Anti-Epiligrin Cicatricial Pemphigoid With Antibodies Against the γ2 Subunit of Laminin 5

Hossein C. Nousari, MD; Adrienne Rencic, MD, PhD; Roger Hsu, MD; Kim B. Yancey, MD; Grant J. Anhalt, MD

Background: Cicatricial pemphigoid (CP) is a scarring subepithelial mucocutaneous blistering disease characterized by anti–basement membrane zone autoantibodies. Anti-epiligrin CP is an uncommon variant that has been recently characterized. Severe laryngeal involvement is infrequently observed in all forms of CP and has been documented in only 2 patients with anti-epiligrin CP.

Observations: We report a case of CP exhibiting extensive laryngeal and ocular involvement. Histological, immunofluorescence, and immunoprecipitation studies confirmed the diagnosis of anti-epiligrin CP. Immunoblotting studies demonstrated the presence of antibodies against the α3 and the γ2 subunit of laminin 5.

Conclusion: This article expands the diversity of the clinical and immunopathologic features of this newly characterized variant of CP.


Cicatricial pemphigoid (CP) is a chronic autoimmune blistering disease that primarily affects mucous membranes and heals with scar formation.1 Cicatricial pemphigoid typically involves oral and/or ocular mucosae, but compromise of the upper aerodigestive tract and anogenital mucosae may also occur.2 Cutaneous lesions occur in approximately 20% of patients, and lesions favor the head, neck, and upper trunk. Laryngeal involvement is a serious complication observed in fewer than 10% of CP cases.3,4 Histological and ultrastructural evaluation demonstrate that blistering occurs within the lamina lucida. Immunopathologic studies have demonstrated the presence of in situ anti–basement membrane zone (BMZ) autoantibody antigens. Circulating anti-BMZ antibodies are found in fewer than 40% of patients.1,5-6

A subset of patients with CP have circulating IgG autoantibodies that bind exclusively to the dermal side of 1-mol/L sodium chloride–split human skin and immunoprecipitate a keratinocyte-derived glycoprotein originally called epiligrin.7-14 Epiligrin is now known to be identical to laminin 5, a heterotrimeric (α3β3γ2) adhesion molecule that is a component of the anchoring filaments.15 Laminin 5 is the major integrin ligand for epidermal cells.

Anti-epiligrin CP occurs in fewer than 10% of antigenically characterized cases of CP, and laryngeal involvement has been documented in only 2 of 14 previous cases.7-14 Virtually all patients with anti-epiligrin CP have pathogenic antibodies that exclusively bind the α3 subunit, and there have been only 2 cases of CP in which the patients’ serum samples were found to bind both the α3 and γ2 subunits of laminin 5. We report one such case of anti-epiligrin CP in a patient with extensive mucosal involvement that includes severe ocular and laryngeal lesions.

RESULTS

HISTOLOGICAL AND IMMUNOFLOUORESCENCE STUDIES

Histological examination of cutaneous biopsy specimens revealed subepidermal blistering with moderate perivascular and interstitial mixed infiltrate in the upper dermis. Direct immunofluorescence demonstrated intense linear ribbonlike IgG deposition along the BMZ. Indirect immunofluorescence demonstrated circulating IgG autoantibodies that bound the dermal side of sodium chloride–split skin at a titer of 320 (Figure 2).
PATIENT AND METHODS

A 69-year-old white man first noted painful ocular irritation that was refractory to treatment with topical corticosteroids and antibiotics. After several months he developed blistering of the palate, oropharynx, and nasopharynx, accompanied by epistaxis and pruritic blistering of the upper back and chest. Skin biopsy specimens revealed a subepithelial blister with mixed inflammatory infiltrate. The patient was treated with prednisone, 40 mg/d, for 4 months, but his condition worsened, leading to progressive and distressing hoarseness. Results of physical examination revealed vesicobullae distributed over the scalp, upper back, and chest. Conjunctival erythema with bilateral obliteration of the inferior fornices, superior and inferior symblephara and left lateral orbital ankyloblepharon without corneal scarring were evident (Figure 1). Ulcerations and scarring in the oropharynx caused near obliteration of the uvula and tonsillar pillar distortion. Video laryngoscopy showed that the laryngeal stri- dor was caused by an anterior laryngeal web without airway obstruction. Diffuse ulceration circumferentially throughout the larynx involving the hypopharynx and epiglottis was also noted. Perilesional skin biopsy specimens from the back were taken.

The patient was treated with prednisone, 80 mg daily, and cyclophosphamide, 200 mg daily, with notable improvement of the cutaneous and oral lesions within 2 months, and without adverse effects. Ocular lesions also improved, although the symblepharon and the ankyloblepharon were unchanged. Laryngeal lesions showed notable improvement in the following 5 months of therapy. A slow taper of the prednisone is in progress.

