

The Value of Type IV Collagen Immunohistochemical Staining in the Differential Diagnosis of Autoimmune Subepidermal Bullous Diseases

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ABSTRACT Autoimmune subepidermal bullous diseases (AISBDs) exhibit various clinical presentations, histological appearances, prognoses, and responses to treatment. Many diagnostic techniques, such as direct immunofluorescence (IF), indirect salt-split skin IF, and enzyme-linked immunosorbent assays, are used in the differential diagnoses of AISBDs. However, these techniques require fresh frozen tissue, expensive laboratory equipment, and sophisticated laboratory techniques. The purpose of this study was to evaluate the value of type IV collagen immunohistochemical (IHC) staining for the differential diagnosis of AISBDs. Paraffin-embedded blocks of skin biopsies were selected from 28 patients with autoimmune subepidermal bullous diseases. Among these 28 cases, 24 patients exhibited bullous pemphigoid (BP), 2 exhibited epidermolysis bullosa acquisita (EBA), 1 exhibited linear immunoglobulin A dermatosis (LAD), and 1 exhibited bullous systemic lupus erythematosus (BSLE). Sections were stained for type IV collagen and examined to determine the location of type IV collagen in the subepidermal blister. Type IV collagen positivity was observed on the base of the subepidermal blister in patients with BP (24 of 24 cases) and LAD (1 of 1 case). Staining was observed on the roof of the blister in patients with EBA (2 of 2 cases) and BSLE (1 of 1 case), and irregular staining was also observed on the base in patients with EBA. In conclusion, type IV collagen IHC staining is a simple and useful diagnostic technique for the differential diagnosis of AISBDs.

KEY WORDS: bullous pemphigoid, epidermolysis bullosa acquisita, type IV collagen

INTRODUCTION

Autoimmune bullous diseases are a heterogeneous group of disorders caused by circulating autoantibodies against distinct adhesion molecules of the skin. Among them, diseases that form blisters on the dermo-epidermal junction and produce autoantibodies to hemidesmosomal components in the

epidermal basement membrane zone are called autoimmune subepidermal bullous disorders (AISBDs) and include bullous pemphigoid (BP), linear IgA dermatosis (LAD), epidermolysis bullosa acquisita (EBA), and bullous systemic lupus erythematosus (BSLE). Providing a specific diagnosis is important due to the

variable prognoses and responses to treatments of these diseases. However, as they are very similar in terms of clinical symptoms and histopathological findings, these diseases often pose a major diagnostic challenge (1). Many diagnostic techniques are employed to differentiate AISBDs, such as direct and indirect immunofluorescence (IF), immunoelectron microscopy, and enzyme-linked immunosorbent assays (ELISAs) (1). However, these techniques require fresh frozen tissues, expensive laboratory equipment, and sophisticated laboratory processes. Type IV collagen is the primary collagen found in extracellular basement membranes and is a major component of the dermal-epidermal junction, where it is mostly found in the lamina densa. Accordingly, its location can be classified as the roof or base according to the separation plane of AISBDs, which may be helpful for the differential diagnosis of these diseases (2-6). In this study, we evaluated the value of type IV collagen immunohistochemical (IHC) staining of formalin-fixed, paraffin-embedded (FFPE) tissue sections for the differential diagnosis of AISBDs.

