 and PF reactive with desmoglein 1. In the skin, desmoglein 1 is strongly expressed throughout the epidermis, being stronger in the superficial epidermis (15). The expression of desmoglein 3 is primarily observed in the basal and suprabasal epidermis. In contrast, in the oral mucosa, desmoglein 1 and 3 are found throughout the entire epithelium, although the expression of desmoglein 1 is much weaker than desmoglein 3. First, why mucosal-dominant lesions are found in PV, while skin-dominant lesions are found in PF? This is essentially due to the fact that in the oral mucosa, anti-desmoglein 3 antibodies in PV disrupt epithelial cell–cell adhesions, which are not compensated by small amount of desmoglein 1, whereas anti-desmoglein 1 antibodies cannot disrupt desmoglein 3-rich epithelial cell. Second, in the skin, anti-desmoglein 3 antibodies cannot disrupt cell–cell adhesion because desmoglein 1 compensates the loss of desmoglein 3-mediated adhesion. In contrast, anti-desmoglein 1 antibodies cause disruption in the upper epidermis, where no desmoglein 3 is present (Fig. 2).

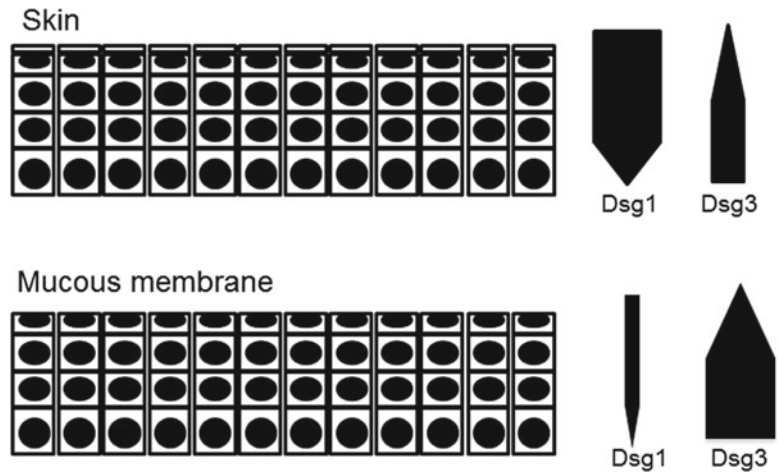


Fig. 3. The distribution of desmogleins in the epidermis for the explanation for desmoglein compensation theory. *Dsg1* desmoglein 1, *Dsg3* desmoglein 3.

5. Pemphigus Vulgaris and Pemphigus Vegetans

In PV, most patients suffer from refractory erosions or ulcers on the oral mucosae including the lips and tongue (12, 16). Some PV patients also show flaccid bullae and erosions on the skin (12). PV patients may also have erosions on other mucosae including larynx, pharynx, esophagus, conjunctiva, and vagina (17–19). Pemphigus vegetans is a variant of PV (13).

The diagnosis of PV and pemphigus vegetans is made by combination method of direct immunofluorescence showing the deposition of IgG and/or C3 at the keratinocyte cell surfaces (20) and enzyme-linked immunosorbent assays for IgG antibodies to desmoglein 3 and desmoglein 1. This is required for the correct diagnosis of all pemphigus group diseases (21). In mucosal-dominant type PV, anti-desmoglein 3 but not anti-desmoglein 1 antibodies are present (21). In contrast, in mucocutaneous type PV, antibodies for both desmoglein 1 and desmoglein 3 are present (21).

6. Pemphigus Foliaceus and Pemphigus Erythematosus

PF is characterized by superficial erosions and bullae and erythema, preferentially on the seborrheic regions (6). Oral mucosal lesions are not present (6). Pemphigus erythematosus is a variant of PF. The butterfly shadow is characteristic of pemphigus erythematosus (13). The diagnosis of PF and pemphigus erythematosus is made via the same methodology as PV, described above.

7. Pemphigus Herpetiformis

Clinically, pemphigus herpetiformis is characterized by small vesicles arranged in an annular fashion on the pruritic erythemas, resembling clinically dermatitis herpetiformis Duhring (22). The diagnosis of pemphigus herpetiformis is exclusively on the basis of its characteristic clinical features associated with histopathological intraepidermal eosinophilic pustules with minimal acantholysis (23). IgG autoantibodies to desmocolins may contribute to pemphigus herpetiformis, although they are not always found (22, 24). The detection of autoantibodies to desmocolins is done by indirect immunofluorescence using desmocolin cDNA-transfected COS7 cells (24).

8. IgA Pemphigus

IgA pemphigus is clinically defined by generalized multiple flaccid pustules or vesicles (25). The hallmark finding of IgA pemphigus is deposition of IgA on keratinocyte cell surfaces by direct immunofluorescence (25). IgA pemphigus is subdivided into subcorneal pustular dermatosis type and intraepidermal neutrophilic IgA dermatosis type (25). The autoantigen of the former is desmocolin 1, but that of the latter is unidentified yet (25). IgA autoantibodies to desmocolin 1 were first detected by indirect immunofluorescence using desmocolin 1 cDNA-transfected COS7 cells (26).

