



# Case Report: Mucous Membrane Pemphigoid With IgG and IgA Anti-Laminin $\gamma$ 1 Antibodies and IgA Anti-Laminin $\alpha$ 5 Antibodies

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### Specialty section:

This article was submitted to  
Autoimmune and Autoinflammatory  
Disorders,  
a section of the journal  
Frontiers in Immunology

Received: 24 March 2022

Accepted: 10 May 2022

Published: 01 June 2022

### Citation:

Kuang W, Qian H, Zhang Q, Li W,  
Hashimoto T, Zeng X and Li X (2022)  
Case Report: Mucous Membrane  
Pemphigoid With IgG and IgA  
Anti-Laminin  $\gamma$ 1 Antibodies and  
IgA Anti-Laminin  $\alpha$ 5 Antibodies.  
*Front. Immunol.* 13:903174.  
doi: 10.3389/fimmu.2022.903174

Mucous membrane pemphigoid (MMP) and anti-laminin (LM)  $\gamma$ 1 pemphigoid, two subtypes of subepidermal autoimmune bullous diseases characterized by autoantibodies against epidermal basement membrane zone proteins, mainly show mucosal and skin lesions, respectively. The known autoantigens of MMP includes BP180, BP230, LM332, integrin  $\alpha$ 6 $\beta$ 4 and type VII collagen, and anti-LM $\gamma$ 1 pemphigoid targets LM $\gamma$ 1. In this study, we present an unique MMP case with oral mucosal lesions, which showed positive IgA signals on basement membrane zone in indirect immunofluorescence using normal human skin and on dermal side in indirect immunofluorescence using salt-split skin, positive IgA autoantibodies against LM $\gamma$ 1 by immunoblotting of epidermal extracts, positive IgA autoantibodies against LM $\alpha$ 5 by immunoblotting of LM521 recombinant protein (rLM521) and positive IgG autoantibodies against LM $\gamma$ 1 by immunoblotting of rLM111 and rLM521 at first visit (Day 0). After therapy, further serological analyses of serum samples collected at Day 30 and Day 50 indicated that IgA autoantibodies against LM $\gamma$ 1 were likely to be pathogenic. These results suggest that LM $\gamma$ 1 is another autoantigen of MMP, and our patient might be the first reported case of anti-LM $\gamma$ 1 MMP.

**Keywords:** mucous membrane pemphigoid, laminin  $\gamma$ 1, laminin  $\alpha$ 5, autoantibody, oral lesion

## INTRODUCTION

Subepidermal autoimmune bullous diseases are a group of rare skin disorders characterized by autoantibodies against epidermal basement membrane zone (BMZ) proteins which include bullous pemphigoid, mucous membrane pemphigoid (MMP), anti-laminin (LM)  $\gamma$ 1 pemphigoid and others (1).

MMP affects one or more mucous membranes and occasionally involves the skin (1). Known MMP-related autoantigens are BP180, BP230, LM332, integrin  $\alpha$ 6 $\beta$ 4 and type VII collagen (2).

Anti-LM $\gamma$ 1 pemphigoid, also called anti-p200 pemphigoid, was first reported in 1996 as unique two cases with IgG autoantibodies to an unidentified 200-kDa protein (p200) present at the dermal side of BMZ (3). In 2009, our group identified this p200 autoantigen as LM $\gamma$ 1 and therefore proposed to rename this disease entity as anti-LM $\gamma$ 1 pemphigoid (4). Clinically, anti-LM $\gamma$ 1 pemphigoid presents mainly skin lesions and occasionally mucosal lesions (5).

In this study, we present a unique MMP case with only oral mucosal lesion, in which our serological studies suggested IgA anti-LM $\gamma$ 1 autoantibodies as pathogenic antibodies. We finally made a tentative diagnosis of anti-LM $\gamma$ 1 MMP for this case.

