Laminin γ3 Chain Binds to Nidogen and Is Located in Murine Basement Membranes

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Recently a novel laminin γ3 chain was identified in mouse and human and shown to have the same modular structure as the laminin γ1 chain. We expressed two fragments of the γ3 chain in mammalian cells recombinantly. The first, domain V/V, consisting of laminin N-terminal (domain VI) and four laminin-type epidermal growth factor-like (domain V) and laminin N-terminal (domain VI) and three laminin-type epidermal growth factor-like (domain V) and laminin N-terminal (domain VI) and self-polymerization into large non-covalent networks, as shown for laminin-1 and laminin-2 (10, 11). This self-assembly is considered to be crucial for the integrity of basement membranes (12). The γ3 chain is a component of four isoforms, laminin-12 (α2β1γ3), -13 (α3β2γ3), -14 (α4β2γ3), and -15 (α5β2γ3) (5, 8). There are interesting theories that these laminins also participate in self-assembly and that they co-polymerize with other isoforms.

Nidogen-1 is a ubiquitously expressed basement membrane protein consisting of three globular domains (G1–G3) connected by a link and a rod-like segment. It has been shown to bind several extracellular proteins through different domains and has been proposed to act as a connecting element for basement membrane assembly (1, 13). The G3 domain of nidogen-1 binds to a single laminin-type epidermal growth factor-like (LE) module of the γ1 chain with high affinity (14, 15), and this binding is thought to be particularly important for basement membrane assembly. Nidogen-2 is another isoform and also binds several matrix proteins with affinities different from nidogen-1 (16, 17), although the binding repertoire is largely overlapping. Site-directed mutagenesis demonstrates the crucial amino acids for the nidogen binding in the laminin γ1 LE module of the γ1 chain with high affinity (14, 15), and this binding is thought to be particularly important for basement membrane assembly. Nidogen-2 is another isoform and also binds several matrix proteins with affinities different from nidogen-1 (16, 17), although the binding repertoire is largely overlapping. Site-directed mutagenesis demonstrates the crucial amino acids for the nidogen binding in the laminin γ1 LE module of the γ1 chain with high affinity (14, 15), and this binding is thought to be particularly important for basement membrane assembly. Nidogen-2 is another isoform and also binds several matrix proteins with affinities different from nidogen-1 (16, 17), although the binding repertoire is largely overlapping. Site-directed mutagenesis demonstrates the crucial amino acids for the nidogen binding in the laminin γ1 LE module of the γ1 chain with high affinity (14, 15), and this binding is thought to be particularly important for basement membrane assembly.

Basement membranes are specialized structures of the extracellular matrix with multiple functions (1). As thin condensed matrices, they divide the cells of the parenchymal tissues from the interstitial matrix. Their main components are collagen type IV variants, laminins, nidogens, and perlecan (2). Laminins constitute a family of heterotrimERIC proteins (αβγ) which are mainly localized in basement membranes and are involved in cell matrix and various other protein interactions. Eleven different chains (α1–α5, β1–β3, γ1–γ3) have been identified and sequenced, and they assemble fifteen different isoforms, laminin-1–15 (3, 4, 5). These chains share a 600-residue domain II/I that oligomerizes into a rod-like coiled-coil structure forming the long arm of laminins. The N-terminal short arms consist of rod-like elements on the basis of tandem arrays of laminin-type epidermal growth factor-like (LE) modules (6) and several globular domains, referred to as laminin N-terminal (LN), L4 modules, and domains IVb (α3B and α5 chains) (IV) (β1 and β2 chains), which have not been classified to date. All α chains share a unique C-terminal G domain, which consists of five laminin G modules (7). Most of these modules are also shared by several other extracellular proteins, such as the proteoglycans perlecan and agrin.

