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REVIEW ARTICLE

Congenital Epidermolysis Bullosa: A Review

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Abstract. Epidermolysis bullosa is a group of hereditary diseases affecting 1 in 17 000 live births worldwide. It consists of blistering of the skin and mucous membranes in response to minimal trauma. The disorder seriously affects the patient's quality of life. Diagnosis is based on immunofluorescence mapping and electron microscopy. Treatment is symptomatic, although new cellular and molecular therapies are currently under investigation. This review covers aspects of the molecular biology, clinical presentation, diagnosis, and treatment of epidermolysis bullosa relevant to improving the care for affected patients.

Key words: epidermolysis bullosa, molecular biology, treatment.

EPIDERMÓLISIS AMPOLLOSA CONGÉNITA: REVISIÓN DEL TEMA

Resumen. La epidermólisis ampollosa (EA) engloba un grupo de enfermedades hereditarias que afectan a uno de cada 17.000 nacidos vivos en el mundo. Consiste en la formación de ampollas ante el menor traumatismo que afectan a la piel y a las mucosas. Esta enfermedad empeora seriamente la calidad de vida. El diagnóstico se realiza principalmente por mapeo por inmunofluorescencia y microscopía electrónica. El tratamiento es sintomático, aunque se están investigando nuevas terapias celulares y moleculares. Esta revisión presentará información relevante sobre la biología molecular, la sintomatología clínica, el diagnóstico y el tratamiento de la EA, con la clara intención de proporcionar un mejor cuidado a los pacientes que padecen esta enfermedad.

Palabras clave: epidermólisis ampollosa, biología molecular, tratamiento.

Definition and Epidemiology

Epidermolysis bullosa (EB) is a heterogeneous group of hereditary disorders characterized by extreme fragility of the skin and mucous membranes, which gives rise to the formation of blisters and ulcers following minor trauma.[1] As the areas of the body most often affected are sites subject to frequent pressure or friction, these conditions are also called mechanobullous disorders.[2] The clinical complexity of this, as yet, incurable disease is further increased by extracutaneous manifestations, which include the involvement of skin adnexa, teeth, and the gastrointestinal, urinary tract, and pulmonary epithelia.

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The reported incidence of this disorder varies from one geographical zone to another, affecting approximately 1 in 17 000 live births with an estimated 500 000 cases worldwide.[3,4] However, in many countries, including Mexico, the actual percentage of children born with EB is unknown.[2] Incidence is not affected by race or ethnic group,[1] and the disease affects both sexes equally. Researchers have identified more than 10 genes implicated in the etiology of EB and have reported over 1000 mutations that can occur *de novo* or be inherited in either an autosomal dominant or an autosomal recessive manner.[5]

There is also an acquired form (EB acquisita) that develops during the fourth or fifth decade of life and is caused by the production of immunoglobulin (Ig) G autoantibodies to collagen VII.[6]

The forms of EB are categorized into the following 3 subtypes: EB simplex, junctional EB, and dystrophic EB. EB simplex is the most common form (92%) and dystrophic EB has the second highest incidence (5%), followed by junctional EB (1%).[7] These 3 subtypes are differentiated according to the level at which the tissue separates and

the blisters form, that is, depending on whether this happens above, within, or below the epidermal basement membrane.[8]

Dermatopathology

The epidermis—a tissue made up entirely of cells—is composed of several layers of keratinocytes in various stages of differentiation, ranging from the deepest layer composed of keratinocytes to the outer layer of corneal cells. The dermis, by contrast, is composed of cells dispersed in an abundant extracellular matrix.[9] The primary function of the epidermal basement membrane, located at the junction between the dermis and the epidermis, is to maintain the adhesion between these two structurally different tissues by means of a complex network of adhesion molecules intricately related one to another (Figure 1).[10]

The cytoskeleton of basal keratinocytes is made up of a cytoplasmic network of intermediate filaments composed mainly of keratin 5 and keratin 14. Through their connection to the desmosomes and hemidesmosomes, these filaments play a role in the organization of the shape of the cell and in maintaining the structural integrity of the epidermis. The epidermal cells communicate with each other through desmosomes and intercellular bridges. Desmosomes are specialized complexes that form tight intercellular junctions between adjacent epithelial cells. The principal components of these intercellular junctions are plakoglobin, plakophilin, and desmoplakin, and the cadherins desmocollin and desmoglein.

Electron microscopy reveals that the epidermal basement membrane is divided into 3 areas: the hemidesmosomes, the lamina lucida, and the lamina densa. Hemidesmosomes—the principal unit of adhesion at the dermoepidermal junction—are composed of an inner cytoplasmic plaque and an outer plaque in continuity with the cell membrane. Keratin filaments attach to the inner plaque of the hemidesmosomes by way of their interaction with 2 proteins of the plakin family: plectin and the 230 kDa bullous pemphigoid antigen (BP230 or BPAG1).

The plakins are in turn associated with 2 transmembrane proteins contained in the outer plaque of the hemidesmosome: α6β4 integrin and the 180 kDa bullous pemphigoid antigen (BP180 or BPAG2), also known as collagen XVII. The extracellular domain of α6β4 integrin extends into the lamina lucida, where it binds to laminin 332 (formerly known as laminin 5).

The extracellular domain of collagen XVII extends into the lamina densa. Laminin 332 and collagen XVII are the anchoring filaments that bind the intermediate filaments to the anchoring fibrils of the papillary dermis.