## CASE DESCRIPTION

A 49-year-old female presented with erythematous and erosive oral mucosal lesions on the gingiva, tongue and buccal mucosa, and white striae on the tongue and buccal mucosa (Figure 1, Day 0) without any lesions on the skin or other mucous membranes

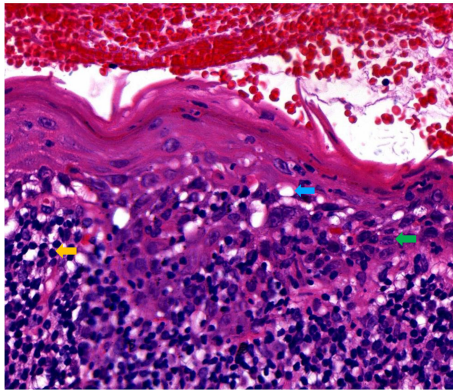
Histopathology of biopsy from white striae on the left buccal mucosa showed atrophic mucosal epithelium with vacuolar degeneration of basal cells, and severe inflammatory infiltration of lymphocytes and plasma cells with formation of lymphoid follicle in the upper dermis (Figure 2). Direct immunofluorescence showed no BMZ deposition for IgG, IgA, IgM and C3 (data not shown).

By indirect immunofluorescence (IIF) using normal human skin, the patient serum taken at Day 0 showed IgA, but not IgG, anti-BMZ antibodies, without antibodies to keratinocyte cell surfaces (Figures 3A, B). By IIF using 1M NaCl-split normal human skin (ssIIF), the patient serum taken at Day 0 showed only IgA binding to the dermal side of the split skin (Figures 3C, D).

We next performed various immunoblotting and ELISA assays for this patient serum taken at Day 0. Immunoblotting of normal human dermal extract detected IgA, but not IgG, autoantibodies against LM $\gamma$ 1 (Figure 4A). Immunoblotting of LM111 recombinant protein (rLM111) detected IgG, but not IgA, autoantibodies against LM $\gamma$ 1 (Figure 4B). Furthermore, IgA anti-LM $\alpha$ 5 and IgG anti-LM $\gamma$ 1 autoantibodies were identified in immunoblotting of rLM521 (Figure 4C). This serum showed negative results in other tests, which are routinely performed in our laboratory for detection of other known autoantibodies in autoimmune bullous diseases, including commercially available



**FIGURE 1** | Changes of clinical features of this patient. The clinical features on the gingivae (left) and buccal mucosa (right) at Days 0, 30 and 50 are shown.



**FIGURE 2** | Histopathological features of this patient. Histopathological features for the biopsy taken from the lesions on the left buccal mucosa (H&E staining, original magnification, x200). Blue, green and yellow arrows indicated the vacuolar degeneration, plasma cell and lymphocyte, respectively.

ELISAs for BP180, BP230, type VII collagen, desmoglein 1 and desmoglein 3 (MBL, Nagoya, Japan), and in house ELISAs using recombinant proteins of LM332, integrin  $\alpha$ 6 $\beta$ 4, integrin  $\beta$ 4 intracellular domain, integrin  $\beta$ 4 extracellular domain, peptides of BP180, desmoglein 1 and desmoglein 3, as well as immunoblotting analyses of normal human epidermal extract, concentrated culture supernatant of HaCaT cells and hemidesmosome-rich fraction prepared from A431 cells.

Based on the clinical features and immunological findings, this patient was first diagnosed as MMP with IgG and IgA

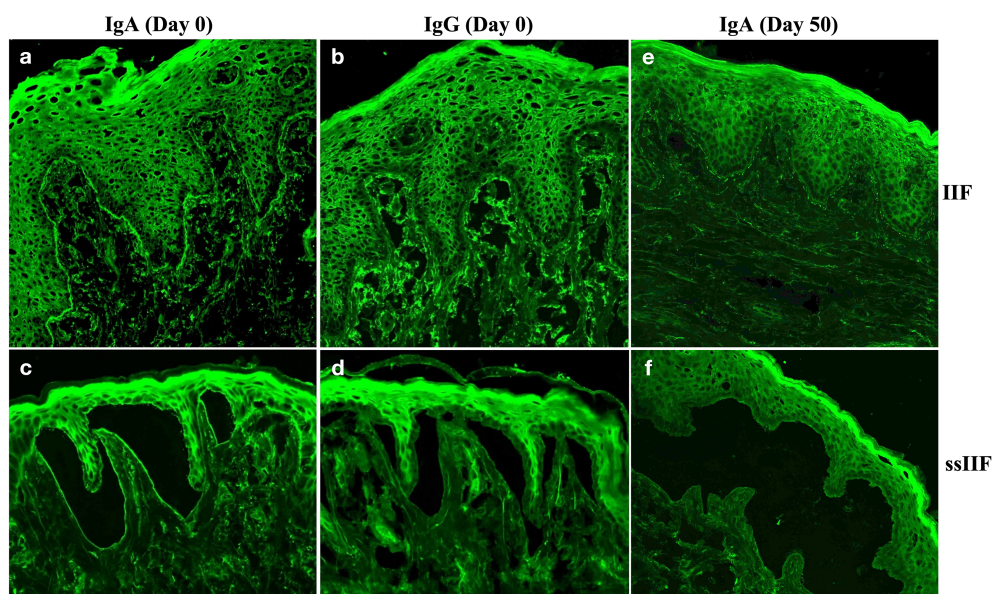
autoantibodies against LM $\gamma$ 1 and IgA antibodies against LM $\alpha$ 5. The treatment with minocycline hydrochloride 100 mg/day was initiated.

