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Epidermolysis Bullosa Acquisita: Autoimmunity to Anchoring Fibril Collagen

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Abstract

Epidermolysis bullosa acquisita (EBA) is a rare and acquired autoimmune subepidermal bullous disease of the skin and mucosa. EBA includes various distinct clinical manifestations resembling Bullous Pemphigus, Brunsting-Perry pemphigoid, or cicatricial pemphigoid. These patients have autoantibodies against type VII collagen, an integral component of anchoring fibrils, which are responsible for attaching the dermis to the epidermis. Destruction or perturbation of the normally functioning anchoring fibrils clinically results in 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 a mouse, the mouse develops an EBA-like blistering disease. Currently treatment is often unsatisfactory, however some success has been achieved with colchicine, dapsone, photopheresis, plasmapheresis, infliximab, rituximab and IVIG.

Introduction

Epidermolysis bullosa acquisita (EBA) is an acquired, subepidermal bullous disease. EBA patients have both tissue bound and circulating IgG autoantibodies to type VII collagen (C7) within anchoring fibrils. Anchoring fibrils (AFs) are structures that attach the epidermis and its underlying basement membrane zone (BMZ) to the dermis. Therefore destruction of AF results in a clinical phenotype of skin fragility, blisters, erosions, scars, milia and nail loss. These IgG anti-C7 antibodies are pathogenic because when injected into a mouse, the mouse develops an EBA-like blistering disease.

Diagnosing EBA is often difficult because of the variety of clinical presentations that often overlap with other blistering skin diseases, such as dystrophic epidermal bullous (DEB), bullous pemphigoid (BP) and cicatricial pemphigoid. DEB is caused by a hereditary defect in the gene that encodes for C7. Thus, like EBA patients, DEB patients have reduced or a complete absence of normal functioning AFs resulting in the same clinical phenotype. However, there are several laboratory tests which assist in diagnosing EBA. Once the diagnosis is established, however, it is challenging to find the optimal treatment for the patient. There have been developments in newer treatment modalities that have achieved some therapeutic success. In this review, we will provide an update on recent progress in the elucidation of the pathogenesis of EBA, the clinical presentations of EBA, clinical and laboratory diagnosis of EBA and potential therapies.

Historical Aspects

The first description of a patient with a bullous disease reminiscent of EB with no known affected family members was reported by Elliot [1] in 1895. Although more cases were described in subsequent years, it is not certain if all of these early reports were truly EBA patients as defined today, but the existence of an acquired form of EB was recognized.

In the early 1970s, Roenigk et al [2] reviewed the EBA world literature, reported three new cases and established the first diagnostic criteria for EBA. They were: (1) spontaneous or trauma-induced blisters resembling hereditary dystrophic EB, (2) an adult onset of the disease, (3) a negative family history for EB, and (4) the exclusion of all other bullous diseases.

In the 1980's Yaoita et al. [3] and Nieboer et al. [4] found that although EBA and bullous pemphigoid (BP) exhibit IgG deposits at the dermal-epidermal junction (DEJ) by direct immunofluorescence, the diseases could be distinguished by immunoelectron microscopy. In EBA, the IgG deposits are found within and below the lamina densa of the basement membrane zone (BMZ), whereas in BP they are located higher, within hemidesmosomes and the lamina lucida. The ability to separate EBA from the BP group has become more relevant with the observation that EBA does not always have the "classic" EB-like clinical presentation and may present with a clinical syndrome highly reminiscent of BP, Brunsting-Perry pemphigoid, or cicatricial pemphigoid (CP) [5–9].

In 1984 Woodley et al. [10] identified the antigenic target for EBA antibodies as a 290 kDa collagenous protein within the DEJ of human skin and later showed this protein was C7 within anchoring fibrils.

Etiology and Epidemiology

EBA is a rare, autoimmune bullous disease with a prevalence of approximately 0.2/million people. The etiology is unknown and as discussed above, it is hypothesized that EBA is most likely an autoimmune phenomenon because the disease involves IgG autoantibodies directed against C7 [11,12]. Similarly, bullous systemic lupus erythematosus (SLE) is an autoimmune bullous skin disease, which exhibits auto-antibodies against C7 [13]. Both EBA and bullous SLE patients often have a common human leukocyte antigen (HLA) major histocompatibility (MHC) class II cell surface protein, HLA-DR2 [14]. This HLA phenotype has been associated with hyperimmunity, which again suggests an autoimmune etiology for EBA.

EBA does not favor any race or gender [15], yet it has recently been suggested that the Korean population has a higher incidence of EBA [16]. The age of onset varies widely from early childhood to late adult life, but most cases begin between the fourth and fifth decades [17,18]. Although it is an acquired disease that usually begins in adulthood, it was placed in the category epidermolysis bullosa (EB) approximately 100 years ago because physicians were struck by how similar the clinical lesions of EBA were to those seen in children with hereditary dystrophic forms of EB.

Pathogenesis

EBA is characterized by IgG autoantibodies directly attacking C7 within AFs; subsequently leading to a shortage of normal AFs. The resulting lack of AFs causes dissociation of the epidermis and BMZ from the underlying papillary dermis. IgG autoantibodies can be visualized at the DEJ, which confirms the likelihood that this disease is an autoimmune process.

The structure of C7 is composed 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) [19]. 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), nine consecutive fibronectin type III-like repeats (FNIII), and a segment with homology to the A domain of von Willebrand factor (VWF). Therefore, the NC1 domain may facilitate the 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 [20].

We and other researchers previously showed that the antigenic epitopes of C7 reside within the NC1 domain [21–23]. 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 [24–26]. 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 in designing tests for EBA autoantibodies. To date, there has been no convincing evidence that differences in epitope specificity of EBA autoantibodies correlate with the various clinicopathologic types (see below), ability of the autoantibodies to bind complement or the subclass of IgG.

Generally EBA presents as a non-inflammatory mechanobullous disease, although it may manifests as an inflammatory, widespread, vesiculobullous disease reminiscent of BP. There are numerous hypothesizes as to how EBA could become an inflammatory disease. One 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 for the dermal-epidermal junction (DEJ). The local inflammation can also cause complement fixation. The consequence of the inflammatory response is blister formation and tissue damage at the DEJ [27]. These mechanisms may explain the inflammatory, BP-like variety of EBA characterized by vesiculobullous lesions on inflamed skin.

Within the spectrum of EBA and autoimmunity to C7, there are several possible mechanisms for autoantibody-induced blister formation leading to a non-inflammatory, mechano-bullous type of EBA. Since 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 compromises the collagen. This could perturb critical direct interactions between C7 and other extracellular components within the dermal – epidermal junction or upper dermis such as type IV collagen, laminin-332, and fibronectin [20, 27]. Also, there may be an interruption in the C7-fibronectin interaction in the collagenous domain, which may be important for the adherence of the basement membrane and the overlying epidermis onto the papillary dermis[28].