HISTOLOGICAL AND IMMUNOFLUORESCENCE STUDIES

Histological examination of skin biopsy specimens from the upper back and direct immunofluorescence of perilesional skin were performed following standard techniques.16 Indirect immunofluorescence was performed on monkey esophagus and 1-mol/L sodium chloride–split human skin.17

IMMUNOBLOT AND IMMUNOPRECIPITATION STUDIES

Laminin 5 was isolated from the extracellular matrix of cultured human keratinocytes and studied by immunoblotting with serum samples from our patient and controls as previously described.9,10 Normal human keratinocytes were radiolabeled with 35S-methionine (New England Nuclear, Boston, Mass). Conditioned media were collected and studied by immunoprecipitation using serum samples from patients and controls as described.18
The incidence of laryngeal involvement in CP is 8% to 9%. Direct laryngoscopy often reveals epiglottic and aryepiglottic erosions and may demonstrate laryngeal stenosis and possibly airway obstruction. Although many cases of CP will respond to prednisone and/or dapsone treatment, laryngeal, esophageal, or ocular involvement necessitates aggressive therapy with a combination of prednisone and cyclophosphamide to prevent irreversible scarring. A high index of suspicion for critical organ involvement should be maintained, since early intervention can prevent the development of the most severe sequelae.

This patient had typical extensive mucosal, laryngeal, and ocular lesions and immunopathologic findings characteristic of anti-epiligrin CP. Histological and direct immunofluorescence findings confirmed the diagnosis of CP, and indirect findings from immunofluorescence demonstrated autoantibodies binding the dermal side of sodium chloride–split skin. Differential diagnoses of disorders showing dermal staining on indirect immunofluorescence include anti-epiligrin CP, epidermolysis bullosa acquisita, and anti-105-kd and anti-200-kd bullous pemphigoidlike disorders (Table). A definitive step in diagnosis of subepidermal blistering diseases is the identification of the autoantigen by immunoprecipitation and/or immunoblotting, which definitively establishes the diagnosis of anti-epiligrin CP. This patient was interesting because of demonstrated autoantibodies binding the α3 and the γ2 subunit of laminin 5. Virtually all patients with anti-epiligrin CP have autoantibodies that bind only the α3 subunit of laminin 5 and laminin 6, but our patient's autoantibodies were directed predominantly against the γ2 subunit.

This observation prompts speculation on the role of individual subunits of laminin 5 in cellular adhesion and the influence of antibodies against these subunits in influencing the course or severity of the disease. The role of anti-α3 vs anti-γ2 antibodies in producing distinct patterns of organ involvement should be tested by animal studies, but this is not feasible owing to the species specificity of human autoantibodies. Human anti-laminin 5 antibodies do not recognize epitopes present on murine laminin 5, so passive transfer into neonatal mice fails to induce lesions. Rabbit polyclonal IgG, produced by immunization with laminin 5, can induce subepidermal blisters in neonatal mice; however, these rabbit autoantibodies bind all subunits of laminin 5. Passive transfer of IgG from patients with α3 subunit–specific autoantibodies into adult mice with severe combined immunodeficiency disorder and bearing human skin grafts does induce blistering in the transplanted human skin. Unfortunately, this model has limitations and cannot demonstrate preferential involvement of various organs or mucosal surfaces. We are therefore limited to speculation, until a better model for testing is developed.

In our patient a rapid diagnosis of laryngeal bullae by video laryngoscopy and initiation of aggressive therapy was decisive.

**Comments**

Morbidity due to CP may be severe, dependent on the organs involved. Laryngeal stenosis may require tracheostomy to maintain airway patency, and esophageal stricture may necessitate bougienage to relieve dysphagia. Ocular involvement results in conjunctival foreshortening, symblepharon, ankyloblepharon, and corneal scarring, causing visual impairment or blindness.

**Figure 3.** A, Serum from a control patient with anti-epiligrin cicatrical pemphigoid immunoblotted the unprocessed (200-kd) α3 subunit of laminin 5 (lane 1). In contrast, our patient's serum immunoblotted the unprocessed γ2 (150-kd) and α3 subunits of laminin 5 (lane 2); reactivity against the α3 subunit was weak. Serum from a healthy control did not immunoblot any specific proteins (lane 3). B, Studies of radiolabeled keratinocyte media shown that the control patient immunoprecipitated laminin 5 (polypeptides of 165, 150, 140, and 105 kd) and laminin 6 (polypeptides of 190 and 200 kd, lane 1). In contrast, serum from our patient with laminin γ2-predominant IgG immunoprecipitated laminin 5 but little laminin 6 (lane 2). Serum from the healthy control did not immunoprecipitate any specific proteins (lane 3).

**Table.**

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<th>Disorder</th>
<th>Antigen</th>
<th>Epitope/Molecular Weight</th>
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<tr>
<td>Anti-epiligrin cicatrical pemphigoid</td>
<td>Laminin 5/6</td>
<td>α3 Subunit/γ2 Subunit (200 kd)</td>
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<tr>
<td>Epidermolysis bullosa acquisita</td>
<td>Type VII collagen</td>
<td>Noncollagenous domain (145 kd)</td>
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<td>Bullous pemphigoid-like disease</td>
<td>Lower lamina lucida component</td>
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with cyclophosphamide and prednisone successfully averted potential airway compromise. Owing to the potentially life-threatening nature of this complication, early recognition and effective treatment is essential. This case further emphasizes that CP is a clinical phenotype comprising several distinct immunological disorders. Accurate classification, assessment for critical organ involvement, and early therapeutic intervention in high-risk subsets is imperative.

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Reprints: Grant J. Anhalt, MD, Division of Dermato-immunology, Johns Hopkins Medical Institutions, 720 Rutland Ave, Room 771, Baltimore, MD 21205.

REFERENCES