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Epidermolysis Bullosa Acquitsita

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Abstract

EBA is a rare, acquired, chronic subepidermal bullous disease of the skin and mucosa characterized by autoantibodies to type VII collagen structures, a major component of anchoring fibrils, that attach the epidermis onto the dermis. EBA patients have tissue-bound as well as circulating anti-type VII collagen autoantibodies that attack type VII collagen and result in a reduction or perturbation of normally functioning anchoring fibrils. Patients with EBA have skin fragility, blisters, erosions, scars, milia, and nail loss: all features reminiscent of genetic dystrophic epidermolysis bullosa. These anti-type VII collagen antibodies are “pathogenic” because when injected into mice, the mice develop an EBA-like blistering disease. In addition to the classical mechanobullous presentation, EBA also has several other distinct clinical syndromes similar to bullous pemphigoid, Brunsting-Perry pemphigoid, or cicatricial pemphigoid. Although treatment for EBA is often unsatisfactory, some therapeutic success has been achieved with colchicine, dapsone, plasmaphoresis, photopheresis, infliximab, and intravenous immunoglobulin.

Introduction

Epidermolysis bullosa acquista (EBA) is an acquired, subepidermal bullous disease with clinical features similar to the genetic forms of dystrophic epidermolysis bullosa (DEB). In DEB there is a hereditary defect in the gene that encodes type VII collagen (C7), the major component of anchoring fibrils. Anchoring fibrils (AFs) are structures that anchor the epidermis and its underlying basement membrane zone (BMZ) onto the dermis. DEB patients have a reduction or complete absence of normal functioning AFs. In EBA, there is also a paucity of AFs, but this reduction is due to the presence of IgG autoantibodies targeted against the C7 within AFs. So, in both cases, either by a hereditary gene defect or by an acquired autoantibody that targets C7 in AFs, the end result is a perturbation of AFs leading to a clinical phenotype featuring skin fragility, blisters, erosions, scars, milia and nail loss. EBA patients have tissue-bound as well as circulating autoantibodies to C7 causing many of the symptoms characterizing the disease. These IgG anti-C7 antibodies are pathogenic because when injected into mice, the mice develop an EBA-like blistering disease. EBA has a variety of clinical presentations that often overlap with other blistering skin diseases such as DEB and bullous pemphigoid (BP), so the diagnosis can often be difficult; however, there are several laboratory tests that assist in confirming the diagnosis of EBA. Nonetheless, once the diagnosis is established, the treatment options are limited. There are newer treatment modalities that have achieved some therapeutic success. In this

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review, we will provide an update on the recent progress in the elucidation of the pathogenesis of EBA, the different clinical presentations of EBA, clinical and laboratory diagnoses of EBA, and potential therapies that may benefit EBA patients.

Etiology and Pathogenesis

EBA is a rare, autoimmune bullous disease with a prevalence of approximately 0.2 per million people and has an unknown etiology. Because the disease features IgG autoantibodies directed against C7, it is thought that EBA has an autoimmune pathogenesis [1, 2]. Another autoimmune bullous skin disease which may exhibit auto-antibodies against C7 is bullous systemic lupus erythematosus (SLE) [3]. Both EBA and bullous SLE patients often have a common human leukocyte antigen (HLA) major histocompatibility (MHC) class II cell surface protein, HLA-DR2 [4]. This HLA phenotype has been associated with hyperimmunity, which again suggests an autoimmune etiology for EBA.

Although there is no racial or gender predilection [5], EBA has recently been suggested to have a higher prevalence in the Korean population [6]. The age of onset varies widely from early childhood to late adult life, but most cases begin between the fourth and fifth decades [7, 8].

EBA is characterized by autoimmunity to C7 present within AFs. The AFs anchor the epidermis and its underlying BMZ to the papillary dermis. Immunoglobulin G (IgG) autoantibodies to C7 are associated with a paucity of normal AFs at the BMZ, separation of the epidermis from the dermis, and poor epidermal-dermal adherence. Although it is an acquired disease that usually begins in adulthood, EBA was placed in the category of epidermolysis bullosa (EB) approximately 100 years ago because physicians were struck by the similarity of the clinical lesions seen in EBA patients and those seen in children with hereditary dystrophic forms of EB. The linkage to autoimmunity is confirmed by the presence of IgG deposits at the dermal-epidermal junction (DEJ).