A newly identified γ3 chain shares the same domain structure as the γ1 chain (8, 9). The domain VI (LN module) of laminin γ3 shows the highest sequence identity with that of the γ1 chain. The LN modules of laminin chains are essential for self-polymerization into large non-covalent networks, as shown for laminin-1 and laminin-2 (10, 11). This self-assembly is considered to be crucial for the integrity of basement membranes (12). The γ3 chain is a component of four isoforms, laminin-12 (α2β1γ3), -13 (α3β2γ3), -14 (α4β2γ3), and -15 (α5β2γ3) (5, 8). There are interesting theories that these laminins also participate in self-assembly and that they co-polymerize with other isoforms.

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the γ3 chain is a non-base membrane-associated laminin chain (8). The laminin isoforms containing the γ3 chain are widely expressed in different compartments of the retina (5).

For this study, we prepared recombinant fragments of the laminin γ3 chain and compared the binding properties with those of the γ1 and γ2 chains. These fragments allowed us to raise specific antibodies that are useful for quantitative analyses and examination of the distribution of the γ3 chain in tissues. We systematically localized the laminin γ1 and γ3 chains during mouse organogenesis from day 12 to day 18 and in adult organs at the light microscopic level as well as at the ultrastructural level and found it to be a true basement membrane component. In addition, we performed immunogold double labeling and showed a co-localization of the laminin γ3 chain and nidogen-1 in basement membranes in vivo.

Experimental Procedures

Sources of Proteins

Mouse (20) and human nidogen-1 (21) and mouse (17) and human nidogen-2 (16) were isolated as recombinant products. Laminin-1 was purified from the mouse Engelbreth-Holm-Swarm tumor (22). The laminin-1 fragments E4 and P1 were purified as described previously (23). The recombinant fragment γ1 III3–5 (14) and γ2 N, γ2 LE4–6, and β3 VI/V (24) have already been described. Other recombinant fragments of mouse laminin included β1 VI/V, β2 VI/V, and γ1 VI/V (Fig. 1), which were prepared by established procedures (25).

Production of Recombinant Proteins

The templates used were mouse γ3 cDNA clone γ3.3PB provided by Prog. Dr. K. Tryggvason for γ3 III5–5 and RNA from mouse testis for γ3 VI/V fragment. The sense and antisense primer for γ3 III3–5 were 5′-GTCAGCTAGCCCATGTGCTGAGCCG-3′ and 5′-GTCACTCG-AGCTGAGAGCAGGCCCTCCC-3′, respectively, and for γ3 VI/V, 5′-GTCACTCTGGCAGCCCCTCCC-3′ and 5′-GTCACTGAGCT-AGTCGAGCCCATCGAG-3′, respectively. They were used for amplification by PCR. These primers introduced at the 5′ end a NheI site and at the 3′ end a stop codon followed by a XhoI site. A NheI/XhoI fragment for γ3 III5–5 as well as that for γ3 VI/V was inserted into the episomal expression vector pCEP-Pu containing the BM-40 signal peptide (26), which was prepared by established procedures (25).

Protein Interaction Assays and Laminin-1 Polymerization Inhibition Assay

Solid phase assays with laminin fragments immobilized on the plastic wells of microtiter plates and soluble nidogens followed published procedures (27). Binding was detected by antibodies specific for each nidogen isoform. The radioligand inhibition assay with [3H]labeled laminin fragment P1 and recombinant nidogen-1 (14) was also used. The laminin-1 polymerization inhibition assay as well as surface plasmon resonance assays (BI-Accore 1000, Stevenage, Hertfordshire, UK) were carried out as described previously (25).

Immunological Assays

Immunization of rabbits, affinity purification of antibodies, enzyme-linked immunosorbent assay titration and inhibition radioimmunoassays were carried out using established protocols. Extraction of mouse tissue was performed with neutral buffer containing 10 mM EDTA followed by the same buffer containing detergents, and both buffers contained protease inhibitors (28).