METHODS

Subjects

This study was approved by the Inje University Ilsan Paik Hospital Institutional Review Board Committee. We retrospectively reviewed patients diagnosed with AISBDs with subepidermal blisters between January 2008 and December 2014 in the Department of Dermatology, Inje University Ilsan Paik Hospital and Ewha University Mokdong Hospital using hematoxylin and eosin (H&E)-stained slides of skin biopsy specimens. The patients were diagnosed with AISBDs based on clinical symptoms, histopathological findings, and the results of direct IF. BP was diagnosed on the basis of clinically pruritic tense blisters without mucosal erosion, scars or milia, histological evidence of bullous pemphigoid with linear deposits of immunoglobulin G (IgG), and/or complement 3 (C3) at the dermal-epidermal junction by direct IF. LAD was diagnosed on the basis of histopathologic findings and linear deposits of immunoglobulin A (IgA) at the dermal-epidermal junction by direct IF with proper clinical features. The diagnosis of EBA was established by typical clinical findings such as blisters and erosions on mucocutaneous areas and dystrophic changes, such as milia, atrophic scars, nail deformities, and histological evidence of subepidermal blisterings with various inflammatory cell infiltration. Direct IF was performed on one patient with EBA. Diagnosis of BSLE was established on the basis of clinical findings and histological evidence of subepidermal blisters with prominent neutrophil infiltration and mucin deposit in the dermis in a patient with

known systemic lupus erythematosus. The research subjects included 24 patients with BP, 2 patients with EBA, 1 patient with BSLE, and 1 patient with LAD.

IHC staining

IHC staining for type IV collagen expression was performed in FFPE tissue sections using a BenchMark XT automated slide stainer (Ventana Medical Systems, Inc., Tucson, AZ, USA) according to the manufacturer's instructions. FFPE tissue from the subject was sliced in 4 µm thick sections. Briefly, the sections were first deparaffinized and rehydrated, and endogenous peroxidase activity was quenched with hydrogen peroxide. The slide was immersed in a container filled with citrate buffer (pH 6.0) and heated for 5 minutes in a microwave oven. It was then cooled at room temperature for 20 minutes. A primary anti-type IV collagen mouse monoclonal antibody (Leica Biosystems, Newcastle, UK: PHM-12) was applied at a dilution of 1:100 for 60 minutes at 25 °C. An Ultra-View™ Universal DAB detection kit (Ventana Medical Systems Inc., Tucson, AZ, USA) was used for visualization of antibody reactions. IHC-stained sections were examined to determine the location of collagen IV at the blister sites by one dermatologist and one dermatopathologist. The location of type IV collagen was classified as the roof and/or base for all of the subepidermal blisters.

RESULTS

In all cases, positive staining for type IV collagen was observed in the basement membrane underlying the epidermis and surrounding the blood vessels and skin appendages (Figure 1). Type IV collagen positivity was observed on the blister bases for all 24 cases of BP (Figure 2. a) and the one case of LAD (Figure 2. b). For the 2 cases of EBA (Figure 3. a), apparent linear staining was observed on the roof, but irregular staining was also observed on the base. In the patient with BSLE, apparent linear staining was observed on the roof of the blister but not on the base (Figure 3. b), (Table 1).

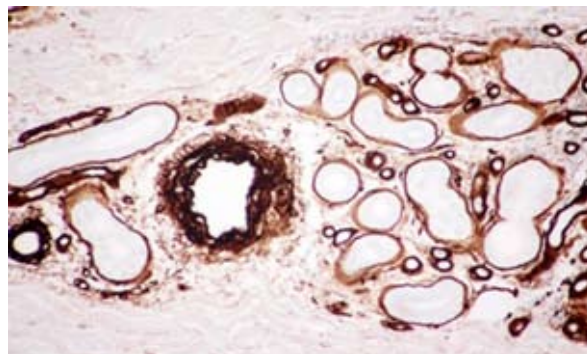


Figure 1. Type IV collagen immunohistochemical staining in the basement membrane of vessels and eccrine glands.

