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LETTER TO THE EDITOR

Anti-β4 integrin autoantibodies in patients with mucous membrane pemphigoid: A retrospective analysis from a tertiary centre in Italy

Editor,

Mucous membrane pemphigoid (MMP) is a rare mucosal-dominant subepidermal autoimmune bullous disease, characterized by autoantibodies targeting different basement membrane zone (BMZ) molecules, including collagen XVII (BP180), laminin 332 and collagen VII.[1] A limited number of studies suggested β4 integrin as a relevant autoantigen of MMP,[2–6] especially of ocular MMP, but the prevalence of these autoantibodies in MMP remains elusive.[1,7,8] Although searching for anti-β4 integrin antibodies is not yet recommended for the diagnosis of MMP, current guidelines strongly advice more investigations to clarify this issue.[9]

In this 3-year monocentric retrospective study started in January 2018 (Ethical approval CEAVC 21730), we retrieved serum samples from 40 patients who were diagnosed with MMP, according to current guidelines.[9] Anamnestic, clinical and immunopathological features of patients are reported in Table 1. Sera from eight healthy donors were included as negative controls.

We tested serum IgG/IgA reactivity to β4 integrin by immunoblotting (IB) of A431 cell lysates. To identify the band corresponding to β4 integrin in A431cell lysates, we performed IB on A431 cell lysates using a commercial antibody targeting the COOH-terminus domain of β4 integrin, and sera from five Japanese ocular MMP patients that had been previously proven positive for anti-β4 integrin autoantibodies.[2] To further confirm the specificity of the assay, we performed IB using cell lysates of A431 cells, *β4 integrin* of which was silenced by specific siRNA (A431 ITGB4-), and compared with non-treated cells.

In accordance with a previous report,[10] the commercial antibody reacted with a major band with an apparent molecular weight of 250 kDa and, albeit not consistently, with a lower molecular weight band of 205 kDa; both bands were drastically reduced in IB of A431 ITGB4—cell lysates (Figure 1a,b). The same reactivity was observed with serum samples of Japanese ocular MMP patients (Figure 1c).

In our cohort, sera of 6 (15%) of 40 MMP patients (four females; two males; mean age 68.5 years, age range

TABLE 1 Demographic, clinical and immunopathological characteristics of 40 patients with MMP

Characteristic	No. (%)
Age at diagnosis	
Mean (range), years	74.6 (43–93)
Gender	
Female	22 (55.0)
Male	18 (45.0)
Sites of the lesions	
Oral mucosa	26/40 (65.0)
Ocular mucosa	22/40 (55.0)
Nasal mucosa	4/40 (10.0)
Genital mucosa	7/40 (17.5)
Pharyngeal	2/40 (5.0)
Laryngeal	1/40 (2.5)
Tracheal	0/40
Oesophageal	1/40 (2.5)
Cutaneous	9/40 (22.5)
Ocular monosite	10/40 (25.0)
Oral monosite	11/40 (27.5)
Multisite	19/40 (47.5)
Antigen specificity	
BP180 NC16A domain (IgG) (ELISA, Euroimmun, Lubeck, Germany)	16/40 (40.0)
BP230 (IgG) (ELISA, Euroimmun, Lubeck, Germany)	0/40
Laminin 332 (IgG/IgA) (IIF on HEK-293 expressing recombinant laminin 332, BIOCHIP, Euroimmun).	0/40
Collagen VII IgG (ELISA)	0/40
β4 integrin (IgG/IgA) (IB)	6/40 (15.0)
Linear deposits of immunoglobulins by DIF	35/37 (94.6)
Linear reactivity with epidermal side of the split by SSS-IIF on human salt-split skin	17/38 (44.7)

Note: Diagnosis of MMP was based on compatible clinical features and direct immunofluorescence. For ocular MMP patients without a positive direct immunofluorescence, the diagnosis was confirmed by compatible light microscopy findings and detection of circulating autoantibodies by either SSS-IIF or antigen-specific techniques.

Abbreviations: ELISA, enzyme-linked immunosorbent-assay; DIF, direct immunofluorescence; SSS-IIF, indirect immunofluorescence on salt-split-skin; IB, immunoblotting.

