State of the Art

Dystrophic Epidermolysis Bullosa

Bibhuti B. Das, MD
Sunati Sahoo, MD

Journal of Perinatology (2004) **24,** 41–47. doi:10.1038/sj.jp.7211019

INTRODUCTION

Epidermolysis bullosa (EB) is a group of inherited blistering disorders that can be potentially life threatening to newborns and infants. An accurate diagnosis in the newborn period can be difficult and should depend on a combination of clinical, histological and molecular techniques. The evolution of clinical lesions (Figures 1 and 2) during the first 30 days of life may confuse physicians. The differential diagnosis of blistering conditions in this age group includes friction blisters, staphylococcal pyoderma, staphylococcal scalded skin syndrome, toxic epidermal necrolysis, epidermolysis bullosa, exfoliative erythroderma, congenital syphilis, intrauterine herpes simplex virus infection, bullous mastocytosis, maternal bullous disease (pemphigus vulgaris), bullous pemphigoid, incontinentia pigmenti and rarely porphyria cutanea tarda. Kindler syndrome, often misdiagnosed as EB, is a rare disorder in which trauma-induced blistering begins at birth or early infancy and then decreases with age. Family history of childhood blistering diseases is often absent, and the clinicians must depend on the findings in the affected patient. Among all the inherited forms of EB, the generalized dystrophic (scarring) type of EB is responsible for most severe debilitating disease. We present a brief review of dystrophic epidermolysis bullosa and highlight the new concepts of management.

DEFINITION AND CLASSIFICATION

EB is a prototype of mechanobullous disease of the stratified squamous epithelium that predominantly affects skin and mucous membrane. The unifying diagnostic feature is fragility of the skin, which manifests in the affected individuals as blistering and erosions secondary to minor trauma. There is considerable phenotypic variability, and the presence of extracutaneous manifestations adds to the complexity of this disorder.

After the first consensus meeting on the diagnosis and classification of inherited EB in 1989,[1] a wealth of new information has been generated about inherited EB. With the availability of new information, in 1999, the second international consensus meeting

Figure 1. DEB with generalized blistering and erosion.

Department of Pediatrics (B.B.D.), The Children's Hospital, Denver, CO, USA; and Department of Pathology (s.s.), University of Louisville, Louisville, KY, USA.

Address correspondence and reprint requests to Bibhuti B. Das, MD, Section of Cardiology, Department of Pediatrics, B #100, The Children's Hospital, Denver, CO 80218, USA.

Journal of Perinatology 2004; 24:41–47
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on diagnosis and classification of inherited EB revised the classification system based on commonly recognized forms and the associated genes involved.[2] Table 1 lists the currently recognized types and subtypes of inherited EB, their mode of inheritance and ultrastructural level of skin cleavage. Apart from the three major subtypes of dystrophic EB (DEB) listed in Table 1, there are additional clinical phenotypes of DEB, particularly those with distinctive distribution of disease activity are still recognized as subtypes in the revised classification system. These include dominant DEB-pretibial, DEB-transient bullous dermolysis of the newborn, dominant DEB-pruriginosa, recessive DEB-inversa, recessive DEB-centripetalis and DEB, autosomal dominant/autosomal recessive heterozygote.[2]

Figure 2. Multiple blisters in a newborn with DEB.

The prevalence for all types of EB is estimated as 19 per million in the US population, and the prevalence of dystrophic form of the EB is 2.4 per million live births in US.[3]

MOLECULAR BASIS OF THE DEB

To understand the molecular basis of EB, one has to be familiar with the attachment structures of cutaneous basement membrane zone (BMZ) that are critical for stable association of the epidermis to the underlying dermis. These structures include hemidesmosomes, anchoring filaments, and anchoring fibrils that form an interconnecting network extending from the intracellular milieu of basal keratinocytes across the dermal–epidermal basement membrane to the underlying dermis.[4] The details of the interconnecting network structure and function of BMZ are beyond the scope of this review. Suffice it to say that aberrations in this network structure due to genetic lesions can result in fragility of the skin at the level of the cutaneous BMZ. A brief summary of molecular genetics of DEB is outlined below.

