Nephrotic Syndrome and Aberrant Expression of Laminin Isoforms in Glomerular Basement Membranes for an Infant With Herlitz Junctional Epidermolysis Bullosa

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ABSTRACT. Herlitz junctional epidermolysis bullosa (H-JEB) is a hereditary bullous disease caused by absent expression of laminin-5, a component of anchoring filaments within the dermal-epidermal basement membrane zone. Affected individuals usually die during the first 1 year of life. We studied an infant with H-JEB who presented with nephrotic syndrome, a previously unreported complication that may contribute to early death in this disease. DNA analysis revealed a compound heterozygote for mutations 2379delG and Q995X in the LAMB3 gene. The patient had massive albuminuria, attributable to failure of the glomerular filtration barrier, and high urinary N-acetylglucosaminidase levels, indicating renal tubular involvement. Electron-microscopic examination of the renal tissue revealed diffuse fusion of the foot processes, irregular swelling of the lamina rara interna, and disappearance of endothelial cell fenestrations. Immunohistopathologic analysis of the patient’s renal tissue revealed compositional changes in laminin isoforms of the glomerular basement membrane and no detectable laminin-5 in the renal tubular basement membrane, which suggests that laminin-5 may play an important role in renal function. Our findings strongly suggest that H-JEB should be considered in the spectrum of congenital nephrotic syndromes. Combination therapy with meticulous skin care and treatment strategies established for congenital nephrotic syndromes may rescue patients with this disease. Pediatrics 2005;116:601–607. URL: www.pediatrics.org/cgi/doi/10.1542/peds.2005-0160; Herlitz Junctional Epidermolysis Bullosa, Laminin-5, Nephrotic Syndrome, Proteinuria, Glomerular Basement Membrane.

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ABBREVIATIONS. H-JEB, Herlitz junctional epidermolysis bullosa; PTC, premature termination codon; GBM, glomerular basement membrane; TBM, tubular basement membrane; NAG, N-acetylglucosaminidase; mAb, monoclonal antibody; PCR, polymerase chain reaction.

Junctional epidermolysis bullosa is a group of autosomal recessive diseases characterized by profound skin fragility, with blister formation resulting from dermal-epidermal disadhesion at the level of the lamina lucida within the basement membrane zone. The most severe form, Herlitz junctional epidermolysis bullosa (H-JEB), manifests with generalized blistering and erosions of the skin, with extracutaneous involvement, and is usually lethal during the first 1 year of life.

Immunohistochemical and molecular genetic studies have demonstrated that the underlying cause of H-JEB is absent expression of laminin-5, a component of anchoring filaments traversing the lamina lucida of the dermal-epidermal basement membrane zone. A characteristic genetic lesion is a premature termination codon (PTC)-causing mutation in both alleles of 1 of the 3 genes (LAMA3, LAMB3, and LAMC2) encoding the subunit polypeptides of laminin-5 (ie, the α3, β3, and γ2 chains, respectively). Because all 3 chains are necessary for the structural assembly of trimeric laminin-5 macromolecules, defects in any of the 3 genes can result in complete absence of laminin-5. Laminin-5 is expressed in multiple tissues, including the skin and the respiratory and gastrointestinal tracts, and its absence in tissues other than skin accounts for the repertoire of extracutaneous manifestations.

Laminins are heterotrimeric extracellular matrix proteins that are composed of α, β, and γ chains. At least 15 isoforms are assembled from 5 α, 3 β, and 3 γ chains. In the kidney, laminin-11 (α5β2γ1) and laminin-1 (α1β1γ1)/laminin-10 (α5β1γ1) are the major laminin components of the glomerular basement membrane (GBM) and the renal tubular basement membrane (TBM), respectively. Findings for laminin β2-null mice showing lethal congenital nephrotic syndrome suggested that laminin-11, which includes the laminin β2 chain, plays an important role in glomerular function.