9. Paraneoplastic Pemphigus

Paraneoplastic pemphigus is characterized by pseudomembranous conjunctivitis and refractory stomatitis (27). The skin symptoms are variable, including erythema, flaccid bullae, tense bullae, erosion, erythema multiforme-like lesions, and/or lichen planus-like lesions (28). Paraneoplastic pemphigus is associated with the presence of internal benign or malignant tumors, including Castleman's disease, malignant lymphomas and other solid cancers (14). If treatment is ineffective, cases with bronchiolitis obliterans are mostly fatal (29). Diagnosis of paraneoplastic pemphigus is now made by the positive IgG reaction by indirect immunofluorescence using rat bladder sections and a double-positive reaction to the 210 kDa envoplakin and 190 kDa periplakin by immunoblotting using normal epidermal extracts (30, 31). In addition, anti-desmoplakin and anti-plectin antibodies are sometimes also found by immunoblotting (14).

10. Autoimmune Diseases Targeting Structures Mediating Cell–Matrix Adhesion

The autoimmune bullous diseases which target the basement membrane zone and their autoantigens are presented in Table 2. Bullous pemphigoid is the most common among all autoimmune bullous diseases (32).

11. Bullous Pemphigoid

Bullous pemphigoid is the most common autoimmune bullous disease seen in the elderly, and is characterized by itchy erythema and tense bullae, caused by IgG autoantibodies to the hemidesmosomal proteins, BP180 and BP230 (33). Bullous pemphigoid patients occasionally develop mucosal lesions (33). Bullous pemphigoid is believed to have higher association of internal malignancy (34). As a pathomechanism, autoantibodies to BP180 cause migration of neutrophils and eosinophils and activation of proteases, resulting in proteolysis of the basement membrane zone (35, 36). The diagnosis of bullous pemphigoid is made by direct and indirect immunofluorescence, immunoblotting and ELISA (37, 38). Direct immunofluorescence using patient skin shows deposition of C3 and/or IgG to the basement membrane zone (37). Indirect immunofluorescence using normal human skin sections detects circulating IgG antibodies to the basement membrane zone (37). Additionally, by indirect immunofluorescence using sections of 1 M NaCl-split normal human skin, patient sera react with the epidermal side of the split (37, 38). By immunoblotting, IgG in the patient sera reacts with BP180 and/or BP230 (33). ELISA with either the recombinant NC16a domain of BP180 and mixture of C- and N-terminal domains of BP230 protein shows a sensitivity of about 85% (39, 40). However, when the both tests were performed, sensitivity raises to 96% (40).

12. Mucous Membrane Pemphigoid

Patients with mucous membrane pemphigoid occasionally develop skin lesions similar to bullous pemphigoid, which tend to heal with scars (41). However, predominant clinical manifestations are erythemas, bullae, and erosions on the oral, nasal, and ocular mucosae (42). Blindness caused by adhesive conjunctivitis is the most severe complication for mucous membrane pemphigoid (43). The two major target autoantigens are the C-terminus of BP180 and

Table 2
Classification, antibody classes, and autoantigens for autoimmune bullous diseases which target keratinocyte–extracellular matrix adhesion

Disease	Ab class	Autoantigen
Bullous pemphigoid	IgG	BP180, BP230
Herpes gestationis	IgG	BP180
Mucous membrane pemphigoid		
anti-BP180 type	IgG, IgA	BP180
anti-laminin-332 type	IgG	Laminin-332
ocular type	IgG	Integrin β 4 subunit
Linear IgA bullous dermatosis		
lamina lucida type	IgA	97/120 kDa LAD-1
sub-lamina densa type	IgA	Unidentified (type VII collagen)
Epidermolysis bullosa acquisita	IgG	Type VII collagen
Bullous SLE	IgG	Type VII collagen
Anti-laminin γ 1 pemphigoid	IgG	Laminin γ 1 subunit
Dermatitis herpetiformis Duhring	IgA	Transglutaminase 3

laminin-332 (44, 45). Diagnosis is made by direct and indirect immunofluorescence and immunoblotting. The findings of direct and indirect immunofluorescence using normal human skin sections and 1 M salt split skin sections for anti-BP180-type mucous membrane pemphigoid are the same as for bullous pemphigoid, except that IgA is also frequently detected (46, 47). The IgG and IgA antibodies react with BP180 in epidermal extracts and recombinant protein of BP180 C-terminus domain by immunoblotting (48). IgG antibodies in the sera of anti-laminin-332 mucous membrane pemphigoid react with dermal side of split skin and with laminin-332 by immunoblotting using human laminin-332 purified from normal human keratinocyte culture media (49). In addition, autoantibodies to α 6 β 4 integrin are reported to be associated with ocular-type mucous membrane pemphigoid (50).

13. Anti-Laminin γ 1 (p200) Pemphigoid

Anti-laminin γ 1 pemphigoid is characterized by tense bullae and erosions and is frequently associated with psoriasis (51). Histopathologically, it is characterized by subepidermal blisters with neutrophilic infiltrations (51). Although direct immunofluorescence and indirect immunofluorescence using normal human skin sections give the same results as bullous pemphigoid,

patient IgG reacts with the dermal side of split skin (50). Immunoblotting using dermal extracts shows IgG reactivity with a 200 kDa protein (50), which was identified as laminin γ 1 (52).

