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Epidermolysis Bullosa Aquisita with Basal Epidermal Cytoplasmic Antibodies

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A 45-year-old woman with epidermolysis bullosa aquisita is presented. The clinical, histological, and immunopathological features were in keeping with the previous reports of this disease. The patient also had anti-basal cell cytoplasmic antibodies at a significant titer, which is considered an unusual finding associated with this disorder. Treatment with a moderate dose of corticosteroid was effective in controlling the bullous lesions.

Key Words: *Epidermolysis bullosa acquisita; Basal epidermal cytoplasmic antibodies.*

INTRODUCTION

Epidermolysis bullosa aquisita (EBA) is an acquired mechanobullous disease of the skin. Patients with EBA usually develop bullae and erosions on acral extensor joint surfaces or points of mechanical stress. The blister is formed by a separation of the skin at the zone of the basement membrane between the epidermis and the dermis. Recently Yaoita and collegues (1981), and Nieboer et al. (1980), demonstrated linear deposits of IgG within the basement membrane of patients' skin, which suggested that blistering may be initiated by an immune process.

There were some problems in diagnosing and differentiating this disease from other bullous disorders, and Roenigk et al. (1971; 1981), established the diagnostic criteria for this disease as, clinical lesions of trauma induced bullae, postinfancy onset, 3) no family history, and 4) exclusion of other bullous diseases by confirming the deposits of immunoreactants at the sub-basal lamina anchoring fibril zone of the basement membrane.

Although it was first described in 1895 by Elliot, fewer than seventy cases have been reported in the English literature so far. The following case study of a patient with EBA reports clinical and immunohistopathological features which had distinct anti-basal cell cytoplasmic antibodies.

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CASE REPORT

A 45-year-old Korean woman presented with a 10-year history of skin fragility characterized by recurring, non-painful blisters on sites of trauma, especially over joints, and some pruritic spontaneously ocurring blisters. These bullous lesions resolved with scarring. Most of the bullous lesions were related to trauma at work and not to sun exposure, medications, diet or other illnesses. Since the onset of her skin disease and for the past 10 years she has taken various doses of corticosteroids to suppress new lesion formation. Family history revealed nothing of note and she has no any family members suffering from cutaneous blistering disorders.

Referral to our service led to the clinical diagnosis of EBA and the following work-up. Examination showed a thin 50 kg, normotensive woman with varied skin lesions distributed over the trauma prone part of the body including the dorsal aspects of both hands and feet, wrists, elbows, ankles, knees (Fig. 1), and oral mucous membrane. Fresh lesions were 3 mm to 1.5 cm tense bullae, on nonerythematous or erythematous skin. The bullae contained clear to slight hemorrhagic fluid. Resolving lesions were dry and crusted, or

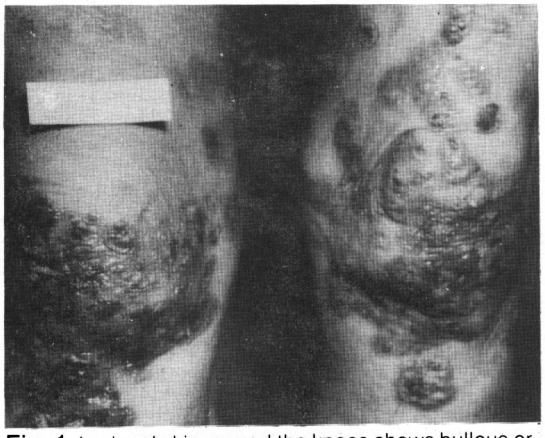

Fig. 1. Lesional skin around the knees shows bullous or ulcerative changes with a few bleeding spots and hyperpigmented scars.

erosive with occasional bleeding, and left multiple superficial atrophic scars, hypo-or-hyperpigmentation, and prominent milia. Mild nail dystrophy, consisting of distal onycholysis were noted on several nails. Diffuse oral erosion and ulcerative changes were noted with a few spots of bleeding. Cicatricial alopecia were seen on the scalp, axillae, and the pubic areas. Light friction trauma with a pencil eraser induced clinical separation of the skin within 20 minutes, and the lesion produced resembled the acquired lesions. Other physical findings were all in normal ranges.

