Anti-laminin 332 mucous membrane pemphigoid in a young woman treated with rituximab

Alexander Gitin[1] BS, Adriana Della Porta[1] BS, Elizabeth Bisbee[2] MD, Katherine Braunlich[2] DO, Kiran Motaparthi[2] MD

Affiliations: [1]University of Florida College of Medicine, Gainesville, Florida, USA, [2]Department of Dermatology, University of Florida Gainesville, Florida, USA

Corresponding Author: Elizabeth Bisbee MD, 4037 NW 86 Terrace, Fourth Floor, Gainesville, FL 32606, Tel: 352-594-1930, Email: elizabethbisbee@gmail.com

Abstract

Mucous membrane pemphigoid, formerly known as cicatricial pemphigoid, is a rare and difficult-to-treat bullous disorder that occurs most commonly in older adults. We describe a 32-year-old woman who was diagnosed with anti-laminin 332 mucous membrane pemphigoid through indirect immunofluorescence for laminin 332 following nonspecific histologic and direct immunofluorescence findings. At 16 weeks following completion of her first cycle of with rituximab 375mg/m^2 weekly for four weeks, her mucosal erosions had resolved. Although not widely available, this case highlights the utility of anti-laminin 332 immunofluorescence for diagnostic confirmation of this entity and the efficacy of rituximab in obtaining disease control.

Keywords: indirect immunofluorescence, laminin 332, mucous membrane pemphigoid, Rituximab

Introduction

Mucous membrane pemphigoid (MMP), formerly known as cicatricial pemphigoid, is a rare immunobullous disease that primarily affects older adults, with a median age of onset of 65 years [1]. The pathogenesis of MMP is related to autoantibody-mediated dysfunction of structural proteins present at the dermoepidermal junction and results in subepidermal blistering. A number of auto-antibodies have been associated with the disease, including those targeting laminin 332, formerly known as epiligrin [2]. Anti-laminin 332 antibodies in MMP have been associated with an increased risk of malignancy [1]. Herein, we present a young woman with anti-laminin 332 MMP confirmed with a novel anti-laminin 332 indirect immunofluorescence.

Case Synopsis

A 32-year-old woman presented with a two-year history of worsening, intermittent oral, gingival, and nasal erosions with involvement of her anal and vaginal mucosa. She described intermittent conjunctivitis, dysphonia, dysphagia, and worsening productive cough. Two previous biopsies of the oral mucosa, performed by her dentist and otolaryngologist, produced nondiagnostic histopathologic and immunofluorescence findings. When she presented to our service, she was being treated with dapsone 100mg daily, methotrexate 15mg weekly, and prednisone 20mg daily with minimal symptomatic improvement. She had previously been on chronic systemic glucocorticoids, with a maximum dose of 60mg daily prednisone. Examination revealed widespread erosions of the oral mucosa (**Figure 1**) with conjunctival injection and early symblepharon (**Figure 2**). Cutaneous involvement was absent (**Figure 3**). Punch biopsy of the mucosal lower lip was performed and revealed ulceration and underlying granulation tissue with a lymphoplasmacytic infiltrate. Direct immunofluorescence (DIF) was noncontributory because of the absence of intact epithelium, which was consistent with the amount of desquamation

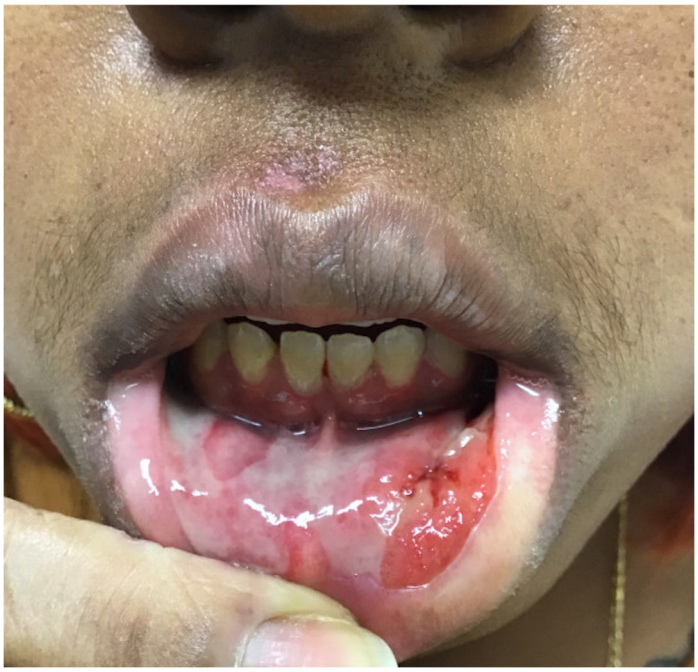

Figure 1. *Eroded plaques of the lower labial mucosa at initial presentation.*

observed in the oral cavity at the time of biopsy. Enzyme-linked immunosorbent assays (ELISAs) for anti-NC16a-BP180, anti-BP230, and anti-collagen VII IgG autoantibodies were negative. Indirect immunofluorescence (IIF) on human 1M NaCl split skin substrate revealed positivity for IgG with dermal localization and a titer of 1:40. Thereafter, a novel indirect immunofluorescence assay utilizing laminin