Although a few studies have shown that laminin-5 is also expressed in the mu-
In this article, we describe an infant with H-JEB complicated by nephrotic syndrome, compositional changes in laminin isoforms of the GBM, and no detectable laminin-5 protein in the renal TBM. This case suggests that laminin-5 may play an important role in renal function and that, in addition to the protein loss from denuded skin and mucous surfaces, massive proteinuria, a previously unreported complication of H-JEB, may contribute to the high mortality rate for H-JEB in early postnatal life.

**CASE REPORT**

A 3500-g male patient, born to unrelated Japanese parents after an uncomplicated term pregnancy, developed widespread blistering of the skin and buccal mucous membranes in areas of friction and trauma within 24 hours after birth. Despite nutritional support and appropriate wound care, the skin lesions progressed, and the patient was transferred to our hospital at 4 months of age.

At the time of admission, extensive denudation and erosion were present on the patient’s occiput, back, buttocks, and extremities, with erosions of the oral mucosa. The fingernails and toenails were absent. Blood examination showed leukocytosis (11 500 cells/mm³ [11.5 × 10⁹ cells per L]), anemia (hemoglobin: 8.6 g/dL [86 g/L]), thrombocytopenia (70 900/mm³ [70 900/L]), and a high level of proteinuria of 2.1 g/d. A high level of hypoalbuminemia (2.5 g/dL [25 g/L] on the 13th hospital day) was present because of suspected sepsis. Despite repeated intravenous antibiotic therapy, nutritional hyperalimentation and repeated intravenous albumin transfusions, massive hypoalbuminemia progressed (1.1 g/dL [11 g/L]) and massive proteinuria continued (albuminuria: 18.9 g/d; urinary NAG level: 3c). Electron-microscopic examination of the renal tissue obtained at autopsy showed glomeruli with obsolescence, with capillary basement membrane wrinkling and segmental shrinking. A wide urinary space was observed frequently. No apparent cell proliferation was noted (Fig 3a). Denudation of the TBM was seen frequently (Fig 3b). In the interstitium, focal mononuclear cell infiltration was observed. Massive luminal dilation of tubules with flattened tubular epithelial cells was observed diffusely (Fig 3c).

To examine expression of laminin-5 in the patient’s kidney, immunohistopathologic analysis was performed on the renal tissue obtained at autopsy. Expression of the laminin β3 and γ2 chains was examined through immunostaining with specific monoclonal antibodies (mAbs) (Fig 5). Although these chains were clearly localized to the TBM in the control specimen (Fig 5, e and f), they were completely absent in the patient’s kidney (Fig 5, d and f), which confirmed the lack of laminin-5 expression at the tissue level. Expression of the laminin α3 chain was also examined through immunostaining with a specific mAb (Fig 5, a and b). Interestingly, the control kidney stained positively for the α3 chain in Bowman’s capsule (Fig 5a), whereas staining in the GBM was apparent in the patient’s kidney (Fig 5b). Because the laminin α3 chain is the subunit polypeptide not only of laminin-5 but also of laminin-6 and laminin-7, expression of the α3 chain in the GBM of the patient’s kidney might indicate the expression of laminin-6 or laminin-7 or the presence of an unreported novel laminin.

Light-microscopic examination of a skin biopsy specimen taken from a newly developed blister on the dorsum of the right foot demonstrated a split at the dermal-epidermal junction, without associated inflammatory cell infiltrates. Electron-microscopic examination showed subepidermal clef formation arising within the lamina lucida, as well as hemidesmosomal hypoplasia (Fig 1).

Two mutations were identified in the LAMB3 gene of the patient, designated 2379delG and Q995X (Fig 2). The parental mutation, 2379delG in exon 17 (Fig 2a), created a frame shift and downstream PTC and predicted premature termination of protein translation. The paternal mutation, Q995X in exon 20 (Fig 2b), caused a stop codon at position 995 of the LAMB3 polypeptide. Both LAMB3 mutations were novel and not reported previously for H-JEB. The results of the electron-microscopic examination of the skin and the PTC mutations in both alleles of the LAMB3 gene, together with the patient’s fatal clinical course, were diagnostic of H-JEB.