Type VII collagen, the anchoring fibril protein, is a homotrimer of three α1(VII) chains, each of which consists of 2944 amino acids. Examination of the cloned type VII collagen cDNA sequences revealed that this collagen consists of 1530-amino-acid collagenous segments characterized by Gly-*X*-*Y* repeat sequences and several interruptions, including a 39-amino-acid noncollagenous segment in the middle of the collagenous domain.[4] The functional importance of the anchoring fibrils has been confirmed by demonstration of a large number of mutations linked to the type VII collagen gene COL7A1, located at 3p21.1, in both recessive (RDEB) and dominant forms of DEB (DDEB).[5–7] However, the mutations in COL7A1 genes are quite distinct in both recessive and dominant forms of DEB.

In RDEB, a number of missense, nonsense mutations and other genetic lesions (small insertions or deletions, or out-of-frame splicing mutations) that cause a frame shift and result in

Table 1 Classification of Common Inherited Epidermolysis Bullosa (EB) Phenotypes

Major types	Mode of inheritance	Major subtypes	Protein/gene involved	Level of skin cleavage
EB simplex (EBS)	AD	EBS-Weber–Cockayne	K5, K14	
		EBS-Kobner		Intraepidermal
		EBS-Dowling–Meara		
		EBS-Muscular dystrophy	Plectin	
Junctional EB (JEB)	AR	JEB-Herliz	Laminin 5	Intralamina densa
		JEB-Non-Herliz	Laminin 5, type XVII collagen	
		JEB-pyloric atresia	$\alpha 6\beta 4$ Integrin*	Intraepidermal and/or intralamina densa†
Dystrophic EB (DEB)	AD	Dominant DEB	Type VII collagen	Sublamina densa
	AR	Recessive DEB-Hallopeau–Siemens		
		Recessive DEB-non-Hallopeau–Siemens		

AD, autosomal dominant; AR, autosomal recessive, K5, keratin 5; K14, keratin 14: *$\alpha 6\beta 4$ Integrin is a heterodimeric protein; mutations in either gene have been associated with the JEB-pyloric atresia syndrome. †Some cases of EB associated with pyloric atresia may have intraepidermal clevage or both intralamina densa and intraepidermal clefts.

premature termination codons (PTC) have been identified. In the majority of cases with the severe, mutilating Hallopeau–Siemens (HS) subtype of RDEB, the genetic lesions consist of PTC mutations in both alleles. These mutations lead to synthesis of truncated type VII collagen polypeptides, which are unable to assemble into functional anchoring fibrils. In addition, as a consequence of PTCs, decay of mRNA is accelerated, resulting in reduced levels of corresponding transcripts.[8] As a result, little if any truncated polypeptides are synthesized, thus explaining the entirely negative immunohistochemical staining for type VII collagen peptides in HS subtype.

A number of glycine substitution mutations have been delineated in the collagenous Gly-*X*-*Y* repeat sequence, required for stable triple-helical conformation of the individual collagen α-chains. These mutations frequently result in DDEB through dominant-negative interference.[9] The mutated polypeptides are full length and thus capable of assembling into triple-helical collagen molecules, but the presence of glycine substitution destabilizes the triple helix. Owing to faulty assembly of mutated molecules, the type VII collagen molecules are apparently capable of forming anchoring fibrils, although they are thin and reduced in number. Nevertheless, the presence of anchoring fibrils explains positive, sometimes slightly diminished reactivity on immunohistochemical staining for type VII collagen as well as relatively mild clinical presentation in DDEB. There appear to be two clusters of glycine substitutions, one in the region of amino acids 1982–2079 and the second around amino acids 2569–2775.[9] It should be noted that not all glycine-substitution mutations result in a dominantly inherited disease even when residing in the collagenous domain of the type VII collagen gene.