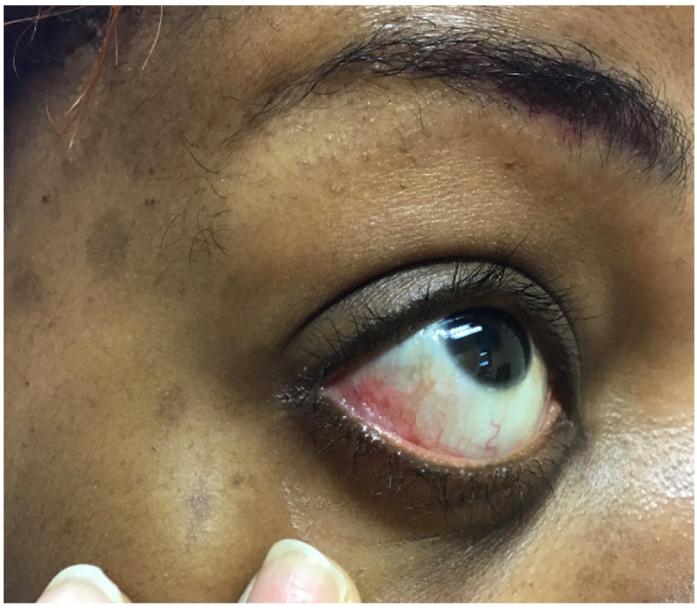

Figure 2. *Symblepharon and conjunctival injection at initial presentation.*

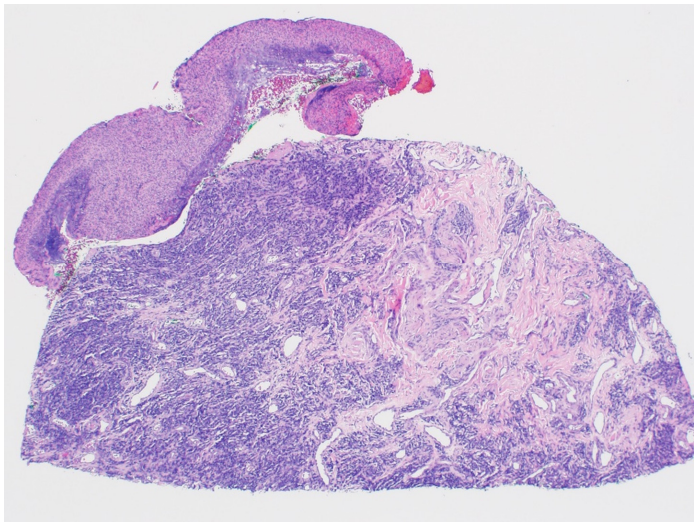

Figure 3. *Punch biopsy of the mucosal lower lip. Ulceration and underlying granulation tissue with a lymphoplasmacytic infiltrate. H&E, 4×.*

332-expressing human embryonic kidney (HEK) 293 cells was performed and was positive with a titer of >1:10, confirming a diagnosis of anti-laminin 332 MMP.

While awaiting definitive diagnosis, the patient was initiated on prednisone 40mg daily with a plan to taper as she was established on rituximab treatment. Methotrexate and dapsone were discontinued at the initial visit. She was re-evaluated after 10 weeks of treatment, at which point her disease had progressed. At 11 weeks after initially presenting to our service, she began treatment with rituximab 375mg/m^2 weekly for four weeks, in addition to continuing prednisone 40mg daily followed by a 4-month taper. On follow-up in 16 weeks, having completed the 4-week treatment course of rituximab, she noted symptomatic improvement with resolution of mucosal erosions (**Figure 4**). A malignancy workup was initiated, and computed tomography (CT) of the abdomen, pelvis, and chest revealed no evidence of malignancy or metastatic disease. A one-time follow-up malignancy screening by CT within 14 months from initial imaging was considered.