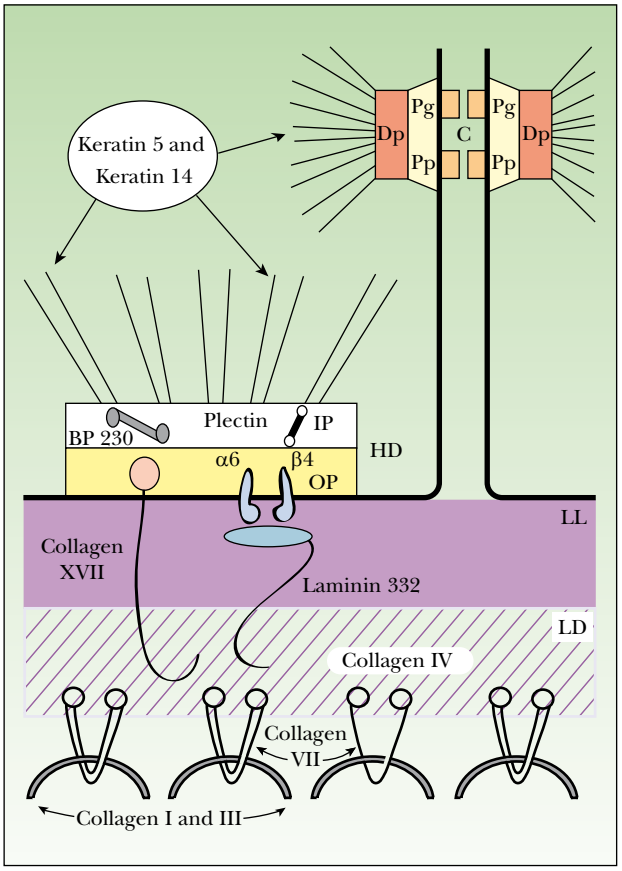

Figure 1. Molecular organization of the epidermal basement membrane. The intermediate filaments (molecules composed of keratin 5 and 14) within the cytoplasm of basal-layer keratinocytes bind to plectin and BP230 in the inner plaque of the hemidesmosomes. These 2 plakins interact with 2 transmembrane molecules, α6β4 integrin and collagen XVII. The α6β4 integrin is the receptor for the extracellular ligand of laminin 332, which in turn binds to collagen VII by way of collagen IV in the lamina densa. Collagen VII is the principal component of the anchoring fibrils and also interacts with collagen XVII. α6 indicates the alpha subunit of a6b4 integrin; b, the beta subunit of integrin α6β4; BP 230, bullous pemphigoid 230 kDa antigen; C, cadherins; Dp, desmoplakin; HD, hemidesmosome; IP, inner hemidesmosomal plaque; LD, lamina densa; LL, lamina lucida; OP, outer hemidesmosomal plaque; Pp, plakophilin; Pg, plakoglobin.

Type VII collagen is the primary component of anchoring fibrils. It forms dimers that aggregate laterally to form semicircular bundles. The ends of these arrays are inserted into the dense layer, where they interact with the anchoring filaments through their association with collagen IV. The semicircular bundles of collagen VII form bands that trap proteins of the dermal matrix, such as type I and type III collagen, and bind them to the basement membrane.[9-13]

Table 1. Genetic Abnormalities in Epidermolysis Bullosa

Type of Epidermolysis Bullosa (EB)	*Gene Affected*	*Protein Encoded*
EB simplex	*PKP1*	Plakophilin 1
	DSP	Desmoplakin
	KRT5	Keratin 5
	KRT14	Keratin 14
	PLEC1	Plectin
	ITGA6, ITGB4	Integrin, α6β4
Junctional EB	*LAMA3, LAMB3, BLAMC2*	Laminin 332
	COL17A1	Collagen XVII
	ITGA6, ITGB4	Integrin, α6β4
Dystrophic EB	*COL7A1*	Collagen VII
Kindler Syndrome	*KIND1*	Kindlin-1

Molecular and Genetic Abnormalities

Genetic defects that give rise to alterations in one or more of the molecules essential to the proper functioning of the dermoepidermal junction will impair the adhesion capacity of the epidermal basement membrane (Table 1).[14] The severity of clinical manifestations varies greatly across the spectrum of different types and subtypes of EB, ranging from the formation of small blisters to the presence of extensive areas of blistered skin and erosions, severe scarring, and fatal complications. It is not possible to attribute these differences to the presence of specific abnormal genes, since the same genetic abnormality can be associated with substantially different clinical manifestations.

In recent decades, advances in molecular genetic analysis techniques have enabled the identification of the specific mutations present in patients with EB. Different types of mutations have been found (deletions, insertions, nonsense, missense, splicing, frameshift, and in-frame) in the genes encoding the adhesion molecules of the epidermal basement membrane. In many cases of EB with an autosomal recessive mode of inheritance, 2 different mutations may even be observed in the same individual (compound heterozygosity). The type and severity of EB in each patient is determined by the types and combinations of mutations present and their effect on transcription into messenger RNA and translation into proteins. This variability in the phenotype explains the wide range of clinical manifestations observed.[15-21]

Classification

In 1962, Pearson developed the first system for classifying EB. Using electron microscopy he defined the 3 main types of EB based on the plane of separation.[5] This classification is still used in clinical practice, education, and research.

During the 1980s, with the advent of immunofluorescence technology, researchers made use of monoclonal and polyclonal antibodies when immunostaining skin samples to demonstrate that the different subtypes of EB could be distinguished by differences in the antigen staining pattern. The first meeting of experts in the field was held in Washington DC in 1988 to attempt to reach an international consensus on the classification of EB using the data generated in the United States of America by the National Epidermolysis Bullosa Registry.

Subsequent developments in mutational analysis techniques enabled identification of the exact molecular and genetic defects associated with each EB subtype. At the second international consensus in Chicago in 1999, participants described new clinical entities and reported the results of mutational analysis studies.

As a great deal has been learned in recent years about the whole spectrum of EB in both clinical and molecular terms, it was deemed necessary to revise the classification system for EB again, taking into account the new information. In May 2007, leading authorities in the field of EB from all over the world met in Vienna for the Third International Consensus Meeting on Diagnosis and Classification of Epidermolysis Bullosa. New clinical forms were added to the spectrum of EB on the basis that they shared characteristics with the well-established forms of this family of disorders and had a hereditary pattern of transmission. The list of new variants included Kindler Syndrome, laryngo-onycho-cutaneous syndrome, lethal acantholytic EB simplex, migratory circinate EB simplex, and plakophilin deficiency. The term "hemidesmosomal EB" was eliminated, and the names of some eponymous subtypes were changed to clinically descriptive names (Table 2).[5]

Clinical Presentation

The characteristic clinical signs and symptoms of the main types of EB (simplex, junctional, and dystrophic) are described below, together with the specific characteristics of certain subtypes. Note that these classic descriptions of the different types and subtypes of EB represent a model and that, far from being rigid or complete descriptions, they are rather diagnostic guides for the physician. Likewise, as the clinical descriptions of the less common subtypes are based on findings observed in a very small number of

Table 2. Classification According to the Third International Consensus Meeting on Diagnosis and Classification of Epidermolysis Bullosa (EB)[a]