C7 is made of three identical alpha chains, each consisting of a 145-kDa central collagenous triple-helical domain, flanked by a large 145-kDa amino-terminal noncollagenous domain (NC1), and a small 34-kDa carboxyl-terminal noncollagenous domain (NC2) [9]. Within the extracellular space, C7 molecules form antiparallel, tail-to-tail dimers, which then aggregate laterally to form AFs. The NC1 domain harbors multiple sub-modules with homology to adhesive proteins, including a segment with homology to cartilage matrix protein (CMP), a segment with homology to the A domain of von Willebrand factor (VWF), and nine consecutive fibronectin type III-like repeats (FNIII). Therefore, the NC1 domain may facilitate binding of C7 to other BMZ and matrix components and stabilize the adhesion of the BMZ to the underlying dermis. Using recombinant NC1 expressed from human cells, we demonstrated that NC1 interacts with various extracellular matrix components including fibronectin, laminin 5, type I collagen, and type IV collagen [10].

We and others have shown that major antigenic epitopes of C7 are located within the NC1 domain; [11-13] however, there has been recent evidence indicating the presence of other domains that are potential targets for EBA autoantibodies. A novel EBA subgroup with a milder clinical presentation was defined in children with tissue-bound and circulating autoantibodies targeting only the triple-helical central domain of C7 [14, 15]. In addition, recent reports showed that the NC2 domain, which may be pertinent in the formation of antiparallel dimers and AF assembly, also contains minor antigenic epitopes. These data suggest that EBA may exhibit a wider, more heterogeneous spectrum of autoantibody reactivities than previously assumed, a fact that should be taken into consideration while designing tests to detect EBA autoantibodies. To date, there has been no convincing

evidence that differences in epitope specificity of EBA autoantibodies correlate with clinicopathologic types, complement fixing abilities, or IgG subclasses in EBA.

EBA does not always present as a non-inflammatory mechanobullous disease reminiscent of DEB. Although less common, EBA may also present as an inflammatory, widespread, vesiculobullous disease reminiscent of BP. It is possible that autoantibody recognition of one or several domains of C7 may invoke an inflammatory cascade which could result in proteolytic degradation of matrix components within the DEJ that are essential for epidermal-dermal adherence. Therefore, within the spectrum of EBA and autoimmunity to C7, there are several possible mechanisms for autoantibody-induced blister formation. First, because EBA often occurs with minimal clinical or histologic inflammation, it is possible that the EBA autoantibodies target certain epitopes on the NC1 domain of C7 and make the function of the collagen compromised. This could perturb critical direct interactions between C7 and other extracellular components within the DEJ or upper dermis such as type IV collagen, laminin-5, and fibronectin [10, 16]. Also, there may be an interruption in the C7-fibronectin interaction in the collagenous domain which may be important for the adherence of basement membrane and the overlying epidermis onto the papillary dermis [16]. A second mechanistic possibility is that EBA autoantibodies may interfere directly with the antiparallel dimer formation of C7 and consequently, anchor fibril assembly [17]. Both mechanisms are attractive possibilities that explain skin fragility and blistering in patients with the classical EBA phenotype who lack significant inflammation in lesions but have defective lamina densa– dermal adherence. Another possibility is that the EBA autoantibody targeting one or more domains of C7 induces an inflammatory response leading to the destruction of essential matrix components in the DEJ. The local inflammation could also induce complement fixation. The consequence of the inflammatory response is blister formation and tissue damage at the DEJ [18]. This latter mechanism may explain the inflammatory, BP-like variety of EBA characterized by vesiculobullous lesions on inflamed skin.

Several independent lines of evidence derived from clinical, histological, and immunological studies have implicated the autoimmune response as a key element in the pathogenesis of EBA. A number of recent passive transfer studies have provided direct evidence for a pathogenic role of EBA autoantibodies in disease induction. Recently, we immunized rabbits and raised a high titer antiserum to the NC1 domain of human C7 and injected this antibody into hairless immunocompetent mice. The mice developed a skin condition that had many of the features of EBA in humans, including subepidermal blisters and nail loss on their paws. They also had circulating NC1 antibodies in their blood from the injections and had anti-NC1 IgG antibody deposits at their DEJ. In addition, like most EBA patients, the mice had murine complement deposits at the DEJ induced by the autoantibody–antigen complex [19].