A second mechanistic possibility is that EBA autoantibodies might interfere directly with the antiparallel dimer formation of C7 and consequently anchor fibril assembly [29]. Both mechanisms are attractive possibilities to explain skin fragility and blisters in patients with

the classic EBA phenotype, which lacks significant inflammation in lesions but have defective lamina densa–dermal adherence.

Several independent lines of evidence derived from clinical, histologic, and immunologic studies have implicated the autoimmune response as a key element in the pathogenesis of EBA. A earlier study showed that autoantibodies from EBA patients, affinity-purified against NC1 domain of human C7, are capable of activating neutrophils at the DEJ and inducing dermal-epidermal separation in cryosections of human skin [30]. A number of recent passive transfer studies have provided direct evidence for a pathogenic role of EBA autoantibodies in the 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 with many of the features of EBA in humans including subepidermal blisters and nails loss on their feet. They also had circulating NC1 antibodies in their blood and anti- NC1 IgG antibody deposits at their DEJ. In addition, like many EBA patients, the mice had murine complement deposits at the DEJ induced by the autoantibody–antigen complex [31].

Sitaru *et al.* performed a similar study showing that mice receiving rabbit polyclonal antibodies to the NC1 domain of mouse C7 also developed subepidermal skin blisters resembling human EBA [32]. Further studies showed that EBA induction in this passive transfer animal model requires complement activation and infiltration of granulocytes into the skin. 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 [33]. By clinical, histological, immunological and ultrastructural parameters, the induced lesions resembled human EBA.

In a more recent study, affinity-purified IgG antibodies against a small N-terminal 227 amino acids 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 demonstrates that EBA patient antibodies against a small domain of the 290 kDa auto-antigenic target are highly pathogenic [34]. This is the first antigenic epitope on C7 proven to be a pathogenic target for EBA autoantibodies.

An active murine 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 [35,36]. However, they found that EBA induction only occurred in certain strains of mice. Taken together, these successful experiments with both passive transfer and active animal models and the observations with bullous SLE strongly suggest that EBA autoantibodies are “pathogenic” and capable of causing epidermal–dermal separation in skin.

Clinical Manifestations

EBA has a diverse spectrum of clinical presentations. 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 or chronic bullous disease of childhood. Although patients with EBA may phenotypically vary, all EBA patients have autoimmunity to C7 and diminished AFs [6,12].

Classic Presentation

The classic presentation is a non-inflammatory mechanobullous disease that heals with scarring and milia formation. It may resemble porphyria cutanea tarda (PCT), without the

full range of symptoms such as hirsutism, photodistribution, scleroderma like changes, and high levels of urinary porphyrins [6,12]. The more severe forms of EBA may be similar to hereditary recessive DEB (RDEB) [2].

Patients present with erosions, blisters, and scars over trauma-prone surfaces such as the dorsal hands, knuckles, elbows, knees, sacral area, and toes. There is often considerable involvement of the oral mucosa with erosions and frank blisters. The bullous and vesicular lesions on glabrous skin appear tense and encompassed by non-inflamed or scarred skin. They can be hemorrhagic and can result in erosions, crusts, and scars. There is also usually a scarring alopecia, milia cysts and nail dystrophy. Patients with severe disease may have many of the same sequelae as those with hereditary forms of recessive dystrophic EB, such as scarring, loss of hair on the scalp, nail loss, fibrosis of the hands and fingers, and esophageal stenosis [37,38]. The histology shows dermal-epidermal separation at the basement membrane and minimal inflammation.

Bullous Pemphigoid-like Presentation

Patients with the Bullous Pemphigoid like presentation manifest with widespread vesiculobullous eruptions involving the trunk, central body, extremities and skin folds, similar to BP or a mixture of EBA and BP [27,39]. 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 classic EBA presentation. Histology reveals aggregates of polymorphic infiltrates of mononuclear cells and granulocytes, more commonly neutrophils than eosinophils.

Cicatricial Pemphigoid-like Presentation

These patients clinically resemble CP and generally have mucosal lesions involving the mouth, upper esophagus, conjunctiva, anus, and vagina with or without similar lesions on the glabrous skin [7,38]. The lesions predominantly involve the mucosa and have even been reported in the trachea [40,41]. Histology shows the presence of subepidermal blisters with microscopic scarring and with a mixed inflammatory infiltrate in the upper dermis.

Brunsting-Perry Pemphigoid-like Presentation

Although these patients have the clinical features of Brunsting-Perry cicatricial BP, the presence of IgG autoantibodies targeting C7 supports the idea that Brunsting Perry Pemphigoid may actually be a clinical presentation of EBA. Brunsting - Perry cicatricial bullous pemphigoid is a chronic, recurrent vesiculobullous eruption, localized to the head and neck. Patients have residual scars, subepidermal bullae, minimal or no mucosa involvement and IgG deposits at the dermal-epidermal junction [8,42]. A group of EBA patients with IgG autoantibodies directed to anchoring fibrils below the lamina densa has been reported with this constellation of findings.

IgA Bullous Dermatitis-like Presentation

These patients have a subepidermal bullous eruption and a linear band of IgA deposits at the BMZ, along with a neutrophilic infiltrate. It closely resembles Linear IgA Bullous Dermatitis (LABD) with IgA deposits at the BMZ when viewed by direct immunofluorescence microscopy. Patients have IgA, IgG or both present in their blood. Currently, clinicians disagree on the diagnosis of patients with IgA autoantibodies against C7 that show linear IgA deposition at the BMZ. Some diagnosticians believe it is LABD [43], while others believe it is a subset of EBA [44,45]. 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 IgG [46]. The clinical

features, such as tense vesicles arranged in an annular pattern along with involvement of mucous membranes, may resemble Chronic Bullous Disease of Childhood [25].

The clinical presentation of a given 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 classic form of EBA. Both the classical and the BP-like forms [38], and the CP-like and BP-like forms of the disease may coexist in the same patient[40]. The clinical phenotype of EBA that is reminiscent of pure CP is probably rarer as it only occurs in fewer than 10% of all EBA cases.

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 [25]. 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 adult EBA [25,47].