Type	*Major Subtype*	*Subtype*
EB simplex (EBS)	Suprabasal	Lethal acantholytic
		Plakophilin-1 deficiency
		EBS superficialis (EBSS)
	Basal	EBS, localized (EBS-loc)[b]
		EBS, Dowling-Meara (EBS-DM)
		EBS, others generalized (EBS, gen-nonDM; EBS, gen-nDM)[c]
		EBS with muscular dystrophy (EBS-MD)
		EBS with mottled pigmentation (EBS-MP)
		EBS with pyloric atresia (EBS-PA)
		Autosomal recessive EBS (EBS-AR)
		EBS, Ogna (EBS-Og)
		EBS, migratory circinate (EBS-migr)
Junctional EB (JEB)	JEB, Herlitz (JEB-H)	
	JEB, others (JEB-O)	JEB, non-Herlitz, generalized (JEB-nH gen)[d]
		JEB, non-Herlitz, localized (JEB-nH loc)
		JEB with pyloric atresia (JEB-PA)
		JEB, inversa (JEB-I)
		JEB, late onset (JEB-lo)[e]
		Laryngo-onycho-cutaneous syndrome (LOC)
Dystrophic EB (DEB)	Dominant dystrophic EB (dDEB)	dDEB, generalized (dDEB-gen)
		dDEB, acral (dDEB-ac)
		dDEB, pretibial (dDEB-Pt)
		dDEB, pruriginosa (dDEB-Pr)
		dDEB, nails only (dDEB-na)
		dDEB, bullous dermolysis of the newborn (dDEB-BDN)
	Recessive dystrophic EB (rDEB)	rDEB, severe generalized (rDEB-sev gen)[f]
		rDEB, generalized other (rDEB-O)
		rDEB, inversa (rDEB-I)
		rDEB, pretibial (rDEB-Pt)
		rDEB, pruriginosa (rDEB-Pr)
		rDEB, centripetalis (rDEB-Ce)
		rDEB, bullous dermolysis of the newborn (rDEB-BDN)
Kindler Syndrome		

[a]Rare variants in italics. [b]Formerly called EBS Weber-Cockayne. [c]Includes patients formerly classified as EBS Koebner. [d]Formerly called generalized atrophic benign EB (GABEB). [e]Formerly called progressive EB. [f]Formerly called Hallopeau-Siemens rDEB.
Source: Fine JD et al.[5]

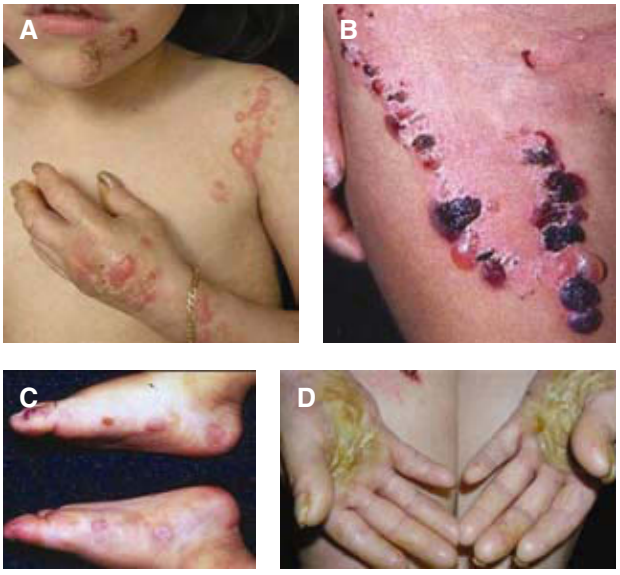

Figure 2. Clinical presentation of epidermolysis bullosa simplex. A and B show a herpetiform annular pattern with scabs and bleeding. C. Walking causes blistering on the feet. D. Marked palmar hyperkeratosis generally occurs in epidermolysis bullosa simplex.

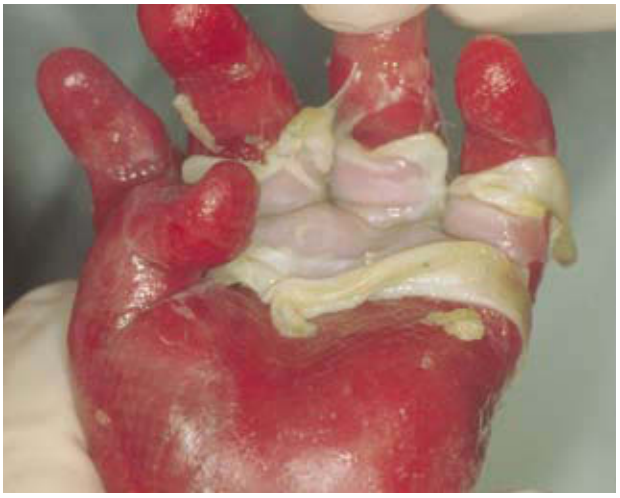

Figure 3. Lethal acantholytic epidermolysis caused by a desmoplakin mutation. Total detachment of the skin in a glove pattern is observed.

patients, the complete range of clinical signs may be more diverse. Finally, we include a brief description of the new forms of EB included in the revised classification drawn up at the Third International Consensus Meeting on Diagnosis and Classification of Epidermolysis Bullosa. A more detailed description of the specific clinical signs and symptoms of the different subtypes of EB can be found in this consensus statement.[5]

Epidermolysis Bullosa Simplex

Most cases of EB Simplex are inherited in an autosomal dominant pattern, although the mode of transmission is recessive in some subtypes, such as autosomal recessive EB simplex, lethal acantholytic EB simplex, plakophilin deficiency, EB simplex with muscular dystrophy, and EB simplex with pyloric atresia. Blisters appear at birth, develop in response to trauma, and often primarily affect the palms of the hands and soles of the feet. They are flaccid and leave a honey-colored crust but do not result in skin atrophy or scarring. Blisters form more frequently in infancy, and their frequency diminishes with age. The most common presentation is localized EB simplex. When blister formation is generalized and associated with palmoplantar keratoderma, EB simplex of the Dowling-Meara type should be suspected with involvement of the mucous membranes and nails, or alternatively non-Dowling Meara generalized EB simplex. EBS Dowling Meara is the most severe form of EB simplex, classically presenting with herpetiform blisters and associated with a higher mortality rate due primarily to sepsis. In rare cases, EB simplex may present with muscular dystrophy or pyloric atresia. EB simplex with mottled pigmentation is another rare form of this type (Figure 2).[5,22,26]

Localized EB simplex is inherited in an autosomal dominant pattern and is caused by a defect in the genes encoding keratin 5 and keratin 14. Classically limited to the hands and feet, this subtype is characterized by blisters with an erythematous halo that are caused by friction and exacerbated by sweating and excessive heat. This form of EB is often underdiagnosed because the clinical manifestations are not sufficiently severe to cause concern in these patients.[5,27]