14. Herpes Gestationis

Herpes (pemphigoid) gestationis occurs during pregnancy and early postpartum, or in patients with hydatidiform moles or chorioepithelioma (53). Clinically, it is characterized by severe pruritus and tense bullae on the urticarial infiltrative erythema (54). The main autoantigen is the NC16a domain of BP180, as in bullous pemphigoid (54–56). Most patients enter remission after pregnancy, but a few cases show a prolonged clinical course (54). Although the reason why herpes gestationis occurs only in pregnancy is not known, some studies have suggested the role of HLA related immunogenetics (57, 58).

15. Dermatitis Herpetiformis Duhring

Dermatitis herpetiformis clinically shows tense vesicles on the periphery of annular infiltrative exudative erythema, which exhibit symmetrical distribution on the knees, elbows, and buttocks (59). In Caucasian, but not Japanese, patients dermatitis herpetiformis Duhring is associated with celiac disease (60). Direct immunofluorescence shows granular deposits of IgA and C3 in the papillary dermis (61). In addition, recently, the target autoantigen has been identified as epidermal transglutaminase (transglutaminase 3) (62, 63).

16. Linear IgA Bullous Dermatosi

Clinically, linear IgA bullous dermatosis develops pruritic small vesicles in the periphery of annular infiltrative erythemas, similar to dermatitis herpetiformis Duhring (64). The linear deposition of IgA at the basement membrane zone seen in direct and indirect immunofluorescence is a hallmark for the diagnosis of the disease and the origin of its name (64). The target autoantigen is 97/120 kDa LAD-1, a shedding product of BP180, excised probably by a protease of ADAM family (65). IgA autoantibodies to LAD-1 are detected by immunoblotting using concentrated HaCaT cell culture media (64). In addition, an ELISA system was developed for the detection of IgA autoantibodies against BP180 in linear IgA bullous dermatosis (66).

17. Epidermolysis Bullosa Acquisita

Epidermolysis bullosa acquisita is divided into inflammatory and non-inflammatory types; the former shows bullous pemphigoid-like skin lesion, and the latter shows non-erythematous blisters leaving scarring and milia (67). The target autoantigen is type VII collagen, a major component of the anchoring fibrils (67). Findings in indirect immunofluorescence using sections of normal human skin and salt-split skin are the same as those in anti-laminin- γ 1 pemphigoid and anti-laminin-332 mucous membrane pemphigoid (67). The detection of autoantibodies to type VII collagen by immunoblotting using normal dermal extracts as substrates is helpful for diagnosis (67).

18. Diagnostic Techniques for the Autoimmune Bullous Diseases

18.1. Immuno-fluorescence

Direct immunofluorescence for IgG, IgA, and C3 is performed in order to distinguish between pemphigus group diseases, various types of pemphigoid, and dermatitis herpetiformis Duhring (6, 33, 68). Deposition of IgG and IgA to the keratinocyte cell surfaces is indicative of the diagnosis of various types of pemphigus (6), and IgA pemphigus (25), respectively. Deposition of IgG and IgA to the basement membrane zone is characteristic for diseases of the pemphigoid group (33), and linear IgA bullous dermatosis (69), respectively. Dermatitis herpetiformis Duhring shows granular or fibrillar deposition of IgA and/or C3 in dermal papillae (68).

By indirect immunofluorescence using normal human skin sections, IgG from pemphigus patients reacts with the keratinocyte cell surfaces, while IgG from pemphigoid patients reacts with the basement membrane zone (6, 33). In mucous membrane pemphigoid, IgG and IgA to the basement membrane zone are frequently negative because of their low titers (70). IgA from linear IgA bullous dermatosis patients reacts with the basement membrane zone, although false-negative reactions are also occasionally seen, due to the low titer of the autoantibodies (71).

Indirect immunofluorescence using salt-split normal human skin sections is used for differential diagnosis of pemphigoid group diseases. 1 M NaCl treatment results in a split at the level of the lamina lucida. Sera from patients with bullous pemphigoid, anti-BP180-type mucous membrane pemphigoid and linear IgA bullous dermatosis react to the epidermal side of the split, while sera from patients with anti-laminin γ 1 pemphigoid, epidermolysis bullosa acquisita, and anti-laminin-332 mucous membrane pemphigoid react with dermal side (33, 52, 64, 67, 72). Complement immunofluorescence is used for diagnosis of herpes

gestationis (55). Moreover, indirect immunofluorescence using rat bladder sections detects anti-plakin antibodies in paraneoplastic pemphigus, and indirect immunofluorescence using COS7 cells transfected with cDNAs of desmocollins 1–3 is used to detect IgA anti-desmocollin 1 antibodies in subcorneal pustular dermatosis type IgA pemphigus and IgG antibodies to desmocollins 1–3 in pemphigus herpetiformis, pemphigus vegetans, or paraneoplastic pemphigus (26, 31).