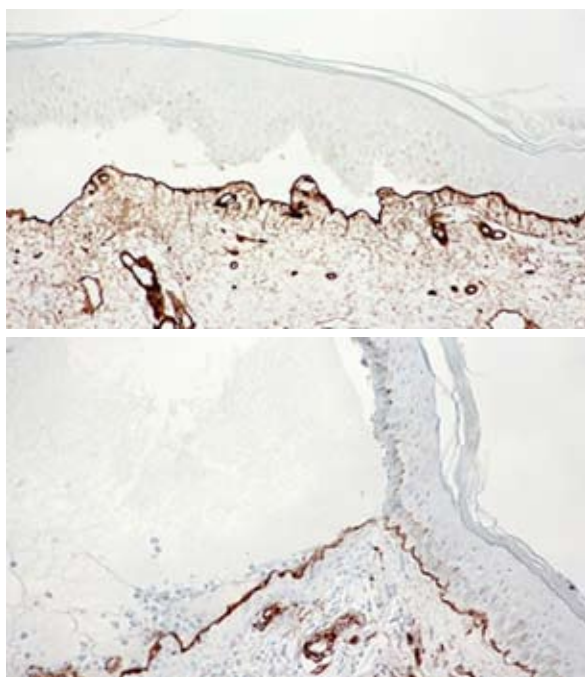


Figure 2. (a, b) Type IV collagen immunohistochemical staining in the floor of subepidermal blisters from patients with bullous pemphigoid (A×200) and linear immunoglobulin A dermatosis (B×200).

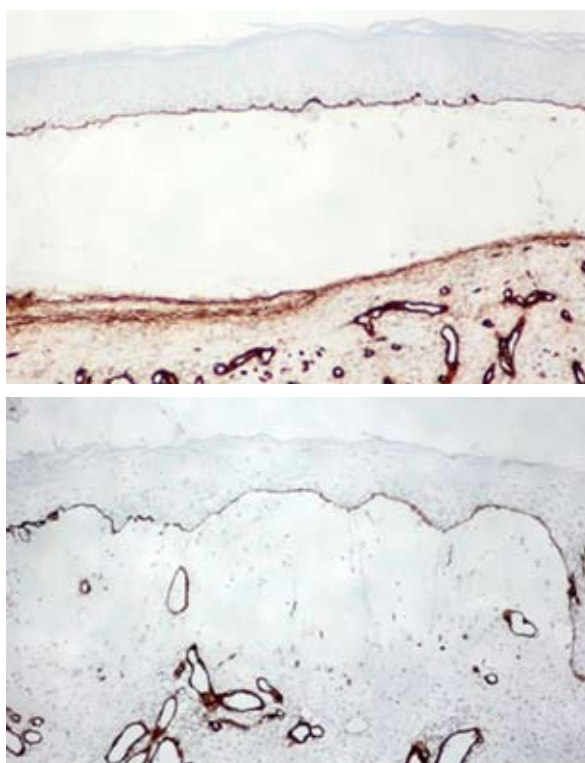


Figure 3. (a, b) Type IV collagen immunohistochemical staining in epidermolysis bullosa acquisita patients showing linear staining on the roof and irregular staining on the base of a blister (A×100). Linear staining is observed on the roof in bullous systemic lupus erythematosus patients (B×100).

Table 1. The results of direct immunofluorescence and immunolocalization of type IV collagen in patients with autoimmune subepidermal blistering diseases

| Disease | DIF | | | Location of type IV collagen IHC staining | |
|---------|-----|-----|-----|---|------|
| | IgG | IgA | C3 | Floor | Roof |
| BP1 | - | - | + | + | - |
| BP2 | + | - | + | + | - |
| BP3 | - | N/A | + | + | - |
| BP4 | + | N/A | + | + | - |
| BP5 | + | N/A | + | + | - |
| BP6 | - | N/A | + | + | - |
| BP7 | + | N/A | + | + | - |
| BP8 | + | N/A | + | + | - |
| BP9 | + | N/A | + | + | - |
| BP10 | + | N/A | + | + | - |
| BP11 | + | N/A | - | + | - |
| BP12 | + | - | + | + | - |
| BP13 | + | N/A | - | + | - |
| BP14 | - | N/A | + | + | - |
| BP15 | + | - | N/A | + | - |
| BP16 | - | - | + | + | - |
| BP17 | - | - | + | + | - |
| BP18 | + | - | + | + | - |
| BP19 | - | - | + | + | - |
| BP20 | + | - | + | + | - |
| BP21 | + | N/A | + | + | - |
| BP22 | + | - | + | + | - |
| BP23 | + | - | + | + | - |
| BP24 | + | - | + | + | - |
| LAD | - | + | - | + | - |
| EBA1 | + | - | + | irregular | + |
| EBA2 | N/A | N/A | N/A | irregular | + |
| BSLE | N/A | N/A | N/A | - | + |

