CASE REPORT

Differentiating antiepiligrin cicatricial pemphigoid from epidermolysis bullosa acquisita by indirect immunofluorescence of skin substrates lacking Type VII collagen or laminin 332: a case report and review of literature

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A B S T R A C T

Antiepiligrin cicatricial pemphigoid (AECP) is a chronic autoimmune subepidermal blistering disease characterized by clinical features of cicatricial pemphigoid and circulating IgG antibasement membrane autoantibodies directed against laminin 332. There is growing evidence of an increased relative risk for solid cancers and lymphomas in AECP patients, especially in the 1st year after the onset of blisters. However, it is difficult to distinguish patients with initially skin-predominant AECP from similar findings of epidermolysis bullosa acquisita merely based on clinical, histopathologic, and immuno-pathologic examinations. This is a report on a case of AECP confirmed by indirect immunofluorescence of type VII collagen- and laminin 332-deficient skin as substrates to differentiate it from epidermolysis bullosa acquisita.

Introduction

Antiepiligrin cicatricial pemphigoid (AECP) is a mucosa-predominant autoimmune subepidermal blistering disease that is clinically indistinguishable from other forms of cicatricial pemphigoid1 (CP). The importance of defining AECP within the spectrum of mucosa-predominant blistering diseases by immunoblot, immunoprecipitation, or indirect immunofluorescence (IF) using antigen-deficient skin substrates2,3 is emphasized by its association with cancer and increased cancer-related morbidity and mortality.3–6 This report is of a case of initially skin-predominant AECP, which is distinguished from epidermolysis bullosa acquisita (EBA) using indirect IF of Type VII collagen- and laminin 332-deficient skin as substrates. The clinical course and histo- and immuno-pathologic findings are presented.

Case report

A 33-year-old male patient presented with a 1-month history of multiple pruritic tense blisters overlying the erythematous bases initially on the dorsum of both hands and feet, then centripetally spreading to both arms, thighs, and trunk without mucosal lesions. His past history was unremarkable except for gout under treatment with colchicine and benz bromazone for several years. Laboratory findings showed negative antinuclear antibody (<1:40), rheumatoid factor, and other autoimmune profiles. The patient also did not fulfill the American Rheumatism Association criteria for systemic lupus erythematosus (LE).

Histological examination of the cutaneous biopsy specimens revealed subepidermal blister with predominant neutrophilic infiltration mixed with some eosinophils (Figure 1). Direct IF showed linear C3 and IgG deposition along the basement membrane zone (BMZ), whereas indirect IF revealed circulating IgG antibasement membrane autoantibodies binding to dermal floor of the 1M NaCl split skin7 (Figure 2). With a tentative diagnosis of EBA, prednisolone 0.7 mg/kg/d was started. However, progressive blisters on the trunk and four extremities with concurrent oral and genital mucosal erosions developed after 2 weeks, and some blisters ruptured with scarring and milia formation (Figures 3A and 3B). Indirect IF on antigen-deficient skin substrates showed positive reactions with the BMZ on Type VII collagen-deficient skin and negative findings on laminin 332-deficient skin3–5 (Figures 4A and 4B), leading to a diagnosis of AECP.
The patient was then shifted to azathioprine 150 mg/d and prednisone 1 mg/kg/d, with good control. Further work-up for malignancy, including serologic tumor markers, chest X-rays, abdominal ultrasound, panendoscopy, and colon fiberscopy, were negative. The patient was in his stable condition during treatment for about half a year, but he was lost to follow-up thereafter.

**Discussion**

CP, also known as mucous membrane pemphigoid, is an autoimmune subepidermal blistering disease predominantly involving the mucous membrane and occasionally involving the skin in up to 30% of patients. Patients with CP exhibit IgG and/or IgA autoantibodies directed against heterogeneous components of the BMZ, including BPAG1, BPAG2, laminin 332, Type VII collagen, α6-integrin subunit, or β4-integrin subunit. Indirect IF studies of 1M NaCl split skin show that IgG autoantibodies from CP patients can bind to the epidermal side, dermal side, and even both sides of test substrates, depending on which autoantigens are the targets.