Jonkman et al[28] described a new form of EB simplex called lethal acantholytic EB simplex. This subtype is caused by an autosomal recessive mutation of the gene encoding desmoplakin, a protein that plays a key role in epithelial and muscle cell adhesion. Epidermolysis is generalized and the clinical signs observed immediately postpartum include skin fragility, universal alopecia, anonychia, and the presence of neonatal teeth. Skin fragility is severe, with a positive Nikolsky sign. This form of EB gives rise to erosive wounds rather than blisters and vesicles. Epithelial regeneration is observed, but the new epithelium detaches again rapidly. The oral cavity and conjunctivas can also be erosive, and the clinical picture may include genitourinary abnormalities, such as pseudophimosis and involvement of the glans penis. Gastrointestinal disorders also occur (Figure 3).[5,28]

Plakophilin deficiency or McGrath syndrome is the result of a mutation of the *PKP1* gene that gives rise to alterations in plakophilin,[1] a structural component of desmosomes. This disorder is also known as ectodermal

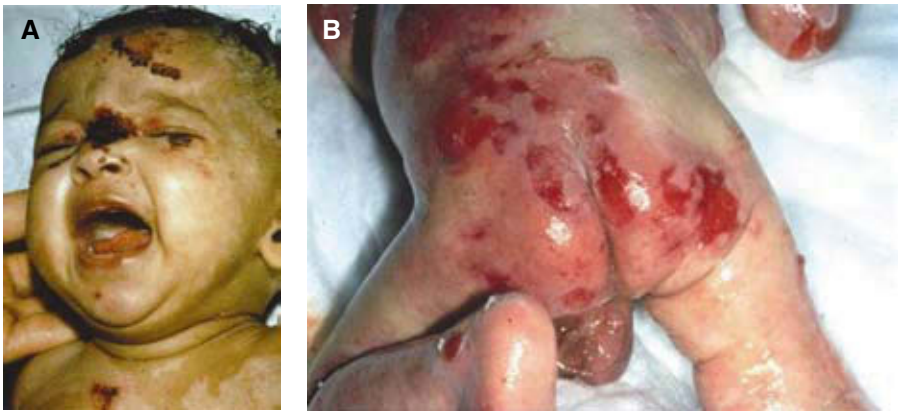

Figure 4. Clinical findings in junctional epidermolysis bullosa (JEB). A. Scabs and erosions on the face of a patient with non-Herlitz type JEB. B. Extensive skinless areas caused by friction in a patient with JEB.

dysplasia/skin fragility syndrome. It manifests at birth when the newborn presents skin fragility, blistering, and generalized erythroderma. These symptoms are accompanied by hypotrychosis, nail dystrophy, and palmoplantar keratoderma. Other symptoms include constipation, esophageal stenosis, blepharitis, fissures on the tongue and around the mouth, and moderate growth delay. The inheritance pattern is autosomal recessive.[5,29]

EB simplex with migratory circinate erythema presents at birth and is caused by a mutation in the keratin gene *KRT5*; the pattern of inheritance is autosomal dominant. Migratory erythema is observed primarily on the limbs (hands, feet, lower legs, and thighs). It is accompanied by vesicles or blisters that heal leaving hyperpigmented brown lesions or hypopigmentated areas but without scarring.[5,30]

Junctional Epidermolysis Bullosa

In junctional EB, cleavage occurs within the lamina lucida at the dermoepidermal junction. This is the least common form of EB (Figure 4). The mode of inheritance is autosomal recessive and the distribution of the lesions can be either localized or generalized. As a general rule, all the subtypes of junctional EB are characterized by blisters, erosions, skin dystrophy, hypoplasia of dental enamel, and caries. On healing, the blisters leave atrophic scars. All variants of this type of EB are manifest at birth with the exception of late-onset junctional EB. The mucous membranes, in particular the oral, gastrointestinal, ocular, and respiratory membranes, are affected mainly in junctional EB of the Herlitz type and in generalized non-Herlitz type junctional EB. Anemia, growth delay, and respiratory abnormalities are found in the subtypes in which the respiratory and gastrointestinal mucosas are affected. Respiratory involvement initially takes the form of stridor or hoarseness and may end in respiratory failure. Sepsis is the primary cause of death in patients with junctional EB, followed by respiratory failure.

Most deaths occur during the first year of life. Junctional EB with pyloric atresia is associated with genitourinary malformations and in some cases aplasia cutis congenita.[5,22-24]

The most common and severest form of junctional EB is the Herlitz type. This variant is characterized by generalized blister formation and the development of hyperplasic granulation tissue on and around the mouth, nose, and nails, and in the sites affected by blistering. There is also involvement of the mucous membranes of the larynx, esophagus, rectum, gallbladder, vagina, urinary tract, cornea, and bronchi. Hypoplasia of the dental enamel gives rise to a higher prevalence of caries. Pseudosyndactyly may occur, but is not common. Patients with Herlitz junctional EB do not usually survive infancy.[31,32]

Laryngo-onycho-cutaneous syndrome is characterized by the presence of chronic granulation tissue in the larynx, nail dystrophy, and skin erosion. This variant is inherited as an autosomal recessive trait caused by a mutation in the *LAMA3A* gene encoding the protein laminin α3A. Clinical signs and symptoms are present at birth and include blistering and ulceration of the skin mainly on the face and neck, hypoplasia of the dental enamel, as well as symblepharon and blindness secondary to conjunctival granulation tissue. Patients may develop respiratory tract abnormalities or anemia as a result of bleeding from erosions.[5,33]

Dystrophic Epidermolysis Bullosa

Dystrophic EB is caused by mutations that affect collagen VII, the protein that forms the anchoring fibrils of the epidermal basement membrane.

Blistering may be localized or generalized depending on the subtype, and the lesions leave dystrophic scars on healing. The inheritance pattern can be autosomal dominant or autosomal recessive; the recessive forms are generally more severe but, fortunately, also less common.