BP: bullous pemphigoid; BSLE: Bullous systemic lupus erythematosus; C3: Complement 3; EBA: Epidermolysis bullosa acquisita; LAD: Linear immunoglobulin A dermatosis; IgA: Immunoglobulin A; IgG: Immunoglobulin G; +: Positive reactivity; -: Negative reactivity, N/A: Not available

DISCUSSION

AISBDs are potentially life-threatening skin diseases, clinically characterized by blisters and erosions on the skin and mucous membranes. AISBDs are classified based on clinical, histopathological, and immunological criteria. However, they are sometimes very similar in terms of clinical symptoms and histopathological findings, and these diseases often pose a major diagnostic challenge (1). BP is caused by autoantibodies directed against two hemidesmosomal antigens, bullous pemphigoid antigen 1 (BPAG1) and bullous pemphigoid antigen 2 (BPAG2). Histopathologically, it is characterized by subepidermal separation at the dermo-epidermal junction and an inflammatory cell infiltration that tends to be rich in eosinophils inside of blisters and the upper dermis. Using direct IF, continuous linear deposition of IgG and/or C3 can be observed along the dermo-epidermal junction (1,7). In LAD, neutrophils mainly infiltrate at the tip of the papillary dermis with subepidermal blister formation. Direct IF reveals a homogeneous linear pattern of IgA deposition along the basement membrane zone (1,7). In patients with BSLE, subepidermal splitting and papillary microabscesses resembling dermatitis herpetiformis (DH) have been observed. Direct IF often shows linear or granular IgG, IgA, IgM, or C3 deposition along the dermo-epidermal junction, and antibodies to type VII collagen have been detected in several patients (8). EBA is a disease that is caused by IgG autoantibodies against type VII collagen, a main component in anchoring fibrils below the lamina densa of the basement membrane (1,3). EBA is clinically and histopathologically indistinguishable from BP. These conditions may be distinguished using indirect IF (with salt-split skin as a substrate), ELISAs, Western immunoblotting of both epidermal and dermal extracts, and direct immunoelectron microscopy studies. However, indirect

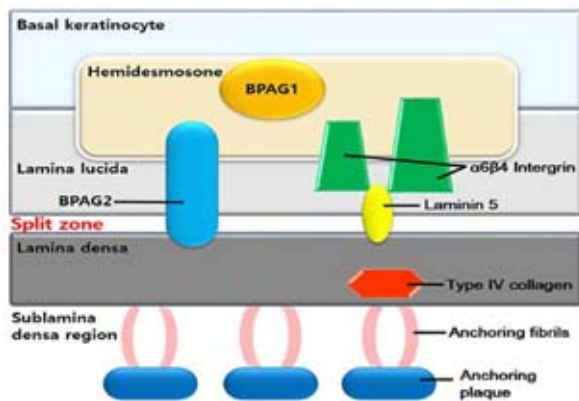


Figure 4. Schematic model of a subepidermal blister in bullous pemphigoid and linear immunoglobulin A dermatosis patients.

IF requires fresh frozen tissue and is difficult to standardize. Immunoblotting and immunoprecipitation can also be used to identify the target antigens. However, they are both time-consuming qualitative techniques. ELISA is a sensitive method for the diagnosis of these conditions and can be completed within one day. However, it requires sophisticated laboratory processing and specific equipment (1,9,10).

Type IV collagen consists of 6 α -chain polypeptides and is a major component of the basement membrane zone, where it forms the lamina densa of the hemidesmosome together with laminin and nidogen. Basement membrane zones are widely distributed in extracellular matrices that interface with the basilar portion of epithelial and endothelial cells and surround muscle, adipose, and Schwann cells. Additionally, type IV collagen is important in wound healing and in embryogenesis (7,10).