CLINICAL FEATURES

There is an overlap of clinical features between the subtypes of DEB. Most patients with DEB have relatively mild dominantly inherited disease and only a minority suffer from severe recessive subtypes.

Dominant Dystrophic Epidermolysis Bullosa (DDEB)

The clinical spectrum of DDEB ranges from a milder phenotype, manifested by localized blistering at sites of maximal trauma to generalized blisters that subsequently heal with scarring. One form of localized DDEB, the pretibial type, is named for the typical anatomic localization of lesions in the pretibial skin and dorsum of the feet. The age of onset is early childhood. Atrophic scarring tends to occur. Nails of both upper and lower extremities tend to be abnormal. No extracutaneous manifestations have been reported.[10]

The previously Pasini and Cockayne-Touraine variants of DDEB have been combined under the more inclusive term DDEB.[2] The usual onset of blistering occurs at birth with a predominantly generalized skin distribution, and is associated with milia, atrophic scarring, and dystrophic or absent nails. The so-called ''albopapuloid lesions'', a hallmark feature of Pasini DDEB, characterized by small hypopigmented or flesh-colored papules, are now found to be both an inconstant and nonspecific finding.[2] Gastrointestinal and other extracutaneous manifestations are reported in a minority of DDEB patients.

An uncommon variant of dystrophic EB called transient bullous dermolysis of the newborn, usually a form of dominant DEB, but less commonly transmitted in an autosomal recessive manner, presents with blistering at sites of trauma that heals after several months without scarring.[11]

Recessive Dystrophic Epidermolysis Bullosa of Hallopeau–Siemens Subtype (RDEB-HS)

Patients with the most severe form of this RDEB have exceedingly fragile skin that blisters at birth. The skin is extremely pruritic and painful, and blisters occur in the mucosa regularly. Extensive scarring leads to contractures or fusions of digital web spaces (pseudosyndactyly, ''mitten deformities''). The oral cavity and esophagus are severely involved with widespread blisters and subsequent development of microstomia, obliteration of oral vestibule, and ankyloglossia. Enamel hypoplasia and cementum disorders are also present. A devastating complication unique to patients with RDEB is the increased propensity for squamous cell carcinoma of the skin.

Recessive Dystrophic Epidermolysis of Non–Hallopeau–Siemens Subtype (RDEB-nHS)

All patients with recessive DEB who lack the cutaneous and extracutaneous features, so characteristic of the Hallopeau–Siemens subtype, are now included under the term RDEB-nHS. In inverse form of RDEB, blisters are present at birth, although they may not be distributed in the classic inverse distribution. Later, cutaneous lesions are localized almost exclusively in skin folds (particularly the lateral neck, groin and axillary vaults). Milia, atrophic scarring, scarring alopecia and nail dystrophy are frequently present. Patients with inverse RDEB usually have severe intraoral blistering and scarring that result in microstomia and ankyloglossia. Moderate to severe involvement of the esophagus leads to localized or diffuse scar formation.[12] In RDEB-centripetalis, lesions occur during infancy and have a progressive centripetal spread. There is no intraoral or other extracutaneous involvement.[13]

''Epidermolysis bullosa pruriginosa'', although included as a dominant form of DEB in the revised classification system,[2] is a genetically diverse type of DEB with a distinctive clinical picture, found to have transmission by both autosomal dominant and recessive means.[14] The cutaneous lesions are characterized by highly pruritic, violaceous, pretibial nodules and plaques that may clinically be confused with hypertrophic lichen planus and nodular prurigo. The onset of skin lesions is variable and occurs as late as 10 years of age.