Laboratory data are as follows. A complete blood count with differential, urinalysis, and stool guaiac examination were within normal limits or negative. Erythrocyte sedimentation rate was elevated at 50mm/hr. Chest roentgenogram and electrocardiogram were not remarkable. The pattern of serum protein electrophoresis showed increased gamma globulin but M-spike was not seen. The serum concentration of IgG was increased of 2,730 mg/dl, but other immunoglobulins were in normal ranges. Total hemolytic complement (CH50), C3 and C4 concentrations, NBT (nitroblue tetrazolium) test score, antinuclear antibody, latex-fixing rheumatoid factor, cryoglobulin, α 1 antitrypsin, urine Bence-Jonce protein, urinary porphyrin, and the values of liver function tests and thyroid function tests, blood urea nitrogen level, anti-streptolysin 0 titer, and VDRL (venereal disease research laboratory) data were all within normal limits or negative. C-reactive protein was found to be strong positive. The proportion of T-cells in the peripheral blood measured by E-rosette techniques was 54% (normal, 65-75% in this study).

Esophagoscopic examination revealed some erosion and signs of esophagitis. Colonosigmoidoscopy showed several areas of hyperemic mucosa, but there was no evidence of ulcerative changes. Roentgenographic upper gastrointestinal contrast studies and small bowel follow-through were normal. Cystoscopic examination revealed slightly hyperemic walls with a few areas of ecchymoses, and the biopsy of the bladder wall showed occasional submucosal vesicles with a mild inflammatory changes. Cervix examination disclosed erosive and hyperemic, and the biopsy showed a few microvesicles between the mucosal epithelium and the stromal tissue. The finding of the bladder and cervix could be the pathologic changes caused by EBA disease processes, but there were no deposits of immunoreactants when studied by immunofluorescence.

Biopsy studies of the early lesional skin on the extensor forearm and buccal mucous membrane showed subepidermal blisters containing many red blood cells, a few neutrophils and eosinophils. A moderate inflammatory infiltrate consisting of neutrophils, eosinophils, and plasma cells was seen in the upper dermis.

Immunofluorescent (IF) studies were performed by the standard methods (Jordon, 1980) with lesional and normal appearing skin. Direct IF showed coarse linear deposits of IgG and C3 at the basement membrane zone of both specimens (Fig. 2). Indirect IF studies of the serum, using normal human skin (blood group

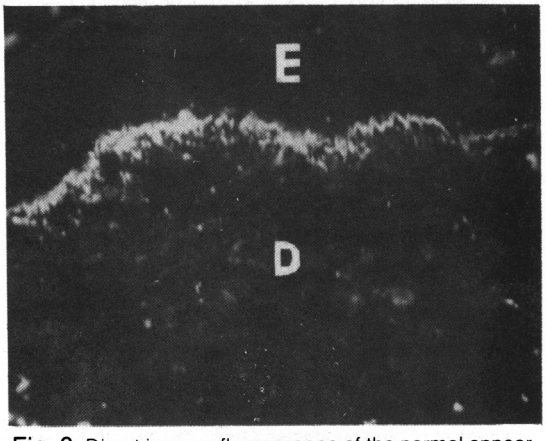

Fig. 2. Direct immunofluorescence of the normal appearing skin shows coarse linear deposits of IgG along the basement membrane zone (E, epidermis; D, dermis, ×200).

O) taken from the flank as the substrates, demonstrated circulating basement membrane zone antibodies (IgG) at a titer of 1:80, and basal cell cytoplasmic antibodies (IgG) at a titer of 1:32.

The biopsy and IF studies with the clinical features were consistent with EBA. The patient was instructed to avoid trauma and instituted with devided doses of prednisolone (40 mg a day). Topical cares were also adjusted for her skin conditions. After four weeks of treatment blistering lesions almost subsided and serum titer of anti-basement membrane zone antibodies decreased to 1:20. Oral lesions also were much improved with no new lesion formations.