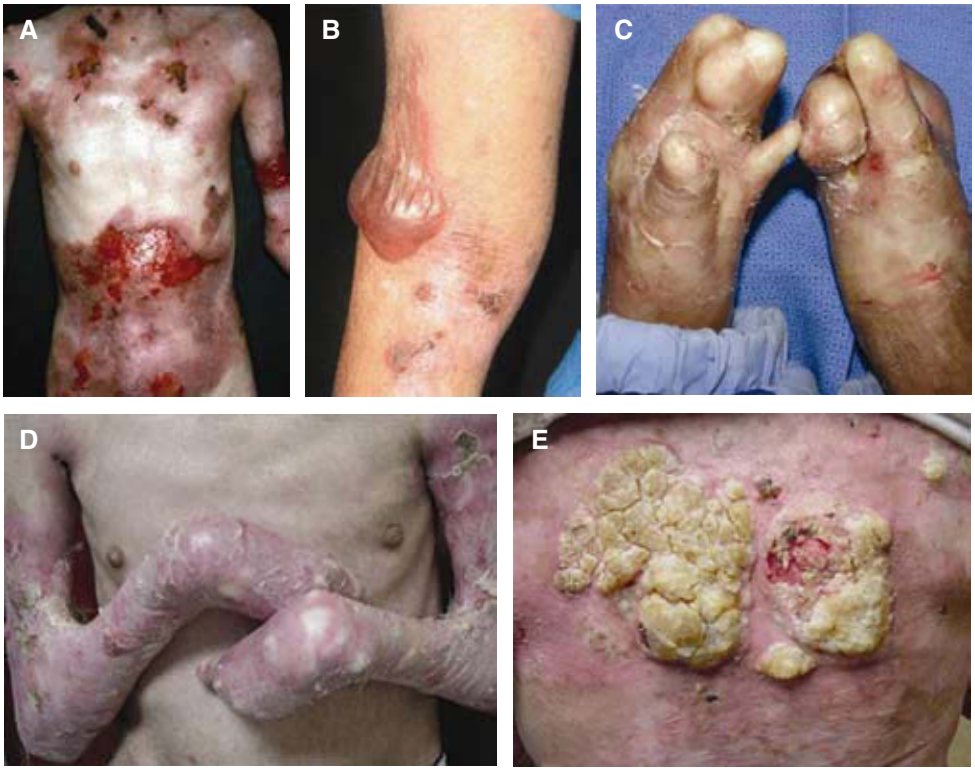

Figure 5. Clinical signs in patients with dystrophic epidermolysis bullosa (rDEB) A. Large skinless areas on the chest. B. Spontaneous blisters may appear anywhere but are more common in areas subject to friction. C. Chronic injury gives rise to scarring and contractures, pseudosyndactyly, joint contractures, and glove keratoderma affecting the hands of a patient with rDEB. D. Extensive joint contractures affecting elbows, hands, and knees limit mobility in these patients. E. Aggressive squamous cell carcinoma on the back of a young patient aged 24 years.

Milia and nail dystrophy are common. The clinical presentation of the dominant forms ranges from localized to generalized disease. In dominantly inherited pretibial dystrophic EB, the blisters appear on the shins and feet, and to a lesser degree on the hands, and there may be lesions that resemble lichen planus. In dominant acral dystrophic EB, blisters appear only on the hands and feet. In the generalized dominant form of dystrophic EB (dDEB-gen, formerly called Cockayne-Touraine and Pasini subtypes), hypopigmentated papules may appear on the torso. These albopapuloid lesions were described as the classic features of these former subtypes. In the generalized forms of the disease, blistering tends to become more localized with age. Dominant EB pruriginosa is characterized by itching. In dominant dystrophic nail-only EB, only the nails are affected and there are no blisters. In the dominant forms, symptoms may be present from birth, but can also appear during infancy. The recessive subtypes also range from mild and localized forms (pretibial recessive dystrophic EB, inverse recessive dystrophic EB, and recessive dystrophic EB centripetalis) to severe and generalized disease (severe generalized recessive dystrophic EB and recessive dystrophic EB, generalized other). The recessive inverse form, which gives rise to localized blistering characterized by intertriginous, lumbosacral, axial, and acral distribution, carries a high risk of external auditory canal stenosis. The recessive centripetal form affects the fingers and toes as well as the pretibial region.

By far the most severe subtype is generalized severe recessive dystrophic EB, formerly known as the Hallopeau-Siemens subtype. This variant is characterized by an extreme fragility of the skin, generalized blistering, extensive dystrophic scarring, and pseudosyndactyly, as well as caries, microstomia, and dystrophy of the nails and teeth.

All the mucous membranes are affected, causing esophageal, anal, and urethral stenosis, corneal ulcers, and phimosis. Anemia and growth delay are also observed. Patients with recessive dystrophic EB of the generalized severe type have a high risk of developing squamous cell carcinoma of the skin (Figure 5). Other possible complications are glomerulonephritis, renal amyloidosis, IgA nephropathy, chronic renal failure, cardiomyopathy, and osteoporosis. Symptoms are apparent at birth.[5,22,24,34,35]

Kindler Syndrome

Kindler syndrome is an autosomal recessive disorder caused by a deficiency of the protein kindlin-1 due to a mutation in the *KIND1* gene. The function of kindlin-1 is to stabilize the dermoepidermal junction by binding the actin microfilaments of the cytoskeleton to the extracellular matrix. Kindler syndrome is characterized by acral skin blistering and photosensitivity during the neonatal period and infancy, and poikiloderma in later

years. The formation of blisters may be spontaneous or associated with trauma, and usually resolves with age. While skin atrophy may be generalized, it most often affects the dorsum of the hands and feet, the knees, and the elbows (Figure 6). Other symptoms reported include palmoplantar keratoderma, nail dystrophy, alopecia, esophageal, anal, vaginal and urethral stenosis, phimosis, syndactyly, and loss of dermatoglyphics.[9,12,26,36]

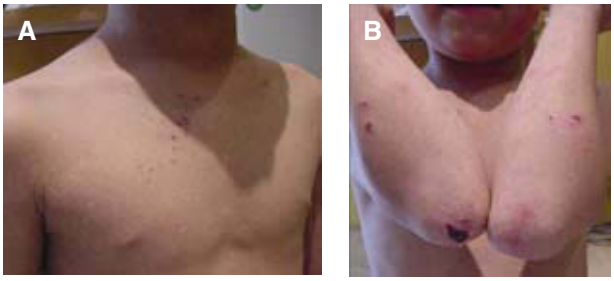

Figure 6. Kindler Syndrome. A. Anterior chest with clinical findings of poikiloderma associated with blisters and abrasions. B. Elbow and forearm with blisters and scabs caused by trauma.