A similar study showed that mice receiving rabbit polyclonal antibodies to the NC1 domain of mouse C7 also developed subepidermal skin blisters reminiscent of human EBA [20]. Further studies showed that EBA induction in this passive transfer animal model requires complement activation and infiltration of granulocytes into the skin. In another study, we affinity-purified anti-C7 antibodies from EBA patients' sera and injected them into hairless immunocompetent mice. The mice developed skin fragility, blisters, erosions and nail loss on their paws – all features of EBA patients [21]. By clinical, histological, immunological and ultrastructural parameters, the induced lesions were reminiscent of human EBA.

In a more recent study, affinity-purified IgG antibodies against a small N-terminal 227AAs CMP homology domain from EBA patient sera were injected into mice. The mice developed blisters and nail loss just like whole EBA patient sera. This shows that EBA patient

antibodies against a small domain of the 290 kDa auto-antigenic target are highly pathogenic [22]. This is the first antigenic epitope on C7 proven to be a pathogenic target for EBA autoantibodies.

An active model for EBA induction has also been reported. Sitaru *et al.* injected a recombinant fragment of the murine NC1 domain into mice and induced an EBA-like disease; however, they found that EBA induction only occurred in certain strains of mice, suggesting that the immunological background and response of the patient are critical to the pathogenesis of the disease [23, 24]. Taken together, these successful experiments on both passive transfer and active animals models strongly suggest that EBA autoantibodies are "pathogenic" and capable of causing epidermal–dermal separation in skin.

Clinical Manifestations

There is great diversity in the clinical presentation of EBA. The common denominator for patients with EBA is autoimmunity to C7 and diminished AFs [2, 25]. The complete range of EBA clinical manifestations is still being elucidated; however, there are 5 main clinical presentations: (1) a 'classical' presentation, (2) a BP-like presentation, (3) a CP-like presentation, (4) a Brunsting-Perry pemphigoid presentation, and (5) a linear IgA bullous dermatosis (LABD)-like disease.

Classical Presentation

The classical presentation is a non-inflammatory bullous disease with an acral distribution that heals with scarring and milia formation. The mild form of this type of presentation is similar to porphyria cutanea tarda (PCT), while the more severe form is similar to hereditary recessive dystrophic epidermolysis bullosa (RDEB) [26]. EBA patients, however, do not have all the symptoms of porphyria-like hirsutism, photosensitivity, scleroderma-like changes, and high levels of urinary porphyrins [2, 25].

Classical EBA is a mechanobullous disease marked by skin fragility over trauma-prone surfaces. These patients typically present with blisters, erosions and scars over the backs of the hands, knuckles, elbows, knees, sacral area, and feet. There is often significant involvement of the oral mucosa with erosions and frank blisters. On the glabrous skin, the vesicles and bullae appear tense on non-inflamed or scarred skin. They can be hemorrhagic and can result in erosions, crusts, scales, scars, scarring alopecia, milia cysts and nail dystrophy. The lesions heal with scarring and frequently with the formation of pearl-like milia cysts within the scarred areas. In severe cases, there may be fibrosis of the hands and fingers and esophageal stenosis [27, 28]. The histology shows dermal-epidermal separation at the basement membrane and minimal inflammation.

Bullous Pemphigoid-like Presentation

These patients present with features characteristic of the autoimmune bullous disease, BP, or a mixture of features characteristic of both BP and classical EBA presentation [18, 29]. This form of EBA manifests with vesiculobullous eruptions that are widespread, typically involving the trunk, central body, extremities, and skin folds usually accompanied by pruritus. The bullae are tense and surrounded by inflamed and/or urticarial skin. Patients frequently have pruritus but lack skin fragility, scarring, or milia formation as seen in the classical EBA presentation. Histology reveals aggregates of polymorphic infiltrates of mononuclear cells and granulocytes. The granulocytes are usually neutrophils, though eosinophils are seen on occasion.

Cicatricial Pemphigoid-like Presentation

The clinical features in these patients closely resemble those considered characteristic of CP. Similar to CP, the lesions and scars usually involve the mouth, upper esophagus, conjunctivae, anus, and vagina [28, 30] with or without similar lesions on the glabrous skin. There has also been evidence of involvement of the trachea in these patients [31] and of mucosal involvement without scarring [32]. Unlike classical EBA, patients with the CP-like presentation often do not show significant skin fragility, evidence of trauma-induced lesions, or a predilection for blistering on extensor skin surfaces. Histology indicates the presence of subepidermal blisters with microscopic scarring and with a mixed inflammatory infiltrate in the upper dermis and at the BMZ.