Diagnostic Tests

Histopathology

Biopsies from lesional skin show variable findings depending on the stage of development. Early lesions typically show vacuolar alteration along the dermal-epidermal junction with subjacent papillary dermal edema. In later stages, there is a subepidermal blister with varying degrees of dermal inflammation that often correlate with the clinical presentation. The classical form shows a sparse inflammatory infiltrate within the dermis as opposed to the inflammatory, bullous pemphigoid-like presentation [48]. The infiltrate in the inflammatory subtypes can be found around vessels, around follicles and in the interstitium. The dermal infiltrate may be rich in neutrophils or mixed with variable numbers of eosinophils and mononuclear cells. Fibrosis can be seen in older lesions, which correlates with scarring seen clinically [49]. 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 anchoring fibrils and an amorphous, electron-dense band just beneath the lamina densa [9,50]. Although the autoantibodies are directed against the anchoring fibrils in the sublamina densa region of the basement membrane zone, the cleavage plane of the blister may be either in the lamina lucida or the sublamina densa region [51]. Immunomapping studies have shown that EBA blisters frequently separate above the immune deposits within the lamina lucida [51]. This is likely because the lamina lucida is the weakest portion of the cutaneous BMZ and is more susceptible to disadherence than the sublamina densa zone [52–54]. Briggaman et al. (1980) have shown that a variety of soluble inflammatory mediators and cytokines can readily induce epidermal disadhesion through the lamina lucida space. 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. Therefore, the location of the cleavage plane of the blister cannot be used to discriminate EBA from other subepidermal bullous diseases.

Distinguishing EBA from other autoimmune subepidermal bullous diseases can be difficult or impossible if patients are evaluated only on the basis of clinical presentation and routine lesional histology. EBA may share clinical, pathologic, and immunohistologic features with BP, CP or LABD. Ancillary tests such as immunofluorescence, salt split skin immunofluorescence, transmission electron microscopy, immunoelectron microscopy, Western blot analysis, enzyme-linked immunosorbent assay (ELISA) and fluorescent overlay antigen mapping (FOAM) are often necessary to establish the diagnosis.

Direct immunofluorescence (DIF)

DIF performed on a perilesional biopsy of EBA patients, shows IgG desposits on the DEJ. Though IgG is the predominant deposit, complement, IgA, IgM, Factor B and properidin have been detected as well[3,55]. The DIF staining should show an intense, linear fluorescent band at the DEJ. Yaoita et al [3] have suggested that a positive DIF and IgG deposits within the sub-lamina densa zone are necessary criteria for the diagnosis of EBA. However, in some LABD – like patients, the deposited antibody is IgA without IgG[43,45].

Patients with PCT may clinically mimic EBA and often have IgG and complement deposits at the DEJ [56]. However, DIF can differentiate PCT from EBA because PCT skin also demonstrates immune deposits around the dermal blood vessels.

Indirect Immunofluorescence (IIF)

IIF can detect autoantibodies targeted against the DEJ from the serum of an EBA patient on the substrate of monkey or rabbit esophagus or human skin [11]. When the IIF is positive, a linear fluorescent line along the DEJ that may be indistinguishable from BP sera is visualized. 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 [57].

Direct Salt Split Skin immunofluorescence (SSSI)

Direct salt split skin immunofluorescence (SSSI) can be used to localize more precisely the immunoreactants and distinguish EBA from BP. A routine DIF is performed on perilesional skin from patients that is fractured at the DEJ, through the lamina lucida zone by incubating the skin biopsy specimen with 1 M NaCl at 4°C for approximately 3 days [58]. This fracture places the BP antigen on the epidermal side of the split and other BMZ components including type VII collagen on the dermal side. The direct SSSI test uses fluorescein-conjugated anti-human IgG to label the IgG deposits in the salt-split skin. If the labeling antibody detects the IgG deposits on the dermal floor of the salt-split skin, the diagnosis of EBA is suggested. In contrast, if the dermal floor is unlabeled and the fluorescent label is observed along the epidermal roof of the salt split skin, EBA is effectively excluded and the diagnosis of BP is suggested [58].

Indirect SSSI

Indirect SSSI is routine IIF executed on fractured human skin substrate to detect anti-BMZ autoantibodies in the serum of a patient. The skin slices are incubated with 1 M NaCl and are fractured through the lamina lucida. 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 [59]. This test may be helpful in distinguishing rapidly EBA patients from BP patients because EBA and BP may have clinical, histological and immunological parameters that are identical [60].

SSSI can lend support to diagnosing EBA versus BP, however several studies have shown the SSSI dermal staining pattern is non-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 [61]. All of the EBA sera (10 patients), however, only showed 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[23], (2) a subset of CP patients who have autoantibodies against laminin 332, a main component of anchoring filaments within lamina lucida [62], (3) a BP-like disease in which the patients have autoantibodies to a 105-kDa lamina lucida glycoprotein that is unrelated to laminin 5[63], and (4) in a newly described subepidermal bullous disease associated with a 200-kDa lower lamina lucida antigen designed “anti-p200 pemphigoid” [64].

Between 50% to 80% of the EBA patients have both tissue-bound and circulating anti-BMZ antibodies [22]. Higher antibody titers are achieved with IIF performed on salt-split skin versus IIF performed on intact human skin [11,39].

IIF Microscopy Using Substrate Deficient in Basement Membrane Molecules

IIF is designed to identify target antigens in immunobullous disorders. In order to perform this test, skin samples directly from donors with inherited EB are required. Skin substrates from Hallopeau-Siemens type of RDEB patients are used because the samples are deficient in C7. EBA sera lack antibody labeling of skin deficient in C7, but are positive on normal or BP-180 or laminin -5 deficient skins [65,66]. This method is a simple and effective technique. However, it is limited by the availability of suitable skin samples from subjects with hereditary dystrophic EB.

Suction Split Immunofluorescence (SSI)

In suction split immunofluorescence, the BMZ layers are separated *in vivo*, instead of *in vitro* as done in SSS assays. The benefit this test includes time and money. SSI takes only hours versus days. Additionally, the skin can be reused for other studies, including molecular biology and immunohistochemical analysis [67].

Transmission Electron Microscopy

Standard transmission electron microscopy (TEM) can be used to diagnose EBA by visualizing the DEJ of the patient's skin. TEM 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 in EBA skin that there is amorphous, electron – dense, ill-defined material lying just beneath the lamina densa. Although never definitively proven, it is likely that this material corresponds to the IgG deposits in the area attached to AFs. This would be suggestive of the diagnosis of EBA.

Immunoelectron Microscopy (IEM)

IEM is the “gold standard” for diagnosing EBA by localizing the immune deposits within the DEJ of the skin of EBA patients [68, 69]. EBA patients have immune deposits within the sub-lamina densa zone of the cutaneous BMZ, which has been demonstrated by Nieboer et al.[4] and Yaoita et al. [3]. 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.

Western Immunoblotting

Western immunoblotting analysis is effective for diagnosing EBA by detecting anti-C7 antibodies in patient sera. Crude protein extracts from the skin basement membrane, amnion or cell culture may be used and subjected to SDS-PAGE and electrophoretically transferred to a membrane. Subsequently, the membranes with immobilized proteins are 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 [11]. 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 [11,12,21,22]. The disadvantage of immunoblot using C7 extracted from skin cells or from conditioned medium of skin cells is the possibility of high background when dealing with low titer sera. 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 better sensitivity with much lower background [20, 70].