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![Fig 1. Electron micrograph of the patient’s skin biopsy specimen, demonstrating cleavage within the lamina lucida and hemidesmosomal hypoplasia.](Image 218x67 to 544x296)
Expression of the laminin α5 chain, a subunit polypeptide of laminin-11, the major laminin component of the GBM, was examined through immunostaining with a mAb specific for this chain (Fig 5, g and h). Expression of the laminin α5 chain was lower in the GBM of the patient’s kidney than in the control kidney, which suggests that the defect in laminin-5 might lead to compositional changes in the GBM by altering the expression of laminin-11, which is essential for the structure of the GBM.

METHODS

Mutation Analysis

Genomic DNA was extracted from the peripheral blood samples obtained from the patient and his parents, after informed consent was obtained. Polymerase chain reaction (PCR) amplification of the LAMB3 exons and flanking intronic sequences, with genomic DNA as a template, was performed with a previously reported strategy.3,12,13 The PCR products were subjected to heteroduplex scanning analysis with conformation-sensitive gel electrophoresis, and all PCR products demonstrating heteroduplexes were sequenced directly with an automated DNA sequencer (ABI 377; Perkin Elmer, Boston, MA). The mutations were verified through digestion with NcoI or PstI restriction enzymes. For detection of the mutation 2379delG, mismatch PCR was performed with a specific primer.

Materials

Renal tissue was obtained from the patient at autopsy and was used with the informed consent of the parents. The renal tissue was fixed in 20% buffered formalin and embedded in paraffin. Sections (4 μm) cut from the paraffin-embedded tissue were used for immunohistochemical detection of the laminin α2 and α5 chains. For immunohistochemical detection of the laminin α3 and β3 chain epitopes, fresh renal tissue was snap-frozen in OCT compound (embedding medium; Sakura Finetek, Tokyo, Japan) and stored at −80°C until use. Renal tissue from a 1-year-old male subject was prepared in a parallel manner and used as a control specimen.

Antibodies

The following mAbs were used in this study: mAb to the laminin α3 chain (P3H9) (Chemicon, Temecula, CA), mAb to the laminin β3 chain (29E), mAb to the laminin γ2 chain (D4B5), and mAb to the
The mAbs 29E and D4B5 were raised against purified human laminin-5 and human recombinant laminin-2 chain (amino acid residues 382–608), respectively, in our laboratory.\textsuperscript{10,14}

**Immunohistochemical Analyses**

For immunohistochemical analyses, the paraffin sections were deparaffinized, rehydrated, immersed in 0.3% hydrogen peroxide-containing methanol for inactivation of intrinsic peroxidase, and treated with protease XXIV (Sigma, St Louis, MO) for 20 minutes at room temperature. The frozen sections were immersed in 0.3% hydrogen peroxide-containing methanol and treated with protease XXIV for 5 minutes.

The paraffin sections were incubated with the anti-\(\gamma_2\) mAb (D4B5) or the anti-\(\alpha_5\) mAb (4C7) at 4°C overnight, whereas frozen sections were incubated with anti-\(\alpha_3\) mAb (P3H9) or anti-\(\beta_3\) mAb (29E) for 20 to 30 minutes at room temperature. The labeled antigen was detected with a HistoFine kit (Nichirei Pharmaceutical, Tokyo, Japan) and observed through the 3,3-diaminobenzidine reaction. Other experimental conditions were as described previously.\textsuperscript{15}

**RESULTS AND DISCUSSION**

Many of the laminin chains are expressed in the GBM and TBM during renal development, under

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Fig 3. (a) Obsolescence (arrowheads) with wrinkling of the capillary basement membrane (arrow), segmental shrinking of the glomerular tuft, and a wide urinary space (periodic acid-Schiff-methenamine-silver stain, counterstained with hematoxylin and eosin; original magnification \(\times400\)). (b) Denudation of the TBM (arrows) (periodic acid-Schiff-methenamine-silver stain, counterstained with hematoxylin and eosin; original magnification \(\times200\)). (c) Massive luminal dilation of tubules with flattened tubular epithelial cells (arrow) (periodic acid-Schiff stain; original magnification \(\times40\)).