Brunsting-Perry Pemphigoid-like Presentation

Brunsting-Perry cicatricial bullous pemphigoid is defined by head and neck vesiculobullous lesions that are chronic and recurrent. In contrast to CP, it has minimal or no mucosal involvement [33, 34]. The lesions are localized to the head and neck and can have residual scars, subepidermal bullae, IgG deposits at the DEJ, and minimal or no mucosal involvement. The antigenic targets for the IgG autoantibodies have yet to be defined, but there is the presence of IgG autoantibodies targeting AFs below the lamina densa in patients with the Brunsting-Perry Pemphigoid-like presentation [33]. The presence of IgG autoantibodies targeting C7 supports the idea that Brunsting-Perry pemphigoid may actually be a clinical presentation of EBA.

LABD-like Presentation

This type of EBA is defined by a subepidermal vesiculobullous eruption, a neutrophilic infiltrate and linear IgA deposits at the BMZ. The clinical features, such as tense vesicles arranged in an anular pattern along with involvement of mucous membranes, may resemble Chronic Bullous Disease of Childhood [15]. The autoantibodies present in the patient are usually IgA, IgG, or both. Currently, there are different views about patients with IgA autoantibodies against C7. Some clinicians believe that these patients have only LABD [35], while others believe that they are a subset of EBA [36, 37]. A study, in which 20 EBA patients' sera were evaluated for serum IgA anti-C7 antibodies by immunoblotting, showed low titers of IgA anti-C7 antibodies in 80% of the patients in addition to presence IgG antibodies [38].

Childhood EBA is a rare disease with variable presentations. In a study in which 14 children with EBA were evaluated, it was found that five of the patients presented as a LABD- like disease, five patients presented with the BP-like form of EBA and the remaining four children presented with the classical mechanobullous form of EBA [15]. Furthermore, eleven out of 14 had mucosal involvement, and all had IgG deposits at the BMZ by direct immunofluorescence (DIF). Indirect immunofluorescence (IIF) was positive in 10 out of 14 patient sera, and the IgG constituted a predominant serum antibody. Although mucosal involvement is frequent and severe in childhood EBA, the overall prognosis and treatment is more favorable than in adult EBA [15, 39].

The clinical presentation of the EBA patient may change during the course of the disease or can show two different presentations simultaneously. About 25% of patients with EBA may appear with the BP-like clinical appearance (unpublished observation). With time, the disease of some patients will eventually smolder into a more noninflammatory classical form of EBA. Both the classical and the BP-like forms [28], and the CP-like and BP-like forms of the disease may coexist in the same patient [31]. The clinical phenotype of EBA reminiscent of pure CP is probably rarer as it only occurs in fewer than 10% of all EBA cases. To date, although the relationship between the epitope profile and the clinical features

(particularly classical non-inflammatory vs. inflammatory EBA) remains to be elucidated, it has been postulated that autoantibodies targeting different epitopes, such as the NC1 domain and other domains, leads to the different clinical phenotypes. This hypothesis is supported by several previous reports showing that some childhood EBA patients with reactivity to the triple-helical collagenous domain, as well as the NC1 and NC2 domains, were of the inflammatory type [40-43].

The Relationship Between EBA and Other Systemic Diseases

A number of published reports suggest that EBA may be associated with various systemic diseases [26] such as inflammatory bowel disease [44], SLE, amyloidosis, thyroiditis, multiple endocrinopathy syndrome, rheumatoid arthritis, pulmonary fibrosis, chronic lymphocytic leukemia, thymoma, diabetes, and other diseases, however, EBA is a relatively rare disease, and most of these are anecdotal reports. It appears that the most frequently associated disease with EBA is inflammatory bowel disease (IBD), with an estimate of 25% of 50 EBA patients reviewed by Chan and associates [45]. It has also been shown that patients with IBD, especially Crohn's disease (CD), have a high prevalence of circulating antibodies against C7 detected in up to 68% of CD patients [46]. Ulcerative colitis also associates with autoantibodies against C7, although with a lower frequency when compared with CD [46]. In addition, C7 was recently shown to be present in the intestinal epithelium [47]. Because C7 is the antigenic target for autoantibodies in patients with EBA, we speculate that autoimmunity to C7, which exists in both gut and skin, may explain why these patients frequently have inflammatory bowel disease. The presence of C7 antibodies in Crohn's disease patients may be an epitope spreading phenomenon. Inflammation originally invoked by Crohn's disease perturbs the intestinal epithelial BMZ such that BMZ components are altered, resulting in an on-going autoimmunity to C7 [48]. Although a remarkable and intriguing association exists between autoimmunity against C7 and IBD, future clinico-epidermiologic, genetic and immunologic studies are necessary to further elucidate the association of IBD with skin blistering by autoantibodies against C7.