CLINICAL EXPERIMENTS

In vitro C3 staining

To examine the complement fixing activity of the IgG autoantibodies, in vitro C3 stainings were carried out using a 2-step method. Normal human skin sections (the same tissue used for indirect IF) were treated with heat inactivated(56°C for 30 minutes)serum dilutions (1:10, 1:20, 1:40, etc). These were prepared with fresh normal human serum diluted 1:10 in veronal buffered saline (PH 7.4) containing 0.5mM of magnesium chloride and 0.15mM of calcium chloride. This resulted in a complement concentration of approximately 10-15 hemolytic unit/ml. In step 1, the normal human skin sections were treated with heat inactivated patient serum dilution plus human complement. In step 2, the tissue were treated with fluorescein labeled anti-human C3. Routine controls as well as experimental controls for in vitro complement IF stainings were performed as previously described (Lee and Jordon, 1980). On the fluorescent microscopy the patient serum yielded positive in vitro C3 staining at a titer of 1:20, all routine controls and experimental controls yielded negative results.

Localization of the tissue bound immune complexes

In stead of using electron microscopy for the localization of the immunoreactants, and for further differentiation from bullous pemphigoid, another system of indirect IF studies using salt-split skin preparations as the substrates (Gammon et al.,1984 a;Woodley et al., 1984) was performed. The differences in the binding site of the anti-basement membrane zone antibodies in bullous pemphigoid and EBA are known, and these substrates, made separation through the lamina lucida by incubation of the normal skin in a 1 M sodium chloride solution for 96 hours at 4°C, was useful for discriminating the site of deposition of autoantigen-immune complexes between these two diseases. Indirect IF was used to examine patient serum, bullous pemphigoid, and normal control sera at a dilution of 1:10. The patient serum was found to bind only to the dermal aspect, below the plane of separation (Fig. 3). Serum samples from the patient with bullous pemphigoid stained the epidermal side of the separated skin and left the dermis unstained. None of the control sera produced any staining patterns at either side of the separation. This indicates that the serum sample from this patient stained a unique region of the basement membrane that was recently recognized by others (Gammon et al., 1984 a; Woodley et al., 1984).

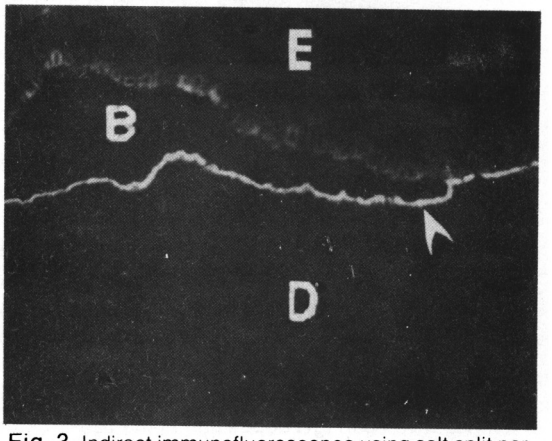

Fig. 3. Indirect immunofluorescence using salt-split normal human skin preparations as the substrates, indicates the binding site of EBA autoantibodies (arrow). Stainings of anti-basal cell cytoplasmic antibodies are also seen (E, epidermis; D, dermis; B, blister space, ×200).

DISCUSSION

EBA is classified as a mechanobullous disease, though it possesses notable features not shared with the other disorders. Immunopathologically this blistering disease is characterized by the presence of circulating complement binding IgG anti-basement membrane zone antibodies, linear deposits of IgG and complement at the sub-basal lamina of the lesional and normal appearing skin, and subepidermal bulla with inflammation in the upper dermis of the lesional skin (Roenigk and Pearson, 1981; Gammon et al., 1984 a; Woodley et al., 1984; Ray et al., 1982). These features suggest that antibody-mediated complement activation may play a role in the pathogenesis of inflammation and dermoepidermal separation in this disease.