Enzyme-linked Immunosorbent Assay

ELISA is valuable for detecting autoantibodies in patients with EBA and bullous SLE. For the development of an enzyme-linked immunosorbent assay (ELISA), we produced milligram quantities of recombinant, purified, posttranslationally modified NC1 in stably transfected human cells [71]. It is superior compared to other techniques, such as IEM; IIF; and immunoblot analysis for autoantibody detection. ELISA is considered quicker, easier to perform with a standardized technique, and more efficient since it requires only about 10–20 μ l of serum. Additionally, ELISA is more sensitive and can detect lower titer EBA sera versus IEM or IIF. ELISA can be completely done in microtiter wells and quantitative measurements can be made with spectrophotometer reader. Furthermore, a standard amount of NC1 can be used to coat the microtiter wells for accurate quantification of anti-NC1 autoantibodies.

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. Therefore, an ELISA using recombinant NC1 is a sensitive, specific, rapid assay for screening EBA and BSLE sera. Initially, NC1 was utilized because it contains the major antigenic epitopes for EBA autoantibodies. However, minor epitopes have now been identified in the NC2 domain as well as the triple helical domain of C7. Consequently, 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 the triple helical domain. The full-length C7 based ELISA is more sensitive than the NC1-based ELISA (unpublished studies).

Fluorescent Overlay Antigen Mapping (FOAM)

FOAM identifies different antigens to components of the BMZ, including C7, by utilizing monoclonal antibodies on perilesional skin from patients with autoimmune skin diseases. Different fluorescent stains are used to stain the immune deposits. Subsequently, a computer analyzes the overlaying stained BMZ antigens over the stained immune deposits, with or without confocal laser scanning microscopy, and reveals the relationship of the immune

deposits with the components of the BMZ. This technique is able to distinguish between 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 [72,73]. FOAM is considered more efficient than ultrastructural studies using immunoelectron microscopy because it is quicker, less expensive, and more accessible.

Diagnosis

Yaoita et al.[3] and Gammon et al.[27] have established guidelines for the diagnosis of EBA with minor 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 with 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, using substrate deficient in basement membrane molecules, Western blotting, FOAM, and ELISA.
 - Salt-split skin IIF and salt-split skin DIF may not be conclusive in the differentiation of true CP from EBA when the clinical presentation of EBA is CP-like. According to the CP (MMP) Consensus Group, they would label such a disease as MMP rather than EBA, a point of semantics [74].

The Relationship Between EBA and Other Systemic Diseases

EBA is linked to numerous systemic diseases [2] including: inflammatory bowel disease [75], SLE, amyloidosis, thyroiditis, multiple endocrinopathy syndrome, rheumatoid arthritis, pulmonary fibrosis, chronic lymphocytic leukemia, thymoma, diabetes, and other diseases. Nonetheless, EBA is a relatively rare disease, and most of these are anecdotal reports.

Considering the combined experience of following over 60 patients from the Universities of North Carolina, Stanford, and Northwestern, it appears that inflammatory bowel disease is the most frequent systemic disease associated with EBA, with 25% of 50 EBA patients reviewed by Chan and associates [76]. Moreover, patients with IBD, more commonly Crohn's disease (CD), have a high prevalence of circulating antibodies against C7 detected in up to 68% of CD patients [77,78]. CD has been described in approximately 30% of EBA patients. Ulcerative colitis has a lower frequency of autoantibodies against C7 when compared with CD [77]. In addition, C7 was recently shown to be present in intestinal epithelium [79]. 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 whereby inflammation originally invoked by Crohn's disease could perturb the intestinal epithelial BMZ such that BMZ components could be altered, resulting in an on-going autoimmunity to C7 [80]. Although a remarkable and intriguing association exists between autoimmunity against C7 and IBD, future clinico-epidermiologic, genetic and immunologic studies are necessary to elucidate at different levels the association of IBD with skin blistering by autoantibodies against C7.

Treatment

Treatment depends somewhat on the clinical presentation of the EBA patient. It is imperative that all EBA patients receive supportive care to reduce the risk of skin trauma and the improve quality of the patient's life. Supportive care includes proper wound care and strategies for avoiding trauma. Patients should be educated about not using harsh soaps, reducing vigorous rubbing of their skin on wash cloths and towels, and to avoid over washing, especially with hot water. Sunscreen should be used because prolonged sun exposure may aggravate or promote new lesions. Lastly, the patient should be educated about identifying superimposed skin infections and when it is appropriate to seek medical care.

The first line treatment in a majority of EBA patients is colchicine due to its effectiveness and low side effect profile. Colchicine is a well-known microtubule inhibitor that also appears to downregulate autoimmunity by the inhibition of antigen presentation to T cells [81]. Its side effect profile is relatively benign compared with other therapeutic choices. High doses of colchicines have been reported to be effective in patients with both classical and inflammatory EBA [82,83]. An unfortunate adverse effect of colchicine is diarrhea, making the drug unusable in EBA patients with IBD [84].

Rituximab is a monoclonal, chimeric antibody that targets CD 20 on both mature and immature B cells. As a consequence of destroying B cells, the number of B cells and circulating antibodies are reduced, resulting in an increase in immunosuppression. A handful of case reports have shown success in refractory EBA patients treated with rituximab as an adjuvant [85–89]. In a study, Rituximab was shown to be effective in helping an EBA patient who was in critical condition as a consequence of the disease. Other similar treatment modalities in EBA that are also currently being considered include anti-tumor necrosis factor-alpha inhibitors like infliximab [84].

Glucocorticoids are limited in their success to inflammatory BP-like EBA. Classical, non-inflammatory, mechanobullous EBA patients are often refractory to high doses of systemic glucocorticoids, and immunosuppressants including, methotrexate, azathioprine, and cyclophosphamide [90–93]. Dapsone and prednisone may benefit some EBA patients with neutrophils in their dermal infiltrate.

Recent studies have shown that a number of EBA patients with severe, refractory EBA responded well to treatment by IVIG [94–100]. IVIG has a limited duration of action and therefore must be administered frequently. The experience with IVIG treatment is fairly limited, so clinicians should be cautious with its use.

Photophoresis was effective in a small group of EBA patients, including one in critical condition [101]. Photophoresis is used in the treatment of Sezary syndrome and mycosis fungoides but has also been helpful in a variety of autoimmune bullous diseases [102]. Photopheresis given monthly reduced the clinical severity of three EBA patients and also improved their dermal-epidermal adherence [103].