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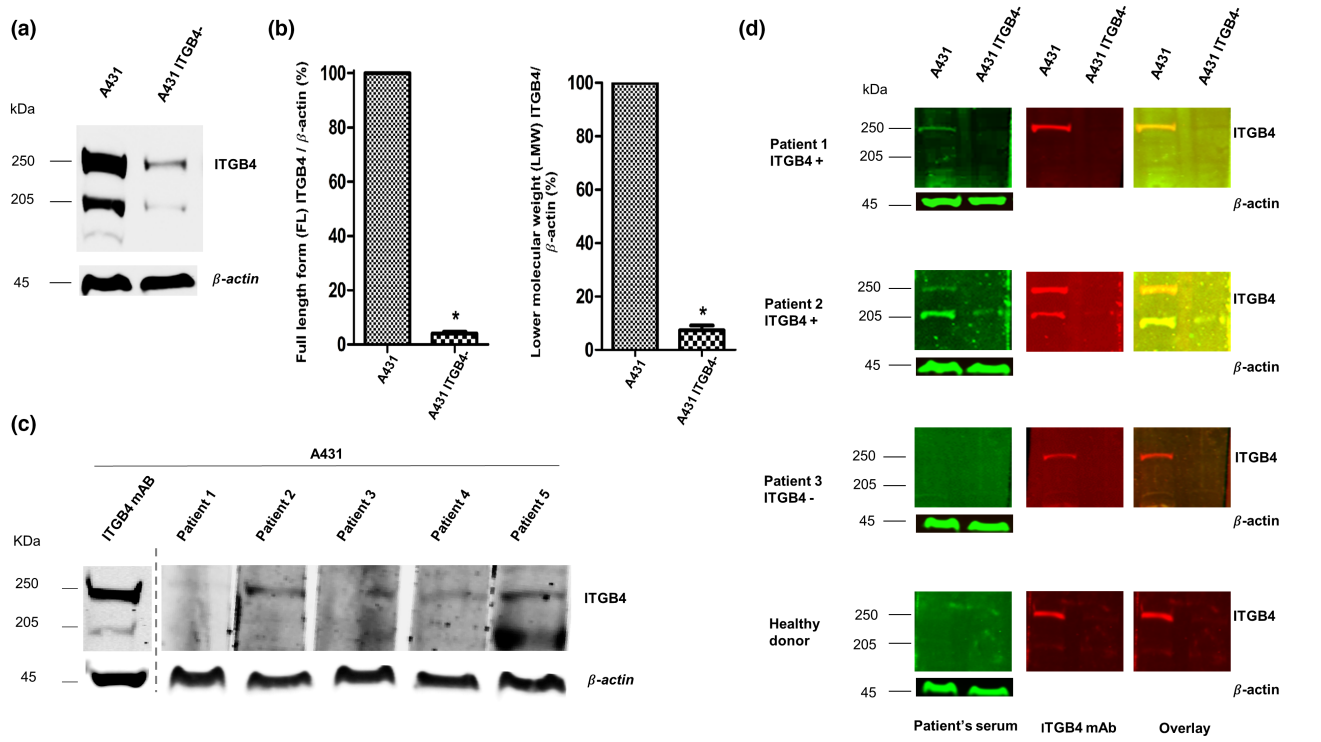

FIGURE 1 (a) Immunoblotting (IB) using lysate of A431 cell (ATCC® CRL-1555™) (cell applications, San Diego, CA, USA) performed with a commercial antibody directed to the C-terminal portion of β4 integrin (B7, Santa Cruz biotechnology, Dallas, TX, USA). A431 cells were transfected either with a negative control siRNA (A431) or with a *β4 integrin*-specific siRNA (*β4 integrin* siRNA sc-35678, Santa Cruz biotechnology) (A431 ITGB4-). Immunoblotting was then performed with the indicated antibodies. β-Actin IB was performed to verify the equality in protein loading. Molecular weight markers are indicated on the left. (b) Quantification and graphical reproduction of the IB signal relative to the higher and lower molecular weight bands of β4 integrin before and after silencing of *β4 integrin*. Data were normalized for β-Actin content. (c) Like the commercial antibody, sera from five Japanese patients with ocular MMP demonstrated reactivity to the 250 kDa band corresponding to β4 integrin in IB of A431 cell lysates. Some sera also demonstrated weakly reactivity to the 205 kDa band. (d) Reactivity to β4 integrin of MMP sera using IB of A431 cell lysates. Representative images from two patients positive for anti-β4 integrin antibodies as well as one patient and a healthy control negative for anti-β4 integrin antibodies were shown. Patients were considered positive for anti-β4 integrin autoantibodies when the major and/or the lower molecular weight bands detected with sera (green) or with the commercial antibody (red) were superposed (yellow). Specificity of the obtained bands is witnessed by their reduction upon *β4 integrin* silencing. Serum of patient 1 reacted with the 250 kDa protein band only, while the serum from patient 2 demonstrated the presence of both the 250 and 205 kDa bands. Like the healthy control, patient 3 demonstrated no antibodies to β4 integrin. ITGB4, β4 Integrin; mAb, Monoclonal antibody.