In this study, we performed type IV collagen IHC staining to differentiate AISBDs. In BP, because autoantibodies bind to BPAG1 and BPAG2, hemidesmosomal protein and type IV collagen of the lamina densa should be found at the base of the blister when subepidermal blistering occurs (Fig. 4) (4-7,11). In LAD, subepidermal blisters are formed by separation at the lamina lucida above lamina densa, and similar to BP, type IV collagen positivity is found on the base of the blister (Figure 4) (4-7,11). In contrast, in EBA and BSLE, subepidermal blistering occurs due to autoantibodies against type VII collagen, an exclusive component of anchoring fibrils that forms loops originating and terminating in the lamina densa. Therefore, the binding of autoantibodies to their target, type VII collagen, results in the separation of dermal anchoring fibrils from the lamina densa, and type IV collagen positivity may be found at the roof of the blister (Figure 5) (4-7,11,12).

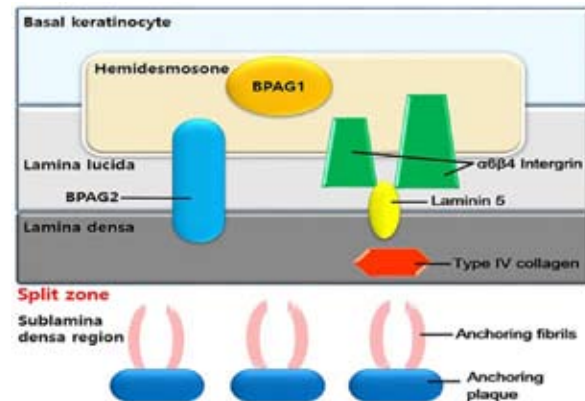


Figure 5. Schematic model of a subepidermal blister in epidermolysis bullosa acquisita and bullous systemic lupus erythematosus patients.

In this study of 28 patients, all cases of BP (24 cases) and LAD (1 case) showed apparent linear staining of type IV collagen on the base of blisters. For BSLE (1 case), linear staining was observed on the roof of subepidermal blisters. However, in patients with EBA (2 cases), linear staining was observed on the roof of the blister and irregular staining was also observed on the base, which was different from BP which showed linear staining only on the base in all cases. Pardo *et al.* (4) conducted type IV collagen IHC staining using FFPE tissue for 45 cases of subepidermal blistering. Their results showed that linear staining was observed on the base of blisters in patients with BP, dermatitis herpetiformis, and porphyria cutanea tarda. However, for patients with EBA, type IV collagen staining was observed on the roof of blisters. In a study of dog skin tissue by Olivry *et al.* (5) in 14 cases of EBA, 6 cases showed type IV collagen staining on the roof of blisters, 4 cases showed staining on the base, and 4 cases showed staining on both sides of blisters. In contrast, in 21 cases of subepidermal bullous diseases other than EBA, no case showed staining on the roof alone. The presence of type IV collagen above subepidermal clefts exhibited a sensitivity of 71%, a specificity of 90%, and a positive predictive value and accuracy of 83% for the diagnosis of canine EBA. Thus, collagen staining was reported to be a less expensive and more useful and convenient method than other diagnosis methods. In some cases of EBA, type IV collagen positivity was observed on both sides of subepidermal blisters. Based on this result, some authors have suggested that type IV collagen may be proteolyzed by inflammatory cells (4,6,13) and exhibit a separation within the lamina lucida, most likely reflecting the formation of a subepidermal blister at the *locus minoris resistentiae* (14). Nonetheless, when type IV collagen IHC staining is present on both the blister roof and floor, it will most often appear irregular and of varying intensity (perhaps digested) on the dermal side of the clefts (5), as also observed in our study.