Cyclosporine, an immunosuppressant, is another drug for consideration in EBA therapy. Studies have shown that a number of EBA patients have responded to cyclosporine [104,105]. In order for the drug to be therapeutic, however, levels greater than 6 mg/kg must be achieved. With continuous usage of high dose cyclosporine, patients experience long term toxicity, which renders it useful only when the patient does not respond to other lines of therapy.

A potentially productive and unexplored approach to EBA therapy is to intravenously administer recombinant C7 fragments to compete for binding with circulating autoantibodies to C7. In this case, the C7 fragments could act as a decoy peptide to nullify the anti-C7 pathogenic antibodies or, alternatively, the fragments and antibodies could form small 7S complexes and be removed by the spleen. The decline in autoantibodies in the patient's sera could then result in decreased binding of C7 to AFs in the DEJ.

Autoreactive epitopes within NC1 domain have been identified and were able to absorb autoantibodies to C7 from patients' sera when they were coupled to affinity matrices [106]. We have generated milligram quantities of recombinant NC1 domain, and this would possibly allow for specific adsorption of C7 antibodies from the EBA patient's plasma. The overall intended effect would be to immuno-absorb out the pathogenic C7 antibodies and reduce the amount of autoantibodies directed against C7 in the circulation.

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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
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 Microscopy
SSSI	Split Skin immunofluorescence
ELISA	Enzyme-linked immunosorbent assay
FOAM	Fluorescent Overlay Antigen Mapping

References

1. Elliott GT. Two cases of epidermolysis bullosa. *J cutan Genitourin Dis.* 1895; 13:10.
2. Roenigk HH, Ryan JG, Bergfeld WF. Epidermolysis bullosa acquisita: Report of three cases and review of all published cases. *Arch Dermatol.* 1971; 103:1–10. [PubMed: 4321800]
3. Yaoita H, Briggaman A, Lawley TJ. Epidermolysis bullosa acquisita: Ultrastructural and immunological studies. *J Invest Dermatol.* 1981; 76:288–292. [PubMed: 7009764]
4. Nieboer C, Boorsma DM, Woerdeman MJ. Epidermolysis bullosa acquisita: immunofluorescence, electron microscopic and immunoelectron microscopic studies in four patients. *Br J Dermatol.* 1980; 102:383–392. [PubMed: 6992836]
5. Gammon WR, Briggaman RA, Woodley DT, et al. Epidermolysis bullosa acquisita – a pemphigoid like disease. *J Am Acad Dermatol.* 1984; 11:820–832. [PubMed: 6392361]
6. Woodley DT. Epidermolysis bullosa acquisita. *Prog Dermatol.* 1988; 22:1.
7. Dahl MG. Epidermolysis bullosa acquisita – a sign of cicatricial pemphigoid? *Br J Dermatol.* 1979; 101:475–483. [PubMed: 389273]
8. Kurzhals G, Stolz W, Meurer M, et al. Acquired epidermolysis bullosa with the clinical feature of Brunsting-Perry cicatricial bullous pemphigoid. *Arch Dermatol.* 1991; 127:391–395. [PubMed: 1998371]
9. Richter BJ, McNutt NS. The spectrum of epidermolysis bullosa acquisita. *Arch Dermatol.* 1979; 115:1325–1328. [PubMed: 389167]
10. Woodley DT, Briggaman RA, O'Keefe EJ, et al. Identification of the skin basement membrane autoantigen in epidermolysis bullosa aquisita. *N Engl J Med.* 1984; 310:1007–1015. [PubMed: 6369131]
11. Woodley DT, Briggaman RA, O'Keefe EJ, et al. Identification of the skin basement-membrane autoantigen in epidermolysis bullosa acquisita. *N Engl J Med.* 1984; 310:1007–1015. [PubMed: 6369131]
12. Woodley DT, Burgeson RE, Lunstrum G, et al. The epidermolysis bullosa acquisita antigen is the globular carboxyl terminus of type VII procollagen. *J Clin Invest.* 1988; 81:683–687. [PubMed: 3278005]
13. Gammon WR, Woodley DT, Dole KC, et al. Evidence that basement membrane zone antibodies in bullous eruption of systemic lupus erythematosus recognize epidermolysis bullosa acquisita autoantigens. *J Invest Dermatol.* 1985; 84:472–476. [PubMed: 3889170]
14. Gammon WR, Heise ER, Burke WA, et al. Increased frequency of HLA-DR2 in patients with autoantibodies to epidermolysis bullosa acquisita antigen: Evidence that the expression of autoimmunity to type VII collagen is HLA class II allele associated. *J Invest Dermatol.* 1988; 91:228–232. [PubMed: 3137269]
15. Gammon WR, Briggaman RA. Epidermolysis bullosa acquisita and bullous systemic lupus erythematosus: disease of autoimmunity to type VII collagen. *Dermatol Clin.* 1993; 11:535–547. [PubMed: 8365038]
16. Lee CW. Prevalences of subacute cutaneous lupus erythematosus and epidermolysis bullosa acquisita among Korean/oriental populations. *Dermatology.* 1998; 197:187. [PubMed: 9732174]
17. Arpey CJ, Elewski BE, Moritz DK, et al. Childhood epidermolysis bullosa acquisita: report of three cases and review of literature. *J Am Acad Dermatol.* 1991; 24:706–714. [PubMed: 1869641]
18. Gammon WR. Epidermolysis bullosa acquisita. *Semin Dermatol.* 1988; 7:218–224. [PubMed: 3153447]