Fig 4. (a) Electron micrograph of the patient’s kidney biopsy specimen, demonstrating diffuse fusion of the foot processes (arrowheads) and irregular swelling of the lamina rara interna (arrows) (original magnification \(\times5000\)). (b) Area defined by the black box in a, showing absent endothelial cell fenestrations (arrow) (original magnification \(\times12000\)).
strict temporal control. For example, during development of the nephron, laminin β1 and γ1 chains are found in all nephron basement membranes. At the capillary loop stage, laminin β2 chain begins to accumulate in the developing GBM; as the GBM matures, laminin β1 is eliminated gradually. Through these developmental transitions of laminin isoforms, laminin-1 (α1β1γ1) becomes the major laminin of the mature GBM. Interestingly, some studies showed that laminin-5 is expressed in fetal and newborn mouse kidney, as well as human fetal and neonatal kidney, but not in human adult kidney. However, the function of laminin-5 in the kidney is unknown.

For the infant with H-JEB, we demonstrated that laminin-5 was completely absent in the TBM, apparently because of the LAMB3 mutations 2379delG and Q995X, in contrast to the apparent expression in the TBM of the control kidney. The laminin α3 chain was also expressed aberrantly in the GBM, compared with expression in Bowman’s capsule in the control kidney. Moreover, we demonstrated diminished expression of the α5 chain of laminin-11 in the GBM, compared with the control kidney. Our immunohistopathologic findings depicting the altered expression of laminin chains in the GBM and the lack of laminin-5 in the TBM of the patient’s kidney may account for the massive albuminuria attributable to failure of the glomerular filtration barrier and the extraordinarily high urinary levels of NAG, indicating renal tubular involvement, respectively. We were unable to detect laminin-5 in the GBM of normal kidney, likely because of the restricted expression in the fetal kidney, as reported previously. We speculate that the laminin-5 defect in fetal nephrogenesis might influence the complex developmental transi-

Fig 5. Immunohistochemical detection of laminin α3, β3, γ2, and α5 chains in renal tissue from a normal control subject and the patient. Frozen sections (a, b, c, and d) and paraffin sections (e, f, g, and h) of renal tissue were immunostained with mAbs specific for laminin α1 (a and b), β3 (c and d), γ2 (e and f), or α5 (g and h) chains. The left panels (a, c, e, and g) represent kidney from the normal control subject, whereas the right panels (b, d, f, and h) represent that from the patient. The TBM of the patient lacked detectable laminin β3 (d) and γ2 (f) chains, in contrast to the TBM of the normal control subject, which had clear expression of laminin β3 (c) and γ2 (e) chains. Expression of the laminin α3 chain was detected primarily in Bowman’s capsule in the control kidney (a), whereas expression of the α3 chain in the patient’s kidney was in the GBM (b). Expression of the α5 chain in the GBM of the patient’s kidney (h) was weaker than that in the GBM of the control kidney (g) (original magnification ×400 in a, b, g, and h; ×200 in c, d, e, and f).
tions of laminin isoforms in the GBM. It is well established that a deficiency of the laminin α2 chain is accompanied by compensatory upregulation of the laminin α4 chain in mouse skeletal muscle cells and a deficiency of the laminin α5 chain causes a striking defect in mouse glomerulogenesis. In addition, it has been shown that laminin β2-null mice exhibit a severe nephrotic syndrome in the first postnatal week; although the GBM appears ultrastructurally normal and, at a molecular level, laminin β1 substitutes for β2 in the GBM, the β1 subunit seems to be functionally inadequate. The findings for the β2-null mice are consistent with our present findings, which suggest that compositional changes in laminin isoforms in the GBM can result in failure of the glomerular filtration barrier. It was reported recently that human laminin β2 deficiency causes congenital nephrosis with mesangial sclerosis and distinct eye abnormalities. Our report is the second case study demonstrating that a defect in a laminin subunit causes dysfunction of human renal basement membranes. It is probable that massive proteinuria among patients with H-JEB has been overlooked, although the possibility that the massive proteinuria for our patient is an unusual presentation of H-JEB has not been excluded completely. Collection of urine samples with urine collection bags is almost impossible, because the bag attachment would hurt the fragile skin. We used soft gauze for collection of urine samples. The histomorphologic findings for our patient’s kidney seem more serious than those observed for the kidneys of laminin β2-null mice or human patients with laminin β2 deficiency, which might result from the additional effects of many infectious, metabolic, and/or pharmacologic insults on the fragile GBM of our patient.