Then, this patient visited us two more times at Day 30 and Day 50. Clinically, erosive oral mucosal lesions improved with time on the therapy (**Figure 1**, Day 30 and Day 50). By both IIF using normal human skin and ssIIF, neither IgG nor IgA anti-BMZ antibodies were detectable for sera collected at Day 30 and Day 50 (**Figures 3E, F** and data not shown). In immunoblotting of normal human dermal extract, IgA reactivity with LM $\gamma$ 1 reduced gradually with time (**Figure 4A**). In immunoblotting analyses of rLM111 and rLM521, IgG reactivities with LM $\gamma$ 1 were not changed with time (**Figures 4B, C**). In immunoblotting of rLM521, IgA reactivity with LM $\alpha$ 5 at Day 30 was similar to that at Day 0, but reduced at Day 50 (**Figure 4C**).

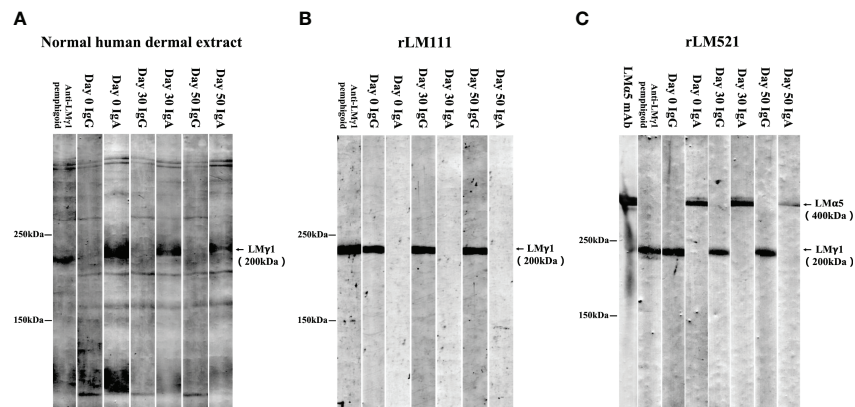
Based on the changes of clinical features, IgA reactivity in IIF studies, and the results of the 3 immunoblotting studies along with time after the therapy, we considered that IgA, but not IgG, autoantibodies against LM $\gamma$ 1 may be pathogenic, while it is currently unknown whether circulating IgA autoantibodies against LM $\alpha$ 5 were involved in the pathogenesis of this disease. Therefore, we finally made a tentative diagnosis of anti-LM $\gamma$ 1 MMP.

## DISCUSSION

In a very recent MMP study for 154 cases, the rates of autoantigen detection for BP180, BP230 and LM332 were 58.4%, 1.9% and 8.4%, respectively (6). In 2017, we reported 721 MMP suspected patients, in which the rates of autoantigen



**FIGURE 3** | Indirect immunofluorescence using normal human skin (IIF) and using 1M NaCl-split normal human skin (ssIIF) for the patient serum taken at Day 0 and Day 50. IgA (**A**), but not IgG of (**B**), anti-BMZ antibodies were detected in IIF for serum taken at Day 0. IgA (**C**), but not IgG (**D**) antibodies bound to the dermal side of the split in ssIIF for serum taken at Day 0. IgA antibodies were not detected in both IIF (**E**) and ssIIF (**F**) for serum taken at Day 50.