19. Keene DR, Sakai LY, Lunstrum GP, et al. Type VII collagen forms an extended network of anchoring fibrils. *J Cell Biol.* 1987; 104:611–621. [PubMed: 3818794]
20. Chen M, Marinkovich MP, Veis A, et al. Interactions of the amino-terminal noncollagenous (NC1) domain of type VII collagen with extracellular matrix components; a potential role in epidermal-dermal adherence in human skin. *J Biol Chem.* 1997; 272:14516–14522. [PubMed: 9169408]
21. Lapiere JC, Woodley DT, Parente MG. Epitope mapping of type VII collagen: identification of discrete peptide sequences recognized by sera from patients with acquired epidermolysis bullosa. *J Clin Invest.* 1993; 92:1831–1839. [PubMed: 7691888]
22. Jones DA, Hunt SW III, Prisayanh PS, et al. Immunodominant autoepitopes of type VII collagen are short, paired peptide sequences within the fibronectin type III homology region of the noncollagenous (NC1) domain. *J Invest Dermatol.* 1995; 104:231–235. [PubMed: 7530271]
23. Woodley DT, O'Keefe EJ, McDonald JA, et al. Specific affinity between fibronectin and the epidermolysis bullosa acquisita antigen. *J Clin Invest.* 1987; 179:1826–1830. [PubMed: 3584471]
24. Park SB, Cho KH, Youn JL, et al. Epidermolysis bullosa acquisita in childhood – a case mimicking chronic bullous dermatosis of childhood. *Clin Exp Dermatol.* 1997; 22:220–222. [PubMed: 9536542]
25. Callot-Mellot C, Bodemer C, Caux F, et al. Epidermolysis bullosa acquisita in childhood. *Arch Dermatol.* 1997; 133:1122–1126. [PubMed: 9301589]
26. Tanaka H, Ishida-Yamamoto A, Hashimoto T, et al. A novel variant of acquired epidermolysis bullosa with autoantibodies against the central triple-helical domain of type VII collagen. *Lab Invest.* 1997; 77:623–632. [PubMed: 9426400]
27. Gammon WR, Briggaman RA, Woodley DT, et al. Epidermolysis bullosa acquisita – a pemphigoid like disease. *J Am Acad Dermatol.* 1984; 11:820–832. [PubMed: 6392361]
28. Lapiere JC, Chen JD, Iwasaki T, et al. Type VII collagen specifically binds fibronectin via a unique subdomain within the collagenous triple helix. *J Invest Dermatol.* 1994; 103:637–641. [PubMed: 7963647]
29. Chen M, Keene Dr, Costa FK, et al. The carboxyl terminus of type VII collagen mediates antiparallel dimer formation and constitutes a new antigenic epitope for epidermolysis bullosa acquisita autoantibodies. *J Biol Chem.* 2001; 276:21649–21655. [PubMed: 11274208]
30. Sitaru C, Kromminga A, Hashimoto T, et al. Autoantibodies to type VII collagen mediate Fcγ-dependent granulocyte activation and induce dermal – epidermal separation in cryosections of human skin. *Am J Pathol.* 2002; 161:301–311. [PubMed: 12107115]
31. Woodley DT, Chang C, Saadat P, et al. Evidence that anti-type VII collagen antibodies are pathogenic and responsible for the clinical, histological, and immunological features of epidermolysis bullosa acquisita. *J Invest Dermatol.* 2005; 124:958–964. [PubMed: 15854036]
32. Sitaru C, Mihai S, Otto C, et al. Induction of dermal-epidermal separation in mice by passive transfer of antibodies specific to type VII collagen. *J Clin Invest.* 2005; 115:870–878. [PubMed: 15841176]
33. Woodley DT, Ram R, Doostan A, et al. Induction of epidermolysis bullosa acquisita in mice by passive transfer of autoantibodies from patients. *J Invest Dermatol.* 2006; 126:1323–1330. [PubMed: 16543893]
34. Woodley DT, Doostan A, Bandyopadhyay P, et al. The cartilage matrix protein subdomain of type VII collagen is a newly recognized antigenic and pathogenic epitope for epidermolysis bullosa acquisita. *American Journal of Pathology.* 2007; 170:2009–2018. [PubMed: 17525268]
35. Sitaru C, Chiriac MT, Mihai S, et al. Induction of complement-fixing autoantibodies against type VII collagen results in subepidermal blistering in mice. *J Immunol.* 2006; 177:3461–3468. [PubMed: 16920988]
36. Sitaru C. Experiment models of epidermolysis bullosa acquisita. *Exp Dermatol.* 2007; 16:520–531. [PubMed: 17518993]
37. Harman KE, Whittam LR, Wakelin SH, et al. Severe, refractory epidermolysis bullosa acquisita complicated by an oesophageal stricture responding to intravenous immune globulin. *Br J Dermatol.* 1998; 139:1126–1127. [PubMed: 9990396]
38. Stewart MI, Woodley DT, Briggaman RA. Acquired epidermolysis bullosa and associated symptomatic esophageal webs. *Arch Dermatol.* 1991; 127:373–377. [PubMed: 1998368]

39. Gammon WR, Briggaman RA, Wheeler CE Jr. Epidermolysis bullosa acquisita presenting as an inflammatory bullous disease. *J Am Acad Dermatol*. 1982; 7:382–387. [PubMed: 6752224]
40. Wieme N, Lambert J, Moerman M, et al. Epidermolysis bullosa acquisita with combined features of bullous pemphigoid and cicatricial pemphigoid. *Dermatology*. 1999; 198:310–313. [PubMed: 10393462]
41. Tokuda Y, Amagai M, Yaoita H, et al. A case of inflammatory variant of epidermolysis bullosa acquisita: Chronic bullous dermatosis associated with non scarring mucosal blisters and circulating IgG anti-type-VII-collagen antibody. *Dermatology*. 1998; 197:58–61. [PubMed: 9693189]
42. Yancey, KB. Cicatricial Pemphigoid. In: Fitzpatrick, TB.; Eisen, AZ., et al., editors. *Dermatology in General Medicine*. New York: McGraw – Hill; 1998. p. 674-679.
43. Hashimoto T, Ishiko A, Shimizu H, et al. A case of linear IgA bullous dermatosis with IgA anti-type VII collagen autoantibodies. *Br J Dermatol*. 1996; 134:336–339. [PubMed: 8746352]
44. Rusenko KW, Gammon WR, Briggaman RA. Type VII collagen is the antigen recognized by IgA anti-sub lamina densa autoantibodies. *J Invest Dermatol*. 1989; 92:510 Abstract.
45. Bauer JW, Schaeppi H, Metze D, et al. Ocular involvement in IgA-epidermolysis bullosa acquisita. *Br J Dermatol*. 1999; 141:887–892. [PubMed: 10583173]
46. Lee CW. Serum IgA autoantibodies in patients with epidermolysis bullosa acquisita: A high frequency of detection. *Dermatology*. 2000; 200:83–84. [PubMed: 10681627]
47. Edwards S, Wakelin SH, Wojnarowska F, et al. Bullous pemphigoid and epidermolysis bullosa acquisita: presentation, prognosis, and immunopathology in 11 children. *Pediatr Dermatol*. 1998; 15:184–190. [PubMed: 9655312]
48. Lever, WF.; Schaumburg-Lever, G. Congenital disease (genodermatoses). In: Lever, WF.; Schaumburg-Lever, G., editors. *Histopathology of the Skin*. Philadelphia: Lippincott; p. 78
49. Ackerman, AB. Histologic disease of inflammatory skin diseases. Bennis, B.; Reichel, M.; Rondall, MB., editors. Vol. Chapter 7. Williams & Wilkins; Baltimore: p. 379-340.
50. Ray TL, Levine JB, Weiss W, et al. Epidermolysis bullosa acquisita and inflammatory bowel disease. *J Am Acad Dermatol*. 1982; 6:242–252. [PubMed: 7037881]
51. Fine JD, Tying S, Gammon WR. The presence of intra-lamina lucida blister formation in epidermolysis bullosa acquisita: Possible role of leukocytes. *J Invest Dermatol*. 1989; 92:27–32. [PubMed: 2642510]
52. Briggaman RA, Wheeler CE Jr. The epidermal-dermal junction. *J Invest Dermatol*. 1975; 65:71–84. [PubMed: 1097542]
53. Briggaman RA, Schechter NM, Fraki J. Degradation of the epidermal-dermal junction by proteolytic enzymes from human skin and human polymorphonuclear leukocytes. *J Exp Med*. 1980; 160:1027–1042. [PubMed: 6384417]
54. Klein GF, Hintner H, Schuler G, et al. Junctional blisters in acquired bullous disorders of the dermal-epidermal junction zone: role of the lamina lucida as the mechanical locus minoris resistentiae. *Br J Dermatol*. 1983; 109:499–508. [PubMed: 6357262]
55. Kushniruk W. The immunopathology of epidermolysis bullosa acquisita. *Can Med Assoc J*. 1973; 108:1143–1146. [PubMed: 4574408]
56. Epstein JH, Tuffanelli DL, Epstein WL. Cutaneous changes in the porphyrias. A microscopic study. *Arch Dermatol*. 1973; 107:689–698. [PubMed: 4702703]
57. Paller AS, Queen LL, Woodley DT, et al. Organ specific, phylogenic and ontogenetic distribution of the epidermolysis bullosa acquisita antigen. *J Invest Dermatol*. 1986; 86:376–379. [PubMed: 3745957]
58. Woodley DT, et al. Localization of basement membrane components after dermal-epidermal junction separation. *J Invest Dermatol*. 1983; 81:149. [PubMed: 6348179]
59. Gammon WR, Briggaman RA, Inman AO. Differentiating anti-lamina lucida and antisublamina densa anti-BMZ antibodies by indirect immunofluorescence on 1.0 M sodium chloride-separated skin. *J Invest Dermatol*. 1984; 82:139–144. [PubMed: 6363567]
60. Ghohestani RF, Nicolas JF, Roussele P. Diagnostic value of indirect immunofluorescence on sodium chloride-split skin in differential diagnosis of subepidermal autoimmune bullous dermatoses. *Arch Dermatol*. 1997; 133:1102–1107. [PubMed: 9301587]