Our study was limited by its retrospective nature and lack of comparison with results from indirect salt-split IF or ELISA. Moreover, fewer cases of diseases other than BP were included due to their low prevalence rates. However, we confirmed the usefulness of type IV collagen IHC staining as a simple method with a high positive predictive value for the differential diagnosis of AISBDs.

CONCLUSION

We performed type IV collagen IHC staining using FFPE tissues from patients with AISBDs. Our results demonstrated that BP and LAD samples exhibited staining on the base of subepidermal blisters in all

cases without staining on the roof, and BSLE samples showed staining on the roof of subepidermal blisters. EBA samples revealed linear positivity on the roof with irregular staining on the base of blister. Type IV collagen IHC staining has high sensitivity and specificity and can be used with FFPE tissue. In addition, this method does not require additional equipment and is simple and low cost. In conclusion, our study confirmed the value of type IV collagen IHC staining for the diagnosis of AISBDs, particularly for the differentiation between BP and EBA.

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References:

1. Kneisel A, Hertl M. Autoimmune bullous skin diseases. Part 2: diagnosis and therapy. *J Dtsch Dermatol Ges.* 2011;9:927-47.
2. Abreu-Velez AM, Howard MS. Collagen IV in normal skin and in pathological processes. *N Am J Med Sci.* 2012;4:1-8.
3. Vassileva S, Drenovska K, Manuelyan K. Autoimmune blistering dermatoses as systemic diseases. *Clin Dermatol.* 2014;32:364-75.
4. Pardo RJ, Penneys NS. Location of basement membrane type IV collagen beneath subepidermal bullous diseases. *J Cutan Pathol.* 1990;17:336-41.
5. Olivry T, Dunston SM. Usefulness of collagen IV immunostaining for diagnosis of canine epidermolysis bullosa acquisita. *Vet Pathol.* 2010;47:565-8.
6. Bowszyc-Dmochowska M, Hashimoto T, Dmochowski M, Nishikawa T. Evaluation of an avidin-biotin-peroxidase method with a monoclonal antibody to type IV collagen in the differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita. *J Dermatol.* 1997;24:217-22.
7. Wu H, Allan AE, Harrist TJ. Noninfectious vesicobullous and vesicopustular diseases. In: Elder DE, Elenitsas R, Rosenbach M, Murphy GE, Rubin AI, Xu X, editors. *Lever's histopathology of the skin.* 11th ed. Philadelphia: Wolters Kluwer; 2015. pp.298-310.
8. Gammon WR, Briggaman RA. Bullous SLE: a phenotypically distinctive but immunologically heterogeneous bullous disorder. *J Invest Dermatol.* 1993;100:285-345.

9. Gan SD, Patel KR. Enzyme immunoassay and enzyme-linked immunosorbent assay. *J Invest Dermatol.* 2013;133:e12.
10. Lee EH, Kim YH, Kim S, Kim SE, Kim SC. Usefulness of enzyme-linked immunosorbent assay using recombinant BP180 and BP230 for serodiagnosis and monitoring disease activity of bullous pemphigoid. *Ann Dermatol.* 2012;24:45-55.
11. Inherited and autoimmune subepidermal blistering diseases. In: Calonje JE, Brenn T, Lazar A, McKee PH, editors. *McKee's pathology of the skin: with clinical correlations*, 4th ed. Philadelphia: Elsevier Saunders; 2012. pp.99-150.
12. LeBleu VS, Macdonald B, Kalluri R. Structure and function of basement membranes. *Exp Biol Med (Maywood).* 2007;232:1121-9.
13. Koskela M, Gaddnas F, Koivukangas V, Oikarinen A, Laurila J, Kallioinen M, *et al.* Dermal expression of laminin-332 and type IV collagen in humans with severe sepsis. *Acta Anaesthesiol Scand.* 2015;59:1009-14.
14. Bernard P, Borradori L. Pemphigoid group. In: Bologna J, Jorizzo JL, Schaffer JV, editors. *Dermatology*, 3rd ed. Philadelphia: Elsevier Saunders; 2012. pp.475-490.