EXTRACUTANEOUS INVOLVEMENT IN DEB

Besides skin and the musculoskeletal system, a variety of other organs may be involved in patients with DEB, especially those with recessive forms of DEB. Apart from blistering, other skin disorders like cutaneous neoplasms, eczema, atopic dermatitis and hayfever are observed in DEB patients. The sites of involvement most commonly seen in DEB are the teeth, gastrointestinal tract, upper respiratory tract, genitourinary tract, eyes and cardiovascular system.[15]

The most common gastrointestinal manifestations include dysphagia, esophageal stricture or stenosis, pyloric stenosis, anal stricture, chronic constipation and fecal impaction.[16] Gingival blisters or erosion, enamel hypoplasia, dental caries, malocclusion and premature loss of teeth are the most commonly reported dental manifestations in DEB patients.[17] The most frequent tracheolaryngeal symptoms or findings include chronic hoarseness, inspiratory stridor and laryngeal stenosis or obstruction.[18] This presents special problems for the anesthesia provider because the equipment used to deliver anesthesia and monitor vital signs may cause serious mechanical complications.[19] The genitourinary symptoms and signs seen in some cases of DEB include urethral meatal stenosis, urinary retention, glomerulonephritis, nephrotic syndrome secondary to renal amyloidosis and menstrual abnormalities.[20,21] Ocular findings range from corneal erosion, corneal scarring, symblepharon, blepharitis, ectropion formation, lacrimal duct obstruction to blindness.[22] Otitis externa, external auditory canal stenosis, chronic otitis media and hearing loss are reported in DEB patients.[23] Anemia and growth retardation are common in patients with the most severe form of DEB. Anemia is attributed to iron deficiency and chronic disease. Growth retardation in DEB patients is a reflection of chronic malnutrition, most likely secondary to continuous disruption of the epithelial lining of the gastrointestinal tract, as well as severe disease activity in the oral cavity.[24] Although the exact mechanism remains unclear, dilated cardiomyopathy has been reported in patients with severe RDEB and may be related in part to carnitine deficiency.[25,26] Other miscellaneous congenital defects may be associated with patients with DEB. Common known causes of death in DEB patients include squamous cell carcinoma, sepsis, pneumonia, respiratory failure, failure to thrive, renal failure, heart disease, stroke, and auto-accidents.

DIAGNOSIS

Evaluation of any patient suspected of having EB should begin with a detailed history, including mapping of the family pedigree. Absence of other known affected family members, however, does not, by itself, establish the mode of transmission as autosomal recessive, because the identification of apparently isolated affected persons may also be the result of spontaneous mutation or the incomplete penetrance of an autosomal dominant trait.[1] Nonmolecular laboratory tests for the diagnosis include transmission electron microscopy (TEM), immunofluorescence antigen mapping and immunohistochemical staining with EB-specific monoclonal antibodies.

TEM permits not only identification of the ultrastructural level of skin cleavage (i.e. intraepidermal, intralaminar lucida, sublaminar densa) but also the appearance of specific structures, most notably keratin tonofilaments, hemidesmosomes, sub-basal dense plates and anchoring fibrils.[27] DEB is characterized by abnormal fibrils and blister formation beneath the lamina densa of the dermoepidermal junction.[28] The ultrastructural findings of skin in DEB often reflect the severity of clinical disease and can be an indicator of prognosis. TEM remains the gold standard whereby all other diagnostic tests must be compared. Its major advantage is the ability to directly visualize and quantitate anchoring fibrils. The major disadvantages are technical difficulties and cost.

Immunofluorescence antigen mapping involves a modified indirect immunofluorescence method using patient's skin and antibodies against three known basement membrane antigens (bullous pemphigoid antigen-1, laminin-1, type IV collagen), which are located in different ultrastructural regions of the dermoepidermal junction.[29] The pattern of antibody binding in induced microscopic clefts in EB skin is characteristic for each major EB type. Since the cleavage occurs beneath the lamina densa in DEB, all three antibodies bind exclusively to the induced blister's roof. The major advantages of this technique are its low cost and relative ease to perform. The major disadvantage is the inability to directly visualize or quantitate the specific ultrastructure of interest in different subtypes of EB.