Laboratory Tests

Histopathology

Lesional skin histology initially shows papillary edema and vacuolar alteration along the DEJ and at a later stage, a subepidermal blister. Various degrees of a dermal inflammatory infiltrate are seen depending upon the clinical presentation. The classical presentation shows little inflammatory infiltrate within the dermis as opposed to the BP-like presentation [49]. The infiltrate can be found around vessels, around follicles and in the interstitium. The BP-like variant of EBA usually has significantly more inflammatory cells within the dermis, and these cells may be a mixture of lymphocytes, monocytes, neutrophils, and eosinophils. The histology of EBA skin specimens obtained from BP-like lesions may be difficult to distinguish from BP itself. Because of the variable clinical and histological presentations, it is difficult to diagnose EBA by clinical and histological parameters alone.

Ultrastructural studies of EBA skin have demonstrated a paucity of AFs and an amorphous, electron-dense band just beneath the lamina densa [50, 51]. Although the autoantibodies are directed against the AFs in the sublamina densa region of the BMZ, it should be noted that the cleavage plane of the blister may be either in the lamina lucida or the sublamina densa region where AFs are located [52]. Immunomapping studies have shown that EBA blisters frequently separate above the immune deposits within the lamina lucida [52]. This is because the lamina lucida is the Achilles' heel of the cutaneous BMZ and is more susceptible to disadherence than the sublamina densa zone [53, 54]. A variety of soluble inflammatory mediators and cytokines can readily induce epidermal dysadhesion through the lamina

lucida space [54]. It is likely that when there is some level of inflammation in EBA, the lamina lucida is much more vulnerable than the sublamina densa area to proteolytic degradation. The cleavage plane of blisters is not a good way to discriminate EBA from other subepidermal bullous diseases.

Transmission Electron Microscopy

Standard transmission electron microscopy (EM) of the DEJ of human skin can suggest the diagnosis of EBA. As mentioned above, EM reveals a decrease in the number of AFs emanating downward into the papillary dermis from the lamina densa compartment of the DEJ. Furthermore, electron microscopists have noted that there is amorphous, electron-dense, ill-defined material lying just beneath the lamina densa in EBA skin. Although never definitively proven, it is likely that this material corresponds to the IgG deposits in the area attached to AFs.

Immunoelectron Microscopy (IEM)

The localization of immune deposits within the DEJ of the skin of EBA patients by immunoelectron microscopy is the “gold standard” for diagnosis. Patients with EBA have immune deposits within the sublamina densa zone of the cutaneous BMZ.[55], [56] This localization is clearly distinct from the deposits in BP, which are higher up in the hemidesmosome area or lamina lucida area of the basement membrane. It is also distinct from CP, which has antigenic targets confined to the lamina lucida.

Direct Immunofluorescence (DIF)

Patients with EBA have IgG deposits within the DEJ of their skin as demonstrated by DIF of a biopsy specimen obtained from a perilesional site. The deposits are predominantly IgG; however, complement, IgA, IgM, Factor B and properidin have also been detected as well [56, 57]. The DIF staining should show an intense, linear fluorescent band at the DEJ. It has been suggested that a positive DIF and IgG deposits within the sublamina densa zone are necessary criteria for the diagnosis of EBA; [56] however, in some LABD- like patients, the deposited antibody is IgA without IgG [35, 37].

Patients with PCT (a syndrome that clinically may mimic EBA) frequently have IgG and complement deposits at the DEJ similar to those seen in EBA patients [58]. The DIF feature that distinguishes PCT from EBA is that PCT skin also demonstrates immune deposits around the dermal blood vessels.

Indirect Immunofluorescence (IIF)

Patients with EBA may have autoantibodies in their blood directed against the DEJ [1] as detected by IIF of the patient's serum on a substrate of monkey or rabbit esophagus or human skin. A positive test gives a linear fluorescent line along the DEJ that may be indistinguishable from BP sera. The autoantibodies in EBA patients will label basement membranes beneath stratified squamous epithelium (skin, upper esophagus, and mucosa of the mouth and vagina) and will not bind to basement membranes within most mesenchymal organs such as blood vessels, liver, or kidney. Therefore, this test could not be used to distinguish between EBA and BP because there is no difference in labeling pattern and distribution between these two autoantibodies [59].