AECP, first described by Domloge-Hultsch et al in 1992, is characterized by clinical features of CP and circulating IgG anti-basement membrane autoantibodies directed against laminin 332. It is estimated to comprise 5–20% of CP cases. It typically affects the elderly, with the median age of onset at 65 years and occurs with equal frequency in men and women. The clinical features of AECP are characterized by erosive and/or vesiculobullous lesions that usually heal with scarring and tissue destruction. Mucosal sites most commonly involved are the mouth and eyes. Other mucosal sites, including the nose, pharynx, larynx, esophagus, and anogenital region, are less commonly involved. However, skin involvement is not a dominant feature. Direct IF studies of perilesional mucous membrane or skin biopsy specimen show linear deposits composed mainly of IgG, and sometimes C3, in the epidermal BMZ. Indirect IF studies reveal binding of IgG anti-basement membrane autoantibodies exclusively to the dermal side of 1M NaCl split skin.
The presence of antilaminin 332 autoantibodies is essential for the diagnosis of AECP. We reviewed the literatures about antilaminin 332 autoantibodies in patients with immunobullous diseases, including bullous pemphigoid (BP), EBA, AECP, and other CPs except AECP, the results are summarized in Table 1. Most of these studies used enzyme-linked immunosorbent assay (ELISA) to detect antilaminin 332 autoantibodies or immunoblotting or immunoprecipitation to detect laminin 332 in human keratinocytes (HKs) extracts. Lazarova et al found that 3 of 34 (8.8%) and 13 of 100 (13%) BP patients' sera were detected with antilaminin 332 IgG4 by ELISA, but none of these patients' sera could immunoblot or immunoprecipitate laminin 332 from HKs extracts. Bekou et al found that 29 of 72 (40.3%) BP patients' sera were detected with antilaminin 332 IgG by ELISA, but the disease severities did not correlate with the levels of antilaminin 332 IgG. Lazarova et al explained that this elevated frequency of false-positive ELISA results among BP patients may be because of elevated serum level of other IgG4 and the use of a conjugated antihuman IgG as a second step antibody—a polyclonal reagent that yielded high background reactivity against laminin 332. None of the patients' sera from EBA or other CPs except AECP could either immunoblot or immunoprecipitate laminin 332 in HKs extracts. Although patients with other undefined immunobullous diseases had been reported to have antilaminin 332 autoantibodies by ELISA, immunoblotting, or immunoprecipitation, these were relatively rare cases reported. And most of these case reports showed circulating IgG and/or C3 binding to epidermal side or both epidermal and dermal side of 1M NaCl split skin by indirect IF. In conclusion, the presence of antilaminin 332 autoantibodies is essential for the diagnosis of AECP and has pathogenic activities in vivo by passive transfer animal models.

It is difficult to distinguish patients with initially skin-predominant AECP from similar findings of EBA based only on clinical and histo- and immunopathologic examinations. Indirect IF with 1M NaCl split skin can differentiate BP from AECP, EBA, and bullous LE because IgG binds to the epidermal roof of 1M NaCl split skin in BP, in contrast to the dermal floor in the other three immunobullous diseases. Clinical information, including the American Rheumatism Association criteria for systemic LE and positive lupus serology, can help differentiate bullous LE from AECP and EBA. The more sensitive techniques, such as ELISA, western immunoblotting, immunoprecipitation, and immunoelectron microscopy, can confirm the final diagnosis of different subepidermal immunobullous diseases. However, these are often time consuming and expensive. Diagnostic indirect IF on antigen-deficient substrates of Type VII collagen-deficient or laminin

Table 1 Detection of antilaminin 332 autoantibodies in patients with immunobullous diseases by ELISA, immunoblotting, or immunoprecipitation.