61. Mayuzumi M, Akiyama M, Nishie W, et al. Childhood epidermolysis bullosa acquisita with autoantibodies against the noncollagenous 1 and 2 domains of type VII collagen: case report and review of the literature. *Br J Dermatol.* 2006; 155:1048–1052. [PubMed: 17034540]
62. Domloge-Hultsch N, Anhalt GJ, Gammon WR, et al. Antiepiligrin cicatricial pemphigoid: A subepithelial bullous disorder. *Arch dermatol.* 1994; 130:1521–1529. [PubMed: 7986125]
63. Chan LS, Fine JD, Briggaman RA, et al. Identification and partial characterization of a novel 105-kDalton lower lamina lucida autoantigen associated with a novel immune-mediated subepidermal blistering disease. *J Invest Dermatol.* 1993; 101:262–267. [PubMed: 8370962]
64. Zillikens D, Ishiko A, Jonkman MF, et al. Autoantibodies in anti-p200 pemphigoid stain skin lacking laminin 5 and type VII collagen. *Br J Dermatol.* 2000; 143:1043–1049. [PubMed: 11069518]
65. Vodegel RM, Kiss M, De Jong MCJM, et al. The use of skin substrate deficient in basement membrane molecules for the diagnosis of subepidermal autoimmune bullous disease. *Eur J Dermatol.* 1998; 8:83–85. [PubMed: 9649654]
66. Jonkman MF, Schuur J, Dijk F, et al. Inflammatory variant of epidermolysis bullosa acquisita with IgG autoantibodies against type VII collagen and laminin α_3 . *Arch Dermatol.* 2000; 136:227–231. [PubMed: 10677099]
67. Feliciani C, Di Muzio M, Mohammad Pour S, et al. ‘Suction split’ as a routine method to differentiate epidermolysis bullosa acquisita from bullous pemphigoid. *J Eur Acad Dermatology & Venereology.* 2000; 10:243–247.
68. Hoang-Xuan T, Robin H, Heller M, et al. Epidermolysis bullosa acquisita diagnosed by direct immunoelectron microscopy of the conjunctiva. *Ophthalmology.* 1997; 104:1414–1420. [PubMed: 9307635]
69. Prost C, Dubertret L, Fosse M, et al. Routine immuno-electron microscopic technique for localizing an auto-antibody on epidermal basement membrane. *Br J Dermatol.* 1984; 110:1–7. [PubMed: 6362707]
70. Chen M, Costa FK, Lindvay CR, et al. The recombinant expression of full-length type VII collagen and characterization of molecular mechanisms underlying dystrophic epidermolysis bullosa. *J Biol Chem.* 2002; 277:2118–2124. [PubMed: 11698408]
71. Chen M, Chan LS, Cai X, et al. Development of an ELISA for rapid detection of anti-type VII collagen autoantibodies in epidermolysis bullosa acquisita. *J Invest Dermatol.* 1997; 108:68–72. [PubMed: 8980290]
72. De Jong MCJM, Bruins S, Heeres K, et al. Bullous pemphigoid and epidermolysis bullosa acquisita: Differentiation by fluorescence overlay antigen mapping. *Arch Dermatol.* 1996; 132:151–157. [PubMed: 8629822]
73. Kazama T, Yamamoto Y, Hashimoto T, et al. Application of confocal laser scanning microscopy to differential diagnosis of bullous pemphigoid and epidermolysis bullosa acquisita. *Br J Dermatol.* 1998; 138:593–601. [PubMed: 9640362]
74. 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. Review. [PubMed: 11902988]
75. Hunderfean G, Neurath MF, Sitaru C. Autoimmunity against type VII collagen in inflammatory bowel disease. *J Cell Mol Med.* 2010; 10:2393–2403. [PubMed: 19878366]
76. Chan, LS.; Woodley, DT. Pemphigoid: Bullous and cicatricial. In: Lichtenstein, LM.; Fauci, AS., editors. *Current Therapy in Allergy, Immunology and Rheumatology.* St Louis: 1996. p. 5-93.
77. Chen M, O’Toole EA, Sanghavi J, et al. Type VII collagen exists in human intestine and serves as an antigenic target in patients with inflammatory bowel disease. *J Invest Dermatol.* 1997; 108:542.
78. Oostingh GJ, Sitaru C, Zillikens D, Kromminga A, Lührs H, et al. Subclass distribution of type VII collagen-specific autoantibodies in patients with inflammatory bowel disease. *J Dermatol Sci.* 2005; 37:182–184. [PubMed: 15734289]
79. Lohi J, Leivo I, Tani T, et al. Laminins, tenascin and type VII collagen in colorectal mucosa. *Histochem J.* 1996; 28:431–440. [PubMed: 8863048]
80. Chan LS, Vanderlugt CJ, Hashimoto T, et al. Epitope spreading: Lessons from autoimmune skin diseases. *J Invest Dermatol.* 1998; 110:103–109. [PubMed: 9457902]