Direct Salt Split Skin Immunofluroescence (SSSI)

This test is a routine DIF performed on patients' perilesional skin that was fractured at the DEJ, through the lamina lucida zone by incubating with 1 M NaCl at 4°C for approximately 3 day [51]. Direct SSSI test employing fluorescein-conjugated anti-human IgG can be used

to easily distinguish BP from EBA patients. EBA patients' immuno-deposits are detected on the dermal side of the separation while BP patients' immuno deposits are on the epidermal roof of the separation.

Indirect SSSI

This test is designed to detect anti-BMZ autoantibodies in the serum of a patient. It is routine IIF performed on human skin substrate previously fractured through the lamina lucida by incubation of the skin slices with 1 M NaCl. This fracture places the BP antigen on the epidermal side of the split and all other BMZ components on the dermal side. When normal human skin is fractured through the DEJ by this method and used as a substrate for IIF to test the sera of patients with primary autoimmune bullous diseases (such as BP and EBA), the EBA autoantibodies bind to the dermal floor of the salt-split skin substrate, while BP autoantibodies bind to the epidermal roof [60]. This test may be helpful in rapidly distinguishing EBA patients from BP patients because EBA and BP may have clinical, histological and immunological parameters that are identical.

While the SSSI can be helpful in distinguishing autoantibodies in patients' sera and making the diagnosis of either BP or EBA, it should be emphasized that the dermal pattern of staining is no longer considered specific for EBA. In one study, a combined dermal-epidermal staining was seen in 5% of 98 BP sera and 45% of 23 CP sera [43]. All of the EBA sera (10 patients), however, showed only dermal staining. Exclusive dermal staining pattern using SSSI assay can be seen in several conditions in addition to EBA. It is seen in (1) bullous SLE [5], (2) a subset of CP patients who have autoantibodies against laminin 5, a main component of anchoring filaments within the lamina lucida [62], (3) a BP-like disease in which patients have autoantibodies to a 105-kDa lamina lucida glycoprotein that is unrelated to lamina-5 [63], and (4) in a newly described subepidermal bullous disease associated with a 200-kDa lower lamina lucida antigen designated “anti-p200 pemphigoid.”

It is estimated that between 50% to 80% of EBA patients have both tissue-bound and circulating anti-BMZ antibodies [5]. IIF performed on salt-split skin is more sensitive than IIF performed on intact human skin and yields higher antibody titers [1, 29]. If a patient's circulating autoantibodies are undetectable by either routine IIF or indirect SSSI and even DIF may be negative, then in this case, direct immunoelectron microscopy is needed to make a diagnosis of EBA [64].

Western Immunoblotting

Western blot analysis can be useful in making the diagnosis of EBA. In a Western blot, extracts of crude protein from skin basement membrane, amnion or cell culture may be used and subjected to SDS-PAGE and electrophoretically transferred onto a membrane. The membrane with immobilized proteins is incubated with EBA and control sera. Antibodies in EBA sera will bind to a 290-kDa band, the alpha chain of C7, whereas sera from all other primary blistering diseases will not [1]. Often a second band of 145 kDa will be labeled with EBA antibodies. This band is the amino-terminal globular NC1 domain of the C7 alpha chain, which contains the major antigenic epitopes of EBA autoantibodies and bullous SLE autoantibodies [1, 2, 11, 12]. Alternatively, both recombinant full-length C7 and the NC1 domain purified from the transfected human cells have been used as substrates for Western analysis and offer excellent sensitivity [10, 65].

Enzyme-linked Immunosorbent Assay

For development of an enzyme-linked immunosorbent assay (ELISA), we produced milligram quantities of recombinant purified posttranslationally modified NC1 in stably transfected human cells [66]. ELISA has advantageous over other techniques, such as