Diagnosis

EB is the suspected diagnosis in individuals with fragile skin, and is manifest by the formation of blisters in response to minor trauma.[37] The differential diagnosis for this disease includes pemphigus vulgaris, bullous pemphigoid, dyshydrotic eczema, linear IgA dermatosis, bullous lupus erythematosus, insect bites, and friction blisters.[22] Once other diagnoses have been ruled out, the suspicion of EB can be confirmed using immunofluorescence techniques or electron microscopy to identify the plane in which separation occurs and blisters form.[15]

The next step in establishing a diagnosis is to obtain a complete medical history and perform an exhaustive physical examination, including assessment of the distribution of the lesions and the presence of other clinical characteristics or complications (Table 3). Genetic analysis of the patient and the parents may be necessary to definitively determine the mode of inheritance.[15,22,23] The aim of the diagnostic process is to determine which EB subtype the patient has in each case because this information is important for defining the clinical and genetic prognosis and for drawing up a suitable treatment plan.[1,38]

Table 3. Medical History and Physical Examination in Epidermolysis Bullosa

Medical history
Family history of blistering diseases or skin fragility
Age of onset
Size, frequency, and location of the bullous lesions
Possible triggering or exacerbating factors
Previous diagnoses and treatments
Quantification of pain and itching caused by the lesions
Growth and development delay
Mucous membrane involvement: oral, nasopharyngeal, ocular, genitourinary, gastrointestinal, and respiratory symptoms
Physical examination
Complete physical examination paying particular attention to the skin as well as the oral, genital, and conjunctival mucous membranes.
Assess the size, location, and characteristics of the blisters
Try to identify at what level the blisters form
Superficial: erosions and scabs
Intraepidermal: flaccid blisters that expand under pressure
Intralaminar: tense blisters that give rise to atrophy but not scars
Intradermal: blisters that leave scars and form milia
Involvement of nails, hair, and teeth

Immunofluorescence Mapping

Immunofluorescence mapping is a useful diagnostic tool because it can be used to determine the plane of separation and to identify the protein affected in each case.[19] Skin should be biopsied at the edge of a spontaneous or friction-induced blister (for example, a blister caused by rubbing the skin with a pencil eraser) and processed fresh; a suitable transport media, such as Michel solution, must be used.[5,19,22,39]

The following antibodies are mapped to determine the plane in which tissue separation occurs on blister formation: antibodies to a hemidesmosomal antigen (such as the bullous pemphigoid antigen BP230) and an antibody to a lamina densa protein (for example collagen IV).

In EB simplex, both antigens are found in the floor of the blister. In junctional EB, the BP230 antigen localizes to the roof of the blister and the collagen IV antigen is located in the floor. In dystrophic EB, both antigens localize to the roof of the blister.[22] The protein affected in a specific case can be identified by analyzing the expression and distribution of antibodies to laminin-332 (formerly known as laminin-5), collagen VII, collagen XVII, plectin, α6β4 integrin, and keratin 14 (Figure 7).

Unlike electron microscopy of skin biopsies, immunofluorescence mapping is a relatively simple, quick, and low cost technique. Another advantage is that the transport medium adequately preserves the skin biopsy for several weeks at room temperature. For these reasons, immunofluorescence mapping is recommended as the primary laboratory study for confirming the diagnosis of EB.[5] However, as this is a semiquantitative analysis technique, it may fail to diagnose disease in individuals in

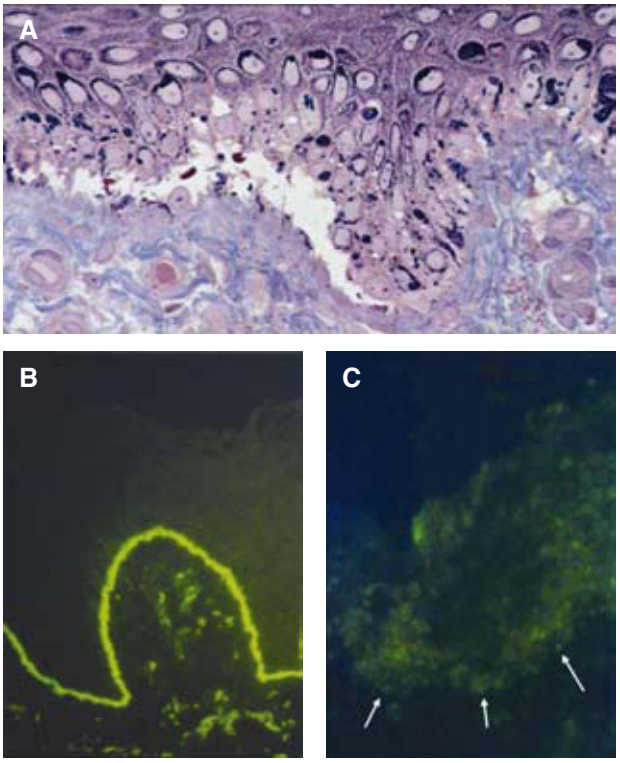

Figure 7. Histologic findings in epidermolysis bullosa. A. A semithin section for electron microscopy colored with methylene blue and Azure II (×60). In epidermolysis bullosa simplex the blister forms in the basal epidermal cell, above the dermoepidermal junction. B. Immunofluorescence mapping of normal skin (×40) showing the presence of collagen VII. C. Skin of a patients with recessive severe dystrophic epidermolysis bullosa in which no trace of collagen VII was identified using the antibody specific for this component of anchoring fibrils (×40).

whom the adhesion protein is deficient (but not absent). When no adhesion protein is present, electron microscopy will be necessary.[19]

Electron Microscopy

Electron microscopy is the gold standard method for classifying EB because it can provide information on both the plane of separation and the ultrastructural abnormalities in the affected proteins (keratin filaments, desmosomes, hemidesmosomes, anchoring fibrils, and anchoring filaments), the number and appearance of which are altered in the different EB subtypes.[5,19] However, at present very few laboratories in the world have the necessary experience to reliably diagnose EB by electron microscopy. Consequently, the diagnostic use of this technique is expected to decrease over time, although it continues to play a key role in research.[5]

Mutational Analysis

Mutational analysis is the ideal method for determining the mode of inheritance and the specific mutation causing EB in each particular case. It plays a key role in prenatal diagnosis and is also an excellent research tool. The future development of gene therapies will be based on identification of the specific mutations present in each subtype. At this time, however, mutational analysis is not considered to be a first line diagnostic tool in EB.[5,22]

Prenatal Diagnosis

To date there is no effective treatment for EB, so that prenatal diagnosis and genetic counseling are very important for couples who are at risk of transmitting this disease. The 1980's saw the advent of prenatal diagnosis of EB using a fetal skin biopsy specimen obtained by fetoscopy.

Today, skin biopsy can be guided by ultrasound and the sample is analyzed by immunofluorescence and electron microscopy. This procedure can be performed after 15 weeks of gestation once the fetal skin has developed sufficiently to permit analysis.

Advances in the study of the molecular pathology of EB have made possible the introduction of prenatal diagnostic techniques based on the analysis of fetal deoxyribonucleic acid (DNA) from amniotic fluid and chorionic villus cells. Amniocentesis and chorionic villus sampling are both associated with a lower risk of miscarriage than fetoscopy and allow diagnosis as early as 10 weeks.

In both fetal skin and fetal DNA analysis, gene mutations must first be analyzed in both the parents and the affected child, and the specific mutations for each family at risk for EB must be identified.