18.2. Enzyme-Linked Immunosorbent Assays

The enzyme-linked immunosorbent assay is used to diagnose and to monitor the clinical course for pemphigus group diseases and bullous pemphigoid (21, 39, 40). The recombinant proteins are prepared by baculovirus expression for desmoglein 1 and desmoglein 3 or by *E. coli* expression for NC16a domain of BP180 and N- and C-terminal domains of BP230 (21, 39, 40). A limitation of current enzyme-linked immunosorbent assays is that the results are not always correlated with disease severity. This is thought to be mostly due to the presence of nonpathogenic antibodies (73). Therefore, future development of enzyme-linked immunosorbent assays specific for the pathogenic epitopes is required. Enzyme-linked immunosorbent assay for envoplakin is now commercially available, too (74). At the experimental stage, ELISA systems for detecting desmocollins, periplakin, type VII collagen, LAD-1, laminin γ 1, and A2ML1 are already available, but are not yet released for routine clinical diagnostic use (42, 66, 75, 76).

18.3. Immunoblot Analyses

Immunoblotting is performed as follows: normal human epidermal or dermal extracts, or keratinocyte cell lysates are electrophoretically separated and then transferred to nitrocellulose or PVDF membranes. Patient sera are then reacted with these membranes. In addition, recombinant proteins for various antigens, purified laminin-332 and concentrated culture medium of HaCaT cells are also used for subepidermal autoimmune bullous diseases. The substrates used in immunoblotting studies for each disease are summarized in Table 3.

19. Conclusions

An algorithm for the differential diagnosis for each disease is shown in Fig. 4. Using this methodology, we can logically diagnose all autoimmune bullous diseases. However, rapid progress in molecular techniques suggests that this algorithm will need sequential modification and updating. In particular, in the near future, diagnostic enzyme-linked immunosorbent assays for the entire list of the aforementioned autoantigens should be introduced for clinical use.

Table 3
The substrates used in immunoblotting studies for autoimmune bullous diseases

Substrates	Diseases
Human epidermal extract	Pemphigus, BP, herpes gestationis
Human dermal extract	EBA, anti-laminin $\gamma 1$ pemphigoid
HaCaT cell culture medium	LABD
Purified laminin-332	Laminin-332 type MMP
Recombinant proteins BP180 NC16a domain BP180 C-terminus BP230 Type VII collagen Envoplakin, periplakin	BP, herpes gestationis BP180 type MMP BP EBA PNP

BP bullous pemphigoid, *EBA* epidermolysis bullosa acquisita, *LABD* linear IgA bullous dermatosis, *MMP* mucous membrane pemphigoid, *PNP* paraneoplastic pemphigus