Animals

Female New Mexico Research Institute (NMRI) mice were kept on a normal day/night cycle and received Altromin commercial food and water ad libitum. The day on which, at 11:00 a.m., a vaginal plug was detected after a mating period of 3 h, was designated as day 0 of gestation. On the respective days of gestation, beginning with day 12, pregnant mice were anesthetized with ether and sacrificed by cervical dislocation. After dissection of the uterine horns, the embryos (day 12) or fetuses (days 14, 16, and 18) were removed. Three embryos or fetuses of each developmental stage were investigated. For light microscopic analysis of adult tissues, the various organs from three different three-month-old NMRI mice were taken.

Immunohistochemistry

Fixation and Preparation of Tissues—For light microscopy, all specimens were fixed by immersion in 4% paraformaldehyde in phosphate buffer, pH 7.2, at 4 °C. They were then dehydrated in an ascending series of ethanol from 30 to 100% and embedded in paraffin (29). Serial sections of 5 μm were cut with a Reichert's microtome. Every fifth section was stained with hematoxylin for topological orientation within the anatomical regions examined, and staging of the embryos or fetuses was achieved by comparison with the appropriate Theiler stages. For the ultrastructural approach, the tissue pieces were fixed in 4% paraformaldehyde and 0.5% glutaraldehyde for 15 min, dehydrated in a graded series of ethanol up to 70%, and embedded in the acrylic resin LR-Gold (London Resin Company, Reading, UK). Semithin (1-μm) and ultrathin (0.8-μm) sections were cut according to procedures previously described in detail (29).

Light Microscopic Immunohistochemistry—For the light microscopic immunohistochemistry, sections were deparaffinized, rehydrated, and rinsed for 10 min in PBS. Endogenous peroxidase was blocked by incubation in 3% H2O2 in methanol for 45 min in the dark. Each of the reaction steps was followed by rinsing for 10 min in PBS. The sections were pretreated for 5 min with 10 μg/ml protease XXIV (Sigma). The anti-laminin γ1 antibody, the anti-laminin γ3 III3–5 antibody, and the laminin γ3 VI/V antibody were used at a dilution of 1:100 for 1 h at room temperature. The anti-nidogen-1 antibody was also used at a dilution of 1:100 for 1 h at room temperature. The peroxidase-anti-peroxidase method followed the previously described procedures (29). As negative controls, normal rabbit IgGs and the corresponding preimmune sera were used instead of the primary antibodies, at similar concentrations. No immunostaining was observed.

Immunogold Histochemistry—For single labeling using immunogold histochemistry, the tissue sections were incubated for 1 h at 4 °C with the antibodies against laminin γ3 VI/V and laminin γ3 III3–5. The sections were rinsed in PBS and incubated with the 16-nm gold-coupled goat anti-rabbit IgG diluted 1:20 in PBS for 16 h at 4 °C. Thereafter, the sections were rinsed with water and stained with uranyl acetate (10 min) and lead citrate (5 min). The sections were examined with a LEO 9106E electron microscope. For double labeling, all sections were incubated for 5 min at room temperature with 1% bovine serum albumin in PBS and then rinsed in PBS. Thereafter, anti-laminin γ3 antibody diluted 1:100 in PBS was applied for 1 h at 4 °C. After a rinse with PBS, the gold-coated (16-nm) goat anti-rabbit antibody diluted 1:200 was applied for 20 min at room temperature. The sections were thoroughly rinsed with PBS. The gold-coated (8-nm) anti-nidogen-1 antibody diluted 1:100 in PBS was then incubated. Colloidal gold particles were prepared and coupled to the antibodies according to our standard protocols (13). All sections were finally rinsed with water and stained with uranyl acetate (10 min) and lead citrate (8 min). As the control for the double labeling experiments, we also applied a monoclonal anti-nido- gen-1 antibody (JF4). This approach avoids possible cross-links between the two rabbit IgGs used in the double labeling described above and yielded identical results.