western analysis, IIF and IEM. First, ELISA is quicker and easier to perform. Second, it is very efficient since it requires only about 10-20 μl of serum. Third, ELISA is also more sensitive than IIF allowing for the ability to detect lower titer EBA sera. ELISA can be done completely in microtiter wells and quantitative measurements can be made with a spectrophotometer reader. Furthermore, a standard amount of NC1 can be used to coat the microtiter wells for accurate quantification of anti-NC1 autoantibodies. Lastly, ELISA is performed under native conditions and can detect autoantibodies that recognize the tertiary and quaternary structure of an antigen (conformational epitope). In immunoblot analysis, the proteins are denatured and thus, this technique does not recognize autoantibodies requiring a tertiary or quaternary structure of the antigen. An ELISA dependent on recombinant NC1 is a sensitive, specific assay effective for rapidly screening EBA and BSLE sera. NC1 is utilized because it contains the major antigenic epitopes for EBA autoantibodies, hvv owever, minor epitopes have been identified in the NC2 domain as well as in the triple helical domain of C7. Therefore, a new ELISA kit has been developed utilizing both recombinant NC1 and NC2 domains of C7 purified from human cells. Furthermore, a full-length C7-based ELISA was recently developed to detect EBA autoantibodies that target all possible antigenic epitopes within NC1, NC2, or triple helical domain. We have shown that the full-length C7 based ELISA is more sensitive than the NC1-based ELISA (unpublished studies).

IIF Microscopy Using Substrate Deficient in Basement Membrane Molecules

IIF is a simple yet effective test to help define target antigens in immunobullous disorders. A collection of skin samples, taken from donors with inherited EB, is used in this method. Skin substrates from Hallopeau-Siemens type of RDEB patients are used because the samples are deficient in C7. EBA sera have a lack of antibody labeling to the skin deficient in C7 along with a positive result on normal or BP-180 or laminin 5 deficient skins [67, 68]; however, this technique is limited by the availability of suitable skin samples from subjects with EB.

Suction Split Immunofluorescence

Suction split immunofluorescence is almost identical to SSSI assays with the exception of the method of separation between the BMZ layers. In this test, the BMZ layers are separated *in vivo*, instead of *in vitro* as done in SSSI assays. This method can take hours instead of days, making it quicker. It is also cheaper because the skin can be reused for other studies, such as molecular biology and immunohistochemical analysis [69].

Fluorescent Overlay Antigen Mapping (FOAM)

FOAM is considered more efficient than ultrastructural studies using immunoelectron microscopy because it is quicker, less expensive, and more accessible. This technique is able to distinguish IgG deposits that are above the lamina densa, as seen in BP patients, from those that are below the lamina densa, as seen in EBA patients [70, 71]. FOAM is able to demonstrate different antigens of the BMZ by using monoclonal antibodies on perilesional skin from patients with autoimmune skin diseases to the different components of the BMZ, such as C7. Immune deposits are then stained with different fluorescent stains. Computer analysis is used to overly the stained BMZ antigens over the stained immune deposits, with or without confocal laser scanning microscopy, revealing the relation of the immune deposits to the components of the BMZ.

Diagnosis

The criteria for the diagnosis of EBA have been established by Yaoita *et al.* [56] and Gammon *et al.* [18] with a few slight modifications:

1. A bullous disorder within the defined clinical spectrum
2. Lack of family history of a bullous disorder
3. Histology revealing a subepidermal blister
4. A positive DIF of perilesional skin showing deposition of IgG deposits within the DEJ
5. IEM of perilesional skin indicating localization of IgG deposits within the lower lamina densa and/or sublamina densa zone of the DEJ
6. Alternative laboratory tests for item 5 include indirect or direct salt-split skin immunofluorescence, IIF using substrate deficient in basement membrane molecules, Western blotting, FOAM, and ELISA

- An SSSI assay may not be conclusive in the differentiation of true CP from EBA when clinical presentation is CP-like

Treatment

Therapy for EBA patients is unpredictable and often ineffective. The therapy modality depends on the presentation of EBA. Supportive therapy is necessary for all EBA patients to help reduce the risk of complications and improve the quality of life. This includes proper wound care and strategies for avoiding trauma. Patients should be warned not to over wash, overuse hot water or harsh soaps, and to avoid prolonged or vigorous rubbing of their skin with a washcloth or towel. Sunscreen should be used because prolonged sun exposure may aggravate or promote new lesions. The patient should be able to identify the presence of superimposed skin infections and to seek medical care and antibiotic therapy immediately.

The first-line drug for EBA therapy is colchicine. High doses of colchicine have been reported to be effective in patients with both classical and inflammatory clinical presentations of EBA [72, 73]. Colchicine has milder side effects relative to other medications. The mechanism of action of colchicine has not been well defined; however, the drug is thought to reduce the production of antibodies and inhibit antigen presentation to T cells. One common side effect of colchicine, however, is diarrhea, which makes it problematic for some patients to obtain a high enough dose for a therapeutic response [74].