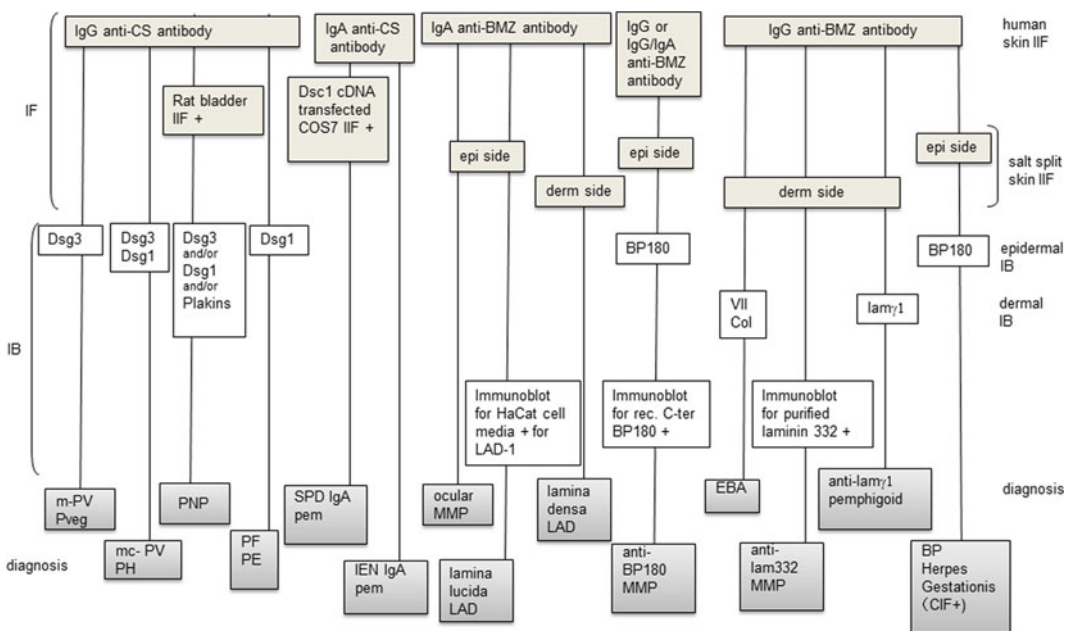


Fig. 4 Algorithm for the diagnosis for all autoimmune bullous diseases. *BMZ* basement membrane zone, *C1F* complement immunofluorescence, *CS* cell surface, *C-ter* carboxy terminus, *der* dermal, *Dsc* desmocollin, *Dsg1* desmoglein 1, *Dsg3* desmoglein 3, *EBA* epidermolysis bullosa acquisita, *epi* epidermal, *IB* immunoblot, *IEN* intraepithelial neutrophilic IgA dermatosis, *IIF* indirect immunofluorescence, *LAD* linear IgA bullous dermatosis, *lam* laminin, *MMP* mucous membrane pemphigoid, *m-PV* mucosal-dominant type pemphigus vulgaris, *mc-PV* mucocutaneous type pemphigus vulgaris, *PE* pemphigus erythematosus, *pem* pemphigus, *PF* pemphigus foliaceus, *PH* pemphigus herpetiformis, *PNP* paraneoplastic pemphigus, *Pveg* pemphigus vegetans, *rec* recombinant, *SPD* subcorneal pustular dermatosis, *VII col* type VII collagen.

Acknowledgment

We greatly appreciate Ms. Ayumi Suzuki, Ms. Takako Ishikawa, and Ms. Sachiko Sakaguchi for technical assistance, and Ms. Akiko Tanaka, Ms. Yasuko Nakayama, Ms. Emiko Hara, Ms. Hanako Tomita, Ms. Mihoko Ikeda, Ms. Kyoko Akashi, and Ms. Nobuko Ishii for secretarial work.

References

1. Lleo A, Invernizzi P, Gao B, Podda M, Gershwin ME (2010) Definition of human autoimmunity-autoantibodies versus autoimmune disease. *Autoimmun Rev* 9:A259–A266
2. Lin YS, Yeh TM, Lin CF, Wan SW, Chuang YC, Hsu TK, Liu HS, Liu CC, Anderson R, Lei HY (2011) Molecular mimicry between virus and host and its implications for dengue disease pathogenesis. *Exp Biol Med* (Maywood) 236:515–523
3. Vasanthakumari R (2007) Autoimmunity. In: Vasanthakumari R (ed) *Textbook of microbiology*, BI Publications Pvt Ltd, New Delhi, pp 154–158
4. Green KJ, Simpson CL (2007) Desmosomes: new perspectives on a classic. *J Invest Dermatol* 127:2499–2515
5. Hatsell S, Cowin P (2001) Deconstructing desmoplakin. *Nat Cell Biol* 3:E270–272
6. Stanley JR (2008) Pemphigus. In: Wolff K, Goldsmith LA, Katz SI, Gilchrist BA, Paller AS, Leffell DJ (eds) *Fitzpatrick's Dermatology in General Medicine*, 7th edn. McGraw Hill, New York, pp 459–468
7. Tsuruta D, Hashimoto T, Hamill KJ, Jones JC (2011) Hemidesmosomes and focal contact proteins: functions and cross-talk in keratinocytes, bullous diseases and wound healing. *J Dermatol Sci* 62:1–7
8. Sterk LM, Geuijen CA, Oomen LC, Calafat J, Janssen H, Sonnenberg A (2000) The tetraspan molecule CD151, a novel constituent of hemidesmosomes, associates with the integrin α 6 β 4 and may regulate the spatial organization of hemidesmosomes. *J Cell Biol* 149:969–982
9. Tsuruta D, Kobayashi H, Imanishi H, Sugawara K, Ishii M, Jones JC (2008) Laminin-332-integrin interaction: a target for cancer therapy? *Curr Med Chem* 15:1968–1975
10. Jones JC, Hopkinson SB, Goldfinger LE (1998) Structure and assembly of hemidesmosomes. *Bioessays* 20:488–494
11. Sitaru C, Zillikens D (2005) Mechanisms of blister induction by autoantibodies. *Exp Dermatol* 14:861–875
12. Hashimoto T (2003) Recent advances in the study of the pathophysiology of pemphigus. *Arch Dermatol Res* 295(Suppl 1):S2–11
13. Huilgol SC, Black MM (1995) Management of the immunobullous disorders. II. Pemphigus. *Clin Exp Dermatol* 20:283–293
14. Zimmermann J, Bahmer F, Rose C, Zillikens D, Schmidt E (2010) Clinical and immunopathological spectrum of paraneoplastic pemphigus. *J Dtsch Dermatol Ges* 8:598–606
15. Amagai M (1999) Autoimmunity against desmosomal cadherins in pemphigus. *J Dermatol Sci* 20:92–102
16. Payne AS, Hanakawa Y, Amagai M, Stanley JR (2004) Desmosomes and disease: pemphigus and bullous impetigo. *Curr Opin Cell Biol* 16:536–543
17. Barnes LM, Clark ML, Estes SA, Bongiovanni GL (1987) Pemphigus vulgaris involving the esophagus. A case report and review of the literature. *Dig Dis Sci* 32:655–659
18. Frohlich (1951) Pemphigus vulgaris of the lower lip, tongue and conjunctiva. *Z Haut Geschlechtskr* 11:xi
19. Onuma K, Kanbour-Shakir A, Modery J, Kanbour A (2009) Pemphigus vulgaris of the vagina—its cytomorphologic features on liquid-based cytology and pitfalls: case report and cytological differential diagnosis. *Diagn Cytopathol* 37:832–835
20. Pohl-Gubo G, Hintner H (2011) Direct and indirect immunofluorescence for the diagnosis of bullous autoimmune diseases. *Dermatol Clin* 29:365–372
21. Ishii K, Amagai M, Hall RP, Hashimoto T, Takayanagi A, Gamou S, Shimizu N, Nishikawa T (1997) Characterization of autoantibodies in pemphigus using antigen-specific enzyme-linked immunosorbent assays with baculovirus-expressed recombinant desmogleins. *J Immunol* 159:2010–2017
22. Tateishi C, Tsuruta D, Nakanishi T, Uehara S, Kobayashi H, Ishii M, Hashimoto T (2010) Antidesmocollin-1 antibody-positive, antidesmoglein antibody-negative pemphigus