Case Discussion

Mucous membrane pemphigoid refers to a group of pemphigoid diseases that primarily affects the

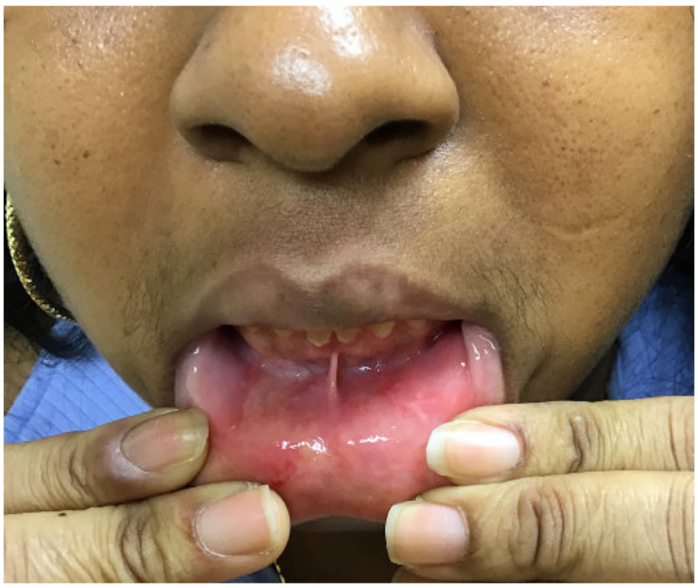

Figure 4. *Resolution of erosions involving labial mucosa following rituximab therapy.*

mucous membranes, with or without skin involvement. Mucous membrane pemphigoid is clinically characterized by waxing and waning erosions and scarring [3]. The most commonly involved anatomical site is the oral cavity, where scarring may lead to restriction in mobility of the tongue [1]. Erosive and/or vesiculobullous lesions of the pharyngeal, respiratory, gastrointestinal, and genitourinary mucosa may also occur [1]. Affected patients may present with epistaxis, chronic sinusitis, hoarseness, dysphonia, strictures, or dysphagia [1]. If the upper airway is affected, repeated inflammation and scarring can be life-threatening, leading to airway obstruction [1]. Chronic ocular involvement in MMP, similarly to the mucosal and cutaneous lesions, is relapsing and remitting. Early ocular involvement consists of conjunctivitis with progressive scarring leading to symblepharon, trichiasis, and eventual corneal ulceration and potential blindness [1]. Mucous membrane pemphigoid is uncommon, and mostly affects older adults with a median age of onset of 65 years [1]. Although the patient described here was younger than most patients with this disease, she presented with classic clinical manifestations of MMP including blistering and erosions primarily limited to the mucous membranes. She also exhibited findings consistent with ocular MMP, namely ocular erythema, conjunctivitis, and symblepharon.

Mucous membrane pemphigoid, like other pemphigoid diseases, is diagnosed through a combination of clinical findings, histopathology, serologies, and immunofluorescence testing. Histopathologic findings in MMP are nonspecific and include a subepithelial blister with a mixed subepithelial inflammatory infiltrate [2]. Direct immunofluorescence typically reveals linear deposition of immunoglobulins and/or complement at the dermoepidermal junction [3]. Direct immunofluorescence in this patient was noncontributory due to absence of intact epithelium amenable to sampling. In patients with MMP, IIF demonstrates circulating IgG autoantibodies that bind to the dermal side of 1M NaCl split skin, unlike most other forms of pemphigoid which bind to the epidermal side of the split [1]. Autoantibodies against several structural proteins found in the dermoepidermal junction, including BP180, BP230, laminin 332, type VII collagen, and $\alpha 6\beta 4$ integrin antigens, have been associated with MMP [2]. It is important to note that the autoantibody profiles of epidermolysis bullosa acquisita, anti-p200 (laminin $\gamma 1$) pemphigoid, and bullous systemic lupus erythematosus are heterogeneous and that these disorders also demonstrate dermal staining on IIF performed on NaCl split skin [2]. Mucosal-predominant disease in a patient who presents with suspected pemphigoid should warrant further antibodies studies, especially if ELISAs for anti-NC16a-BP180 and anti-BP230 autoantibodies are not strongly positive.

Our diagnosis was aided through application of a novel IIF assay first described by Goletz et al. The in vitro specificity of this assay for detecting anti-laminin 332 IgG was reported as >99.5%. The authors utilized laminin 332-expressing HEK293 cells as a substrate for sera from anti-laminin 332 subjects, as determined by a combination of clinical presentation, DIF findings, and immunoblotting (IB) with extracellular matrix of cultured human keratinocytes (ECM), and controls. They found that anti-laminin 332 IgG was detected in only one of 430 control sera, which included 180 pemphigoid sera [4]. The authors also studied the correlation between disease activity and serum anti-laminin 332 levels in five patients with anti-laminin 332 MMP whose sera

was subjected to IIF on NaCl split skin, IB with ECM, and this novel IIF assay. Although the results were comparable between the three assays, serial dilution was not performed on the sera of the patient presented here due to the small sample size and potential for false negative results [4].