The disadvantage of diagnosing EB through analysis of fetal skin and fetal DNA is that the only solution in both cases is termination of the pregnancy. In vitro fertilization is another alternative for at risk parents as this method allows genetic analysis of the embryo prior to implantation in the uterus, and thus before the start of pregnancy. Genetic diagnosis can be performed before implantation by extracting a cell for mutational analysis from an embryo at the 8-cell stage. If analysis reveals a normal genotype, the original blastocyst is then implanted in the uterus, otherwise it is discarded. Although this technique has been used in other skin-fragility diseases, it has not yet been used successfully in EB.

Other less invasive diagnostic methods are currently under development, including the use of 3-dimensional ultrasound and analysis of fetal DNA taken from a maternal blood sample.[19,22,34,40,41]

Treatment

There is presently no definitive cure for EB and the objective of treatment is to alleviate symptoms and provide supportive measures. Therapy is therefore focused on the prevention of lesions and complications. The complexity of the therapeutic strategy will depend on the severity of the patient's lesions. Optimum management of this disease can only be achieved by a multidisciplinary team, which should include the following specialists: dermatologist, surgeon, nutritionist, dentist, physiotherapist, nurse, psychologist, pain specialist, and geneticist (Table 4). The treatment plan must be individualized, and optimal communication among team members is a vital factor in obtaining good results. Oral health will foster a healthy mucous membrane and improve chewing, swallowing, nutrition, breathing, and speech. A daily rehabilitation routine will prevent the formation of joint contractures. Psychological support for parents and family members is vital, and overprotection of the patients should be avoided. EB is not a contraindication for any vaccination

Education of patients with EB and their families is a cornerstone of treatment. Physicians should encourage them to access support groups such as the *Dystrophic Epidermolysis Bullosa Research Association* (DebRA) to obtain the correct information in Spanish on patient care. Clinicians should also provide a letter explaining the non-contagious nature of the disease to be used when necessary by the patient.[42-59]

Dystrophic Epidermolysis Bullosa Research Association

Voluntary non-profit organizations all over the world offer support and information to individuals with EB, their families, and the medical teams involved in their care. DebRA is an organization with groups in over 32 countries offering excellent informative manuals about the care of patients with EB. They are also in contact with doctors who have experience in the care of these patients. Many of these organizations also contribute to basic and clinical research on EB (Table 5).[4]

Research and Future Therapies

Today, the race the find a cure for this genodermatosis has attracted great interest all over the world. The aim of an ideal treatment for EB would be to correct the deficiency or lack of the specific anchoring proteins at the dermoepidermal junction.

This is the aim of several protein, cell, and gene therapies currently under investigation.[9] The main lines of research are continuously evolving. For example, gene therapies based on the use of creams or epidermal cell grafts have been replaced by others using intradermal injections of fibroblasts or the patient's own stem cells. Recent research has focused on bone marrow transplants from immunologically compatible healthy siblings, a technique that appears to offer some hope to these patients.

Protein Therapy

Protein therapy is based on the direct application of the missing protein, which is obtained using recombinant methods. Topical application of human collagen VII on mice with an inactivated *COL7A1* gene has been shown to promote faster wound healing. Intradermal or intravenous administration of human collagen VII in mice with an inactivated *COL7A1* gene generated elevated levels of this protein in the basement membrane and anchoring fibrils, accelerating wound healing and even prolonging survival.

Similar results have been obtained using a minicollagen produced by removing a portion of the collagenous domain of collagen VII, thereby making the protein less susceptible to protease digestion.[58] Following intraepidermal injection of this product in mice with an inactivated *COL7A1* gene, researchers detected the expression of collagen VII and its incorporation into anchoring fibrils, an improvement in the phenotype, and higher survival rates.[20,58]

The central focus of current research is on dystrophic EB and the restoration of adequate collagen VII levels in these patients. Some experiments have, however, been aimed at replacing laminin 332, another protein that is deficient or missing in junctional EB.[9] All the current research is in murine models.

Cell Therapy

Cell therapy is based on local or systemic treatment with cells that will either produce the proteins needed for proper adhesion or differentiate into other cell lines that will produce the required cells. Stem cells and fibroblasts are currently the most promising options.

Following bone marrow transplantation in mice, researchers have observed that hematopoietic stem cells migrate to the skin and differentiate into epidermal stem cells, which in turn produce keratinocytes. Besides representing a possible source for the huge quantities of stem cells that would be needed to sustain the healing process throughout the lives of patients with EB, these findings also point towards the use of bone marrow transplantation as a therapeutic tool for the treatment of EB.[3,58] The aim of a study currently underway in the University of Minnesota is to assess the use of chemotherapy followed

Table 4. Table 4. Management and Treatment of Epidermolysis Bullosa and its Complications

Ophthalmology	Preventive	Initial ophthalmologic examination, repeated annually if indicated
		Artificial tears containing methyl cellulose to prevent corneal abrasions
		Soft contact lenses to prevent corneal erosions
	Treatment	Urgent examination in the case of red eye, photophobia, or a foreign body sensation
		Use of lanolin-free ocular lubricants for xerophthalmia
		Topical antibiotics and eye patches in the case of corneal erosions
		Ocular lubricants and moisture chambers to treat chronic blepharitis
Odontology	Preventive	Early and aggressive dental intervention
		Dental cleaning and fluoride treatment once or twice a year
		Oral hygiene with a small soft-bristle toothbrush previously soaked in hot water
		Exercises to prevent microstomia and ankyloglossia
	Treatment	Sucralfate mouthwash to reduce the pain associated with oral lesions and to prevent blistering
		Restoration of dentition under general anesthesia when necessary
Nutrition	Preventive	Use of a feeding bottle designed for cleft palate in babies
		Monitor growth parameters, especially body mass index/growth curves
		Involve a clinical nutritionist from the outset
		Follow-up every 6 to 12 months with the following laboratory tests: complete blood count, urea, electrolytes, liver function tests, erythrocyte sedimentation rate, C-reactive protein, iron, ferritin, calcium, phosphate, vitamin D, zinc, and selenium
	Treatment	Frequent meals with a high calorie content and soft consistency. Food should be bland and not hot.
		Protein and calorie requirements are double those recommended for the patient's age or based on Birge's formula
		Multivitamin supplements at 1 or 2 times the recommended dose
		Calcium and vitamin D supplements for osteopenia-osteoporosis
		Oral iron supplementation (with caution as this can cause gastrointestinal symptoms and constipation), intravenous iron and erythropoietin, blood transfusions for anemia
Surgery	Management of surgical procedures	Never place adhesives directly on the skin!
		The patient should be placed on a shock-absorbing surface that minimizes pressure points
	Gastrostomy	Open or percutaneous placement, using endoscopic techniques in some cases This is replaced by a gastrostomy button.
	Pseudosyndactyly	Surgical release of pseudosyndactyly and joint contractures as soon as these conditions cause any serious functional impairment
		The patient should follow a strict postoperative regimen, including rehabilitation, gloves, and rigid splints.
		Functional recovery will not be optimal
		Recurrence is inevitable within 1 to 3 years
	Squamous cell carcinoma	Excision of malignant lesions