- herpetiformis. *J Am Acad Dermatol* 63:e8–10
23. Maciejowska E, Jablonska S, Chorzelski T (1987) Is pemphigus herpetiformis an entity? *Int J Dermatol* 26:571–577
 24. Kozłowska A, Hashimoto T, Jarzabek-Chorzelska M, Amagai A, Nagata Y, Strasz Z, Jablonska S (2003) Pemphigus herpetiformis with IgA and IgG antibodies to desmoglein 1 and IgG antibodies to desmocollin 3. *J Am Acad Dermatol* 48:117–122
 25. Tsuruta D, Ishii N, Hamada T, Ohyama B, Fukuda S, Koga H, Imamura K, Kobayashi H, Karashima T, Nakama T, Dainichi T, Hashimoto T (2011) IgA pemphigus. *Clin Dermatol* 29:437–442
 26. Hashimoto T, Kiyokawa C, Mori O, Miyasato M, Chidgey MA, Garrod DR, Kobayashi Y, Komori K, Ishii K, Amagai M, Nishikawa T (1997) Human desmocollin 1 (Dsc1) is an autoantigen for the subcorneal pustular dermatosis type of IgA pemphigus. *J Invest Dermatol* 109:127–131
 27. Niimi Y, Kawana S, Hashimoto T, Kusunoki T (2003) Paraneoplastic pemphigus associated with uterine carcinoma. *J Am Acad Dermatol* 48:S69–72
 28. Frew JW, Murrell DF (2011) Paraneoplastic pemphigus (paraneoplastic autoimmune multiorgan syndrome): clinical presentations and pathogenesis. *Dermatol Clin* 29:419–425
 29. Anhalt GJ (2004) Paraneoplastic pemphigus. *J Invest Dermatol Symp Proc* 9:29–33
 30. Lee SE, Kim HR, Hashimoto T, Kim SC (2008) Paraneoplastic pemphigus developed shortly after resection of follicular dendritic cell sarcoma. *Acta Derm Venereol* 88:410–412
 31. Niimi Y, Ohyama B, Di Zenzo G, Calabresi V, Hashimoto T, Kawana S (2010) Paraneoplastic pemphigus presenting as mild cutaneous features of pemphigus foliaceus and lichenoid stomatitis with antidesmoglein 1 antibodies. *Dermatol Res Pract pii*:931340
 32. Kulthanan K, Chularojanamontri L, Tuchinda P, Sirikudta W, Pinkaew S (2011) Prevalence and clinical features of Thai patients with bullous pemphigoid. *Asian Pac J Allergy Immunol* 29:66–72
 33. Stanley JR (2008) Bullous pemphigoid. In: Wolff K, Goldsmith LA, Katz SI, Gilchrist BA, Paller AS, Leffell DJ (eds) *Fitzpatrick's dermatology in general medicine*, 7th edn. McGraw Hill, New York, pp 475–480
 34. Ogawa H, Sakuma M, Morioka S, Kitamura K, Sasai Y, Imamura S, Inaba Y (1995) The incidence of internal malignancies in pemphigus and bullous pemphigoid in Japan. *J Dermatol Sci* 9:136–141
 35. Ujiie H, Shibaki A, Nishie W, Shimizu H (2010) What's new in bullous pemphigoid. *J Dermatol* 37:194–204
 36. Liu Z, Giudice GJ, Swartz SJ, Fairley JA, Till GO, Troy JL, Diaz LA (1995) The role of complement in experimental bullous pemphigoid. *J Clin Invest* 95:1539–1544
 37. Schmidt E, Della Torre R, Borradori L (2011) Clinical features and practical diagnosis of bullous pemphigoid. *Dermatol Clin* 29:427–438
 38. Barnadas MA, Gelpi C, Curell R, de Moragas JM, Alomar A (1999) Repeat direct immunofluorescence (DIF) test, using, 1 M NaCl treated skin, in the subepidermal autoimmune bullous diseases that contain IgG at the dermal epidermal junction. *J Cutan Pathol* 26:37–41
 39. Kobayashi M, Amagai M, Kuroda-Kinoshita K, Hashimoto T, Shirakata Y, Hashimoto K, Nishikawa T (2002) BP180 ELISA using bacterial recombinant NC16a protein as a diagnostic and monitoring tool for bullous pemphigoid. *J Dermatol Sci* 30:224–232
 40. Yoshida M, Hamada T, Amagai M, Hashimoto K, Uehara R, Yamaguchi K, Imamura K, Okamoto E, Yasumoto S, Hashimoto T (2006) Enzyme-linked immunosorbent assay using bacterial recombinant proteins of human BP230 as a diagnostic tool for bullous pemphigoid. *J Dermatol Sci* 41:21–30
 41. Egan CA, Yancey KB (2000) The clinical and immunopathological manifestations of anti-epiligrin cicatricial pemphigoid, a recently defined subepithelial autoimmune blistering disease. *Eur J Dermatol* 10:585–589
 42. Groth S, Recke A, Vafia K, Ludwig RJ, Hashimoto T, Zillikens D, Schmidt E (2011) Development of a simple enzyme-linked immunosorbent assay for the detection of autoantibodies in anti-p200 pemphigoid. *Br J Dermatol* 164:76–82
 43. Fleming TE, Korman NJ (2000) Cicatricial pemphigoid. *J Am Acad Dermatol* 43:571–591, quiz 591–574
 44. Zillikens D (2002) BP180 as the common autoantigen in blistering diseases with different clinical phenotypes. *Keio J Med* 51:21–28
 45. Dainichi T, Takeshita H, Moroi Y, Urabe K, Yoshida M, Hisamatsu Y, Komai A, Duan H, Koga T, Hashimoto T, Furue M (2005) Cicatricial pemphigoid with autoantibodies against the laminin 5 gamma 2 subunit. *Eur J Dermatol* 15:189–193
 46. Balding SD, Prost C, Diaz LA, Bernard P, Bedane C, Aberdam D, Giudice GJ (1996)