Results

Expression and Purification of Recombinant Proteins—Previous recombinant studies with the LN module of the α1 (30) and the β1 and γ1 chains have shown that this module does not represent an autonomous folding unit and requires the addition of several adjacent, rod-like LE modules from domain V to achieve efficient production in mammalian cells. Therefore, we prepared the LN module of the γ3 chain together with the complete domain V (four LE modules) (position 29–488), as already performed for the γ1 VI/V fragment. Domain γ3 III3–5 (three LE modules) corresponds to amino acid 766–927 of the

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2. T. Sasaki, unpublished data.
laminin \(\gamma_3\) chain, a region homologous to the laminin \(\gamma 1\) chain exhibiting a high affinity binding site for nidogen-1. These fragments were produced in human 293-EBNA cells transfected with episomal expression vectors (26). These cells secreted the recombinant fragments in sufficient amounts into serum-free culture media, which were used for purification by conventional chromatography. The purified fragments showed a single 55-kDa band for domain \(\gamma 3\) VI/V (Fig. 1, lane 1) and a 23-kDa band for domain \(\gamma 3\) III3–5 (Fig. 1, lane 5) in SDS-polyacrylamide gel electrophoresis. The purity was also confirmed by a single N-terminal ADMGS(C)YDGV sequence for the 23-kDa band for domain \(\gamma 3\) III3–5, the first four residues being derived from the signal peptide cleavage region of the vector.

**Inhibition of Laminin-1 Polymerization**—A major function of LN modules is the promotion of self-assembly of laminins into non-covalent quasihexagonal networks in a Ca\(^{2+}\)-dependent fashion, as shown for laminin-1 (10) and subsequently for \(\alpha 2\) chain-containing laminins (11). In an earlier experiment, we developed a quantitative inhibition radioimmunoassay to study the interference of the different \(\alpha\) chain fragments (25). In the present study, we used this assay for the \(\beta\) and \(\gamma\) chain fragments. The assay was designed to allow the polymerization of 30–38% of laminin-1 (subsequently set to 100%), which was reduced to only a few percent in the presence of EDTA (Table I). The inhibitors were added in stoichiometric amounts (0.32 \(\mu\)M) or in five-fold molar excess (1.6 \(\mu\)M) prior to starting the polymerization. Lower activities for \(\beta 2\) VI/V and \(\gamma 3\) VI/V and distinctly low activity for \(\beta 2\) VI/V at both concentrations were observed (Table I). As a positive control, we used the laminin E4 fragment corresponding to \(\beta 1\) VI/V, which was used in previous inhibition studies (10), and found activity approximately identical to that of recombinant \(\beta 1\) VI/V and \(\gamma 1\) VI/V. Surface plasmon resonance assays were used to examine the binding to immobilized laminin-1. This demonstrated a similar binding of \(\gamma 1\) VI/V (\(K_d = 0.245 \text{ \(\mu\)M}\)) and \(\beta 1\) VI/V (\(K_d = 0.275 \text{ \(\mu\)M}\)), and lower affinities were observed for \(\beta 3\) VI/V (\(K_d = 1.05 \text{ \(\mu\)M}\)) and for \(\gamma 3\) VI/V (\(K_d = 1.75 \text{ \(\mu\)M}\)). No binding was detected for \(\beta 2\) VI/V up to the highest concentration used (4 \(\mu\)M).