Another drug for consideration in EBA therapy is cyclosporine, an immunosuppressant. Studies have shown that a number of EBA patients have responded to cyclosporine [75, 76]. For effective therapy, however, patients need high doses of the drug (>6mg/kg). The long term toxicity of the drug renders it useful only when the patient does not respond to other lines of therapy.

High doses of systemic glucocorticoids may have some effectiveness in EBA patients with the inflammatory BP-like disease. Other immunosuppresants may also be tried including methotrexate, azathioprine, and cyclophosphamide [77-80]. Dapsone and prednisone can help some EBA patients, especially those with neutrophils in their dermal infiltrate and/or children with EBA. Studies have reported that EBA patients with the classical mechanobullous presentation are usually refractory to systemic glucocorticoids.

Rituximab is a chimeric, monoclonal antibody directed against the cell surface marker CD20, which is primarily expressed on pre-B cells and mature B cells. Rituximab kills off B cells with the CD20 marker through cytotoxicity, mediated by the complement system and antibodies, and apoptosis. The resulting effect is a reduction in immunosupression by decreasing the number of circulating B cells and the amount of antibody production. Rituximab was shown to be effective in helping an EBA patient who was in critical

condition as a consequence of the disease. Furthermore, a small number of case reports have reported the successful treatment of treatment-refractory EBA patients with rituximab as an adjuvant agent [81-85]. Other similar treatment modalities in EBA that are also currently being considered include anti-tumor necrosis factor-alpha inhibitors like infliximab [74].

Intravenous immunoglobulins (IVIG) is another treatment modality for EBA patients [86-89]. Recent studies have shown that a number of EBA patients with severe, refractory EBA responded well to treatment by IVIG [86, 90-95]. IVIG must be administered frequently due to the limited duration of the therapeutic effect. The experience with IVIG treatment is fairly limited, so clinicians should be cautious with its use.

Photophoresis, used in the treatment of a variety of autoimmune bullous diseases; Sezary syndrome; and mycosis fungoides [96], was dramatically effective in an EBA patients who was refractory to all other treatment modalities and in a very critical condition. In another open study, three EBA patients responded well to photophoresis and had improved dermal-epidermal adherence [97]. Plasma exchanges via plasmaphoresis and lowing plasma anti-C7 autoantibodies has also been reported as a treatment for EBA patients.

One possible, yet unexplored, approach to EBA therapy is the use of recombinant C7 fragments to compete for binding with circulating autoantibodies to C7. The absorption of autoantibodies from the patient's sera could potentially reduce the amount of autoantibodies binding to C7 within the AFs in the DEJ. The rationale for this approach, particularly in patients with the inflammatory phenotype, is supported by two lines of evidence. First, there is a relationship between autoantibody levels and the development of inflammation at the DEJ in EBA and bullous SLE. Second, there is a close correlation between circulating and tissue-deposited C7 autoantibodies. Autoreactive epitopes within the NC1 domain have been identified and were able to absorb autoantibodies to C7 from patients' sera when they were coupled to affinity matrices [98]. We have generated milligram quantities, in human 293 cells, of the recombinant NC1 domain, allowing for the ability to utilize affinity plasmapheresis for EBA therapy. The overall intended effect for affinity plasmapheresis is to reduce the amount of autoantibodies directed at C7, causing the reduction in the development of inflammation due to decreased circulating and tissue-bound autoantibodies.

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Abbreviations

EBA	Epidermolysis bullosa acquista
DEJ	Dermal-epidermal junction
DEB	Dystrophic epidermolysis bullosa
C7	Type VII collagen
AFs	Anchoring fibrils
BMZ	Basement membrane zone
SLE	Systemic lupus erythematosus
HLA	Human leukocyte antigen
EB	Epidermolysis bullosa
IgG	Immunoglobulin G
BP	Bullous pemphigoid
NC1	Noncollagenous domain 1
NC2	Noncollagenous domain 2
CMP	Cartilage matrix protein
FNIII	Fibronectin type III like repeat
VWF	von Willebrand factor
CP	Cicatricial pemphigoid
DIF	Direct immunofluorescence
IIF	Indirect immunofluorescence
IBD	Inflammatory Bowel Disease
CD	Crohn's Disease
IVIG	Intravenous immunoglobulins

EM	Electron microscopy
IEM	Immunoelectron m
SSSI	Salt split skin immunofluroescence
ELISA	Enzyme-linked immunosorbent assay
FOAM	Fluorescent Overlay Antigen Mapping