- Cicatricial pemphigoid autoantibodies react with multiple sites on the BP180 extracellular domain. *J Invest Dermatol* 106:141–146
47. Horvath B, Niedermeier A, Podstawa E, Muller R, Hunzelmann N, Karpati S, Hertl M (2010) IgA autoantibodies in the pemphigoids and linear IgA bullous dermatosis. *Exp Dermatol* 19:648–653
 48. Kobayashi K, Tanaka M, Nakajima S, Ito H, Harada T, Hashimoto T (2009) Simultaneous occurrence of anti-BP180 mucous membrane pemphigoid and mucosal-dominant pemphigus vulgaris. *Clin Exp Dermatol* 34:e785–e788
 49. Lazarova Z, Salato VK, Lanschuetzer CM, Janson M, Fairley JA, Yancey KB (2008) IgG anti-laminin-332 autoantibodies are present in a subset of patients with mucous membrane, but not bullous, pemphigoid. *J Am Acad Dermatol* 58:951–958
 50. Bhol KC, Dans MJ, Simmons RK, Foster CS, Giancotti FG, Ahmed AR (2000) The autoantibodies to alpha 6 beta 4 integrin of patients affected by ocular cicatricial pemphigoid predominantly epitopes within the large cytoplasmic domain of human beta 4. *J Immunol* 165:2824–2829
 51. Dilling A, Rose C, Hashimoto T, Zillikens D, Shimanovich I (2007) Anti-p200 pemphigoid: a novel autoimmune subepidermal blistering disease. *J Dermatol* 34:1–8
 52. Dainichi T, Kurono S, Ohya Y, Ishii N, Sanzen N, Hayashi M, Shimono C, Taniguchi Y, Koga H, Karashima T, Yasumoto S, Zillikens D, Sekiguchi K, Hashimoto T (2009) Anti-laminin gamma-1 pemphigoid. *Proc Natl Acad Sci U S A* 106:2800–2805
 53. Halkier-Sorensen L, Beck HI, Sogaard H (1985) Herpes gestationis in association with neoplasma malignum generalisata. A case report. *Acta Derm Venereol Suppl (Stockh)* 120:96–100
 54. Intong LR, Murrell DF (2011) Pemphigoid gestationis: pathogenesis and clinical features. *Dermatol Clin* 29:447–452
 55. Murakami H, Amagai M, Higashiyama M, Hashimoto K, Chorzelski TP, Bhogal BS, Jenkins RE, Black MM, Zillikens D, Nishikawa T, Hashimoto T (1996) Analysis of antigens recognized by autoantibodies in herpes gestationis. Usefulness of immunoblotting using a fusion protein representing an extracellular domain of the 180 kD bullous pemphigoid antigen. *J Dermatol Sci* 13:112–117
 56. Matsumura K, Amagai M, Nishikawa T, Hashimoto T (1996) The majority of bullous pemphigoid and herpes gestationis serum samples react with the NC16a domain of the 180-kDa bullous pemphigoid antigen. *Arch Dermatol Res* 288:507–509
 57. Semkova K, Black M (2009) Pemphigoid gestationis: current insights into pathogenesis and treatment. *Eur J Obstet Gynecol Reprod Biol* 145:138–144
 58. Al-Fouzan AW, Galadari I, Oumeish I, Oumeish OY (2006) Herpes gestationis (Pemphigoid gestationis). *Clin Dermatol* 24:109–112
 59. Rose C, Brocker EB, Zillikens D (2010) Clinical, histological and immunopathological findings in 32 patients with dermatitis herpetiformis. *J Dtsch Dermatol Ges* 8: 265–270, 265–271
 60. Marks J, Shuster S, Watson AJ (1966) Small-bowel changes in dermatitis herpetiformis. *Lancet* 2:1280–1282
 61. Zone JJ, Meyer LJ, Petersen MJ (1996) Deposition of granular IgA relative to clinical lesions in dermatitis herpetiformis. *Arch Dermatol* 132:912–918
 62. Rose C, Armbruster FP, Ruppert J, Igl BW, Zillikens D, Shimanovich I (2009) Autoantibodies against epidermal transglutaminase are a sensitive diagnostic marker in patients with dermatitis herpetiformis on a normal or gluten-free diet. *J Am Acad Dermatol* 61:39–43
 63. Asano Y, Makino T, Ishida W, Furuichi M, Shimizu T (2011) Detection of antibodies to epidermal transglutaminase but not tissue transglutaminase in Japanese patients with dermatitis herpetiformis. *Br J Dermatol* 164:883–884
 64. Horiguchi Y, Ikoma A, Sakai R, Masatsugu A, Ohta M, Hashimoto T (2008) Linear IgA dermatosis: report of an infantile case and analysis of 213 cases in Japan. *J Dermatol* 35:737–743
 65. Franzke CW, Bruckner-Tuderman L, Blobel CP (2009) Shedding of collagen XVII/BP180 in skin depends on both ADAM10 and ADAM9. *J Biol Chem* 284:23386–23396
 66. Csorba K, Schmidt S, Florea F, Ishii N, Hashimoto T, Hertl M, Karpati S, Bruckner-Tuderman L, Nishie W, Sitaru C (2011) Development of an ELISA for sensitive and specific detection of IgA autoantibodies against BP180 in pemphigoid diseases. *Orphanet J Rare Dis* 6:31
 67. Ishii N, Hamada T, Dainichi T, Karashima T, Nakama T, Yasumoto S, Zillikens D, Hashimoto T (2010) Epidermolysis bullosa acquisita: what's new? *J Dermatol* 37:220–230
 68. Cardones AR, Hall RP 3rd (2011) Pathophysiology of dermatitis herpetiformis: a model for cutaneous manifestations of gastrointestinal inflammation. *Dermatol Clin* 29: 469–477