(See over)

Table 4. Management and Treatment of Epidermolysis Bullosa and its Complications *(continuation)*

Wounds	Blisters	Puncture the blister at the base with a sterile needle and apply pressure with a gauze dressing to drain fluid and prevent extension of the blistering; the blister roof should not be removed.
	Erosions and ulcers	Should be treated and dressed daily; apply a topical antibiotic and a non-adherent dressing, such as petroleum-jelly gauze, and cover with dry gauze.
		Moisten or soak dressings before removing them.
Infection	Prevention	Change dressings daily
		Use topical agents: antibiotics, acetic acid, chlorine, silver compounds, hydrogen peroxide, or honey.
		Monthly rotation of topical antibiotics to prevent the development of resistance
	Treatment	Systemic antibiotics
		Wound cultures and antibiogram to guide treatment
Gastroenterology		Constipation should be managed with laxatives and stool softeners. Apply petroleum jelly to the perianal area to prevent fissures.
		Balloon dilation to remedy esophageal stenosis and gastrostomy if dilation fails. Antegrade dilations or retrograde via the gastrostomy
Rehabilitation		To prevent joint contractures, microstomia, and ankyloglossia
		Use of elastic gloves to prevent pseudosyndactyly
Psychology		Psychological support for the patient and family. Particular attention should be paid to promoting acceptance of the disease and preventing the overprotection and isolation of the patient.
		Management of crises, such as depression and suicide attempts
Anesthesia	Pain management	Oral analgesics for the pain caused by acute blisters
		Surgery. Use local or regional anesthesia whenever possible.
		In the case of general anesthesia involving tracheal intubation, great care should be taken of the oral and pharyngeal mucosa during intubation and extubation.

by transplantation of hematopoietic stem cells from an allogeneic donor to treat patients with dystrophic EB.[60,61] The preliminary results are promising.

Fibroblasts are an attractive option in cell therapy because they are simple to culture and expand, they are more stable, and they can be frozen, manipulated, and used more simply than keratinocytes.[9,20] It has also been reported that dermal fibroblasts themselves can generate collagen VII at the dermoepidermal junction.[62]

After administering allogeneic fibroblasts by intradermal injection to patients with recessive dystrophic EB, Wong et al[63] observed an increase in the production of collagen VII with the abnormal morphology typical of the patient and the formation of rudimentary new anchoring fibrils, giving rise to improved adhesion at the dermoepidermal junction. They suggest that autologous fibroblasts are capable—by way of a paracrine signal—of increasing synthesis of the mutant collagen VII, which, when produced in sufficient quantities, can reduce blister formation.[63]

Table 5. Some Organizations Affiliated to the Dystrophic *Epidermolysis Bullosa Research Association* Worldwide

DebRA Internacional	www.debra-international.org
DebRA United Kingdom	www.debra.org.uk
Information in Spanish	
DebRA Mexico	www.debra.org.mx
DebRA America	www.deba.org
DebRA Chile	www.debrachile.cl
DebRA Spain	www.aebe-debra.org
DebRA Costa Rica	www.debracr.org

The use of mesenchymal stem cells is also being investigated, and it has been reported that their use in patients with recessive dystrophic EB resulted in improved

wound healing and the expression of collagen VII at the dermoepidermal junction.[58]

Gene Therapy

Gene therapy involves direct correction of the defective genotype through the transfer of one or more external genes into the cell. These genes may or may not become part of the genome. The aim is to add, replace, or suppress functions which, when unaltered, cause the EB phenotype. For gene therapy to be a realistic option the following issues still need to be addressed: a) what cells should be treated, b) what approach should be used (ex vivo or in vivo), and c) what is the ideal vector type (viral or nonviral).[64]

In in vivo approaches, the genetic material is introduced directly through the skin using a topical vehicle, injection, electroporation, or biolistically with a gene gun. The disadvantage of in vivo techniques is the low efficiency of the gene transfer and the short duration of the synthesis of the desired product. The ex vivo method involves in vitro expansion of a skin biopsy, the introduction of genetic material into the cells, and regrafting of the genetically modified and expanded skin onto the patient.[20,64]

Gene transfer can also be achieved by using viral and nonviral vectors. Plasmids and transposons are nonviral vectors capable of transferring long segments of DNA. However, the chief disadvantages of this method are the low transduction efficiency and transient expression achieved.[3] Viral vectors are more effective, and retroviruses and lentiviruses are currently the most promising possibilities. The disadvantages associated with the use of viral vectors include their inability to transfer long DNA segments, and the fact that they are potentially oncogenic and immunogenic.[9,20,62,65]

It has been reported that gene transfer using cotransfection of plasmids encoding fC31 integrase and collagen VII or laminin 332 resulted in the in vitro expression of collagen VII in dystrophic EB keratinocytes and laminin 332 in junctional EB keratinocytes.[9] Transfer of the *COL7A1* gene using lentiviral vectors into keratinocytes and fibroblasts of the dystrophic EB genotype in vitro resulted in the restoration of the basement membrane structure and collagen VII expression. Gene transfer using retroviral vectors has been moderately successful in animal models of junctional EB with *LAMB3, ITGB4*, and *COL17A1* mutations and in canine models of dystrophic EB.[3,20] The successful transfer of a recombinant *COL7A1* minigene which achieved in vitro expression of the protein has also been reported.[20]

The use of gene therapy as a treatment for junctional EB in humans has recently been approved. Epidermal stem cells were obtained from an adult patient with junctional EB caused by a defective β3 chain of laminin 332. *LAMB3* complementary DNA was transferred to these cells using a retroviral vector and the cells were then cultured to prepare skin grafts. These grafts were later implanted in the patient. Subsequent histological analysis of skin biopsies showed normal levels of laminin 332 and proper adhesion of the epidermis, which remained stable during the 1-year follow-up period.[66]

Scientific advances and the enthusiasm of the physicians and researchers who have dedicated much of their lives to the study of the different forms of EB will surely bear fruit, leading to new discoveries that will provide a better quality of life and increased life expectancy in patients with this disease.

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Conflict of Interest
The authors declare no conflicts of interest.

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