Several antibasement membrane monoclonal antibodies have been developed for immunohistochemical studies that can bind along the dermoepidermal junction and help in the diagnoses of various subtypes of EB.[30] The best studied anti-type VII collagen antibody, LH 7:2, typically shows absence of staining with RDEB-HS specimens and stains weakly in specimens from other subtypes of RDEB except in skin from inverse-RDEB, in which the staining is normal.[31] LH 7:2 is also an excellent marker for transient bullous dermolysis of the newborn, demonstrating the intracytoplasmic retention of type VII collagen in keratinocytes, if performed during clinically active disease. The advantage of antibody study is that it can be performed on the same biopsy specimen taken for immunofluorescence antigen mapping studies. If skin biopsies are obtained solely for monoclonal antibody studies, they should preferably be taken from an intact rather than blistered-skin to reduce the risk of false-negative staining. There are no disadvantages of this study, although the only limitation is the lack of complete sensitivity and specificity, only if such results are used in the absence of other confirmatory data from either immunofluorescence antigen mapping or TEM.

PRENATAL DIAGNOSIS

The purpose of prenatal diagnosis is not only to identify fetal anomalies but also to provide choices to parents at risk of having a child with EB. Health-care professionals involved in this process include but are not limited to genetic counsellors; clinical geneticists; obstetricians; dermatopathologists; research scientists in cytogenetics, biochemistry and molecular biology; and ultrasonographers. The ability to diagnose EB in utero dates back to 1980 when the technique of fetal biopsy made it possible to obtain samples of fetal skin at 17 to 21 weeks of gestation for light and electron microscopic examination and immunohistochemical evaluation. Features that suggest diagnosis of DEB include: dermoepidermal junction separation below the lamina densa where lamina densa forms the blister roof, absent or poorly developed anchoring fibrils, and reduced or absent expression of type VII collagen. The drawback of this method is that it can only be performed fairly late in the pregnancy with a high rate of miscarriage.

With the advances in molecular biology, the underlying gene defects and the linkage of various forms of EB with certain genes have provided a basis for direct mutation detection and indirect linkage analysis in affected families.[32] This information has allowed for the development of a relatively simple, fast, and reliable DNA based test for first-trimester prenatal diagnosis using DNA from chorionic villi and amniotic fluid as early as 10 weeks' gestation. Direct methods include Southern blotting to detect gene deletions or rearrangements, Southern blotting and restriction enzyme analysis to detect point mutations that alter restriction sites, allele-specific oligonucleotide hybridization to detect previously characterized mutations, and polymerase chain reaction (PCR) amplification to detect deletions or previously characterized mutations. Indirect methods include DNA polymorphism within or near disease loci to detect mutations in a particular gene and DNA polymorphism linked to disease loci to diagnose unknown mutations in a particular gene whose map position is known. Prenatal diagnosis can be performed with more than 98% accuracy when pregnancy screening for mutations or informative markers allow timely and accurate diagnosis in the fetus.[33]

Mutation-based DNA testing of RDEB must be designed specifically for each family. Haplotype analysis using informative single-base polymorphisms or microsatellite markers has to be undertaken with the recognition that rare de novo mutations or parental germline mosaicism can lead to false-positive prediction of the fetal genotype. Ideally, however, prenatal testing, when possible, should be based on direct demonstration of the presence or absence of the mutations in both COL7A alleles.[34]

In embryos resulting from in vitro fertilization, preimplantation diagnosis can be performed at the eight-cell stage before the embryo is implanted in the uterus.[35] One of the eight cells from the blastomere is removed by micromanipulation and the DNA is used as a template for PCR amplification for specific mutation(s) or polymorphisms in the candidate gene. If the diagnosis reveals a normal or carrier genotype, the original blastocyst can be implanted into the uterus, whereas embryos that have an affected genotype are discarded. The advantage of this technique is that an affected embryo is identified before implantation, thus preventing a pregnancy rather than terminating one.