69. Yanagihara S, Mizuno N, Naruse A, Tateishi C, Tsuruta D, Ishii M (2011) Linear immunoglobulin G/immunoglobulin A bullous dermatosis associated with Vogt-Koyanagi-Harada disease. *J Dermatol* 38:798–801
70. Murakami H, Nishioka S, Setterfield J, Bhogal BS, Black MM, Zillikens D, Yancey KB, Balding SD, Giudice GJ, Diaz LA, Nishikawa T, Kiyokawa C, Hashimoto T (1998) Analysis of antigens targeted by circulating IgG and IgA autoantibodies in 50 patients with cicatricial pemphigoid. *J Dermatol Sci* 17:39–44
71. Kharfi M, Khaled A, Karaa A, Zaraa I, Fazaa B, Kamoun MR (2010) Linear IgA bullous dermatosis: the more frequent bullous dermatosis of children. *Dermatol Online J* 16:2
72. Challacombe SJ, Setterfield J, Shirlaw P, Harman K, Scully C, Black MM (2001) Immunodiagnosis of pemphigus and mucous membrane pemphigoid. *Acta Odontol Scand* 59:226–234
73. Yokouchi M, Saleh MA, Kuroda K, Hachiya T, Stanley JR, Amagai M, Ishii K (2009) Pathogenic epitopes of autoantibodies in pemphigus reside in the amino-terminal adhesive region of desmogleins which are unmasked by proteolytic processing of prosequence. *J Invest Dermatol* 129:2156–2166
74. Schmidt E, Zillikens D (2010) Modern diagnosis of autoimmune blistering skin diseases. *Autoimmun Rev* 10:84–89
75. Saleh MA, Ishii K, Kim YJ, Murakami A, Ishii N, Hashimoto T, Schmidt E, Zillikens D, Shirakata Y, Hashimoto K, Kitajima Y, Amagai M (2011) Development of NC1 and NC2 domains of Type VII collagen ELISA for the diagnosis and analysis of the time course of epidermolysis bullosa acquisita patients. *J Dermatol Sci* 62:169–175
76. Probst C, Schlumberger W, Stocker W, Recke A, Schmidt E, Hashimoto T, Zhu XJ, Zillikens D, Komorowski L (2009) Development of ELISA for the specific determination of autoantibodies against envoplakin and periplakin in paraneoplastic pemphigus. *Clin Chim Acta* 410:13–18



<http://www.springer.com/978-1-62703-226-1>

Molecular Dermatology

Methods and Protocols

Has, C.; Sitaru, C. (Eds.)

2013, XII, 460 p., Hardcover

ISBN: 978-1-62703-226-1

A product of Humana Press