**Binding to Nidogens**—The mouse laminin \(\gamma 1\) chain fragment \(\gamma 1\) III3–5 was shown to bind to nidogen-1 with high affinity (14). Most of the amino acids that interact with the nidogen G3 domain are conserved in human and mouse laminin \(\gamma 3\) chains (19), suggesting that the \(\gamma 3\) chain also binds to nidogens. We prepared the mouse laminin \(\gamma 3\) III3–5 fragment to compare the binding to nidogen isoforms with that of \(\gamma 1\) and \(\gamma 2\) chains. Solid phase assays were used to test the binding of nidogen-1 and nidogen-2 to immobilized \(\gamma 1\) III3–5 (Fig. 2A) and \(\gamma 3\) III3–5 (Fig. 2B). Both mouse nidogen isoforms bound better to \(\gamma 1\) III3–5, and the half-maximal binding for mouse nidogen-1, human nidogen-1, mouse nidogen-2, and human nidogen-2 were 0.2–0.3, 0.4–0.5, 1–2, and 3–4 nM, respectively. The concentrations of half-maximal binding to the \(\gamma 3\) III3–5 fragment were calculated, 0.8–1 nM for both types of nidogen-1 and 20–30 nM for both types of nidogen-2. No nidogen isoforms showed any binding to the \(\gamma 2\) LE4–6 fragment, the homologous region of laminin \(\gamma 2\) chain in the solid phase assay. The inhibitory activity of these \(\gamma\) chain fragments on the interaction between laminin fragment P1 and nidogen-1 was analyzed by radioligand inhibition assays (Fig. 3). The inhibitory activity of the \(\gamma 1\) III3–5
fragment was identical to the P1 fragment, and the concentration required for 50% inhibition (IC_{50}) was found to be 0.08 nM. The IC_{50} of γ3 III3–5 was 20 nM and that of γ2 LE4–6 was >10 μM. Surface plasmon resonance assays were performed using III3–5 fragments as immobilized ligands. Nidogens showed high affinity binding to γ1 III3–5 but no binding was seen to γ3 III3–5 (data not shown).

Immunological Assays—Rabbit antisera were prepared against recombinant γ3 VI/V and γ3 III3–5 fragments. These antisera had high titers against the antigen used for immunization and were only marginally cross-reactive with the fragments obtained from γ1 and γ2 chains by enzyme-linked immunosorbent assay (data not shown) and by inhibition radioimmunoassays (Fig. 4). Several tissues from laminin γ3 knock-out mice tested for both antibodies did not show any staining (data not shown).

In inhibition radioimmunoassays, the half-maximal inhibitions were achieved at 0.1 nM for γ3 VI/V (Fig. 4A) and at 0.06 nM for γ3 III3–5 (Fig. 4B). A >1000-fold excess of homologous fragments obtained from other γ chains did not show inhibition. Some adult mouse tissues were extracted with EDTA and detergents and examined by this assay together with the γ1 VI/V assay. However, mouse tissues showed inhibitions in the γ3 VI/V assay but not in the γ3 III3–5 assay. Therefore, the amount of the γ3 chain was quantified by the γ3 VI/V assay. The much lower amounts of the γ3 chain (1–6% of the γ1 chain of laminin) were found in the tissue extracts examined (Table II).

Immunolocalization of Laminin γ1 and γ3 Chains during Embryonic Development and in Adult Tissues—From day 12 to day 18, and in adult mouse tissue, staining for the laminin γ1 chain was seen in almost all epithelial basement membrane zones in all consecutive stages of development, as already known for a long time (Table III). In the skin, for example, the dermal-epidermal basement membrane zone exhibited staining, as did the basement membrane zones of hair follicles, but neither the fibroblasts nor the keratinocytes showed any staining (Fig. 5A). In all stages of kidney organogenesis, only basement membrane zones of the consecutive stages of glomeruli development (comma, S-shaped, early glomeruli) and those of the tubules were positive for the γ1 chain (Fig. 5B). In the small and large intestine, the epithelial cells were not stained, whereas the basement membrane zones underlying the developing epithelium were stained for the γ1 chain (Fig. 5C).

From day 12 to day 18, and in adult mouse tissue, staining for the laminin γ3 chain was seen in basement membrane zones of capillaries, neuroectoderm, and choroid plexus in all consecutive stages of brain development. In addition, the endothelial basement membrane zones of capillaries and larger blood vessels revealed staining for the protein. In the skin, the dermal-epidermal basement membrane zone and the basement membrane zones of hair follicles were positive for the γ3 chain (Fig. 5D). The endoneurium of peripheral nerves also stained positive for the γ3 chain. In the kidney, the basement membrane zones of developing glomeruli (comma, S-shaped, early

![Image](http://www.jbc.org/)

**Table II**

| Tissue   | γ1 VI/V | γ3 VI/V |
|----------|---------|---------|
| Skin     | 42      | 2.6     |
| Lung     | 145     | 1.8     |
| Stomach  | 163     | 2.0     |
| Intestine| 132     | 1.3     |
| Kidney   | 69      | 2.1     |
glomeruli) and tubules were positive for the γ3 chain (Fig. 5E).