The importance of GBM composition for glomerular function is also illustrated by Alport’s syndrome, a human hereditary glomerular nephritis in which a mutation in the collagen α3, α4, or α5 chain gene leads to progressively altered glomerular function. It has been shown that type IV collagen isolated from human Alport’s syndrome kidney containing α1 and α2 chains is more susceptible to endoproteolysis than type IV collagen isolated from normal kidney containing α1 to α6 chains. This observation suggests that the Alport’s syndrome GBM could be more vulnerable to deterioration than the normal GBM. It is likely that the GBM in H-JEB could also be more susceptible to many insults to the patient, such as sepsis, disseminated intravascular coagulation, anemia, malnutrition, hypoxia, and antibiotics, compared with that of normal kidney. Throughout the patient’s clinical course, blood urea nitrogen and creatinine levels, as well as creatinine clearance, remained at normal levels despite massive albuminuria. It is of interest to note that compositional changes in laminin isoforms and collagen isoforms in the GBM cause different types of nephropathy (ie, nephrosis and nephritis, respectively), but the precise mechanisms remain to be explored. Studies of mutant mice that genetically lack laminin-5 could elucidate the underlying mechanism.

Proteinuria and mild hypoalbuminemia (not in the nephrotic range), secondary to focal segmental glomerulosclerosis, have been reported in epidermolysis bullosa with pyloric atresia, an autosomal recessive disease caused by mutations in the genes encoding either 1 of the 2 subunits of α6β4 integrin, which are expressed in the hemidesmosomes of a variety of epithelial tissues, including human skin and the gastrointestinal tract. In the case of epidermolysis bullosa with pyloric atresia, reduced β4 integrin expression was demonstrated in glomerular podocytes. The finding that α6β4 integrin functions as a receptor for laminin-5 supports the functional importance of laminin-5 in human kidney. It has been shown that both laminin-5 and laminin-binding integrins play a role in kidney development and ureteric bud branching morphogenesis in embryonic rats.

From the therapeutic point of view, the observations for our patient strongly suggest that H-JEB should be considered not only a hereditary skin disease but also one of the congenital nephrotic syndromes. Currently, H-JEB among affected infants is invariably lethal within the first 1 year of life. A potential approach for treatment may be combination therapy with meticulous skin care and the treatment protocol proposed for infants with congenital nephritistic syndromes, ie, massive intravenous albumin supplementation and nutritional support (to make normal growth and development possible), followed by bilateral nephrectomy and peritoneal dialysis (to stop protein loss from the kidney) and finally by renal transplantation. Because keratinocyte gene therapy for H-JEB is now under investigation, combination therapy with gene therapy for skin lesions and the aforementioned treatment protocol for congenital nephrotic syndromes might be worth investigating for H-JEB in the near future.

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