TREATMENT

At present, no specific therapy is available for DEB. The approach to the management of patients with DEB must be multifactorial and be based on a series of principles. Therapy should be directed toward prevention of skin trauma to avoid new blister formation, prevention of secondary bacterial infection, aggressive treatment of infection when it occurs, measures to improve wound healing, maintenance of good nutrition, treatment of all correctable complications and, finally, rehabilitation. Prevention of blisters is attempted by gentle handling of the infant, use of soft and loose-fitting clothes, padding the bony prominences, sheepskin pads for the crib, and avoidance of skin friction, excess heat and adhesives on the skin. Prevention of infection is achieved by changing dressings daily, applying topical antibiotics to lesions and nonstick dressings to denuded areas, and draining the blisters. Early, aggressive, dental interventions should be undertaken in all children with DEB since the premature loss of primary teeth may have a profound effect on nutritional intake and on the development of secondary bony and soft tissue changes in the oral cavity.[17] Methyl-cellulose containing artificial tears are useful to prevent ocular abrasions. Corneal erosions may be treated conservatively with topical antibiotics and pressure-patch dressings.[22]

Severe pain is usually not a major clinical problem, despite the extent of skin denudation. Although pain reduction is appropriate, narcotic analgesics should be avoided whenever possible in order to decrease future risk of narcotic dependency. Topical analgesics are ineffective for control of pain when applied to skin lesions but are helpful in the management of painful anal fissures and erosions.[36] Stool softeners and psyllium-containing fiber preparations are useful for management of chronic constipation.[36] Oral prophylaxis with sucralfate prevents blister formation and provides comfort for mouth lesions.[37] Some patients with generalized DEB will benefit from whirlpool therapy. Care must be taken to maintain the whirlpool temperature equal to body temperature in order to prevent blister formation. Nonhealing areas of the skin can be treated with split-thickness skin grafts. Keratinocyte-cultured autografts have been successful in severe non-healing erosions.[38] Careful use of hand splints help prevent development of mitten deformities.[39] Any surgical procedure, including circumcision results in delayed wound healing. A multidisciplinary approach is required to deal with problems such as esophageal strictures, contractures, pseudodactyly, laryngeal disorders, urethral meatal stenosis, conjunctival scarring and psychosocial problems.[40,41]

A nutritionist should be consulted about the nutritional needs of an infant with DEB. Protein and calorie requirements are twice that recommended for size because of ongoing skin and mucosal losses. Occasionally total parenteral nutrition is used to improve nutritional status. Anemia occasionally becomes unresponsive to iron therapy and requires blood transfusion.

Much support is required for parents and patients who suffer from this chronic, frustrating and debilitating disease. When indicated, psychiatry referral is exceedingly important since severe depression and even suicide may eventuate in some severely affected patients who are not diagnosed and treated sufficiently early. Rehabilitation therapy is very important in DEB patients, and referral to a physiotherapist plays an important role in overall management of these patients. The lay support group DEBRA of America has excellent information available for care of patients with DEB.

Finally, with the identification of mutations responsible for DEB and with the isolation of the corresponding normal alleles, gene therapy can become a future hope for cure.[42] In general, there are three requisites for gene therapy to be a viable option: (1) expression of the normal allele in a mutant cell must correct the mutant phenotype; (2) permanent or long-term expression is essential; and (3) technical advances for efficient in vivo gene transfer or less destructive methods for transplanting ex vivo modified cells must be developed. Despite the fact that keratinocytes offer an attractive target for therapeutic gene delivery, there are currently no approved human trials involving this cell type. As the field of epidermal gene therapy advances, we can look forward to a new dimension in the treatment of DEB.

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