The same was true for the basement membrane zones underlying the developing epithelium of the small and large intestine (Fig. 5F). In all stages of testis organogenesis, only basement membrane zones of tubuli seminiferi were stained for the γ3 chain.

Ultrastructural Localization of Laminin γ3 Chain during Mouse Development and in Adult Tissues—Affinity-purified antibodies specific for γ3 VI/V and γ3 III3–5 were also used for ultrastructural localization of laminin γ3 chain in mouse tissues by immunogold staining (Fig. 6). The staining was observed in the basement membranes of developing brain arteriole (Fig. 6A), in those of developing tubules in the kidneys (Fig. 6B), and also in muscle basement membranes (Fig. 6C).

We also performed double labeling for a potential binding partner, nidogen-1. Laminin γ3 chain was co-localized with nidogen-1 in the basement membranes of the adult mouse kidney (Fig. 7). A co-localization was classified as a potential molecular contact when the distance between the different sizes of gold label (8 or 16 nm) was <30 nm (31). Fig. 8A demonstrates the mean values of nidogen-1 (Fig. 8A, lane a), laminin γ3 chain (lane b), and co-localization of nidogen-1 and the γ3 chain (lane c). The γ3 chain is nearly always co-localized with nidogen-1 (Fig. 8A, lane c). Fig. 8B visualizes the mean values of unspecific (Fig. 8B, lane a) and specific (Fig. 8B, lane b) reactions. The difference between the co-localization rates of the laminin γ3 chain with nidogen-1 was statistically significant with p values < 0.001 (Fig. 8B, lane b, asterisk).

DISCUSSION

Recently, the γ3 chain of laminin was identified by protein chemistry (8) and by a homology search from the sequence tag data base (9). Sequence comparison with the laminin γ1 chain predicts that they have similar functional activities. We have now expressed two fragments of the γ3 chain to compare them...
with those of the γ1 and γ2 chains. These fragments were also used to raise specific antibodies against the γ3 chain.

The important step for the supramolecular organization of basement membranes is the formation of two independent networks of collagen type IV and laminins, which are connected to each other and stabilized by interactions with nidogens, perlecans, and other proteins (1, 4, 12). The polymerization was initially shown for laminin-1 from the Engelbreth-Holm-Swarm tumor. This process is dependent on the concentration (>1 μM), temperature (>30°C), and Ca²⁺ and could be reversed by EDTA and cooling, demonstrating a non-covalent interaction (32, 39). At high concentrations (>1 μM), laminin-1 forms a quasisixagonal network similar to those also found in situ. The N-terminal globular domains of all three laminin-1 chains are involved in the polymerization, as shown in inhibition studies with proteolytic fragments (10, 34–36). Later, it was shown that α2 chain-containing laminins (laminin-2 and -4) can also self-assemble and co-polymerize with laminin-1, but laminin-5 (α3β3γ2) and -6 (α3β1γ1) cannot (11). Up to now, 10 more laminin isoforms are known, but they are not available to perform such analysis. Therefore, we have used domain VI/V fragments to evaluate their activity in laminin-1 polymerization inhibition assays. The previous study on VI/V fragments from different α chains demonstrated a high activity of α3B VI/V and α5 VI/V fragments, 50–60% inhibition of laminin-1 polymerization being achieved at equimolar concentrations of laminin-1. However, a five-fold molar excess was required for α1 VI/V, α2 VI/V, and β1 VI/V to produce comparable effects (25). The present data show that β2 VI/V can inhibit only ~10%, and 30–40% inhibitions are found for β3 VI/V and γ3 VI/V at five-fold molar excess. The data on β1 and γ1 fragments agree well with previous data (10, 11, 35). These data suggest that laminin-13, -14, and -15, consisting of β2 and γ3 chains together with different α chains (α3 chain for laminin-13, α4 chain for laminin-14, and α5 chain for laminin-15) may not co-polymerize, at least not with laminin-1. Recently, recombinant VI/V fragments from eight laminin chains (but not the α3B chain) have been expressed, and homophilic and heterophilic interactions have been analyzed using various techniques (37). It has been shown that most of the LN domains derived from α chains and β chains interact with each other homotypically, except for the β1 chain-derived LN domain, which exhibits no self-interaction. However, the γ chain LN domains showed limited interactions without self-interactions and especially the γ3 LN domain interacted only with β2 and β3 LN domains. Our inhibition data indicated that β2, β3, and γ3 LN domains have no binding or only low binding to α1, β1, and γ1 LN domains. In this respect, our data contradict earlier assumptions (37). According to the three-arm interaction model for laminin self-assembly, they speculate that the γ3 chain-containing laminins may not form tight networks. This possibility should be tested with isolated laminin isoforms. In Table II, the sum of the γ1 and γ3 chains extracted sequentially with neutral buffer containing EDTA followed by detergents is shown. 70–80% of the γ1 chain-containing laminins were extracted by EDTA, but only 30–40% of the γ3 chain was extracted in the same extract, except from skin and intestine (69–75%). These data suggest that the γ3 chain-containing laminins may integrate into tissues by a different mechanism.