<table>
<thead>
<tr>
<th>Method</th>
<th>AEC (n, %)</th>
<th>EBA (n, %)</th>
<th>CP (except AEC) (n, %)</th>
<th>BP (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-laminin 332 autoantibodies ELISA</td>
<td>29/32 (90.6)&lt;sup&gt;8&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>3/34 (8.8)&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Immunoblotting</td>
<td>30/32 (93.8)&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0/16 (0)&lt;sup&gt;10&lt;/sup&gt;</td>
<td>0/3 (0)&lt;sup&gt;10&lt;/sup&gt;</td>
<td>13/100 (13)&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td>Immunoprecipitation</td>
<td>32/32 (100)&lt;sup&gt;8&lt;/sup&gt;</td>
<td>0/4 (0)&lt;sup&gt;11&lt;/sup&gt;</td>
<td>0/5 (0)&lt;sup&gt;11&lt;/sup&gt;</td>
<td>29/72 (40.3)&lt;sup&gt;9&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as n (%).

AEC — antiepiligrin cicatricial pemphigoid; BP — bullous pemphigoid; CP — cicatricial pemphigoid; EBA — epidermolysis bullosa acquisita; ELISA — enzyme-linked immunosorbent assay.

Figure 4 Indirect immunofluorescence revealed (A) positive reaction on Type VII collagen-deficient skin and (B) negative reaction on laminin 332-deficient skin (x > 160).
332-deficient skin from patients with inherited dystrophic and junctional epidermolysis bullosa, respectively, can provide a rapid and simple test to help clinicians differentiate between AECP and EBA. If indirect IF on antigen-deficient skin substrates show positive reaction on Type VII collagen-deficient skin and negative finding on laminin 332-deficient skin, this demonstrates that the patient’s serum contains antilaminin 332 autoantibodies instead of anti-Type VII collagen autoantibodies. Hence, AECP is diagnosed and vice versa. Two added indirect IF tests can provide a double check and improve the specificity and sensitivity of diagnosis.

It is of significant importance to distinguish AECP from other immunobullous diseases because of increased relative risk (RR) of developing solid cancers or lymphomas, especially in the 1st year after the onset of blisters. A cohort study of 35 patients with AECP in a 12-year follow-up period showed a RR of 6.8, with 10 patients having a solid cancer. The RR increased up to 15.4 in the 1st year after the blister onset. All the solid cancers reported in this cohort study were adenocarcinoma of visceral origin, including the lungs, stomach, colon, and uterus. The 10 AECP deaths were cancer related, occurring within 21 months of cancer diagnosis. Another review study revealed two patients with AECP having diffuse large B-cell lymphoma and cutaneous T-cell lymphoma, respectively. Thus, patients with AECP have a higher morbidity and mortality rate than other immunobullous diseases because of their association with cancers. Age-appropriate cancer screening is therefore recommended in patients diagnosed with AECP.

The mechanism of association of AECP with cancer is still unknown, but laminin 332 seems to play a central role. Laminin 332, a heterotrimeric adhesion protein consisting of α3β3γ2 subunits, is expressed in the basement membrane of normal epithelia and in the extracellular matrix of various malignancies. AECP is considered a paraneoplastic syndrome, a condition characterized by an autoimmune response to laminin 332 present in both normal skin and the underlying malignancy. This is supported by observations that clinical disease activity of AECP correlates with total tumor mass. That is, the clinical disease activity of AECP is markedly reduced when the primary tumor is partially or completely removed, and metastasis or cancer recurrence is associated with recurrent or high disease activity.

Treatment of all subsets of CP, including AECP, is similar. It depends on disease location, extent, and severity. In limited mucosal or cutaneous lesions, topical or intralesional glucocorticoids with topical anesthetic agents and oral hygiene may suffice. While in extensive or progressive disease, systemic agents are warranted as following. Dapsone and tetracycline have been reported to be effective in some patients. If there is poor response to both agents, systemic glucocorticoid in combination with immunosuppressive agents, such as cyclophosphamide, azathioprine, or mycophenolate mofetil, are indicated. High dose intra-venous immunoglobulin is reserved for patients who are refractory to the above-mentioned therapies. During treatment of AECP, early detection of solid cancer or lymphoma is important to decrease the cancer-related morbidity and mortality.

In conclusion, this is a report of a middle-aged male patient with an initial skin-predominant AECP, which was distinguished from EBA using indirect IF of Type VII collagen- and laminin 332-deficient skin. The patient had good response to systemic treatment with azathioprine and prednisolone. The relationship of AECP with cancers and the effect on prognosis warrant long-term follow-up.

References