Clinically the most dramatic features of this disease is the extreme fragility of the skin, and the vesicula-

tion which is most prominent on the extremities prone to trauma as were seen in our patient. Although blisters are usually present on the extremities and other trauma susceptible sites, they may also occur spontaneously on the trunk or other sites, as other autoimmune bullous dermatoses.

This disorder is frequently associated with systemic diseases, usually of an autoimmune nature. Inflammatory bowel diseases have been noted in approximately 30% of cases (Ray et al., 1982; Raab et al., 1983; Gammon et al., 1982). Other less commonly reported diseases associated with EBA include systemic lupus erythematosus, thyroiditis, diabetes mellitus, amyloidosis, multiple myeloma, and cryoglobulinemia (Raab et al.,1983; Palestine et al.,1981; Krivo and Miller, 1978). In this patient, extensive investigations have failed to see any of the other associated diseases.

The criteria for the diagnosis of EBA which most rely on is proposed by Roenigk et al (Roenigk et al; 1971; Roenigk and Pearson, 1981), but the problem was the "exclusion of other bullous diseases". Recently Kushniruk (1973) demonstrated in vivo bound IgG deposited in a linear fashion along the basemen t membrane zone of skin of patients with this disease, which was a similar feature found in pemphigoid. Futhermore Yaoita et al.,(1981) and Nieboer et al. (1980) have seen IgG deposition in the upper dermis just beneath the basal lamina by electron microscopy, and this clearly differentiate EBA from pemphigoid in which the IgG are located to the lamina lucida (Holuba et al., 1975). The results of the above clinical experiments to discriminate the binding site of serum autoantibodies substantially fulfilled the immunopathologic criterion in this patient. The IgG autoantibodies in the serum of this patient also had the ability to fix complement.

There is no satisfactory explanation at the present time of the pathogenesis of EBA. The leukocyte attachment assay performed by Gammon et al. (1984b) have shown that EBA immune complexes formed in vivo are capable of activating complement and mediating the attachment of leukocytes to the basement membrane zone as in pemphigoid. The mechanism of leukocyte mediated dermoepidermal separation is not clear but presumably leukocyte derived proteases or reactive oxygen intermediates may play some roles. However, this may not be relevant to all EBA lesions. The deposition of immunoreactants may interfere, physically or sterically, with the anchoring fibrils' association with the basal lamina and underlying collegen bundles, and which may contribute to the blister formation. Pass and Dobson (1965) had shown that there was a decrease in hydroxyproline content in their patients with EBA. Sasai (1965) suggested with his histochemical studies that there was a decrease in the mature collagen fibers in the upper dermis. Thus it could be possible that the dermal fragility which may be due to an abnormal synthesis of collagen, can also participate in the production of mechanobullous lesions. Another hypothesis, proposed by Medenica (1981), is that of increased collagenase activity, resulting in damage to sub-basal fibrous components that may become antigenic and bind IgG and complement.

Antibodies directed against cytoplasmic antigen of normal keratinocytes have been known for many years to be present in some normal sera as well as in the sera of patients with various cutaneous and noncutaneous disorders as an autoimmune phenomenon. But as in this patient, antibodies directed only to the cytoplasm of the basal cells have been described far less often. As has been reported, the occurence of anti-basal cell cytoplasmic antibodies at a significant titer could be found in those patients with immune disorders involving the skin such as pemphigus, pemphigoid, allergic drug reaction, or burn victims as well as in cutaneous malignancies (Bystryn, 1977; Paluch and Bloch, 1982), however is rarely found in patients with EBA (Palestine et al., 1981). The nature of this antigenic moiety is unknown, but it might be differentiation antigens of the epidermis (Hinter et al., 1983).

Medications that have been shown variable success in the treatment of EBA include dapsone, corticosteroids, azathioprine, gold sodium thiomalate, vitamin E, sulfasalazine, and phenytoin (Raab et al., 1983). There is no proved effective therapy, and these drugs have provided inconsistent benifit. In our patient corticosteroid of moderate dosage was effective in controlling this blistering disease.

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