The laminin γ1 chain has a high affinity binding site for nidogen-1 (14), and the interface between the γ1 IILE modules and nidogen-1 G3 domain was revealed by the crystal structure of these complexes (19). The γ1 IILE4 module docks to an amphitheatre-shaped concave surface on the pseudo-six-fold
axis of the β-propeller in nidogen-1 with a complementary shape and a small interface. The LE3 module binds over its rim. The key interactions are mediated by the LE4 module as predicted from mutagenesis (18), and the contribution of the LE3 module to the binding is rather small (14). The interaction sites in the LE4 module are Asp-800, Asn-802, Val-804, Arg-809, and Arg-816, and the first three amino acids occupy about 60% of the interface. Asn-802 and Val-804 are changed to Ser in the human chain, and Val-804 is changed to Ser in the mouse chain, showing a low binding to nidogen-1 or none at all (21, 24, 38). These amino acids, except for the mutation R809P, are conserved in human and mouse chains, and the interaction sites are also conserved in nidogen-1 and -2 from human and mouse, which is a reason to predict that the γ3 chain binds to both nidogens with affinities similar to the γ1 chain.

Contrary to the previous report that the laminin γ3 chain is only localized apically (8), the systematic light microscopic localization of the laminin γ3 chain from day 12 to day 18 and in adult tissues shows that the protein is very tissue- and cell-specific and can always be localized in close proximity to basement membranes. The laminin γ3 chain can be localized in basement membrane zones of brain, skin, kidney, testis, and endoneurium (Table II). However, basement membranes are ultrastructures and only an ultrastructural method can undoubtedly confirm whether or not a protein is a true basement membrane component. Electron microscopic immunohistochemistry revealed that the laminin γ3 chain is localized in tissue- and cell-specific basement membranes in adult tissues, as well as during development.

We performed immunogold double labeling of the laminin γ3 chain and nidogen-1. This semi-quantitative method revealed
that ~98% of the detectable laminin γ3 chain is co-localized with nidogen-1 in basement membranes in vivo. In general, these results indicate that the laminin γ3 chain has similar cell biological functions as the laminin γ1 chain and might form laminin networks via nidogen-1 and -2 (29). However, despite the proposed similar functions of the laminin γ1 and γ3 chains, during early developmental stages, the laminin γ3 chain cannot compensate for the absence of the laminin γ1 chain, as laminin γ1 knock-out mice die at a very early embryonic stage (39).

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