Although clinically indistinguishable from other forms of MMP, the importance of diagnosing anti-laminin 332 MMP is reflected by the strong association with internal malignancy [1,2]. In one cohort of 35 patients with anti-laminin 332 MMP, 10 patients had solid cancers; 8 patients developed cancer after onset of their bullous disease. The mean time from blister onset to the diagnosis of malignancy was 13.2 months and the average age of patient in this cohort was 65 years. The study showed an increased risk of malignancy in this cohort of patients with respect to incidence of all cancers as reported in the National Cancer Institute registry, with a statistically significant risk ratio of 6.8 overall and 15.4 within the first year after blister onset [1,5]. At present, no uniform guidelines exist to inform malignancy screening of patients with anti-laminin 332 MMP. However, based on the available evidence from this cohort, screening for malignancy should be considered within 14 months from time of blister onset. In the patient presented here, the onset of blistering occurred two years prior to presentation. Therefore, she was screened via CT at time of diagnosis. Additional malignancy screening 14 months after initial imaging was considered for this patient specifically in part due to her young age when compared to the average age of this cohort.

Treatment of MMP is based on sites of mucosal involvement and severity. In high-risk patients with ocular involvement, treatment should be aggressive and initiated early to prevent irreversible fibrosis [2]. Immunosuppressants such as glucocorticoids, mycophenolic acid, dapsone, and cyclophosphamide are the mainstay of therapy [2]. B cell depletion therapy, such as rituximab, represents a more recent approach to treatment and has been used as an adjunct to conventional immunosuppressive regimens. Although data is limited, in patients with refractory disease rituximab has been shown to be effective in this role as demonstrated in our patient. A recent retrospective cohort study of 49 patients with moderate MMP demonstrated disease control in all patients treated with rituximab as an adjunct to conventional immunosuppression compared to only 40 percent of patients treated with conventional therapy alone [6]. Of note, none of the subjects in the conventional therapy group were treated with cyclophosphamide. Disease control was also achieved three times faster with rituximab and, although the result did not achieve statistical significance, the rate of serious adverse events was 15% lower in the rituximab group [6]. Subjects in the rituximab group were divided into two treatment protocols including a lymphoma protocol, which consisted of 375mg/m^2 weekly for four weeks, and a rheumatoid arthritis protocol, which consisted of two infusions of 1000mg given 15 days apart. The mean time from first infusion to disease control was reported as 10.17 months, with a mean number of 5.25 infusions [6]. Le Roux-Villet et al. had previously reported a median of 12 weeks from first infusion to disease control in patients with severe, refractory MMP treated with the lymphoma protocol as an adjunct to previous ongoing treatment, which included topical corticosteroids, dapsone, and sulfasalazine [7]. In this report, 68% of subjects achieved disease control after one cycle of treatment [7]. Disease control in our patient was obtained 16 weeks following completion of her first cycle of the lymphoma protocol in conjunction with a concomitant systemic corticosteroid taper, similar to this study.

Conclusion

This case highlights the importance of considering anti-laminin 332 MMP in patients presenting with mucosal erosions and scarring with ocular symptoms and nonspecific direct and indirect IF findings, even in younger patients. This variant has been associated with a significantly increased risk of malignancy. The utilization of a novel IIF assay for laminin 332 aided in the diagnosis of this rare pemphigoid disease. The addition of rituximab to conventional immunosuppressive therapy may improve disease control. Because of the serious implications of unrecognized and untreated anti-laminin 332 MMP,

correct and timely diagnosis of this cohort of patients is critical.

Potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Egan CA, Lazarova Z, Darling TN, Yee C, Yancey KB. Anti-Epiligrin Cicatricial Pemphigoid. *Medicine (Baltimore)*. 2003;82:177-186. [PMID: 12792304].
2. Schmidt E, Zillikens D. Pemphigoid diseases. *Lancet*. 2013;381:320-332. [PMID: 23237497].
3. 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. [PMID: 11902988].
4. Goletz S, Probst C, Komorowski L, et al. A sensitive and specific assay for the serological diagnosis of antilaminin 332 mucous membrane pemphigoid. *Br J Dermatol*. 2019;180:149-156. [PMID: 30216412].
5. Egan CA, Lazarova Z, Darling TN, et al. Anti-epiligrin cicatricial pemphigoid and relative risk for cancer. *Lancet*. 2001;357:1850-1851. [PMID: 11410196].
6. Maley A, Warren M, Haberman I, et al. Rituximab combined with conventional therapy versus conventional therapy alone for the treatment of mucous membrane pemphigoid (MMP). *J Am Acad Dermatol*. 2016;74:835-840. [PMID: 26936298].
7. Le Roux-Villet C, Prost-Squarcioni C, Alexandre M, et al. Rituximab for Patients With Refractory Mucous Membrane Pemphigoid. *Arch Dermatol*. 2011;147:843-849. [PMID: 21422323].