**FIGURE 4** | Immunoblotting analyses. Immunoblotting analyses of normal human dermal extract **(A)**, rLM111 **(B)** and rLM521 **(C)** were performed for IgG and IgA autoantibodies against LM $\gamma$ 1 and LM $\alpha$ 5 in patient sera collected at Days 0, 30 and 50. The serum of an anti-LM $\gamma$ 1 pemphigoid patient and anti-LM $\alpha$ 5 monoclonal antibody (LM $\alpha$ 5 mAb) were used as positive controls.

detection for BP180 alone, LM332 alone and both of BP180 and LM332 were 38.5%, 20.9% and 7.5%, respectively (7). In another MMP study for 78 cases, the rates of autoantigen detection for BP180, BP230, LM332 and type VII collagen were 33.3%, 11.5%, 11.5% and 3.8%, respectively (2). Although large cohort studies of antibodies to integrin  $\alpha$ 6 $\beta$ 4 in MMP have not yet been performed, we reported that 27 (62.8%) of 43 pure ocular MMP patients targeted integrin  $\beta$ 4 (8). The results of these studies suggests that BP180 is the major autoantigen of MMP, followed by LM332 and integrin  $\alpha$ 6 $\beta$ 4, whereas some unidentified autoantigen(s) may still exist.

The present study suggested that LM $\gamma$ 1 is also an autoantigen of MMP. Anti-LM $\gamma$ 1 antibodies were detected in previously reported MMP cases with known MMP autoantibodies (9). However, our patient was the first case with clinical features of MMP, which showed antibodies to LM $\gamma$ 1 (and LM $\alpha$ 5) but not any known MMP autoantibodies, suggesting that LM $\gamma$ 1 is one of the MMP autoantigens. Therefore, it is necessary to screen autoantibodies against LM $\gamma$ 1 in MMP suspected cases, particularly when autoantibodies against BP180, LM332, integrin  $\alpha$ 6 $\beta$ 4, BP230 and type VII collagen were negative. Our case is also the first case with IgA antibodies to LM $\alpha$ 5, although their pathogenic relevance is currently unclear and future studies for the similar cases are needed to clarify it.

For detection of IgG autoantibodies against LM $\gamma$ 1 in the sera of anti-LM $\gamma$ 1 pemphigoid cases, we usually use immunoblotting analyses of normal human dermal extract and LM $\gamma$ 1-containing LM trimer such as rLM521 and rLM111, and all of these tests are positive in most cases (4, 9). In addition, anti-LM $\gamma$ 1 pemphigoid cases rarely show IgA anti-LM $\gamma$ 1 antibodies (5). However, although all these immunological studies were repeatedly performed in our anti-LM $\gamma$ 1 MMP case, immunoblotting of dermal extract always detected IgA anti-LM $\gamma$ 1 autoantibodies, and those of rLM521 and rLM111 detected IgG anti-LM $\gamma$ 1 antibodies. The true reason for this discrepant reactivity with LM $\gamma$ 1 is currently unknown. However, the different reactivity of

IgG and IgA antibodies may be due to the different epitopes within LM $\gamma$ 1 molecule for IgG and IgA antibodies.

On clinical features, our anti-LM $\gamma$ 1 MMP case showed only oral lesions without skin lesions. In contrast, anti-LM $\gamma$ 1 pemphigoid mainly shows skin lesions with occasional concurrence of oral mucosal lesion.

Considering the positive IgA anti-BMZ antibodies detected by IIF and sIIF, the negative results of direct immunofluorescence might be false negative. For diagnosis of autoimmune bullous diseases, there is a certain false negative rate of direct immunofluorescence, particularly for MMP. Actually, in our recently published paper, the positive rate of direct immunofluorescence in anti-LM332-type MMP was 89.8% (88 in 98 cases) (10).

There is a limitation of this study that minocycline hydrochloride might simply be acting as a protease inhibitor, and therefore serum autoantibody titers might not be correlated with disease response.

In summary, we reported for the first time that LM $\gamma$ 1 may also be another autoantigen of MMP, and propose a new disease entity, anti-LM $\gamma$ 1 MMP. Significance of IgA antibodies to LM $\alpha$ 5 should be clarified in the future studies for the similar cases.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Dermatology Hospital of Jiangxi Province. The patients/participants provided their written informed consent to

participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

## AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. WK, XZ, and XL had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

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## FUNDING

This work was supported by the National Natural Science Foundation of China (NSFC) (Grant Nos. U19A2005) and CAMS Innovation Fund for Medical Sciences (CIFMS) (Grant Nos. 2020-I2M-C&T-A-203).

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