43–89 years), but none of the healthy donors, showed the reactivity against the 250 kDa and/or 205 kDa β4 integrin bands. Again, silencing of *β4 integrin* confirmed the specificity for both bands. A complete overlap of IB signals was demonstrated when serum samples and the control antibody were simultaneously incubated with cell lysates of either A431 cells or A431 ITGB4- cells (Figure 1d).

Unexpectedly, among the six patients, whose sera reacted with β4 integrin proteins, four patients exclusively showed oral disease, while two patients were multisite MMP including ocular involvement. Three (50%) of six patients had also antibodies against BP180 NC16A domain, while the other three patients showed negative results in either indirect immunofluorescence of salt-split-skin or BP180 NC16A ELISA.

The frequency of serum reactivity to β4 integrin observed in our cohort was significantly high, although it was lower than the study by Oyama et al.[4] The present study also question the previous assumption that β4 integrin represents a site-specific autoantigen of ocular involvement in MMP.

We showed that IB of A431 cell lysates is a reliable and accurate test for the detection of anti-β4 integrin antibodies, which was further confirmed by comparison of IB patterns between intact A431 cells and A431 ITGB4- cells.

The study limitations include the retrospective design, the small number of ocular MMP patients and the fact that we did not perform IB for detecting antibodies against other MMP antigens.

To conclude, we found serum antibodies against β4 integrin in 15% of patients with MMP. Our results suggest that these antibodies may be relevant in the diagnosis of MMP, especially in cases without detectable antibodies against more common autoantigens. Multicentric prospective investigations will help strengthen this preliminary observation.

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CONFLICT OF INTEREST

None declared.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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REFERENCES

1. Du G, Patzelt S, van Beek N, Schmidt E. Mucous membrane pemphigoid. Autoimmun Rev. 2022;21:103036.
2. Li X, Qian H, Sogame R, Hirako Y, Tsuruta D, Ishii N, et al. Integrin β4 is a major target antigen in pure ocular mucous membrane pemphigoid. Eur J Dermatol. 2016;26:247–53.
3. Rashid KA, Gurcan HM, Ahmed AR. Antigen specificity in subsets of mucous membrane pemphigoid. J Invest Dermatol. 2006;126:2631–6.
4. Oyama N, Setterfield JF, Powell AM, Sakuma-Oyama Y, Albert S, Bhogal BS, et al. Bullous pemphigoid antigen II (BP180) and its soluble extracellular domains are major autoantigens in mucous membrane pemphigoid: the pathogenic relevance to HLA class II alleles and disease severity. Br J Dermatol. 2006;154:90–8.
5. Letko E, Bhol K, Foster SC, Ahmed RA. Influence of intravenous immunoglobulin therapy on serum levels of anti-beta 4 antibodies in ocular cicatricial pemphigoid. A correlation with disease activity. A preliminary study. Curr Eye Res. 2000;21:646–54.
6. Bhol KC, Colon JE, Ahmed AR. Autoantibody in mucous membrane pemphigoid binds to an intracellular epitope on human beta4 integrin and causes basement membrane zone separation in oral mucosa in an organ culture model. J Invest Dermatol. 2003;120:701–2.
7. Jonkman MF, Groot AC, Slegers TP, Jong MC, Pas HH. Immune diagnosis of pure ocular mucous membrane pemphigoid: indirect immunofluorescence versus immunoblot. Eur J Dermatol. 2009;19:456–60.
8. Gaudin O, Seta V, Alexandre M, Bohelay G, Aucouturier F, Mignot-Grootenboer S, et al. Gliptin accountability in mucous membrane pemphigoid induction in 24 out of 313 patients. Front Immunol. 2018;9:1030.
9. Schmidt E, Rashid H, Marzano AV, Lamberts A, di Zenzo G, Diercks GFH, et al. European guidelines (S3) on diagnosis and management of mucous membrane pemphigoid, initiated by the European academy of dermatology and venereology - part II. J Eur Acad Dermatol Venereol. 2021;35:1926–48.
10. Moch M, Windoffer R, Schwarz N, Pohl R, Omenzetter A, Schnakenberg U, et al. Effects of Plectin depletion on keratin network dynamics and organization. PLoS One. 2016;11:e0149106.