81. Mekori YA, Chowder Y, Ducker I, et al. Inhibition of delayed hypersensitivity reaction by colchicine: Colchicine inhibits interferon-gamma-induced expression of HLA-DR on a epithelial cell line. *Clin Exp Immunol*. 1989; 78:230–232. [PubMed: 12412754]
82. Megahed M, Scharfetter-Kochnek K. Epidermolysis bullosa acquisita-successful treatment with colchicine. *Arch Dermatol Res*. 1984; 286:35–46. [PubMed: 8141610]
83. Cunningham BB, Kirchmann TT, Woodley DT. Colchicine for epidermolysis bullosa (EBA). *J Am Acad Dermatol*. 1996; 34:781–784. [PubMed: 8632074]
84. Ishii N, Hamada T, Dainichi T, et al. Epidermolysis bullosa acquisita: What's new? *J Dermatol*. 2010; 37:220–230. [PubMed: 20507385]
85. Saha M, Cutler T, Bhogal B, et al. Refractory epidermolysis bullosa acquisita: successful treatment with rituximab. *Clin Exp Dermatol*. 2009; 34:979–980.
86. Schmidt E, Benoit S, Brocker EB, Zillikens, et al. Successful adjuvant treatment of recalcitrant epidermolysis bullosa acquisita with anti-CD20 antibody rituximab. *Arch Dermatol*. 2006; 142:147–150. [PubMed: 16490841]
87. Crichlow SM, Mortimer NJ, Harman KE. A successful therapeutic trial of rituximab in the treatment of a patient with recalcitrant, high-titre epidermolysis bullosa acquisita. *Br J Dermatol*. 2007; 156:194–196. [PubMed: 17199602]
88. Neidermeier A, Eming R, Pfütze M, et al. Clinical response of severe mechanobullous epidermolysis bullosa acquisita to combined treatment with immunoadsorption and rituximab (anti-CD20 monoclonal antibodies). *Arch Dermatol*. 2007; 143:192–198. [PubMed: 17309999]
89. Sadler E, Schafleitner B, Lanschuetzer C, et al. Treatment-resistant classical epidermolysis bullosa acquisita responding to rituximab. *Br J Dermatol*. 2007; 157:417–419. [PubMed: 17596155]
90. Hallel-Halevy D, Nadelman C, Chen M, et al. Epidermolysis bullosa acquisita: update and review. *Clin Dermatol*. 2001; 19:712–718. [PubMed: 11705680]
91. Engineer L, Ahmed AR. Emerging treatment for epidermolysis bullosa acquisita. *J Am Acad Dermatol*. 2001; 44:818–828. [PubMed: 11312431]
92. Mutasim DF. Management of autoimmune bullous diseases: Pharmacology and therapeutics. *J Am Acad Dermatol*. 2004; 51:859–877. [PubMed: 15583576]
93. Kirtschig G, Murrell D, Wojnarowska F, et al. Interventions for mucous membrane pemphigoid cicatricial pemphigoid and epidermolysis bullosa acquisita: a systematic literature review. *Arch Dermatol*. 2002; 138:380–384. [PubMed: 11902989]
94. Kofler H, Wambacher-Gasser B, Topar G, et al. Intravenous immunoglobulin treatment in therapy – resistant epidermolysis bullosa acquisita. *J Am Acad Dermatol*. 1997; 36:331–335. [PubMed: 9039213]
95. Caldwell JB, Yancey KB, Engler RJ, et al. Epidermolysis bullosa acquisita: efficacy of high-dose intravenous immunoglobulins. *J Am Acad Dermatol*. 1994; 31:827–828. [PubMed: 7929940]
96. Mohr C, Sunderkotter C, Hildebrand A, et al. Successful treatment of epidermolysis bullosa acquisita using intravenous immunoglobulins. *Br J Dermatol*. 1995; 132:824–826. [PubMed: 7772494]
97. Harman KE, Black MM. High-dose intravenous immunoglobulin for the treatment of autoimmune blistering diseases: an evaluation of its use in 14 cases. *Br J Dermatol*. 1999; 140:864–874.
98. Gourgiotou K, Exadaktylou D, Aroni K, et al. Epidermolysis bullosa acquisita: treatment with intravenous immunoglobulins. *J Eur Acad Dermatol Venereol*. 2002; 16:77–80. [PubMed: 11952298]
99. Campos M, Silvente C, Lecona M, et al. Epidermolysis bullosa acquisita: diagnosis by fluorescence overlay antigen mapping and clinical response to high-dose intravenous immunoglobulin. *Clin Exp Dermatol*. 2006; 31:71–73. [PubMed: 16309488]
100. Segura S, Iranzo P, Martinez DE, et al. High-dose intravenous immunoglobulins for the treatment of autoimmune mucocutaneous blistering diseases: evaluation of its use in 19 cases. *J Am Acad Dermatol*. 2007; 56:960–967. [PubMed: 17368865]
101. Miller JK, Stricklin GP, Fine JD, et al. Remission of severe epidermolysis bullosa acquisita induced by extracorporeal photochemotherapy. *Br J Dermatol*. 1995; 133:467–471. [PubMed: 8547007]

102. Rook AH, Jegasothy BV, Heald P, et al. Extracorporeal photochemotherapy for drug-resistant pemphigus vulgaris. *Ann Intern Med.* 1990; 112:303–305. [PubMed: 2297209]
103. Gordon KB, Chan LS, Woodley DT. Treatment of refractory epidermolysis bullosa acquisita with extracorporeal photochemotherapy. *Br J Dermatol.* 1997; 136:415–420. [PubMed: 9115929]
104. Crow LL, Finkle JP, Gammon WR, et al. Cleaning of epidermolysis bullosa acquisita on cyclosporine. *A J Am Acad Dermatol.* 1988; 19:937–942.
105. Connolly SM, Sander HM. Treatment of epidermolysis bullosa acquisita with cyclosporine. *J Am Acad Dermatol.* 1987; 16:890. [PubMed: 3571559]
106. Gammon WR, Murrell DF, Jenison MW, et al. Autoantibodies to type VII collagen recognize epitopes in a fibronectin-like region of the noncollagenous (NC1) domain. *J Invest Dermatol.* 1993; 100:618–622. [PubMed: 7684054]